

OLYMPUS®

CONFIGURATION

- I . SAFETY GUIDE (Another manual)
- II . SPECIFICATIONS (Another manual)
- III . SYSTEM OVERVIEW
- IV . OPERATION INSTRUCTIONS
- V . MAINTENANCE
- VI . TROUBLESHOOTING

User's Manual

FLUOVIEW FV300

CONFOCAL LASER SCANNING BIOLOGICAL MICROSCOPE

Ver 4.3a

Petition

This user's manual is for the software to be run on Olympus FLUOVIEW FV300 Confocal Laser Scanning Biological Microscope. To ensure safety, obtain optimum performance and familiarize yourself fully with this product, we recommend that you study this manual thoroughly before operation.

This user's manual is composed of two volumes including "SYSTEM OVERVIEW", "OPERATION INSTRUCTIONS", "MAINTENANCE" and "TROUBLESHOOTING". Together with this manual, please also read the "User's manual FLUOVIEW FV300" and the instruction manual of the microscope in order to understand overall operation methods. To ensure the safety operation of laser system, we recommend you to study the manual of each laser and the light source equipment besides this manual. Retain this manual in an easily accessible place near a system for future reference.

AX6338

CAUTION

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FLUOVIEW MANUAL CONFIGURATION

The FLUOVIEW system uses two manuals including this “User’s Manual” and the on-screen manual built into the software (“Online Help”).

The User’s Manual is composed of the five following volumes and subject matter:

I. SAFETY GUIDE (Another manual)

Describes notes and cautions on using the FLUOVIEW system and on types of warning labels.



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2 WARNING LABELS I 2-1

3 CAUTION FOR SYSTEM OPERATION I 3-1

4 USER’S SAFETY PROTECTION MEASURES ACCORDING TO IEC60825-1:1993+A1:1997+A2:2001 “LASER PRODUCT RADIATION SAFETY STANDARD” I 4-1

II. SPECIFICATIONS (Another manual)

Describes the specifications of the FLUOVIEW system.

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III. SYSTEM OVERVIEW (This manual)

Describes the outline of the FLUOVIEW system.

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IV. OPERATION INSTRUCTIONS (This manual)

Describes the operation procedures of the FLUOVIEW system, for example, methods for image acquisition and various image processing.

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V. MAINTENANCE (This manual)

Describes maintenance of the FLUOVIEW system.

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VI. TROUBLESHOOTING (This manual)

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For Online Help, please see “1-3 Online Help” in “OPERATION INSTRUCTIONS” of this manual.

NOTATIONS IN THIS MANUAL

NOTATIONS IN THIS MANUAL

This manual complies with the following notations.

✧ Notation of Caution, Notes and Tips

Notation	Description
	Caution to prevent injuries to the user or damage to the product (including surrounding objects).
	Note for the user.
	Hint or one-point advice for user reference.

✧ Notation of panel, Command Buttons and Dialog Boxes

Notation	Description
[Acquire] panel	The name of a panel, dialog box, list box or check box is enclosed inside square brackets ([]).
<OK> button	The name of a button in a panel or dialog box is enclosed inside angled brackets (< >).
<Open File> button	

✧ Notation of Mouse Operations

Notation	Description
clicking	Action of pressing, then immediately releasing the mouse button.
double-clicking	Action of clicking the mouse button twice in quick succession.
dragging	Action of moving the mouse while holding down the mouse button, then releasing the mouse button at the desired destination.

(Note) In this manual, clicking, double-clicking and dragging involves pressing the left button of the mouse, unless otherwise specified.



✧ Notation of key operations

Notation	Description
<input type="text" value="Enter"/>	The name of a key is enclosed inside <input type="text" value=""/> .
<input type="text" value="Alt"/> + <input type="text" value="F1"/>	The positive sign (+) expresses the combination of more than one key operation. For example, <input type="text" value="Alt"/> + <input type="text" value="F1"/> refers to pressing the <input type="text" value="F1"/> key while holding the <input type="text" value="Alt"/> key down.
Direction keys	Generic names given to the <input type="text" value="→"/> , <input type="text" value="←"/> , <input type="text" value="↑"/> and <input type="text" value="↓"/> keys.

✧ Notation of system-specific terms

Notation	Description
XY observation (Other observations)	Refers to observation with XY scanning. (The same principle also applies to other observations such as XZ, Xt, XYZ, XYt and XYZt.)

Note that some of the panels and dialog boxes shown in this manual are not the precise reproductions of the originals. Some windows are resized to facilitate the reading and some grayed-out characters are printed in readable characters.

III. SYSTEM OVERVIEW

On This Volume

This volume describes the outline of the FLUOVIEW FV300 system.

Please read this volume so that you can understand the system before use.



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1 SYSTEM OVERVIEW

Olympus FLUOVIEW is a confocal scanning type laser reflected fluorescence microscope with high resolution, high contrast and drastically improved resolution in the light axis direction thanks to the use of confocal optics. It offers researchers features for sectioning, 3D construction and time-series observation as well as a variety of image processing and analysis functions.

This section describes the principle and characteristics of the system and the functional configuration of the software used with the system.

1-1 Principle

A laser-scanning microscope concentrates a laser beam on a very small spot using an objective and scans the specimen in the X-Y directions.

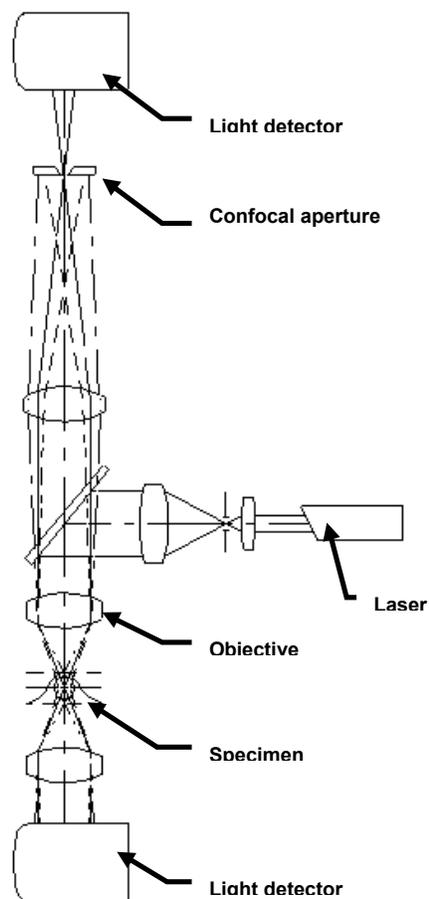
It then detects the fluorescence and transmitted light from the specimen using light detector and outputs the image of the specimen on an image monitor.

The confocal optics place a confocal aperture at a position which is optically conjugate with the focusing position (i.e. confocal plane) in order to eliminate light from parts other than those for the focusing position. As a result, the part corresponding to the eliminated light is darkened in the image, making it possible to optically slice a thick tissue specimen.

On the other hand, with ordinary optical microscopes, light from a part other than the focusing position are overlapped from the image-forming light on the focusing position, and the overall image tends to be vague.

Transmitted light images can be obtained by use of a transmitted light detector, but the images will not be confocal image, because there is no confocal aperture in front of the detector.

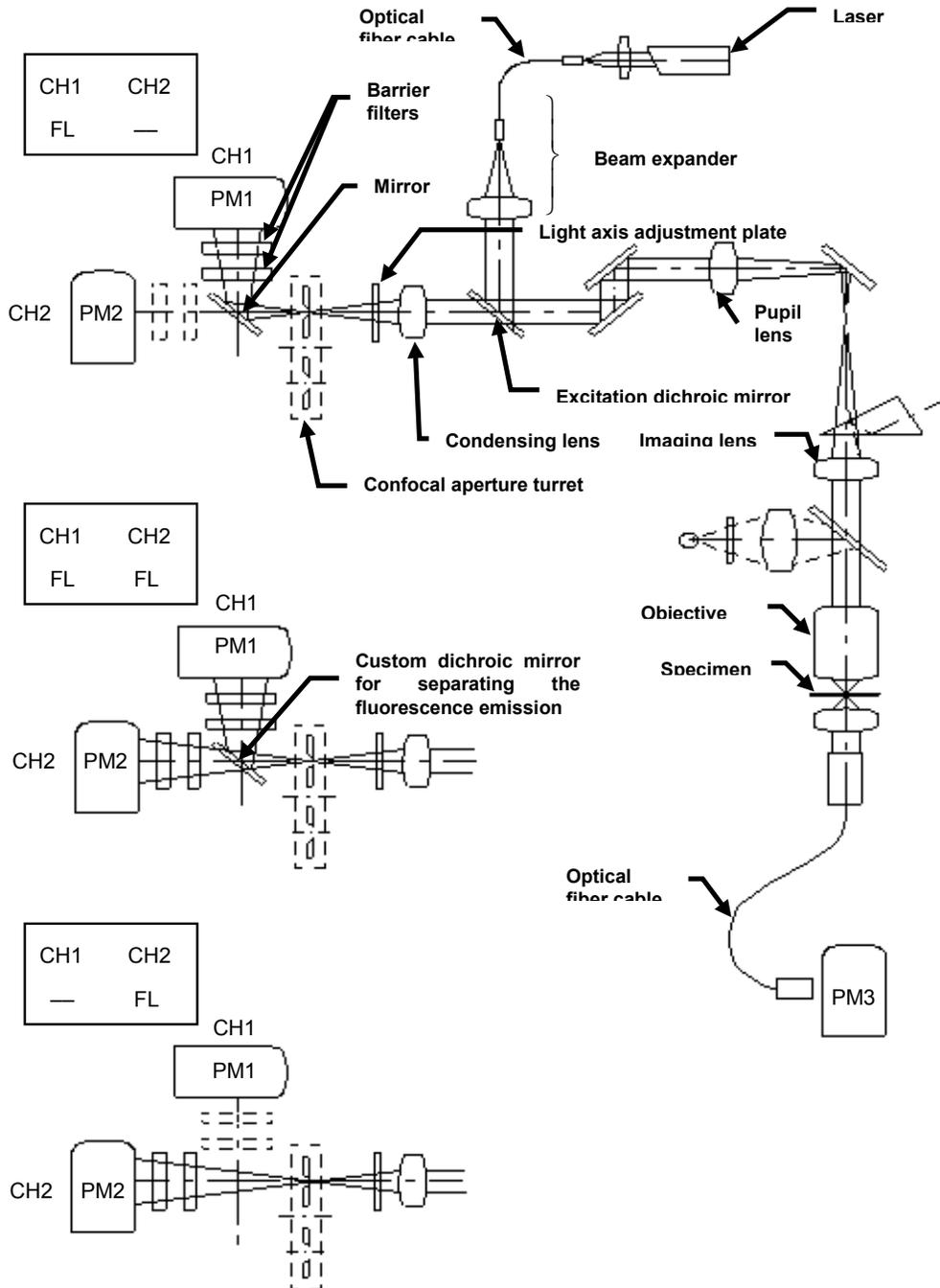
The transmitted light image can be used by combination with fluorescence image to observe fluorescent localization.



1-2 Features of FLUOVIEW FV300

1. The detector has 12-bit resolution and is capable of detecting very small changes in fluorescence inside cells.
2. The system has high resolution of a maximum 2048 x 2048 pixels. The output video signal is non-interlaced to provide a clear image without flickering.
3. The transmitted light is detected by a photomultiplier to offer a bright, sharp transmitted image.
4. The auto gain adjustment feature eliminates the need for complicated gain adjustment operation.
5. A total of 3 image channels, including up to 2 fluorescence images and a transmitted image, can be acquired simultaneously.
6. The confocal aperture can be selected from 5 positions so the optimum confocal aperture for each objective can be selected with one-touch operation.
7. High-speed scanning acquires 4 images per second (512 x 512 pixels).
8. Sequential scan for acquiring images without crosstalk.
9. In addition to raster scanning, scanning modes such as vector scanning and oblique scanning are provided to meet a wide range of applications.
10. When using the system with AOTF (FV5-COMBA), the following functions are available.
 - Line Kalman scanning
 - Line sequential scanning
 - Image acquisition in the REX/Bleach mode(FRAP experiment)
11. One of the two DMs can be selected using the excitation DM selector knob.
12. The ports for introducing the fiber are provided on two positions, that is, on the rear and left side panels of the scan unit. (The port on the left side panel accepts only the cable for laser wavelengths of 430 nm or less.)

1-3 Light Path Diagram



1-4 System Configuration

1-4-1 System Component Units and Their Functions

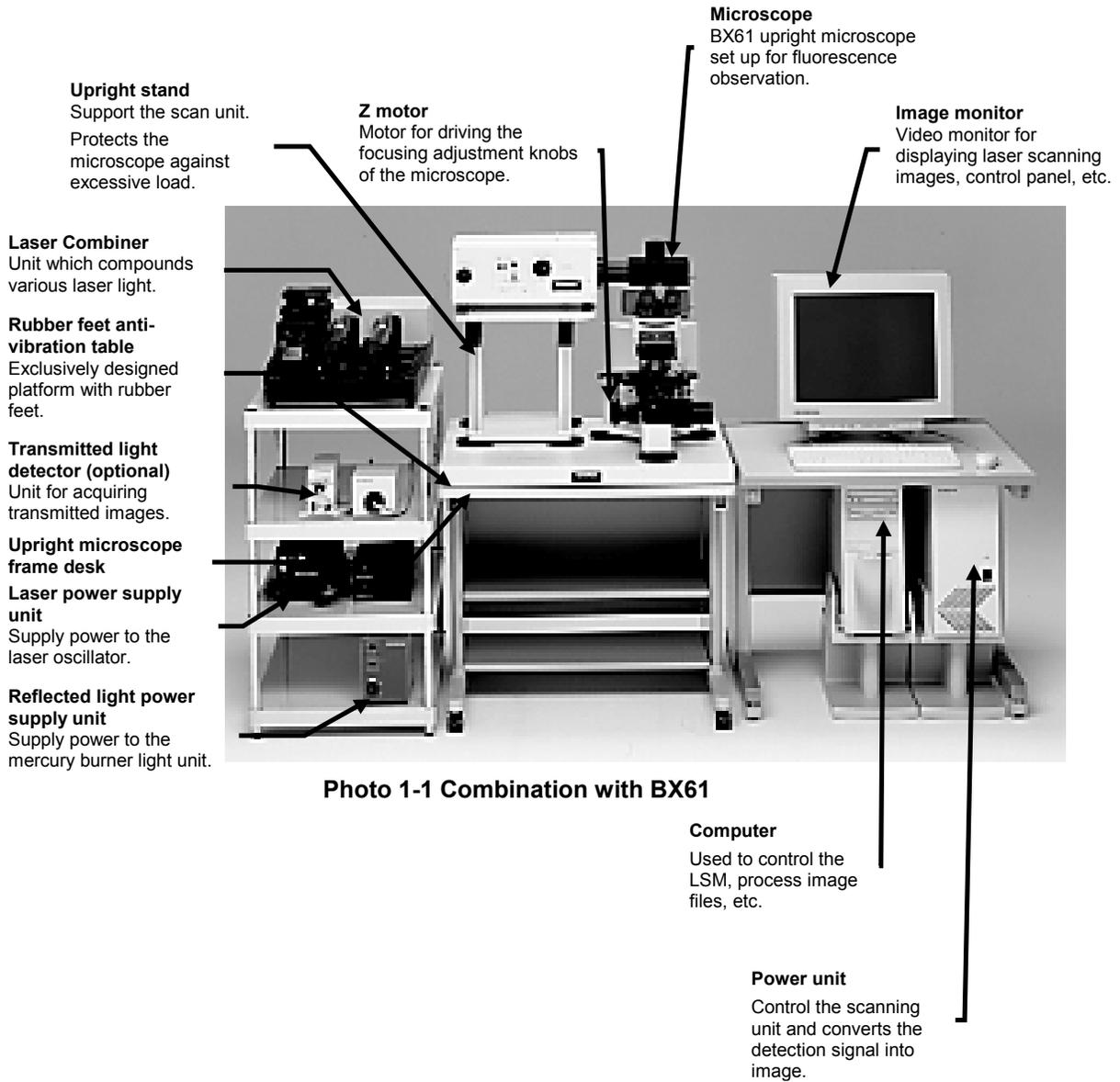


Photo 1-1 Combination with BX61

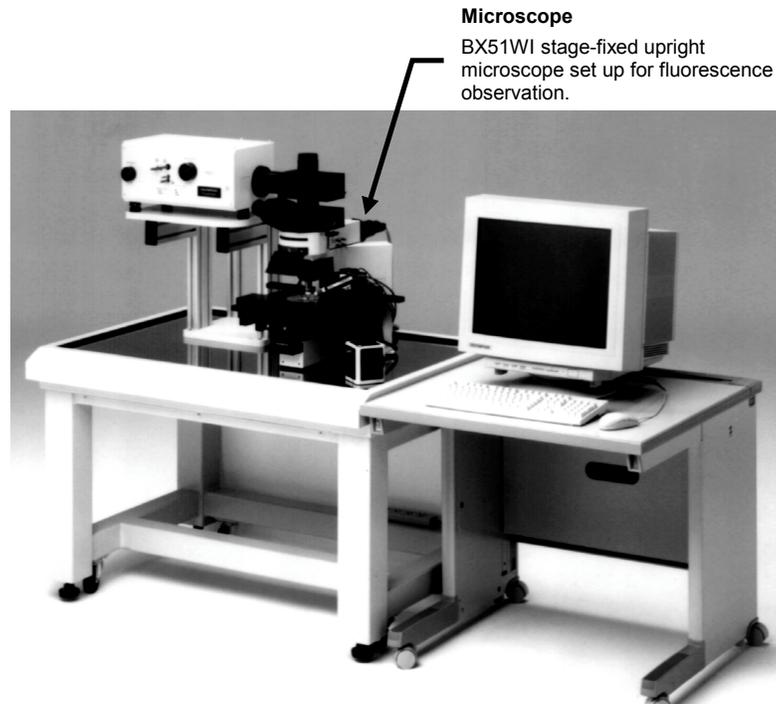


Photo 1-2 Combination with BX51WI

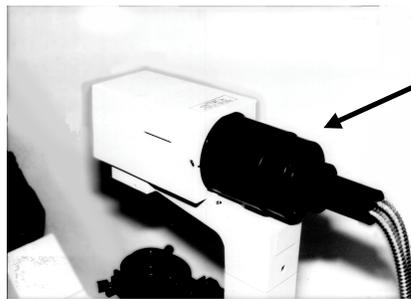


Microscope

IX81 inverted microscope set up for fluorescence observation.



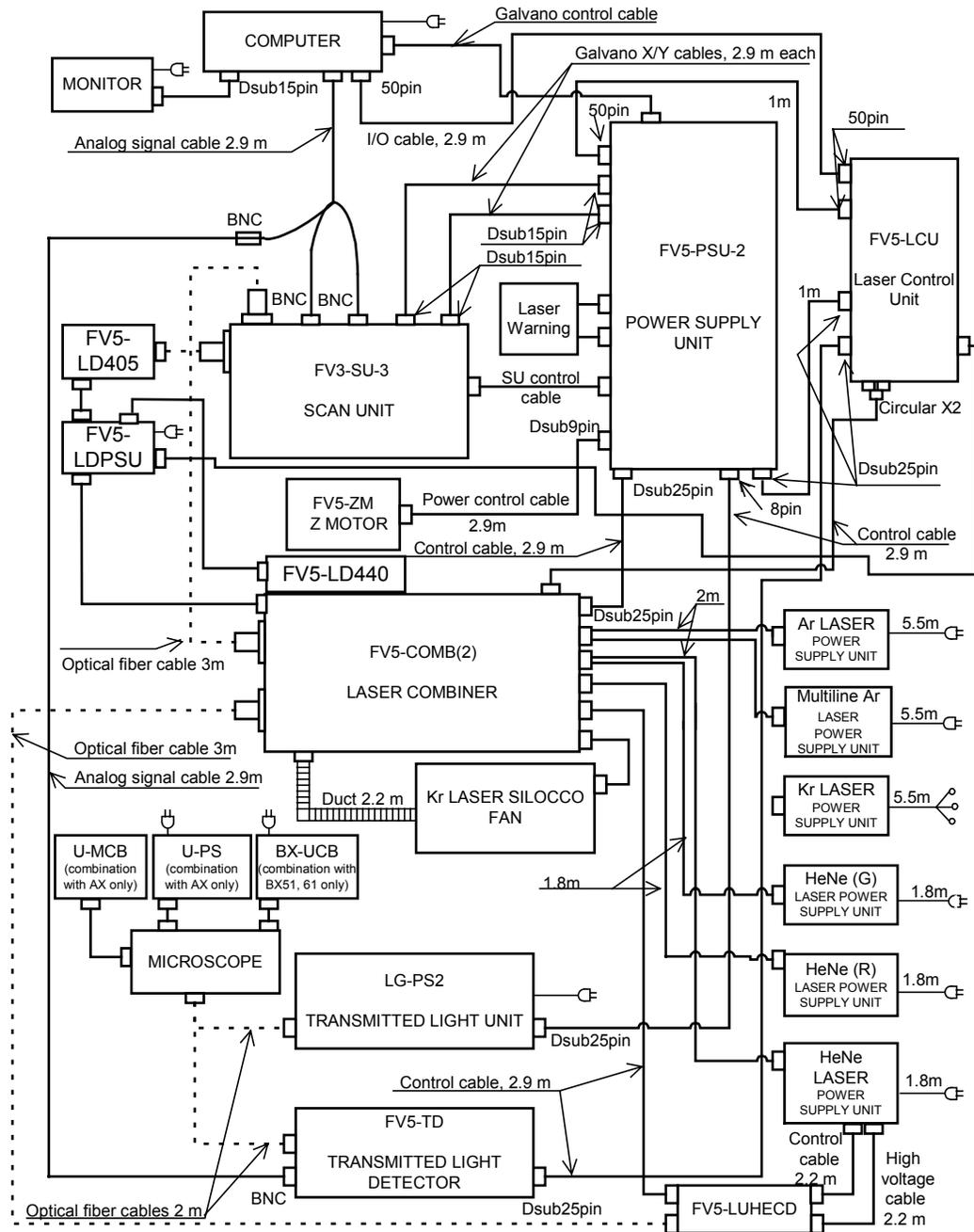
Photo 1-3 Combination with IX



Transmitted light detector (optional)

Unit for acquiring transmitted images.

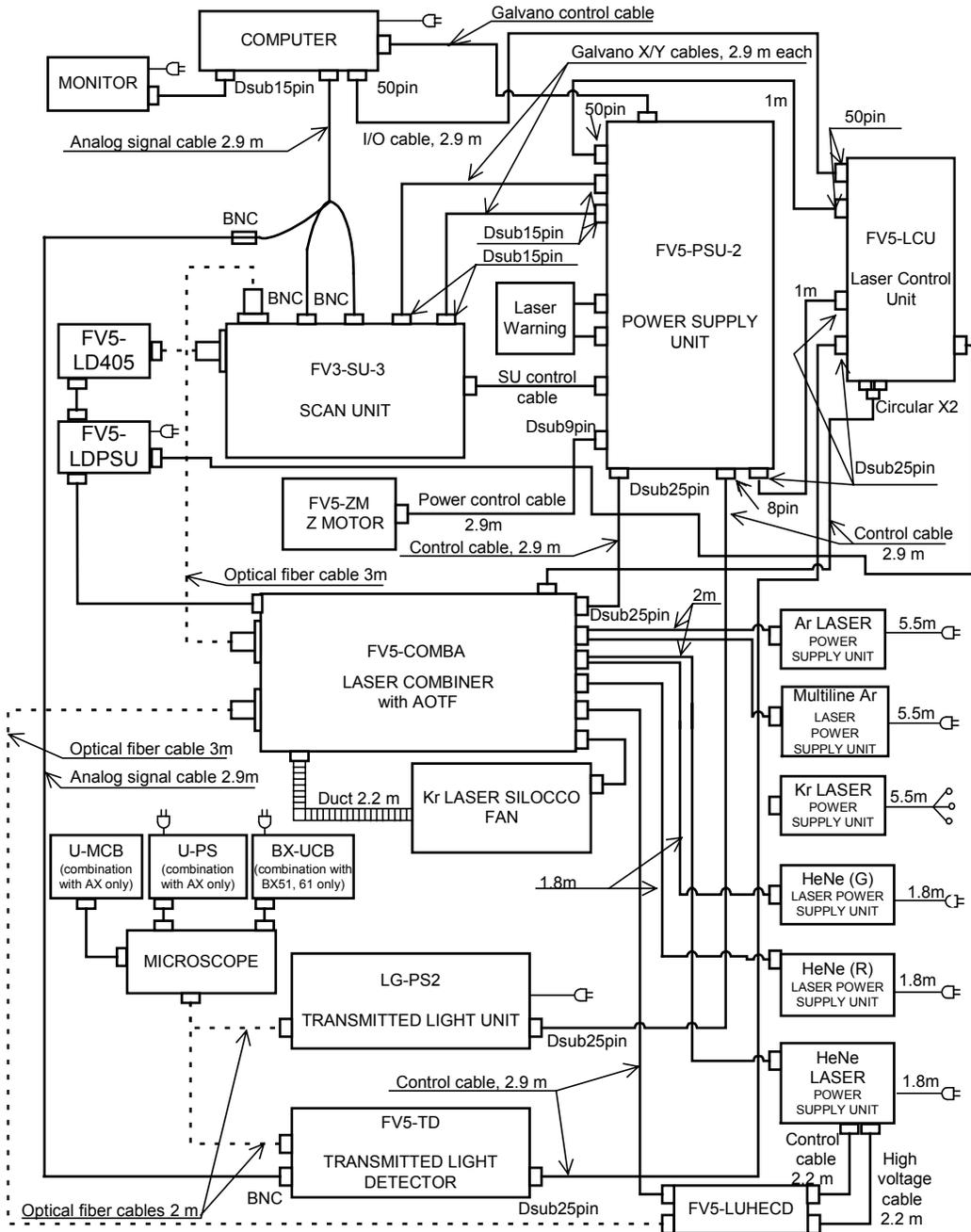
1-4-2 Block Diagram

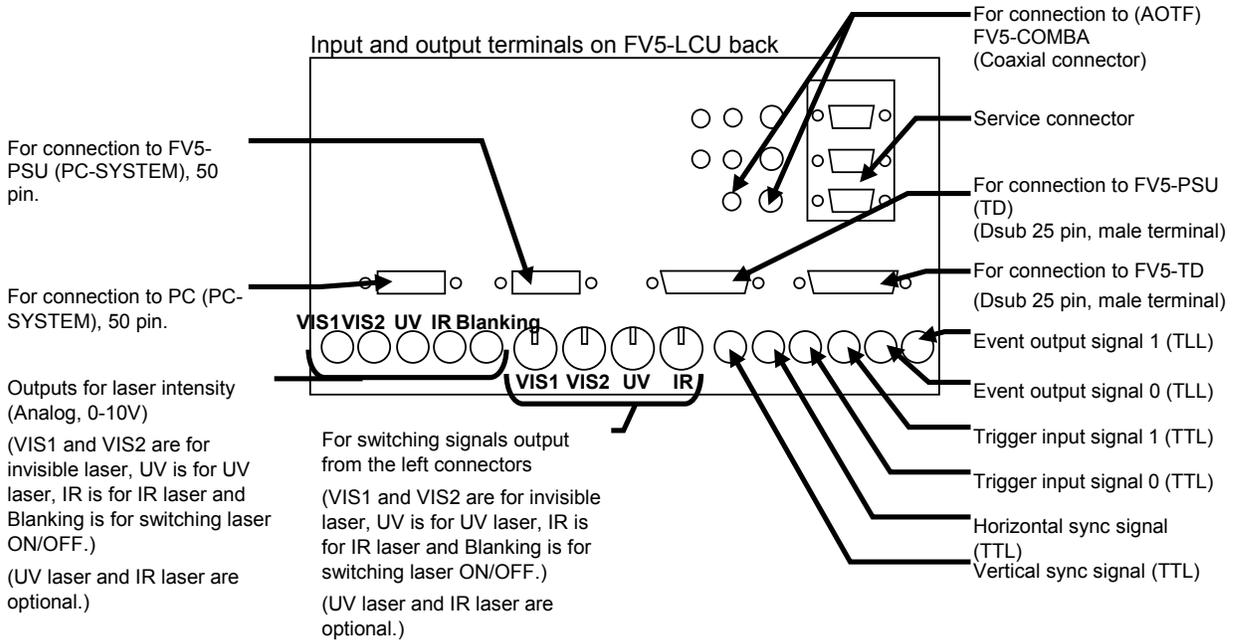


Either the Kr laser or HeNe(G) laser can be used.

SYSTEM OVERVIEW/System Configuration

The block diagram in AOTF (FV5-COMBA) use.





1-5 Software Functional Configuration

This software uses panel-type windows.

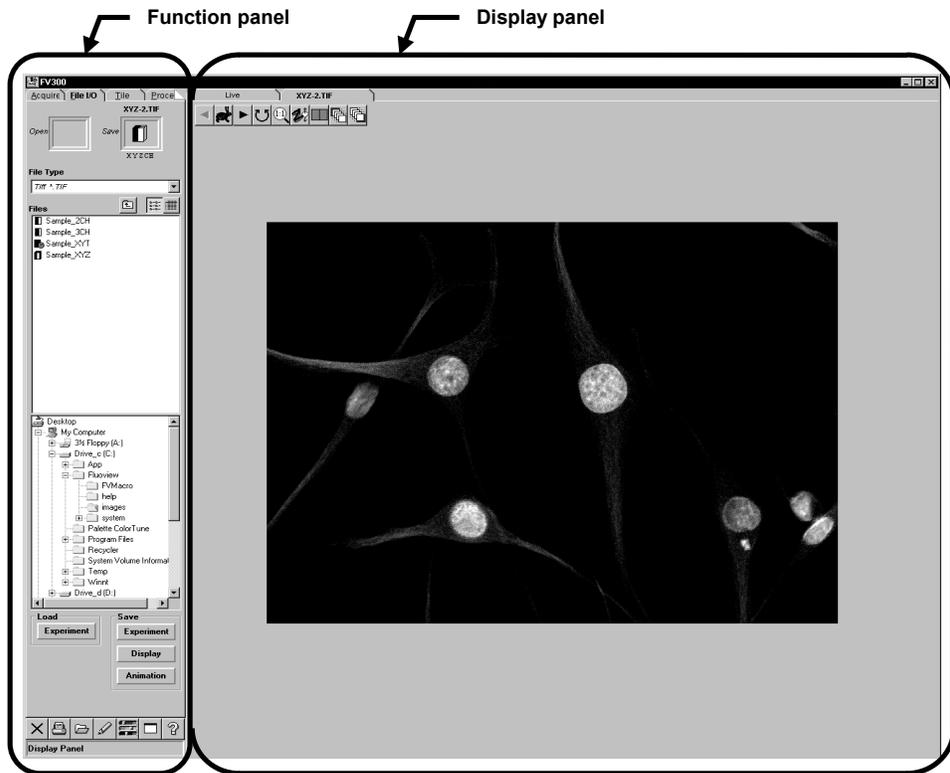
Usually, it is required to “select a menu then select the command to be executed” in order to execute a function provided by software. With the panel system, a software function can be executed easily by “selecting the panel page tab of the function to be executed”, just like when using a system notebook or file folder.

1-5-1 Function Panel and Display Panel

The FLUOVIEW software is organized by two kinds of panels, the function panels and display panel.

The function panels include the [Acquire], [File I/O], [Tile], [Process], [Analyze] and [Visualize] panels.

The display panel shows either the [Live] panel or the panel image loaded from a file ([[filename]] panel).

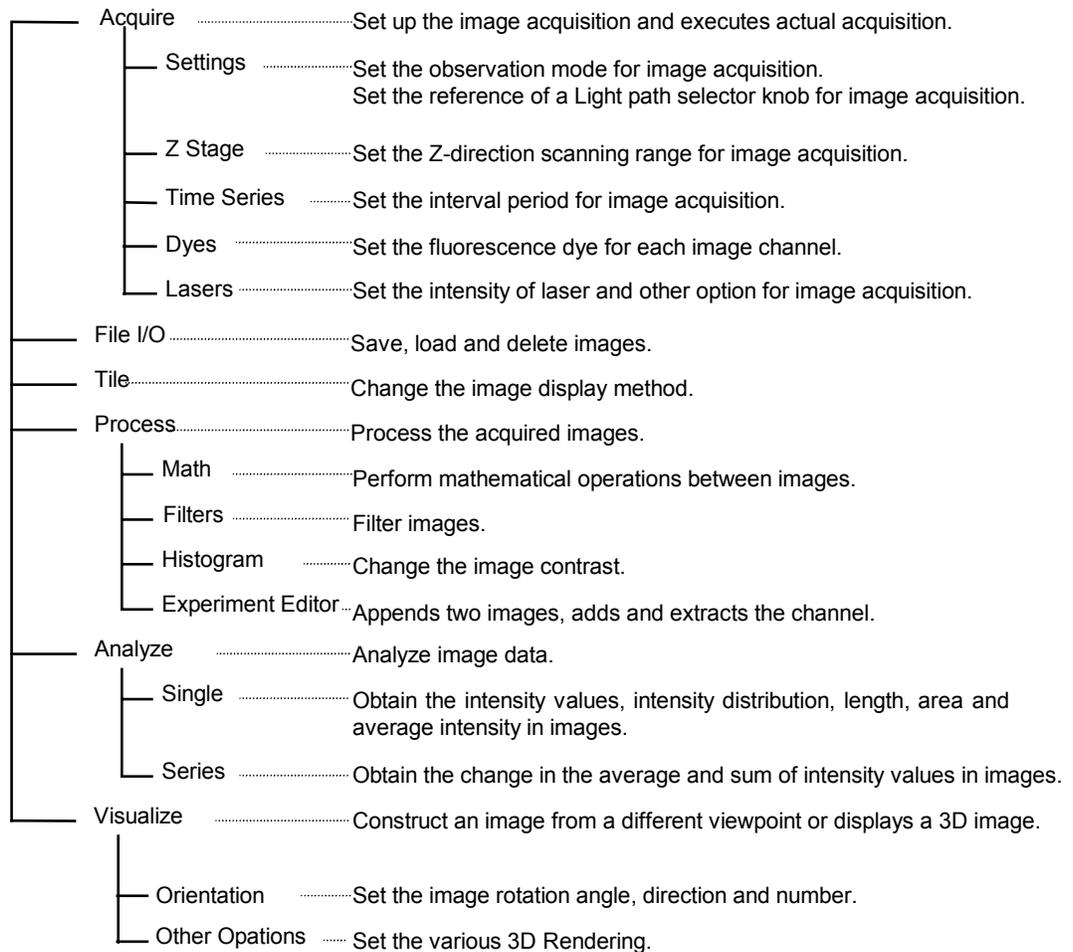


In this manual, the function panels are referred to simply using their page tabs.

Namely, the [File I/O] panel of function panel is referred to simply as the [File I/O] panel.

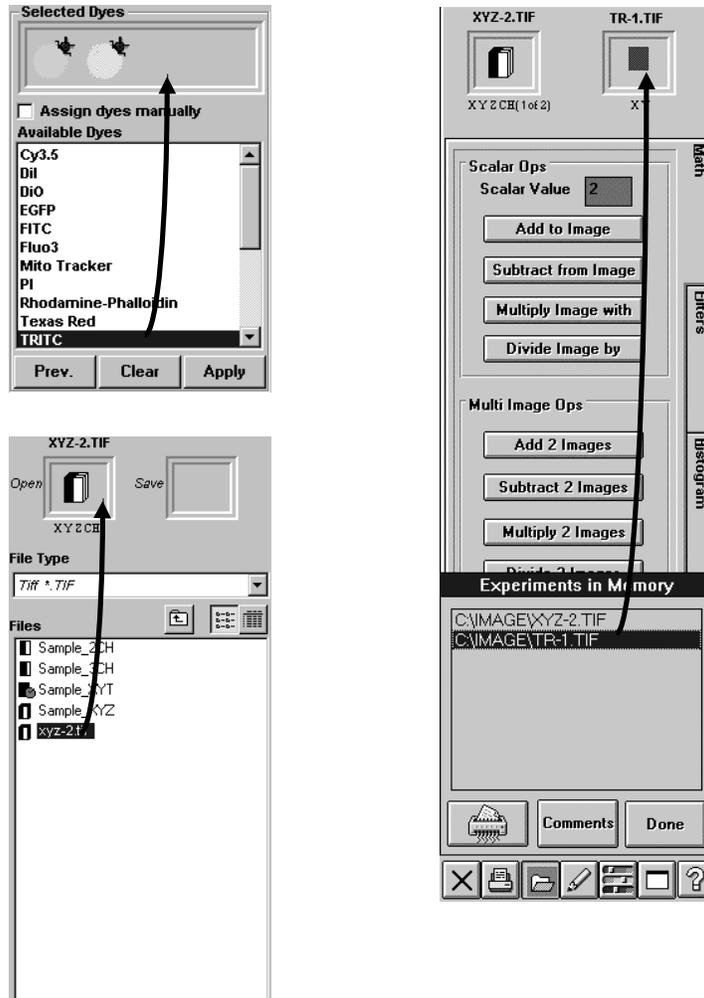
1-5-2 Panel Structure of the Software

This software cannot show the page tabs of all function panels at a glance, but uses scrolling to display the desired page tab. Please use the following list of the panels as reference in scrolling.



1-5-3 Icons Executed by Dragging & Dropping

This software selects image files and observation methods (dye name) by means of dragging & dropping. This allows simple selection based on an intuitive operation of “selecting an icon (image file or observation method), dragging it to the desired position and dropping it there”.



1-5-4 Identification of Images Depending on the Observation Methods

Image Icon	Significance
	XZ observation
	XZ observation, 2-channel mode
	Xt observation
	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
	Point Scan
	Animation image
	Stereo 3D image: Image to be viewed with color eyeglasses.
	3 or more channels

In many occasions, FLUOVIEW displays image icons to allow identification of the observation method used when each image is acquired. (See table on the left.)

When the [File I/O], [Tile], [Process], [Analyze] or [Visualize] panel is selected, the icon of the image selected in the [Display] panel is displayed in a frame at the top of the function panel. The image icons are also displayed in the [Icon] field in the [Files] list box in the [File I/O] panel or during dragging of an image file.

Use these icons to identify the observation methods used in image acquisition.



In all observation modes, the icons for 3 or more channels are identical.

IV. OPERATION INSTRUCTIONS

On This Volume

This volume describes the operating procedures of the FLUOVIEW FV300 system.

“Getting Started FLUOVIEW” contains information on the basic operation flow until acquisition of XY images.

“APPLIED OPERATIONS” provides detailed operating procedures of the system.

Please read this volume so that you can understand the system before use.

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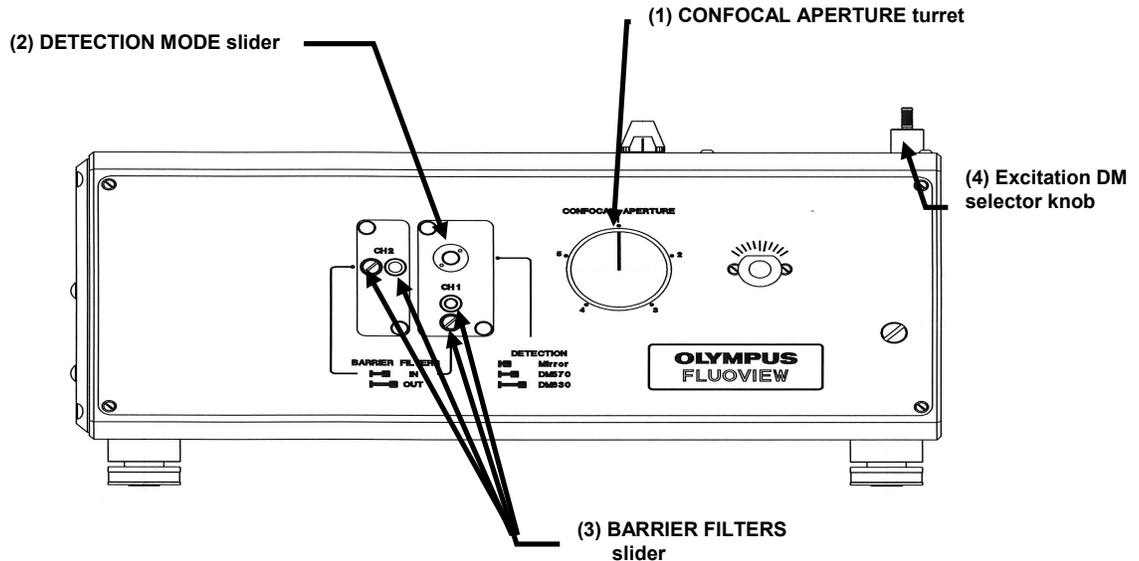
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1 Getting Started FLUOVIEW

1-1 Basic Operations

1-1-1 Scan Unit



(1) Confocal aperture switching

The confocal aperture includes 5 positions, any of which can be selected by rotating the turret.

Rotate the CONFOCAL APERTURE turret to select the optimum confocal aperture for the objective in use.

Select the optimum confocal aperture number for the objective being displayed in the control panel displayed by the computer ([Acquire] panel).

When the acquired image is bright enough and noise is not noticeable even when the HV of photomultiplier is increased, an even better image may be obtained by selecting a smaller confocal aperture size by rotating the CONFOCAL APERTURE knob.

The actual sizes of the confocal aperture positions are as listed below.



- 1: 60 μ m
- 2: 100 μ m
- 3: 150 μ m
- 4: 200 μ m
- 5: 300 μ m

} The confocal aperture sizes recommended for objectives are 1, 2 or 3 as shown in the following table.

} Use these sizes when the observation image is dark and a brighter image is required. Confocality is compromised in this case.

Basically, the resolution in the Z-axis direction cannot be improved by selecting a smaller confocal aperture than the confocal aperture size recommended for each objective. However, this sometimes improves the resolution in the X-Y plane. The results vary depending on many conditions such as the specimen's reflectivity and light scattering properties.

The following table shows the objectives and the confocal aperture numbers recommended for them.

Objective	N.A.	Confocal Aperture Number
PLAPO 40X	0.95	2
PLAPO 60XO	1.4	2
PLAPO 100XO	1.4	3
PLAPO 40XWLSM	0.9	2
PLAPO 60XWLSM	1.0	3
PLAPO 60XOLSM	1.1	3
UPLAPO 10X	0.4	1
UPLAPO 20X	0.7	1
UPLAPO 20XO	0.8	1
UPLAPO 40X	0.85	2
UPLAPO 40XOI	1.0	2
UPLAPO 60X	0.9	3
UPLAPO60 X W	1.2	2
UPLAPO 60XWPSF	1.2	2
UPLAPO 100XOI	1.35	3
UPLFL10X	0.3	1
UPLFL20X	0.5	2
UPLFL40X	0.75	3
UPLFL60XOI	1.25	2
UPLFL100XO	1.3	3
UPLFL100XOI	1.3	3
UMPLFL10XW	0.3	1
UMPLFL20XW	0.5	2
LUMPLFL40XW	0.8	2
LUMPLFL60XW	0.9	3

(2) Detection mode setting

The detection modes can be switched over by pushing or pulling the DETECTION MODE slider (2).

This switch allows you to set the detection mode of the 2 or 1 fluorescence channel by selecting one of the 3 positions. It switches section A shown in Fig. (I) on the next page.

- When the slider switch is in the pushed-in position

The mirror is engaged in the light path as shown in Fig. (I) on the next page. As a result, 100% of the fluorescence is introduced in CH1 (PM1).

Use this mode when observing a fluorescence specimen having a spectral characteristic with a shorter wavelength than 570 nm, such as FITC.

- When the slider switch is pushed-in half way to the position

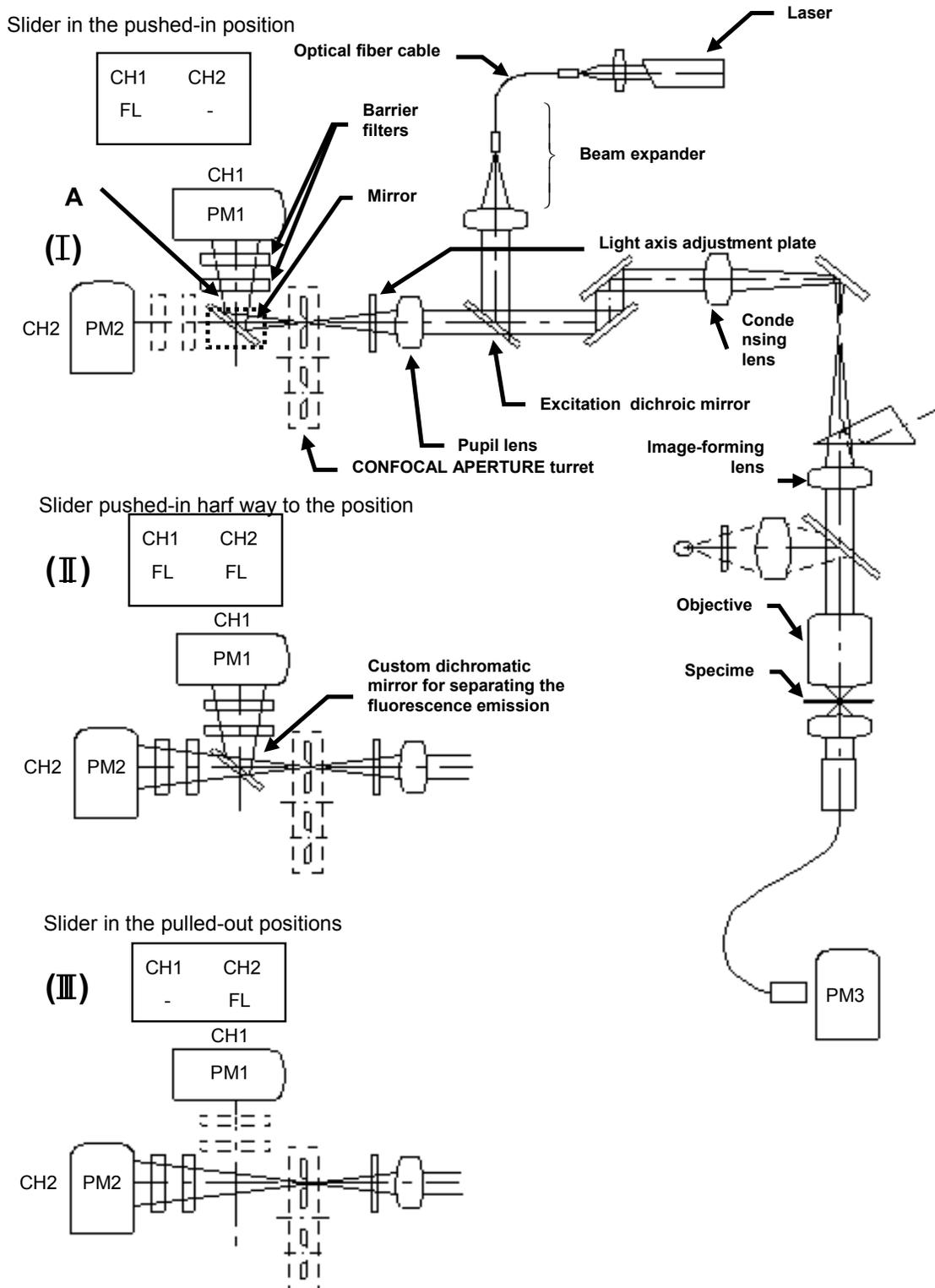
The custom dichromatic mirror for separating the fluorescence emission is engaged in the light path as shown in Fig. (II) on the next page. This dichroic mirror reflects light with wavelengths shorter than about 570 nm and transmits light with longer wavelengths. As a result, it can separate fluorescence into CH1 (PM1) and CH2 (PM2).

Use this mode when observing a double-labeled specimen such as FITC+TRITC.

- When the slider switch is in the pulled-out position

Nothing is engaged in the light path as shown in Fig. (III) on the next page. As a result, 100% of the fluorescence is introduced in CH2 (PM2).

Use this mode when observing a fluorescence specimen having a spectral characteristic with a wavelength longer than 570 nm, such as TRITC.



(3) Barrier filter setting

The barrier filters can be switched over by pushing or pulling the BARRIER FILTERS slider (3).

Up to 2 barrier filters can be mounted for each of CH1 and CH2. The filters are engaged in the light path when the slider switch is pushed in and disengaged when it is pulled out.

When combination is not known, see section 1-3-2-3, "Configuring the Filters" in this volume and follow instructions in the [Optical System Configuration] window.



If the slider switch is set pushed-in half way to the position by mistake, no images can be observed at all. Be sure to set it to the fully pulled-out or pushed-in position

(4) Switching the excitation DM

One of the two DMs can be selected using the excitation DM selector knob ④. As the light axis may be deviated when the excitation DM is switched with the excitation DM selector knob, adjustment of the detection light axis is required after switching.

For details on detection light axis adjustment, see section 1-2-12, "Adjustment of Detection Light Axis" in the Main Volume.



Overlapping the image before excitation DM switching and that after it may result in deviation between images. To prevent this, it is recommended to use excitation DMs that can be excited simultaneously.

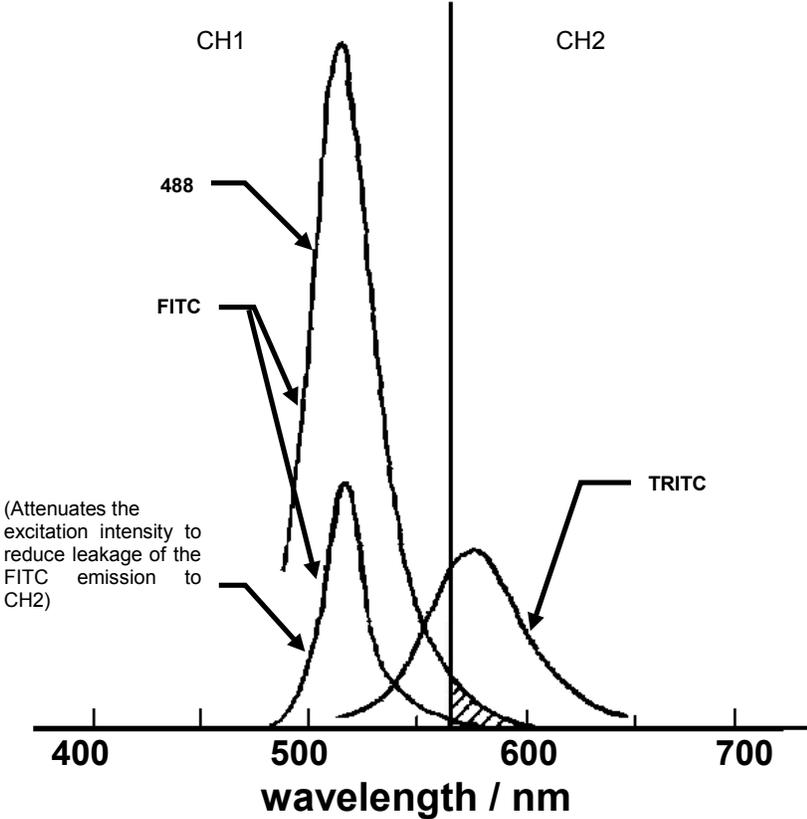
(5) Kr/Ar laser line filter switching (Available only with the Kr/Ar laser combination)

The 488 nm and 568 nm excitation wavelengths of the Kr/Ar laser can be selected according to applications. This switch has 5 positions which can be selected with a rotary knob.

- 1) 488: Excites the specimen at 488 nm. Use this position when observing a single-labeled specimen such as FITC.
- 2) 568: Excites the specimen at 568 nm. Use this position when observing a single-labeled specimen such as TRITC and PI.
- 3) 568
+
488
AT6: Excites the specimen at 488 nm and 568 nm, but the excitation output at 488 nm is attenuated to 6% of the normal level. Use this position to prevent interference of FITC emission on PI emission when observing a double-labeled specimen such as FITC+PI.
- 4) 568
+
488
AT25: Excites the specimen at 488 nm and 568 nm, but the excitation output at 488 nm is attenuated to 25% of the normal level. Use this position to prevent interference of FITC emission on PI emission when observing a double-labeled specimen such as FITC+PI.
- 5) 568
+
488: Excites the specimen at 488 nm and 568 nm. Use this position when observing a double-labeled specimen such as FITC+PI.



The FITC emission tails longer than 570 nm. As a result, the FITC emission tends to be detected by CH2 and interferes with the PI emission (see diagram below). This problem may be solved ideally by cutting off the FITC emission using OFFSET in the control panel ([Acquire] panel), but if this is not possible, it is necessary to attenuate the intensity of the 488 nm wavelength exciting the FITC emission in order to balance the excitation emissions. Otherwise, you can also use sequential scanning to obtain the same result.

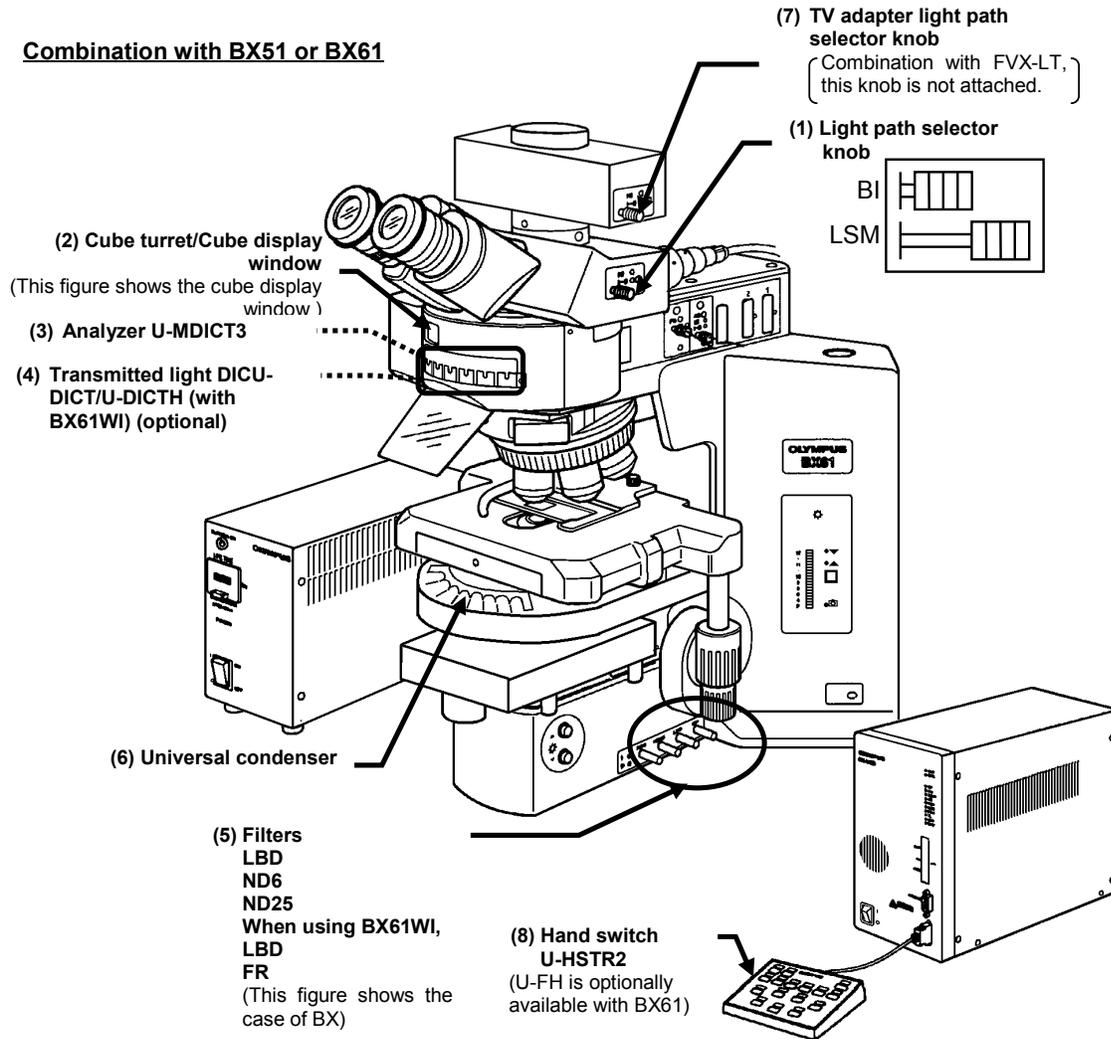


1-1-2 Microscope

The following figure shows the major controls of a microscope. The configuration of the modules including the specimen stage, revolving nosepiece and lighting equipment may differ from those shown below.

For detailed operation procedure of the microscope, refer to the instruction manual of your microscope.

Combination with BX51 or BX61



(1) Light path selector knob

Select the light path between direct observation and laser microscopy.

- Set to the pushed-in position for direct observation.
- Set to the pulled-out position for laser microscopy.

(2) Cube turret

Select the fluorescence observation tube by rotating the turret.

- Engage the desired cube in the light path for direct fluorescence observation.
- For laser microscopy or for direct transmitted light observation, rotate the turret to page tab . (This sets the cube turret so that no cube is engaged.)

(3) Analyzer U-MDICT3

Polarizing plate for use in differential interference observation and polarized light observation.

- Rotate the turret to engage the U-MDICT3 in the light path for direct transmitted light differential interference observation or transmitted polarized light observation.
- For laser microscope, always disengage the U-MDICT3. Engaging the U-MDICT3 in the light path degrades the image quality.

(4) Transmitted light DIC U-DICTS/WI-DICTHRA (when using BX61WI) (optional)

This is the prism for use in differential interference observation.

- Engage the transmitted light DIC in the light path for laser differential interference observation or direct transmitted light differential interference observation.

Leaving the transmitted light DIC engaged during laser fluorescence observation will degrade the image quality somewhat. We recommend disengaging the transmitted light DIC from the light path when simple laser fluorescence observation is required.

NOTE

With transmitted light differential interference observation using an immersion objective, set the microscope's field diaphragm so that it circumscribes the field of view. Otherwise the contrast may degrade. (This applies to both direct observation and laser differential interference observation.)

(5) Filters

These filters are used to adjust transmitted light.

- Be sure to disengage any filter from the light path for transmitted observation using laser. Leaving a filter engaged in the light path will degrade the image quality.

When you perform transmitted observation using laser with BX61WI, use the filter knob to disengage the LBD from the light path and engage the FR (Frost) into the light path. Disengaging the FR (Frost) from the light path may generate interference fringes on an image.

(6) Universal condenser

Used for transmitted lighting. In addition, the rotary turret for the transmitted light DIC slider and the polarizing plate for differential interference observation (polarizer) are also provided.

- To perform differential interference observation, engage the transmitted light DIC slider (optional) matching the objective in use in the light path. (For both direct observation and laser differential interference observation)
- To perform direct differential interference observation or laser differential interference observation, engage the polarizing plate in the light path.

(7) TV adapter light path selector (Combination with FVX-LVT)

Select the light path between laser microscopic observation and TV/photography observation.

- Set to the pushed-in position for TV or photography observation.
- Set to the pulled-out position for laser microscopic observation.

(8) Hand switch U-HSTR2 (U-FH is optionally available with BX61)

This is the hand switch to operate the BX motorized system.



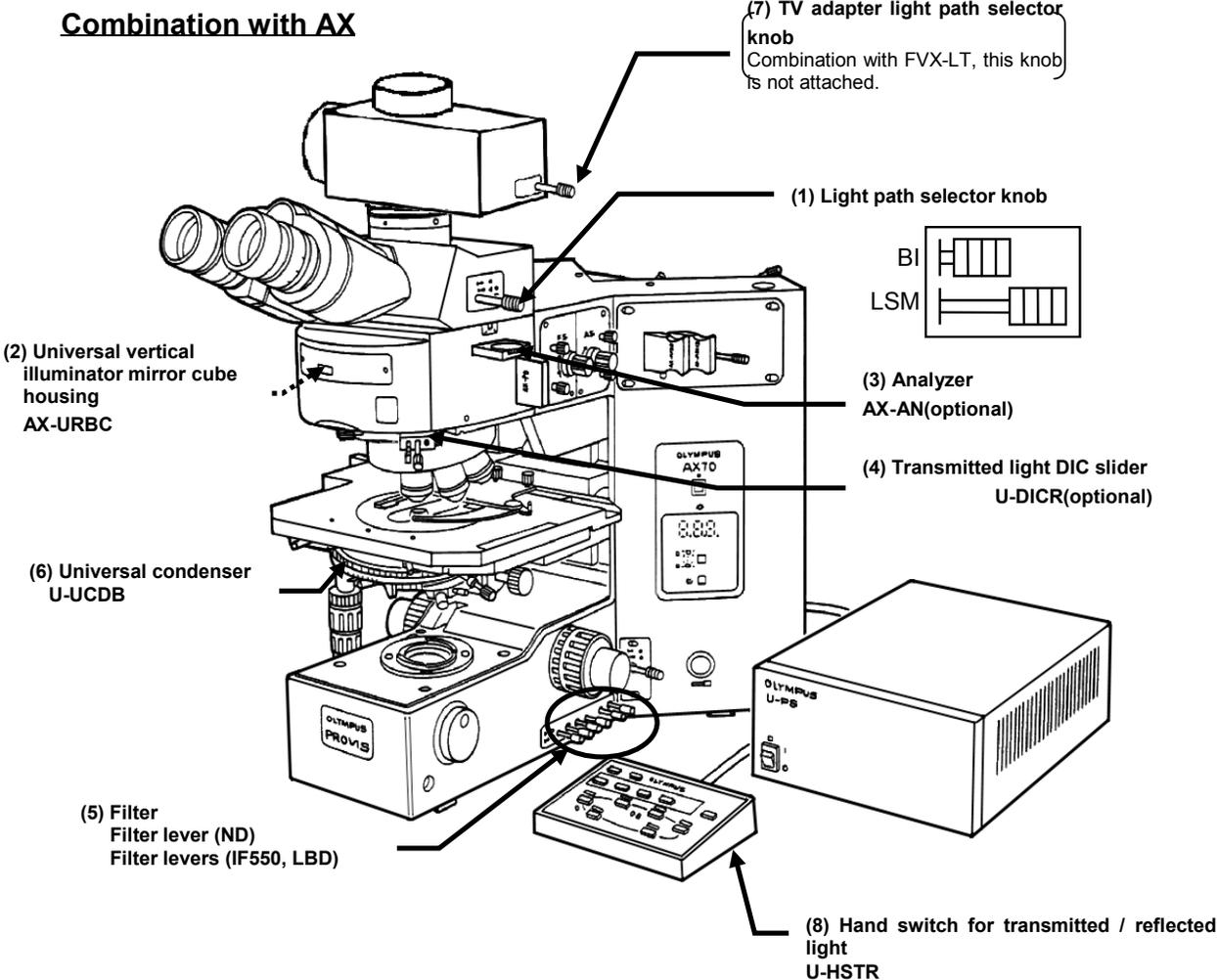
NOTE

Connect the filter wheel of BX to connector of the following in the back of UCB.

- FW0: FW1
- FWR: FW2
- FWT: FW3



Combination with AX



(1) Light path selector knob

Select the light path between direct observation and laser microscopy.

- Set to the pushed-in position for direct observation.
- Set to the pulled-out position for laser microscopy.

(2) Universal vertical illuminator mirror cube housing

The turret for the cube for reflected light observation can be switched with the hand switch for transmitted/reflected light.

- Engage the desired cube in the light path for direct fluorescence observation.
- For laser microscopy or for direct transmitted light observation, push BF button in cube selector buttons in Hand switch for transmitted/reflected light.

(This sets the cube turret so that no cube is engaged.)

(3) Analyzer AX-AN(optional)

Polarizing plate for use in differential interference observation and polarized light observation.

- Set to the pushed-in position to engage the AX-AN in the light path for direct transmitted light differential interference observation or transmitted polarized light observation.
- Set to the pulled-out position to disengage the U-MDICT3 for laser microscopy. Engaging the AX-AN in the light path allows improvement of the acquired image quality.

(4) Transmitted light DIC slider U-DICR(optional)

This is the prism for use in differential interference observation.

- Engage the transmitted light DIC Slider in the light path for laser differential interference observation or direct transmitted light differential interference observation.

Leaving the transmitted light DIC slider engaged during laser fluorescence observation will degrade the image quality somewhat. We recommend disengaging the transmitted light DIC slider from the light path when simple laser fluorescence observation is required.



NOTE

With transmitted light differential interference observation using an immersion objective, set the microscope's field diaphragm so that it circumscribes the field of view. Otherwise the contrast may degrade. (This applies to both direct observation and laser differential interference observation.)

(5) Filters

These filters are used to adjust transmitted light.

- Be sure to disengage any filter from the light path for transmitted observation using laser. Leaving a filter engaged in the light path will degrade the image quality.

(6) Universal condenser U-UCDB

Used for transmitted lighting. In addition, the rotary turret for the transmitted light DIC slider and the polarizing plate for differential interference observation (polarizer) are also provided.

- To perform differential interference observation, engage the transmitted light DIC slider (optional) matching the objective in use in the light path. (For both direct observation and laser differential interference observation)
- To perform direct differential interference observation or laser differential interference observation, engage the polarizing plate in the light path.

(7) TV adapter light path selector (Combination with FVX-LVT)

Select the light path between laser microscopic observation and TV/photography observation.

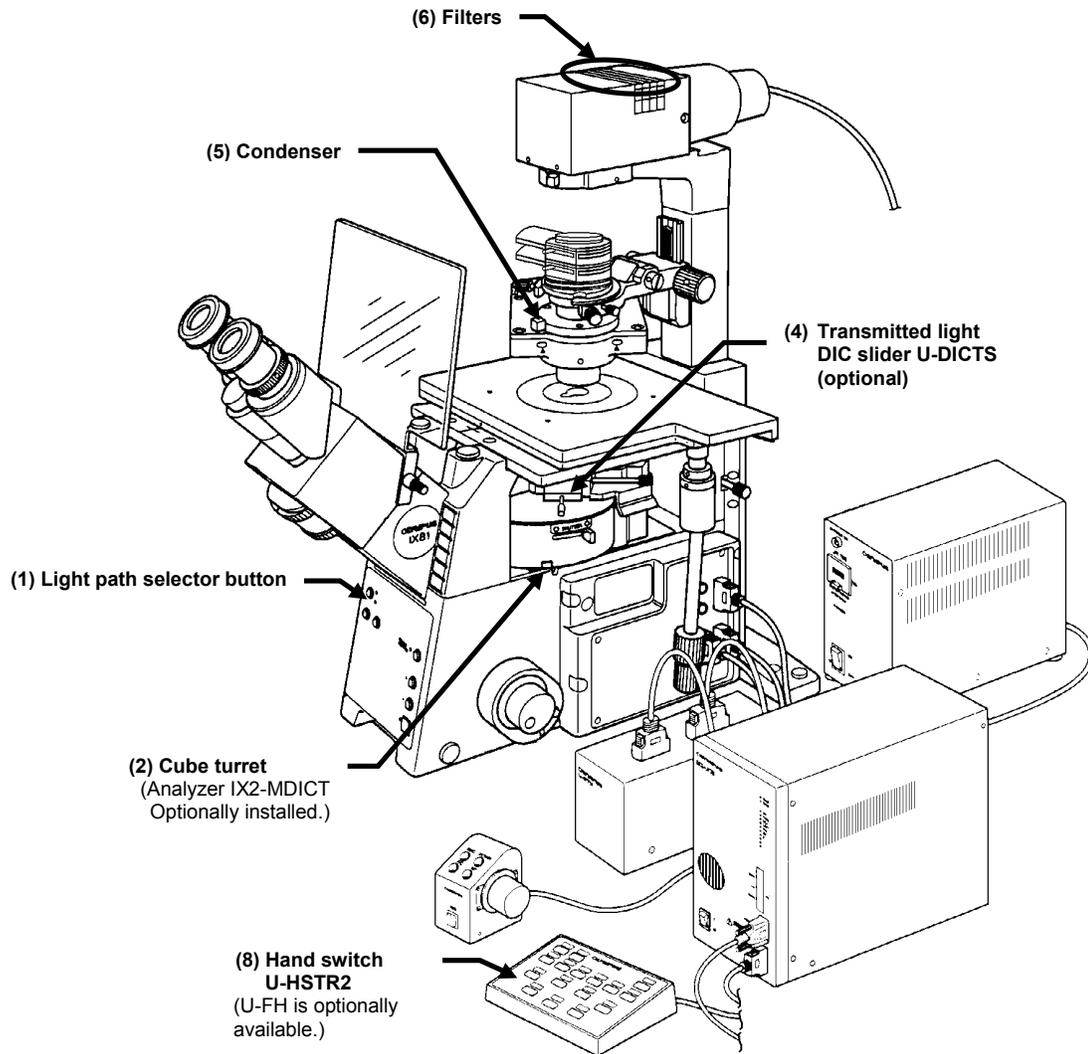
- Set to the pushed-in position for TV or photography observation.
- Set to the pulled-out position for laser microscopic observation.

(8) Hand switch for transmitted / reflected light U-HSTR

Switch objective and cube.



Combination with IX81 FVF



(1) Light path selector button

Select the light path between laser microscopic observation and direct observation.

- When LED is illuminated, the light path is switched for laser microscopic observation.
- When LED is not illuminated, the light path is switched for direct observation.

(2) Fluorescence mirror unit

Select the fluorescence observation tube by rotating the turret.

- Engage the desired cube in the light path for direct fluorescence observation.
- For laser microscopy or for direct transmitted light observation, rotate the turret to page tab . (This set the turret so that no cube is engaged.)

(3) Analyzer IX2-MDICT (optional)

Polarizing plate for use in differential interference observation and polarized light observation.

- Rotate the cube turret to engage the IX2-MDICT analyzer into the light path for direct transmitted light differential interference observation or transmitted polarized light observation.
- Be sure to disengage the IX2-MDICT analyzer for laser microscopy. Engaging the IX2-MDICT analyzer into the light path allows the acquired image quality to improve.

(4) Transmitted light DIC slider U-DICTS (optional)

This is the prism for use in differential interference observation.

- Engage U-DICTS in the light path for laser differential interference observation or direct transmitted light differential interference observation.
Leaving U-DICTS engaged during laser fluorescence observation will degrade the image quality somewhat. We recommend disengaging U-DICTS from the light path when simple laser fluorescence observation is required.

NOTE

With transmitted light differential interference observation using an immersion objective, set the microscope's field diaphragm so that it inscribes the field of view. Otherwise the contrast may degrade. (This applies to both direct observation and laser differential interference observation.)

(5) Condenser, polarizing plate

Used for transmitted lighting.

In addition, the rotary turret for the transmitted light DIC slider and the polarizing plate for differential interference observation (analyzer) are also provided.

- To perform differential interference observation, engage the transmitted light DIC slider (optional) matching the objective in use in the light path. (For both direct observation and laser differential interference observation)
- To perform direct differential interference observation or laser differential interference observation, engage the polarizing plate in the light path.

(6) Filters

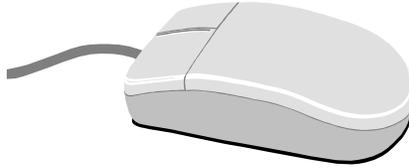
These filters are used to adjust the transmitted light.

- For transmitted observation using laser, disengage the LBD filter from the light path and engage the FR (frost) filter in the light path by operating the filter levers. If the FR filter is disengaged from the light path, the image may be marred by stripe interference.

(7) Hand switch U-HSTR2 (U-FH is optionally available.)

This is the hand switch to operate the IX motorized system.

1-1-3 General Mouse Operation Procedures



Use the mouse to select a command, character string or button. Use the left button of the mouse unless otherwise specified.

To select or execute something: Clicking

To click the mouse, place the mouse pointer on the desired function and press the mouse button once.

(Pressing the right button of the mouse is referred to as right-clicking.)

To select something and execute its function: Double clicking

To double-click, place the mouse pointer on the desired function and press the mouse button successively twice.

To move something: Dragging

To drag, place the mouse pointer on the desired function, and while pressing and holding the mouse button, move the mouse to the desired destination. At the desired destination, release the mouse button.

(Dragging by pressing the right button of the mouse is referred to as right-dragging.)

One Point!

When the mouse is moved, the picture of arrow on the screen moves accordingly. The picture which moves on the screen as the mouse is moved is referred to as the mouse pointer.

1-1-4 Names of Major Panel and Window Controls and Their Functions

The window as shown below is displayed when FLUOVIEW starts up. FLUOVIEW uses panel-type windows.

This section describes the names of the major controls displayed in panels and windows by taking the [Acquire] panel and [Microscope Configuration] window as examples.

Page tab

Click to switch the panel for executing the indicated processing function.
Right-clicking a page tab displays the pop-up menu of all items under it so the desired one can be selected.

Page tab scroll marking

When there are a large number of panels, it is not possible to display all of them. In this case, clicking this marking scrolls the panels one by one.

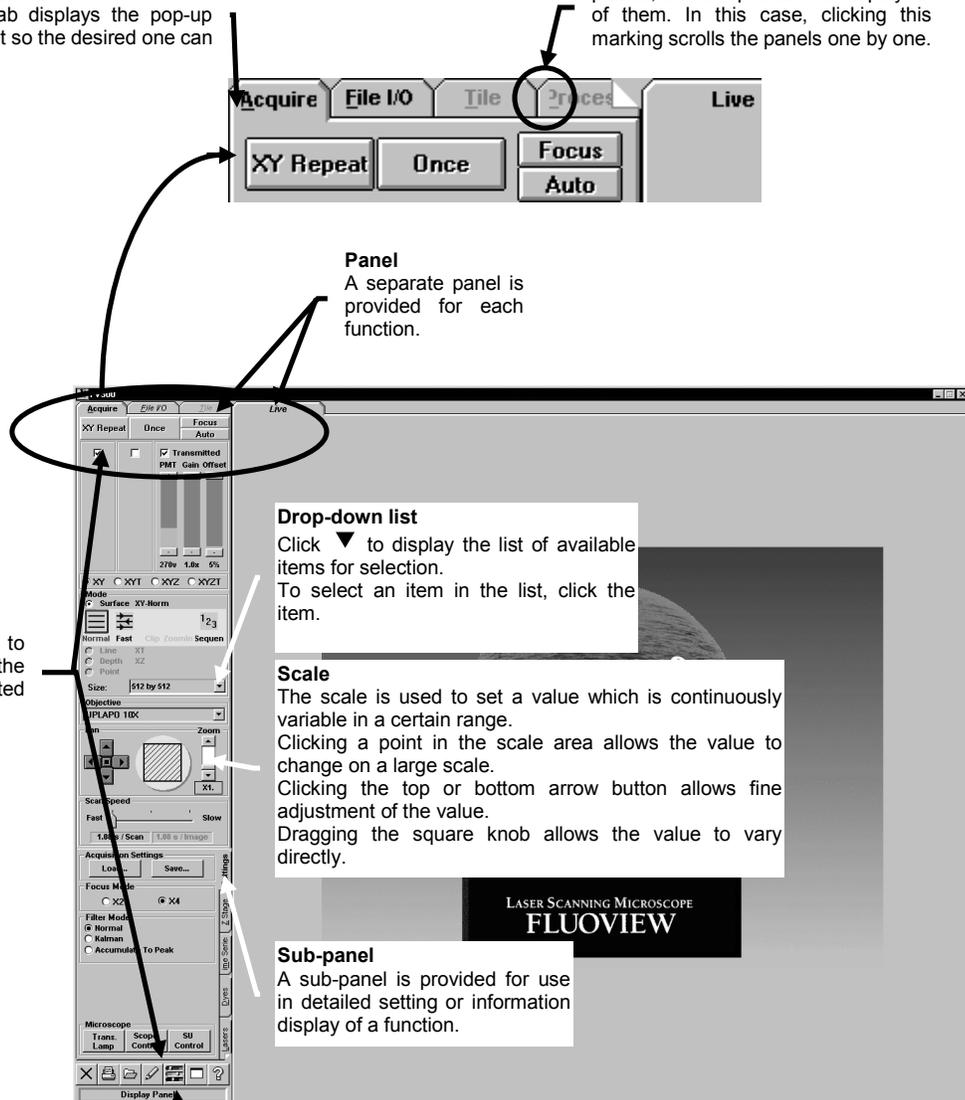
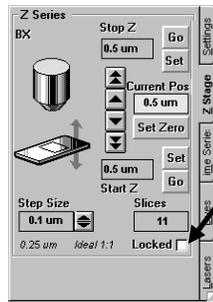


Fig. 1-1 Panel and Major Functions

Status bar

Shows the description of the command being pointed by the mouse pointer. During processing, which takes a long time, the status bar shows the progress of the processing.



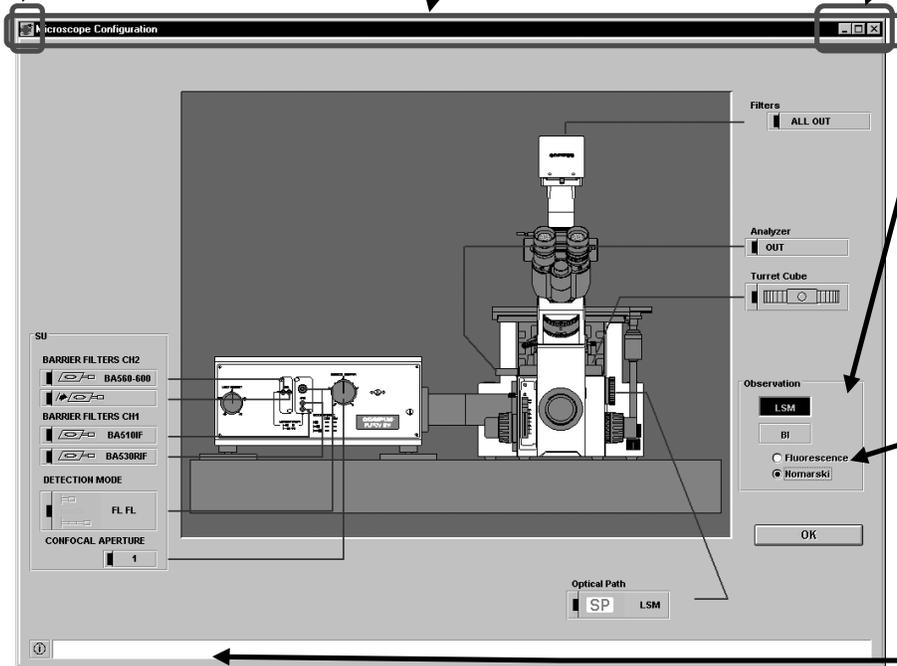
Check box
Clicking this box enables or disables the indicated item. The item is enabled when the check box is checked (x).

Control menu box
Clicking this box displays a control menu, which contains the commands for use in controlling the window.

Title bar
Shows the title of the window. The title bar of a window that is active is displayed in a color different from that of other windows.

Minimize button
Click to turn the window into an icon.

Original size button
Click to return the maximized window to its original size.

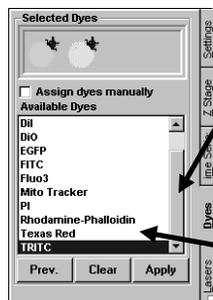


Group box
The group box is organized by grouping functions with the specific meanings and enclosing them in a frame.

Option buttons
Groups multiple items when only one of them can be selected. Clicking one of the round buttons selects the corresponding item. The option button of the selected item is displayed with a black dot in the center of it.

Information
Shows information on operations and meanings of functions.

Fig. 1-2 Window and Major Functions

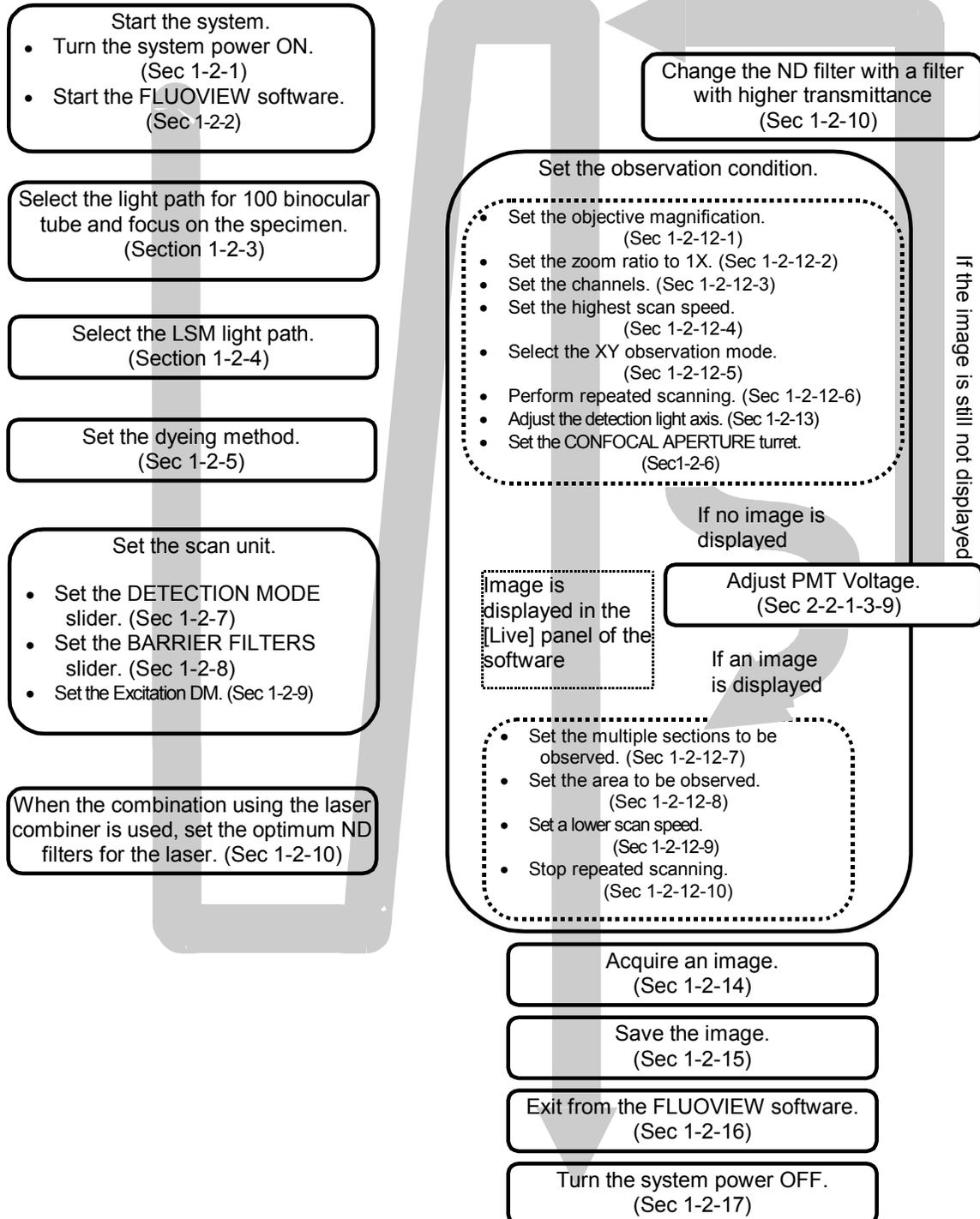


Scroll bar
The scroll bar is displayed when there are too many data items to be displayed in a field at once and used to display the data items outside the field. Clicking a point in the scroll area allows scrolling of the data items in large steps. Clicking the top or bottom arrow button allows fine scrolling of the data items. Dragging the square knob allows direct scrolling.

List box
Shows the list of available items for selection. All items in the list can be displayed by scrolling. All items in the list can be displayed by the item. To select an item in the list, double-click or drag the item.

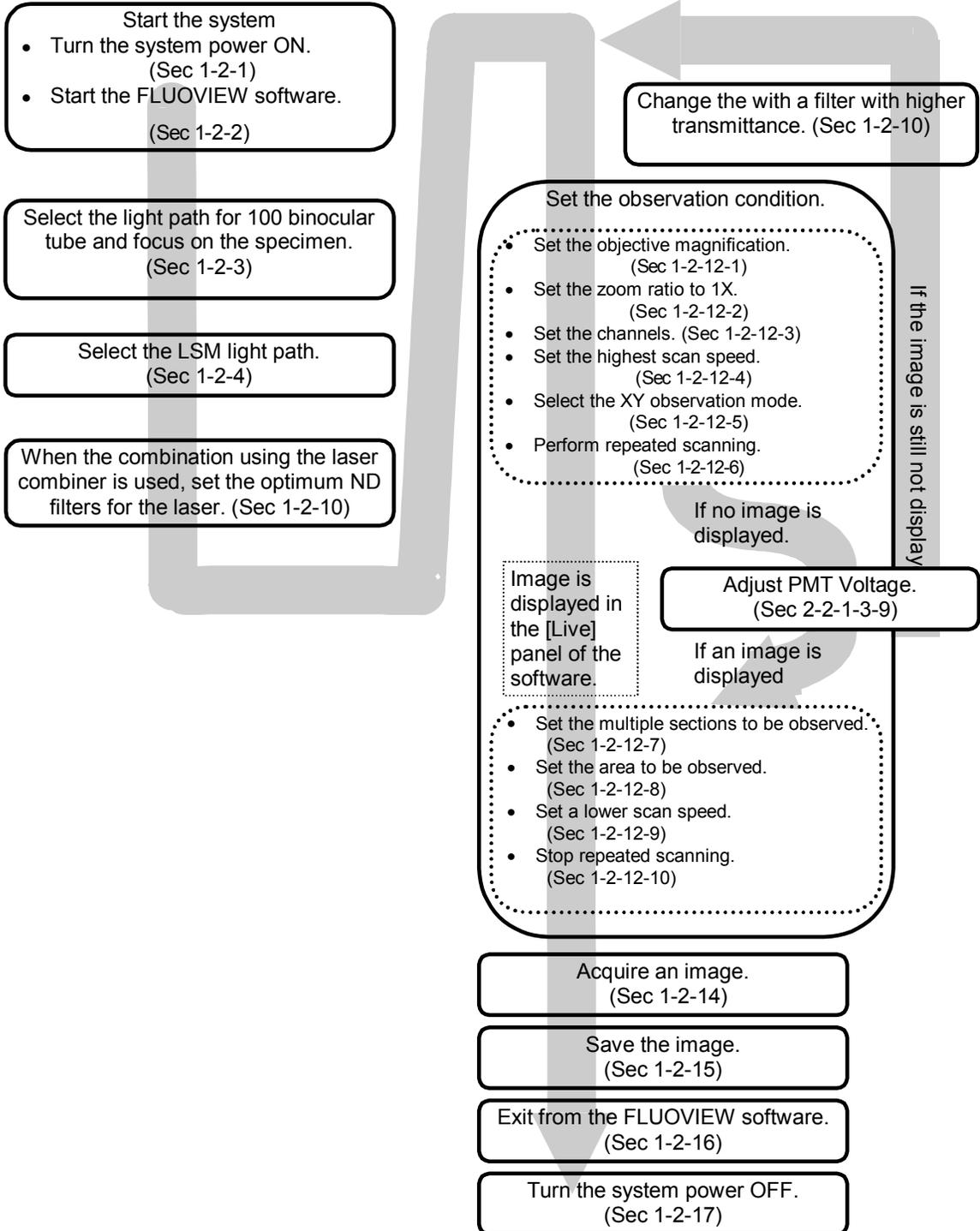
1-2 Outline of LSM Observation Procedures

- **Fluorescence observation procedure**





• **Transmitted light observation procedure**



1-2-1 Turning Power On

Set the power switch of each unit to ON.

When it is necessary only to read data, it is not necessary to turn the laser power supply and reflected light power supply units ON.

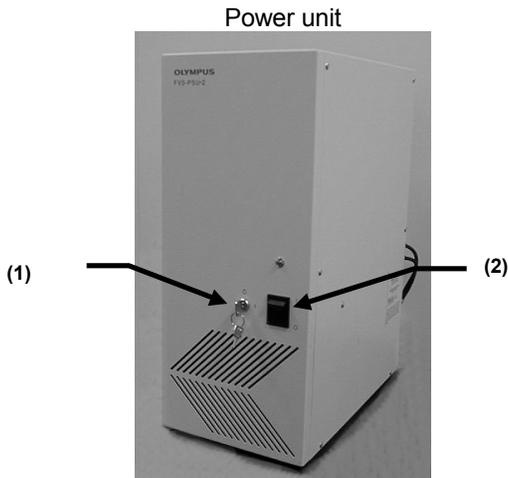
To stabilize the laser beam output, we recommend leaving the system for warm-up after turning the laser power ON. The warm-up period should be more than 10 minutes when the laser system in use uses an Ar laser or more than 30 minutes when it uses a Kr laser or Green HeNe laser.

- Turning the computer (PC) ON

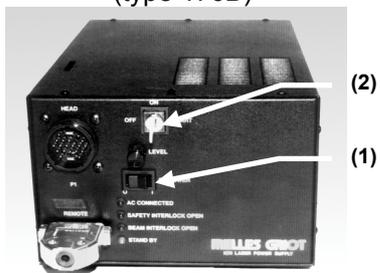
When the power outlet unit is used, turning the computer power ON also turns ON the power supplies to the microscope, monitor, power supply unit, hard-copy unit and transmitted light unit (when the transmitted light detector is used).

- Turning the power unit ON
 - (1) Turn the key to the ON position.
 - (2) Set the power switch to ON.

As this unit should be connected to the power outlet unit, leave its power switch in the ON position.



Ar laser power supply
(type 176B)



- Turning the laser power ON

Ar laser, Multiline Ar laser (type 176B and type 300):

- (1) Set the power switch to ON.
- (2) Turn the key to the ON position. (The laser fan will start.)

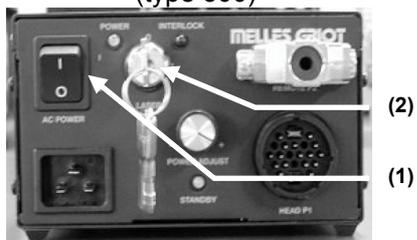
After the key is set to ON, it takes tens of seconds before the laser oscillation starts.

Kr laser:

- (1) Set the power switch to ON.
- (2) Turn the key to the ON position. (The laser fan will start.)

After the key is set to ON, it takes tens of seconds before the laser oscillation starts.

Ar laser power supply
(type 300)

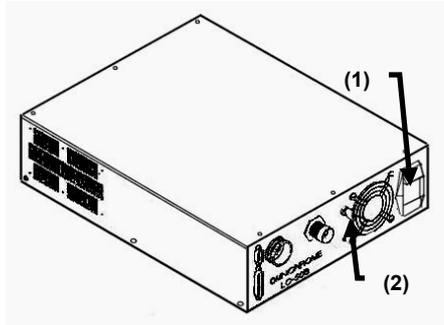


HeNe (Green/Red) laser:

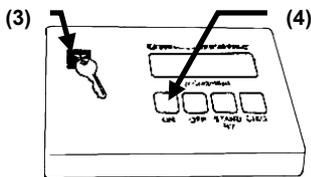
- (1) Turn the key to the "I" (ON) position.

HeNe (Green/Red)
laser power supply

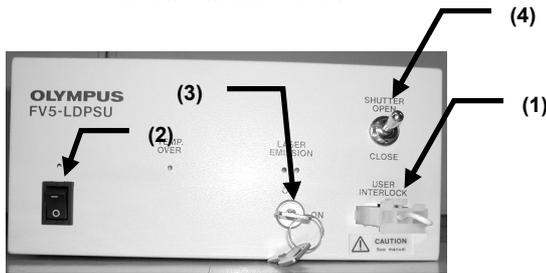




Laser controller



Remote interface module



LD405/440 laser power supply

HeCd laser.

- (1) Turn the rocker switch(1) of laser controller to "I" (ON).
- (2) Turn the key switch(2) of laser controller to the ON position.
- (3) Turn the key switch(3) of remote interface module to the ON position.
- (4) Turn the <ON> button of the remote interface module ON.

After the key is set to ON, it takes tens of seconds before the laser oscillation starts.

LD405/440 laser.

- (1) Confirm that the provided shorting plug is attached to the remote interlock (1) or that the user's equipment is connected to the remote interlock and interlocking released.
- (2) Set the power switch (2) to "I" (ON).
- (3) Turn the Key (3) to the ON position.
- (4) Set the shutter switch (4) to OPEN .



If the LASER EMISSION LED of the LD405/440 laser power supply is lit in red, the laser is oscillating. In this case, simple setting the shutter switch (4) to OPEN may cause the laser beam to be output depending on setups.



Rotary switch



(In case of LD405)



(In case of LD440)



Change right side rotary switch only. Do not touch left side rotary switch. If you change left rotary switch, you may not be able to get optimum performance of this system.

When you use LD405, align arrow mark of right side rotary switch with 5.

When you use LD440, align arrow mark of right side rotary switch with 4.



To stabilize the laser beam output, we recommend leaving the system for warm-up after turning the laser power ON. The warm-up period should exceed 10 minutes when the laser system in use uses an Ar laser or exceed 30 minutes when it uses a Kr laser, Green HeNe laser or HeCd laser.



- Turning the reflected light power supply unit
 - (1) Set the power switch to ON.



Once the reflected light power supply unit is turned OFF, do not turn it ON again for at least 10 minutes. Otherwise the service life of the mercury burner will be shortened.

- Turning the monitor ON
 - (Refer to the instruction manual of your monitor.)
 - As this unit should be connected to the power outlet unit, leave its power switch in the ON position.
- Turning the microscope ON
 - (Refer to the instruction manual of the microscope combined with the system.)
 - As this unit should be connected to the power outlet unit, leave its power switch in the ON position.
- Turning the transmitted light unit (when the transmitted light detector is used)

1-2-2 Starting the Software

NOTE

Before starting up this software, wait for more than 2 minutes after turning the microscope and power supply unit ON.
Allow the microscope and power supply unit to initialize themselves for about 2 minutes after they are turned on.

1. Input the user name and password, and start Windows NT or Windows 2000.

NOTE

Input the user name authorized as an Administrator.



2. Double-click the [FLUOVIEW] icon on the desktop.

TIP

When more than one user uses the FV300, each user shall log into it in different ways. See Appendix G, "User Registration of FV300" in this Volume.

TIP

It takes 20 to 30 seconds to start up software since double-clicking the [FLUOVIEW] icon.

3. The following window appears when the FLUOVIEW software starts.

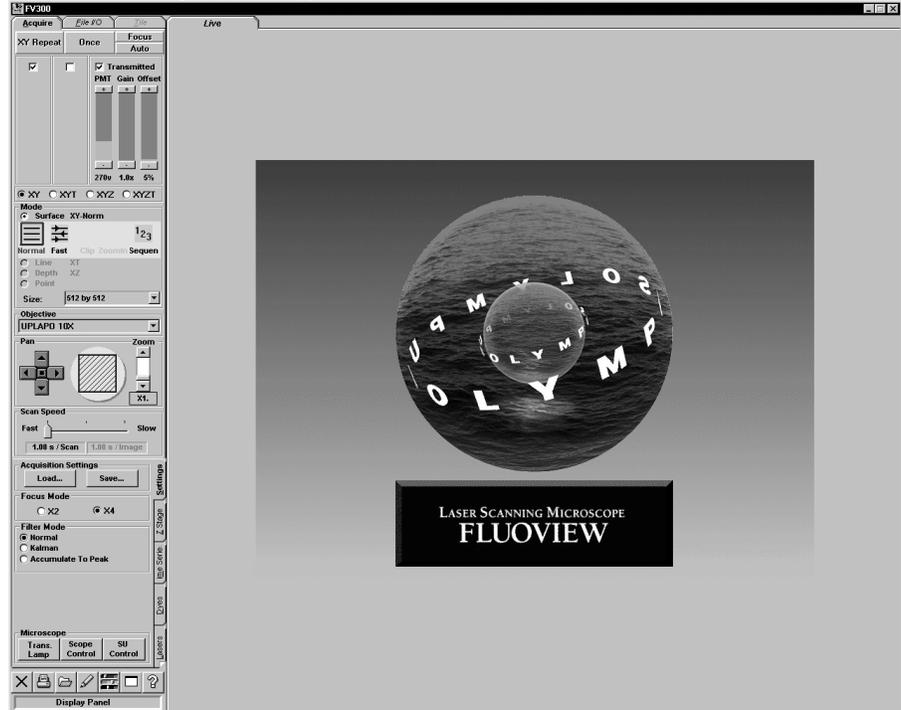
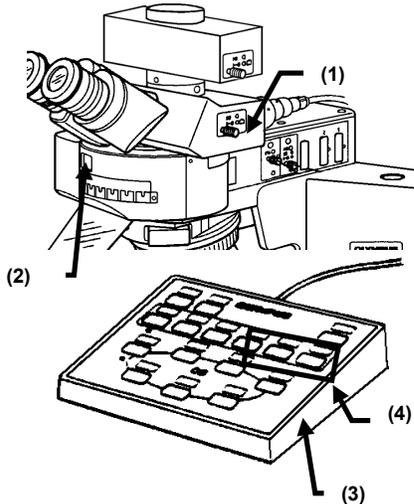


Fig. 1-3 Window at Start-up

1-2-3 Focusing on the Specimen

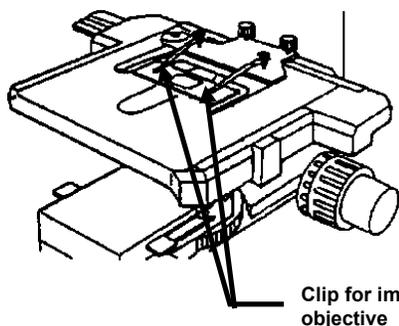
1-2-3-1 Combination with BX



1. Select the light path for 100% eyepiece by pushing in the light path selector knob (1) on the trinocular tube fully to the stop position.
2. Push the cube button (4) of the hand switch (3) to engage the optimum cube for specimen dye. In the cube display window (2), the selected cube is displayed (when using the Motorized microscope). Engage the optimum cube for specimen dye by operating the cube turret(2)(when using the Manual microscope).
3. Focus on the specimen by looking into the eyepiece. Be sure to adjust the diopter of the eyepiece in advance. (Refer to the instruction manual of the BX microscope.)



When the Z motor is in use, clear the check mark in the [Locked] check box in the [Z Stage] sub-panel in the [Acquire] panel (see section 2-2-1-3-7 of this volume), then focus on the specimen by operating the fine focus adjustment knob of the microscope. Be sure not to operate the fine focus adjustment knob while the [Locked] check box is checked, for this may damage the Z motor.



NOTE

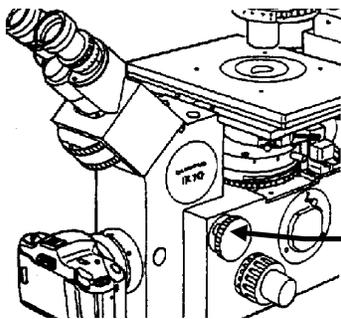
The specimen may float during oil-immersed observation. In this case, prepare an optional clip for immersion objective (BH2-SCB-3) and attach as shown on the left.



If you want to use a differential interference unit in transmitted light observation, refer to the instruction manual of your microscope.

NOTE With transmitted light differential interference observation using an immersion objective, set the microscope's field diaphragm so that it circumscribes the field of view. Otherwise the contrast may degrade.

1-2-3-2 Combination with IX



1. Turn the light path selector (1) on the right side of the microscope to  (when using the Manual microscope)

From the page tabs on the bottom right of the [Acquire] panel, select the [Settings] sub-panel (when using the Motorized microscope).

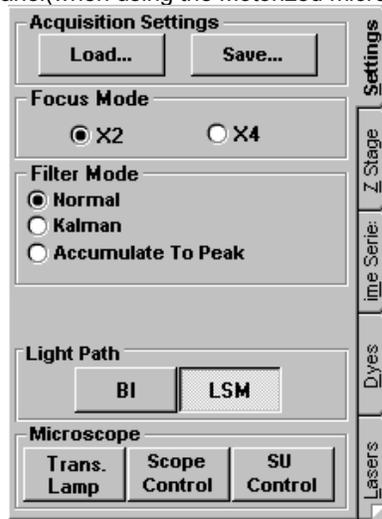
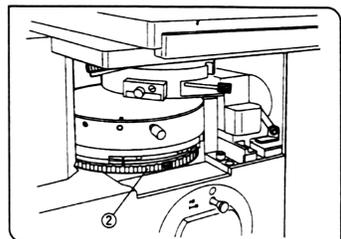
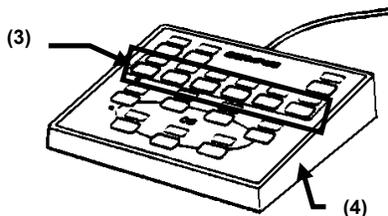


Fig. 1-4 [Settings] Sub-panel



2. Select the <BI> button in the [Light Path] group box (when using the Motorized microscope).

The <BI> button looks pushed in to indicate that it is selected.



3. Engage the optimum cube for specimen dye by operating the cube turret (2) (when using the Manual microscope)

Engage the optimum cube for specimen dye by pressing the cube button (3) on the hand switch (4).



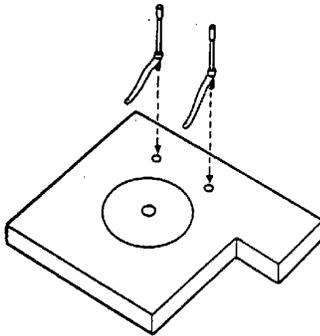
4. Focus on the specimen by looking into the eyepiece.
Be sure to adjust the diopter of the eyepiece in advance. (Refer to the instruction manual of the IX70/81 microscope.)



When the Manual microscope and the Z motor are in use, clear the check mark in the [Locked] check box in the [Z Stage] sub-panel in the [Acquire] panel (see section 2-2-1-3-7 of this volume), then focus on the specimen by operating the fine focus adjustment knob of the microscope. Be sure not to operate the fine focus adjustment knob while the [Locked] check box is checked, for this may damage the Z motor.



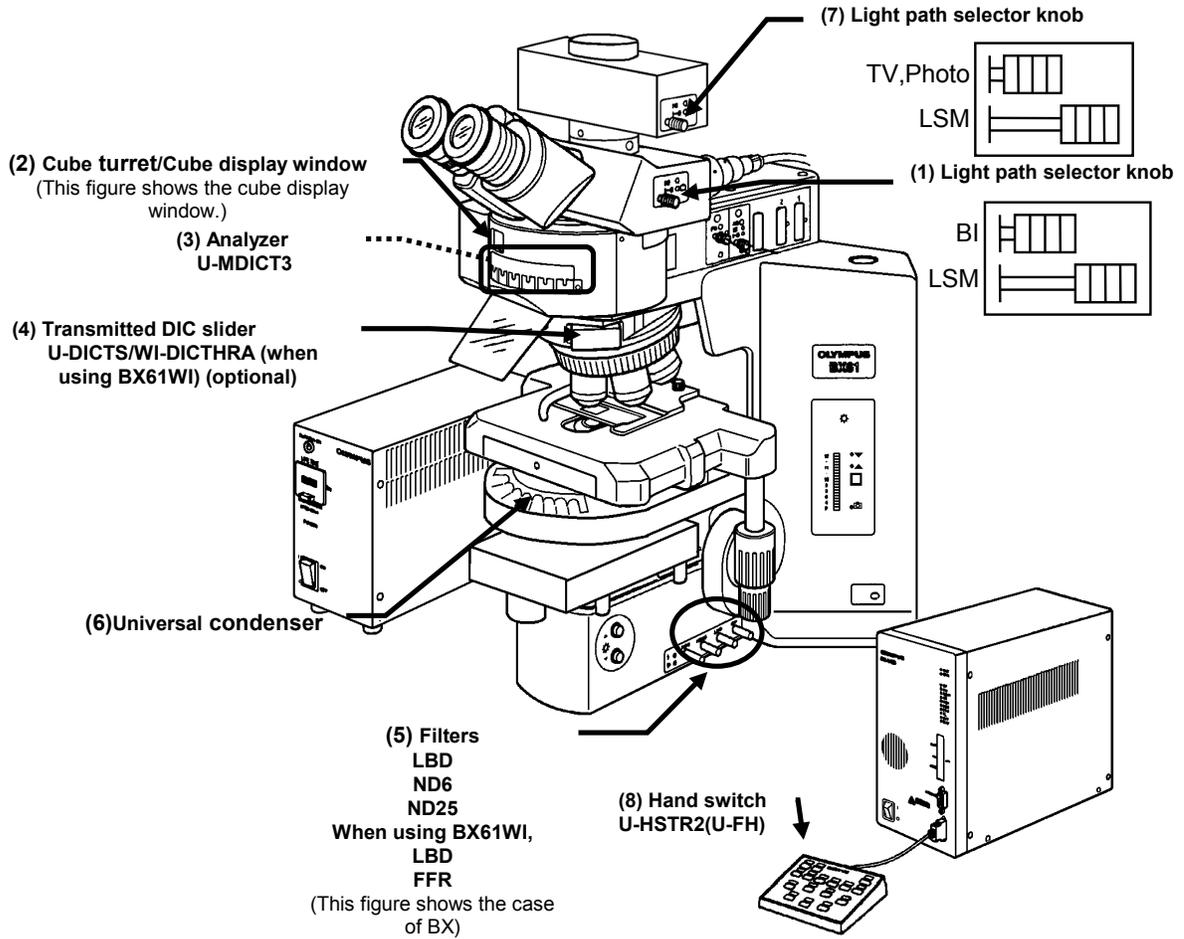
The specimen may float during oil-immersed observation. In this case, prepare a stage clip (U-SCL) and attach it to the microscope as shown on the left.



1-2-4 Setting the LSM Light Path

1-2-4-1 Combination with Upright Microscope (BX)

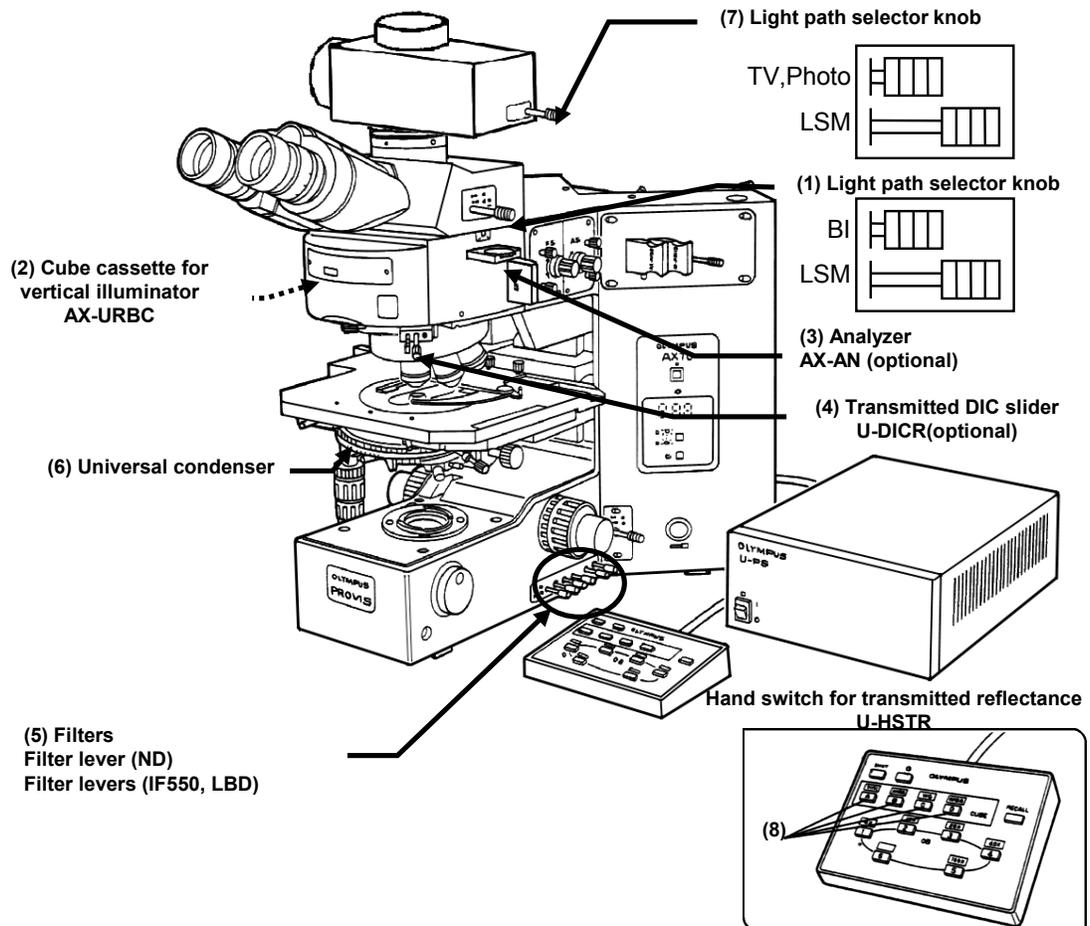
1. Pull out the light path selector on the trinocular tube fully to the stop position.
2. Pull out the light path selector (7) on the TV adapter fully to the stop position.
3. Push the hand switch button to (8) set  to be displayed in the cube display window (2) on the reflected light fluorescence vertical illuminator (when using the Motorized microscope).
Rotate the cube turret (2) on the reflected light fluorescence vertical illuminator to  when using the Manual microscope).
4. **When the U-MDICT3 analyzer (3) is in use, disengage it from the light path by setting the switch.**
5. When only fluorescence observation is required, disengage the U-DICTHR/WI-DICTHRA transmitted light DIC (4) by setting the switch to the pulled-out position. When transmitted light differential interference observation or simultaneous fluorescence + transmitted light differential interference observation is required, engage the transmitted light DIC and the optimum transmitted light DIC in the light path by operating the universal condenser (6).
6. For transmitted light observation, disengage any filter (5) from the light path. When you perform transmitted observation using laser with BX61WI, use the filter knob to disengage the LBD from the light path and engage the FR (Frost) into the light path. Disengaging the FR (Frost) from the light path may generate interference fringes on an image.





1-2-4-2 Combination with Erected Microscope(AX)

1. Pull out the light path selector (1) on the trinocular eyepiece fully to the stop position.
2. Pull out the light path selector (7) on the TV adapter fully to the stop position.
3. Select the cube in the cube cassette (2) for the vertical illuminator by pressing the required cube conversion button (8) on the U-HSTR hand switch.
4. **When the U-AN analyzer (3) is in use, disengage it from the light path by setting the switch to the pulled-out position.**
5. When only fluorescence observation is required, disengage the U-DICR transmitted DIC slider (4) by setting the switch to the pulled-out position. When transmitted light differential interference observation or simultaneous fluorescence + transmitted light differential interference observation is required, engage the U-DICT and the optimum transmitted light DIC slider for the objective in the light path by operating the universal condenser (6).
6. For transmitted light observation, disengage any filter (5) from the light path.



1-2-4-3 Combination with Inverted Microscope (IX81)

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Settings] sub-panel.

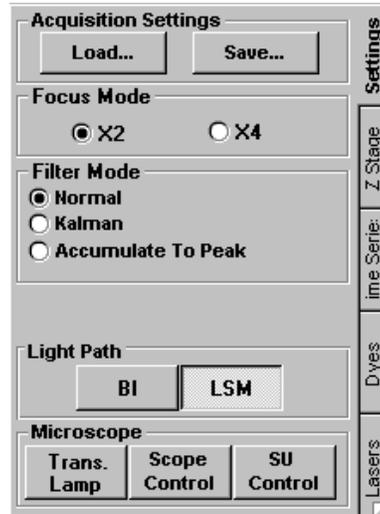
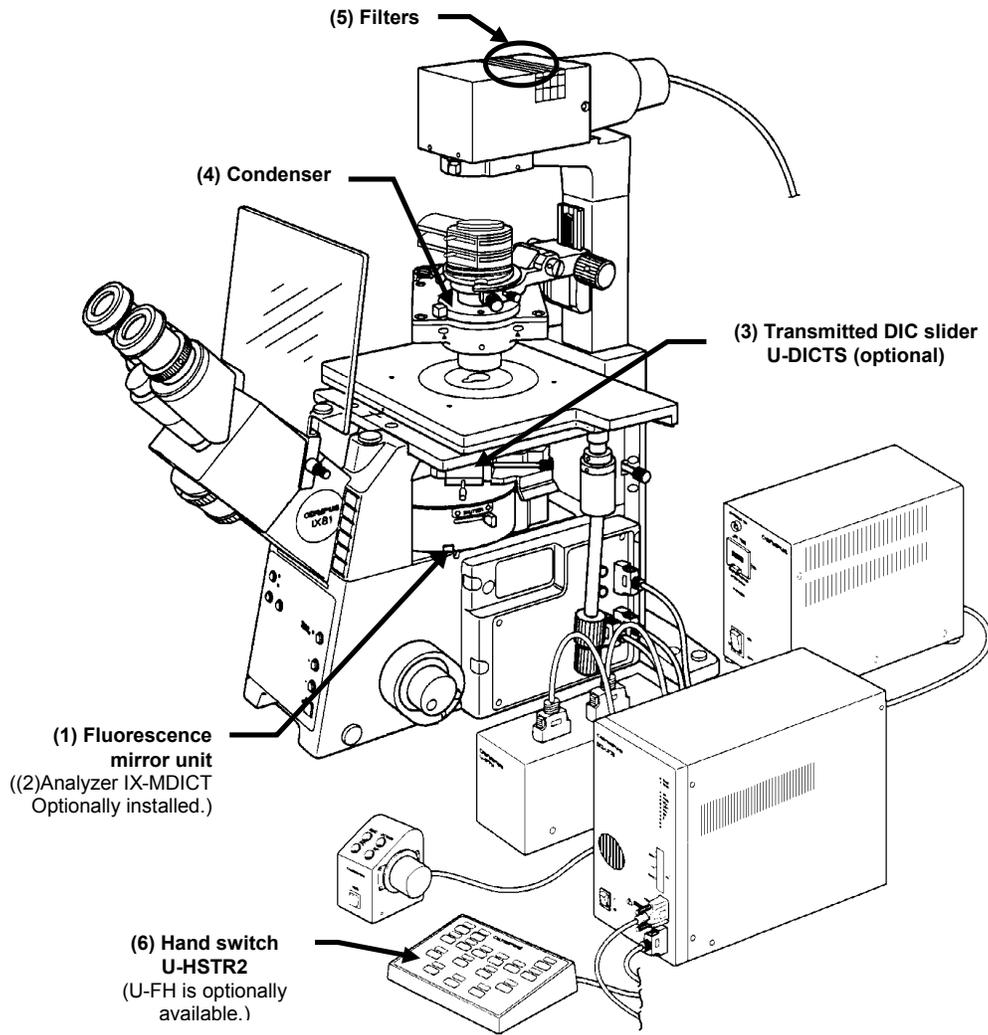


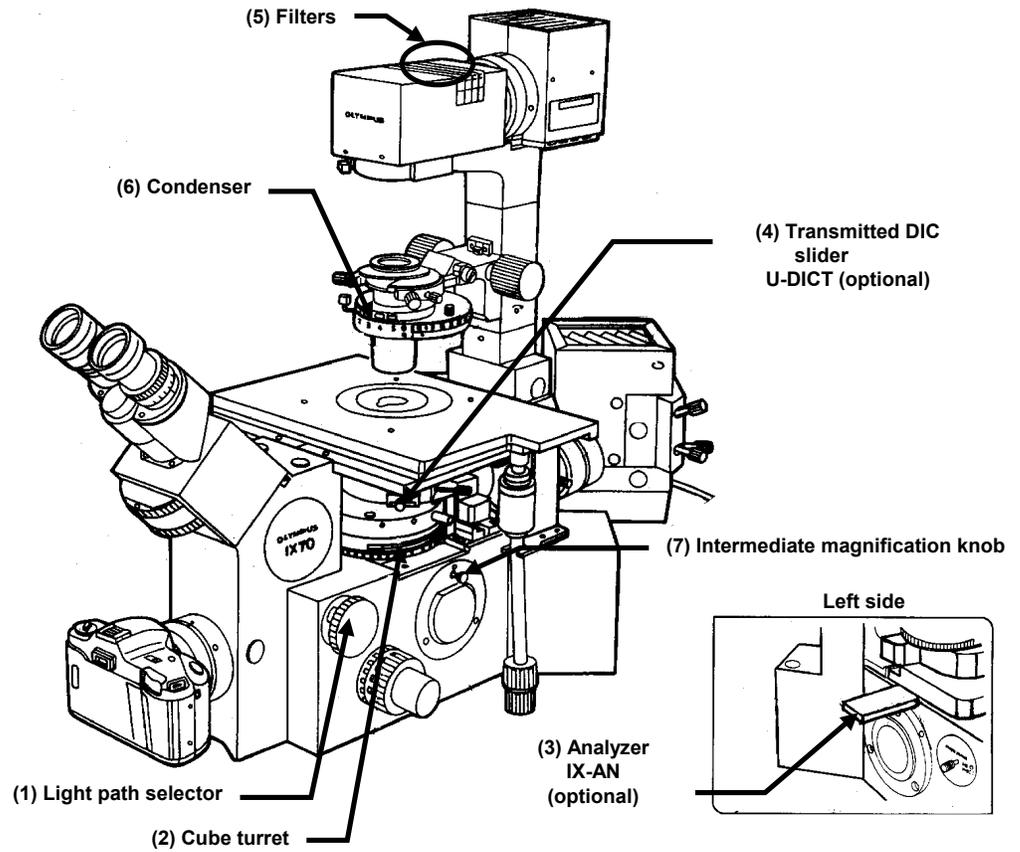
Fig. 1-5 [Settings] Sub-panel

2. Select the <LSM> button in the [Light Path] group box.
The <LSM> button looks pushed in to indicate that it is selected.
(When scanning is started while the <BI> button is selected, the LSM light path is selected automatically. It is switched back to the visual observation automatically when scanning completes.)
3. Push the hand switch button (6) to set reflected light fluorescence unit to .
4. **When the IX2-MDICT analyzer (3) is in use, disengage it from the light path by pressing the button (6) on the U-HSTR2 hand switch.**
5. When a fluorescence observation alone is required, disengage the U-DICTS transmitted DIC slider (3) by setting the switch to the pulled-out position. When a transmitted light DIC observation or a simultaneous fluorescence & transmitted light DIC observation is required, engage the U-DICTS and the optimum transmitted DIC slider for the objective into the light path by operating the universal condenser (4).
In a simultaneous fluorescence & transmitted light differential interference observation, leaving the U-DICTS within the light path degrades the fluorescence image resolution to some extent.
6. During the transmitted light observation, be sure to disengage the filter (5) from the light path.



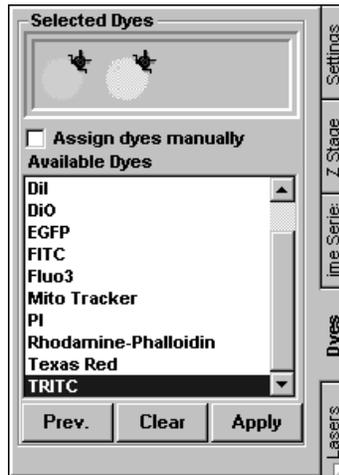
1-2-4-4 Combination with Inverted Microscope (IX70)

1. Turn the light path selector (1) to .
2. Set the intermediate magnification knob (7) to 1X. (The 1.5X position cannot be used.)
3. Rotate the cube turret of the reflected light fluorescence unit to .
4. **When the IX-AN analyzer (3) is in use, disengage it from the light path by setting the switch to the pulled-out position.**
5. When only fluorescence observation is required, disengage the U-DICT transmitted DIC slider (4) by setting the switch to the pulled-out position. When transmitted light differential interference observation or simultaneous fluorescence + transmitted light differential interference observation is required, engage the U-DICT and the optimum transmitted light DIC slider in the light path by operating the universal condenser (6). With simultaneous fluorescence + transmitted light differential interference observation, leaving the U-DICT engaged in the light path will degrade the fluorescence image resolution somewhat.
6. For transmitted light observation, disengage the LBD filter from the light path and engage the FR (frost) filter in the light path by operating the filter levers. If the FR filter is disengaged from the light path, the image may be marred by stripe interference.



1-2-5 Setting the Dyeing Methods

- From the page tabs on the bottom right of the [Acquire] panel, select the [Dyes] sub-panel.



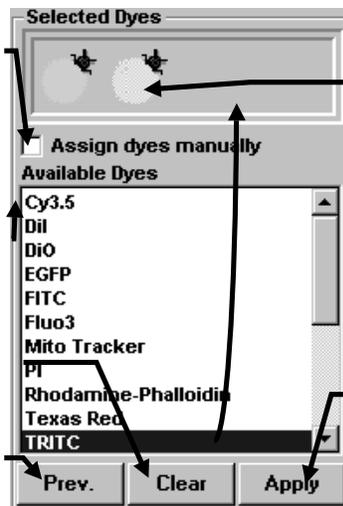
- Select the specimen dyeing method by dragging [FITC] and [TRITC] in the [Available Dyes] list box in the [Selected Dyes] group box to the field immediately above the list box.

[Assign dyes manually] check box
Checking this enables the manual setting. Dragging the dyeing method in the list directly to the [Ch] group box assigns the dye to the desired channel.

[Available Dyes] list box
Lists the available dyes. Select the desired items from this list and drag them to the field above it to select the dyeing method.

<Clear> button
Clear the set dyeing method.

<Prev.> button
Sets the dyeing method which was set last time by clicking the <Apply> button.



Place the pointer on the icon displayed in the [Selected Dyes], and the dyeing method is shown in the pop-up display.

<Apply> button
Applies the dyeing method dragged in the [Selected Dyes] group box to the [Channel 1]/[Channel 2] group box in the [Acquire] panel.

- Click the <Apply> button to apply the selected dyeing method to the [Channel1] / [Channel 2] group box on the upper part of the [Acquire] panel.

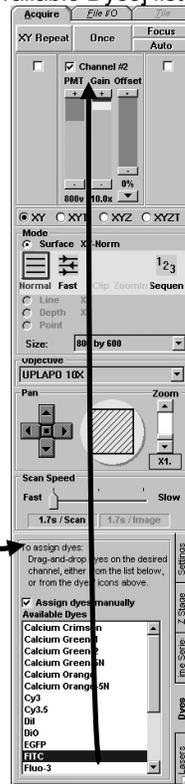
TIP When the dyeing method is selected in the [Available Dyes] list box and the <Apply> button is clicked, the dyeing method will be set automatically to the optimum channels.

The confocal aperture is also set automatically to the optimum channel for the wavelength and the objective being selected in the [Acquire] panel.

One Point!

The [Assign dyes manually] check box can also be used to set the dyeing method to the desired channel.

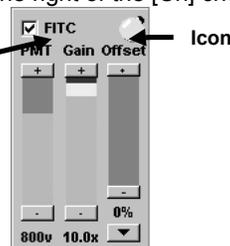
1. Check the [Assign dyes manually] check box in the [Dyes] sub-panel.
2. Select the dyeing method in the [Available Dyes] list box and drag it directly to the field of the [Ch] check box.



[Assign dyes manually] check box

3. After dragging, the icon appears on the right of the [Ch] check box and the dyeing method is set.

The dyeing method is set



Icon

Dragging the icon to the out of the [Ch] check box field cancels the setting of the dyeing method.

1-2-6 Selecting the CONFOCAL APERTURE

Set the CONFOCAL APERTURE knob (1) to select the optimum confocal aperture number for the objective, that is displayed on the control panel.

(Refer to section 1-1-1, "Scan Unit" in this volume.)

1-2-7 Selecting the DETECTION MODE

Set the DETECTION MODE slider to the optimum position according to the dye used with the observed specimen.

If the optimum setting cannot be identified, see section 1-3, "Online Help" in this volume and follow instructions in the [Microscope Configuration] window.

Reference Examples

Dye in Use	Detected Channels	DETECTION MODE slider
FITC Lucifer Yellow, etc.	CH1	 (Pushed-in position, mirror)
FITC + TRITC FITC + PI, etc.	CH2	 (pushed-in half way to the position,SDM560)
TRITC, PI	CH1/CH2	
FITC + CY5 TRITC+CY5	CH1/CH2	 *(Pulled-out position, SDM630)
CY5, etc.	CH2	

(See Section 1-1-1, "Scan Unit" in this volume.)

1-2-8 Setting the Barrier filters

Engage barrier filters in the light path according to the dye used with the observed specimen. (For the barrier filter selection method, see section 1-1-1, "Scan Unit" in this volume.)

The barrier filters are engaged in the light path when the BARRIER FILTERS slider (3) is set to the pushed-in position and disengage when the switch is set to the pulled-out position.



NOTE If the slider switch is set pushed-in half way to the position by mistake, no images can be observed at all.

Be sure to set the switch to the fully pulled-out or pushed-in position. If the optimum setting cannot be identified, see section 1-3-2-3, "Configuring the Filters" in this volume and follow instructions in the [Microscope Configuration] window.

The following table shows the possible combinations of the barrier filters and excitation dichroic mirrors.

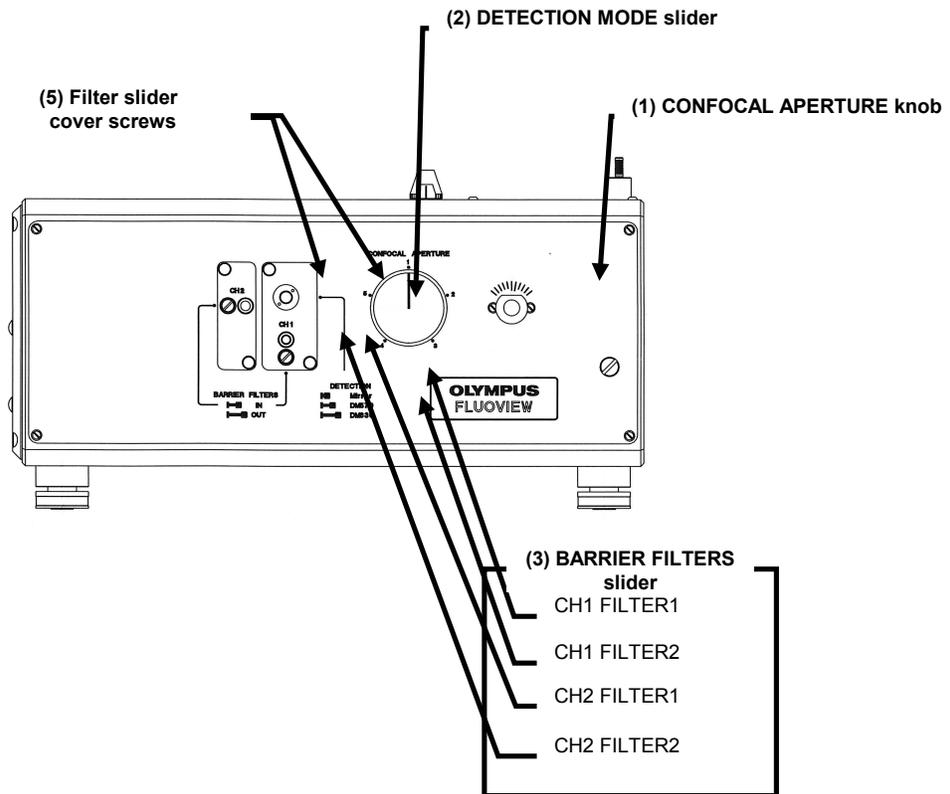
Combinations of Barrier Filters, Excitation Dichroic Mirrors, and Beam Splitter
(Example)

Lasers Combination	Excitation Dichroic Mirror	Beam Splitter	Barrier Filter 1	Barrier Filter 2
Ar 488 (2 channels)	(1) DM488	(1) Mirror (2) SDM570 (3) SDM630	(1) BA510IF	(1) BA565IF
Ar, HeNe(G) 488, 543 (2 channels)	(1) DM488/543	(1) Mirror (2) SDM570 (3) SDM630	(1) BA510IF (2) BA530RIF	(1) BA565IF
Ar, Kr 488, 568 (2 channels)	(1) DM488/568	(1) Mirror (2) SDM570 (3) SDM630	(1) BA510IF (2) BA550RIF	(1) BA585IF
Ar, HeNe(R) 488, 633 (2 channels)	(1) DM488/568/633	(1) Mirror (2) SDM570 (3) SDM630	(1) BA510IF (2) BA550RIF	(1) BA660IF
Ar, HeNe(G), HeNe(R) 488, 543, 633 (2 channels)	(1) DM488/543/633	(1) Mirror (2) SDM570 (3) SDM630	(1) BA510IF (2) BA530RIF	(1) BA560-600 (2) BA660IF
Ar, Kr, HeNe(R) 488, 568, 633 (2 channels)	(1) DM488/568/633	(1) Mirror (2) SDM570 (3) SDM630	(1) BA510IF (2) BA550RIF	(1) BA585-615 (2) BA660IF
Multiline Ar 458/488/515 (2 channels)	(1) DM488 (2) DM458/515	(1) Mirror (2) SDM570 (3) SDM630 (4) SDM515	(1) BA510IF (2) BA480-495	(1) BA565IF (2) BA535-565
Multiline Ar, HeNe(G) 458/488/515, 543 (2 channels)	(1) DM488/543 (2) DM458/515 (3) BS20/80	(1) Mirror (2) SDM570 (3) SDM630 (4) SDM515	(1) BA510IF (2) BA530RIF (3) BA480-495	(1) BA565IF (2) BA535-565
Multiline Ar, Kr 458/488/515, 568 (2 channels)	(1) DM488/568 (2) DM458/515 (3) BS20/80	(1) Mirror (2) SDM570 (3) SDM630 (4) SDM515	(1) BA510IF (2) BA550RIF (3) BA480-495	(1) BA585IF (2) BA535-565
Multiline Ar, HeNe(R) 458/488/515, 633 (2 channels)	(1) DM488/568/633 (2) DM458/515	(1) Mirror (2) SDM570 (3) SDM630 (4) SDM515	(1) BA510IF (2) BA550RIF (3) BA480-495	(1) BA660IF (2) BA535-565
Multiline Ar, HeNe(G), HeNe(R) 458/488/515, 543, 633 (3 channels)	(1) DM488/543/633 (2) DM458/515 (3) BS20/80	(1) Mirror (2) SDM570 (3) SDM630 (4) SDM515	(1) BA510IF (2) BA530RIF (3) BA480-495	(1) BA560-600 (2) BA660IF (3) BA535-565
Multiline Ar, Kr, HeNe(R) 458/488/515, 568, 633 (3 channels)	(1) DM488/568/633 (2) DM458/515 (3) BS20/80	(1) Mirror (2) SDM570 (3) SDM630 (4) SDM515	(1) BA510IF (2) BA550RIF (3) BA480-495	(1) BA585-615 (2) BA660IF (3) BA535-565

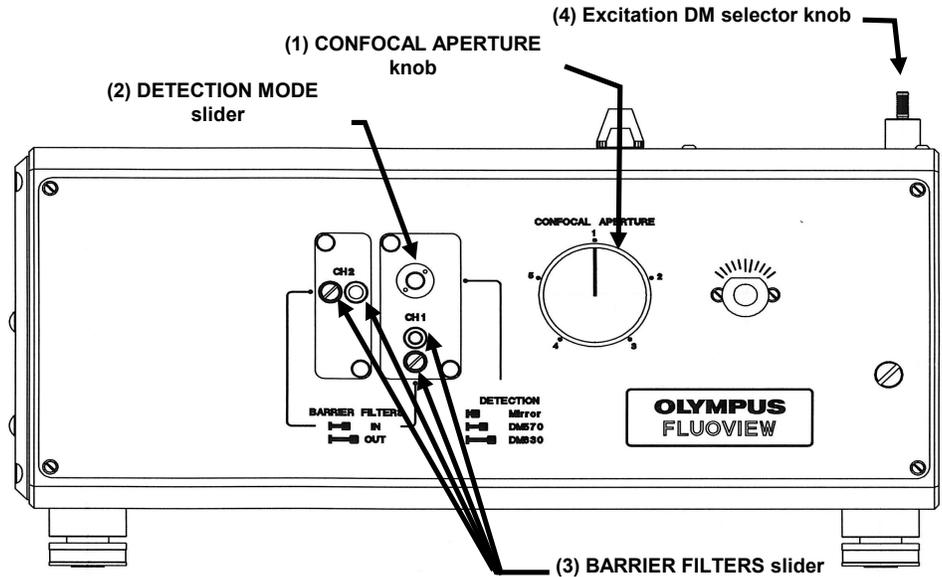


(1), (2), and (3) of the beam splitter are equipped as the factory configuration.
Up to two types of barrier filter can be equipped per channel.

If the equipment of another filter set for laser configuration is required, please contact your local Olympus representative.



1-2-9 Setting the Excitation DM



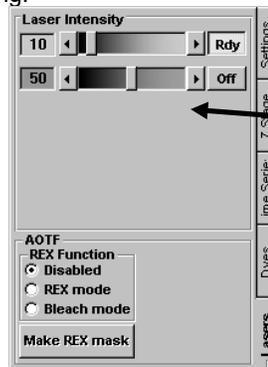
Using the excitation DM selector knob (4), select the excitation DM according to the wavelength of laser in use.

1-2-10 Setting the ND Filters of the Laser Combiner

When you use the laser combiner, you can set the laser intensity by setting ND filter on the laser combiner.

Display the [Acquire] panel.

Set each laser intensity by sliding the scale bar in the [Laser Intensity] group box of the [Lasers] sub-panel, in accordance with specimen's brightness, fluorescence crosstalk and photo-bleaching.



[Laser Intensity] group box
Set the laser intensity value by the scale bar.
The number of the displayed laser intensity sliders varies depending on that of channels setting for the acquisition.

While using the HeNe green laser, try out the laser power 50% by setting the [Intensity] scale bar in the [Laser Intensity] group box.

For other lasers, try the laser power 10%.

1-2-11 Selecting the Laser Line Filters (Combination Using the Kr/Ar Laser)

Select the laser line filters using the laser line filter turret.

Reference Examples

Dye	Laser Line Filters		
FITC Lucifer Yellow, etc.	488		
FITC+TRITC FITC+PI, etc.	568 +	or	568 +
	488		488
			AT25
PI TRITC, etc.	568		

(See item (5), "Kr/Ar laser line filter switching" in section 1-1-1, "Scan Unit" in this volume.)

1-2-12 Setting the Observation Condition

1-2-12-1 Setting the Objective Magnification

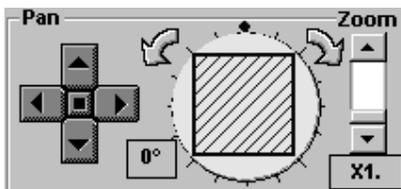
From the drop-down list on the center of the [Acquire] panel, select the objective being used with the microscope.



If the magnification of the objective in use and that set here do not match, the measurement results will be inaccurate.

1-2-12-2 Setting the Zoom Ratio to 1X

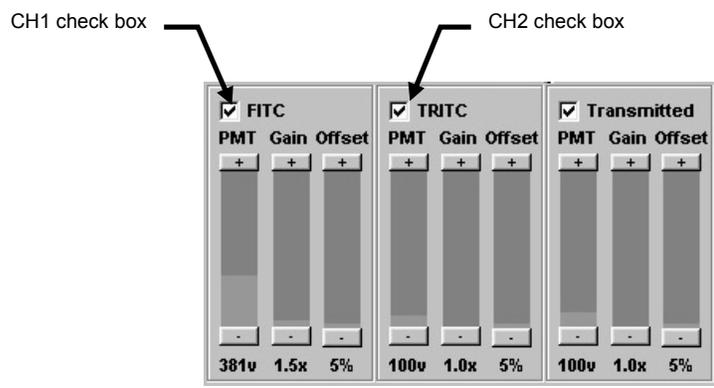
From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Settings] sub-panel. Then use the [Zoom] scale to set the zoom ratio to "X1".





1-2-12-3 Setting the Channels

1. In the Channel 1 group box, check the check box showing the applicable dyeing method to make the image acquisition ready.
2. In the Channel 2 group box, check the check box showing the applicable dyeing method to ready the image acquisition.



TIP To display the information on all channels simultaneously, right-click the boundary between channel display boxes. Click the boundary again to return to the original display.

1-2-12-4 Setting the Highest Scan Speed

1. Set the scan speed to the fastest speed by using the scale in the [Scan Speed] group box in the [Acquire] panel

[Scan Speed] group box
Set the scan speed by clicking a point on the scale line.



TIP

The focus mode makes it possible to increase the line skipped scan speed. From the page tabs on the bottom right of the [Acquire] panel, select the [Settings] sub-panel.

Select either option button in the [Focus Mode] group box.

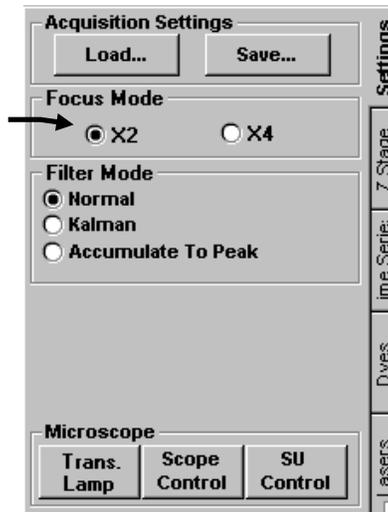
[Focus Mode] group box
[X2] option button

Acquires image at twice the highest speed.

[X4] option button

Acquires image at 4 times the highest speed.

Increasing the number of divided images in the [Display] panel, line skipped scan at 4 times (Focus) cannot be done.



The focus mode is enabled when acquiring images using the <Focus> button.

NOTE

The Focus function reduces the scanning time by line skipped scan. As a result, the acquired images become coarse.



1-2-12-5 Setting the XY Observation Mode

1. In the [Mode] group box in the [Settings] sub-panel, select the [Surface] option button.
2. In the [Mode] group box in the [Acquire] panel, select [800 by 600] from the [Size] drop-down list.
3. On the center of the [Acquire] panel, select the XY observation mode option button.

1-2-12-6 Repeated Scanning Operation



<XY Repeat> button



<Focus> button

1. Select the <XY Repeat> button. The acquired image will be displayed in the [Live] panel.



Use the <FOCUS> button to acquire image at an even higher speed. If the specimen is already being scanned, stop scanning with the <STOP SCAN> button before selecting the <XY Repeat> button.



The Focus function reduces the scanning time by line skipped scan. As a result, the acquired images become coarse.

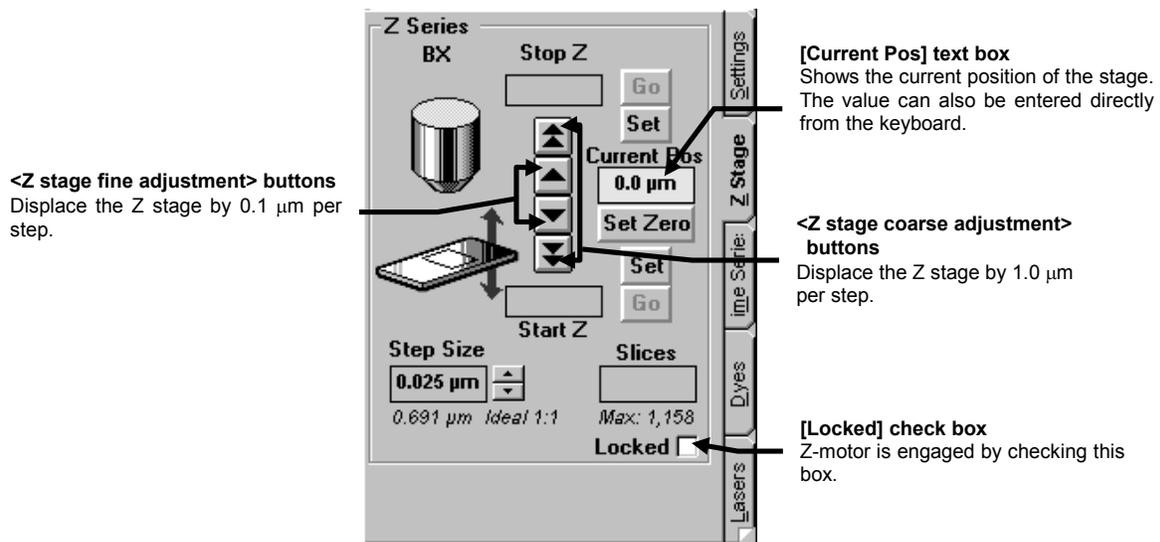


Do not move FLOUVIEW FV300 Menu while acquiring an image.

1-2-12-7 Setting the Multiple sections to be Observed

While acquiring image, move the Z stage to select the multiple sections to be observed.

From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.



1. Check the [Locked] check box in the [Z Stage] sub-panel.



Do not turn the fine focus adjustment knob while the [Locked] check box is checked, for this may damage the Z motor.

2. While observing the image in the [Live] panel, locate the plane to be observed by displacing the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

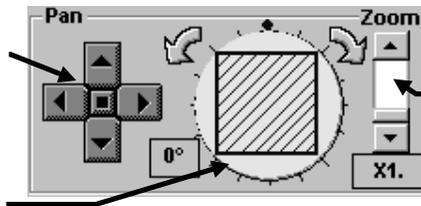


1-2-12-8 Setting the Area to be Observed

When the observation targets are concentrated in a narrow area or when it is necessary to observe the detail of a specific area, the image of a limited area can be acquired selectively.

The 4 buttons represent directions, and clicking a button moves the acquired image area in the direction indicated by the button. Clicking the square button on the center returns the acquired image area to the center.

Click a point inside the circle to change the position of the acquired image area directly.



Clicking a point in the scale area allows the value to be changed on a large scale. Clicking the top or bottom arrow button allows fine adjustment of the value. Dragging the square knob allows the value to vary directly.

1. Increase the zoom ratio using the [Zoom] scale in the [Acquire] panel.

1-2-12-9 Setting a Lower Scan Speed

The scan speed can be decreased using the scale in the [Scan Speed] group box on the center of the [Acquire] panel.

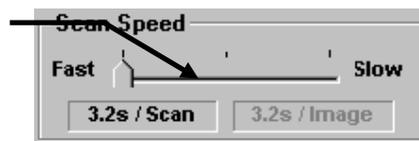


In general, setting a lower scan speed allows the acquired image quality to be improved. However, a low scan speed also lengthens the time required for image acquisition.



When the scan speed is decreased during fluorescence observation, the saturation of fluorescence may darken the image of certain types of specimens. In this case, increase the scan speed and increase the PMT Voltage or use accumulation in scanning.

[Scan Speed] group box
Set the scan speed by clicking a point on the scale line.



1-2-12-10 Stopping Repeated Scanning

After the brightness and gain have been adjusted, select the <STOP SCAN> button in the [Acquire] panel to stop scanning temporarily.



1-2-13 Adjusting the Detection Light Axis



<XY Repeat> button

Adjust the detection light axis for efficient acquisition of the detection light.

This adjustment is particularly important after the excitation DM has been switched using the excitation DM selector knob because this operation tends to deviate the light axis.

1. Select the <XY Repeat> button in the [Acquire] panel. The image appears in the [Live] panel.
2. If no image appears in the [Live] panel, increase the confocal aperture number by 1 using the confocal aperture rotary knob (1).
3. While observing the image in the [Live] panel, adjust the detection light axis adjustment knobs on the top and front of the scan unit to maximize the brightness of the image in the [Live] panel.
4. Decrease the confocal aperture number by 1 using the confocal aperture rotary knob (1).
5. Repeat steps 1 to 4 until the optimum confocal aperture number is set. For the confocal aperture number recommended for each objective, see section 1-1-1.



If the detection light axis adjustment is difficult due to dark image, adjust the PMT Voltage as described in section 2-2-1-3-9.

1-2-14 Acquiring Image



<Once> button

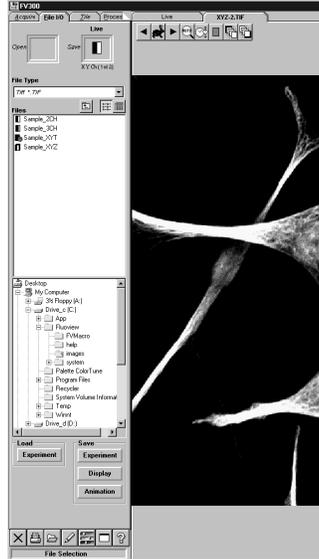
Select the <Once> button. The acquired image will be displayed in the [Live] panel.



Fig. 1-6 Image Acquired in the [Live] Panel

1-2-15 Saving Image

1. Display the [File I/O] panel.



2. When saving images acquired with more than one channel, it is possible to select whether images from more than one image are saved simultaneously or only one of the images is saved.

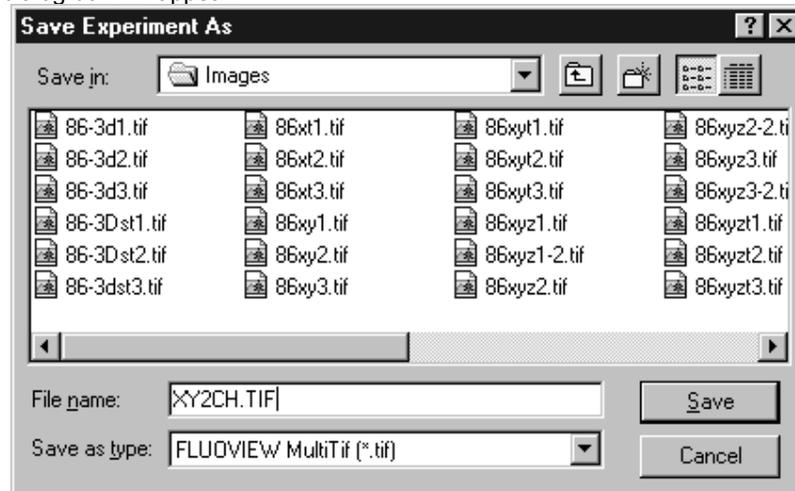


<Display channel switch> buttons

Use the <Display channel switch> buttons to select the images to be saved. The selected images will be saved under the conditions set for each channel.

Example) When only the image of Channel 1 is displayed, only the image of Channel 1 will be saved.

3. Select the <Experiment> button in the [Save] group box. The [Save Experiment As] dialog box will appear.

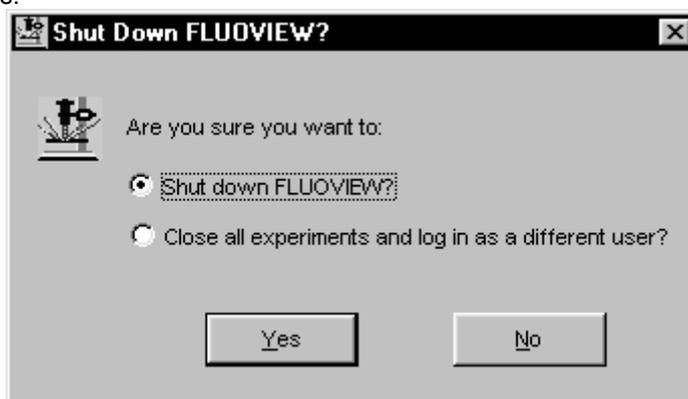


4. Enter the file name in the [File name:] text box.
5. Select "FLUOVIEW MultiTif" from [Save as type:]
6. Select the <Save> button.

1-2-16 Exiting from the Software



1. Click the <Exit> button in the toolbar at the bottom of the screen. This will allow you to exit the software. The [Shut Down FLUOVIEW?] dialog box as shown below appears.



2. To exit from this software, select the [Shut Down FLUOVIEW?] option button and click the <OK> button. Then this software finishes.
3. To logout from this software, select the [Close all experiments and log in as a different user?] option button and click the <Yes> button. The dialog box asking whether to save the observation data or not appears. If you want to save the data, click the <Yes> button, or the <No> button if saving is unnecessary. It returns to the FLUOVIEW login screen.
4. To shut down Windows, select the Windows <Start> button to display the [Start] menu, and select the [Shut Down] command from the menu.
5. When the [Shut Down Windows] dialog box appears, select the [Shut down the Computer?] option box and select the <Yes> button.

Wait until message "It is now safe to turn off your Computer" is displayed

1-2-17 Turning Power Off

1. Turn off the power supply to the units (reflected light power supply, power outlet unit).
2. Turn the laser power OFF.
 - Ar laser, Multiline Ar laser:

Turn the key to the OFF position. The fan of laser head will stop automatically in a few minutes, i.e. when the laser has cooled down.

Then set the power switch to OFF.

The fan of laser supply will stop.

(Refer to the instruction manual of your laser unit.)
 - Kr laser:

Turn the key to the OFF position then set the power switch to OFF. The fan will stop automatically in a few minutes, i.e. when the laser has cooled down.

(Refer to the instruction manual of your laser unit.)
 - HeNe (Green / Red) laser:

Turn the key to the OFF position.
 - HeCd laser:
 1. Turn the <OFF> button of remote interface module OFF.

Wait till "LASER OFF" message appears on digital display. (For about 5 minutes to cool it down.)
 2. Turn the key switch of remote interface module to the OFF position.
 3. Turn the key switch of laser controller to the OFF position.
 4. Turn the rocker switch of laser controller to "0" (OFF).



NOTE

Turning the laser power off by force may damage the laser tube.

Never omit the step 1 (the process for cooling down) except for emergency.

- LD405/440 laser
 1. Set the shutter switch to CLOSE.
 2. Turn the key switch to the OFF position.
 3. Set the power switch to "O" (OFF).

1-3 Online Help

The FLUOVIEW application comes with the online help facility which allows you to reference the function and operating procedure description while controlling the application.

This section describes a simple method of displaying and consulting online help.

1-3-1 Referencing Method



<Help> button



Finger pointer

The figure below shows the initial display (table of contents) of the FLUOVIEW Online Help window. To display this window, click the <Help> button in the toolbar at the bottom left of the screen.

Some words in the information display are shown in enhanced display (underscored or colored green). Clicking on one of these words allows you to “jump” to the meaning of the word or to further information about its meaning.

TIP

When the mouse pointer is placed on a word in enhanced display, the mouse pointer turns into a “finger pointer”.

Enhanced display

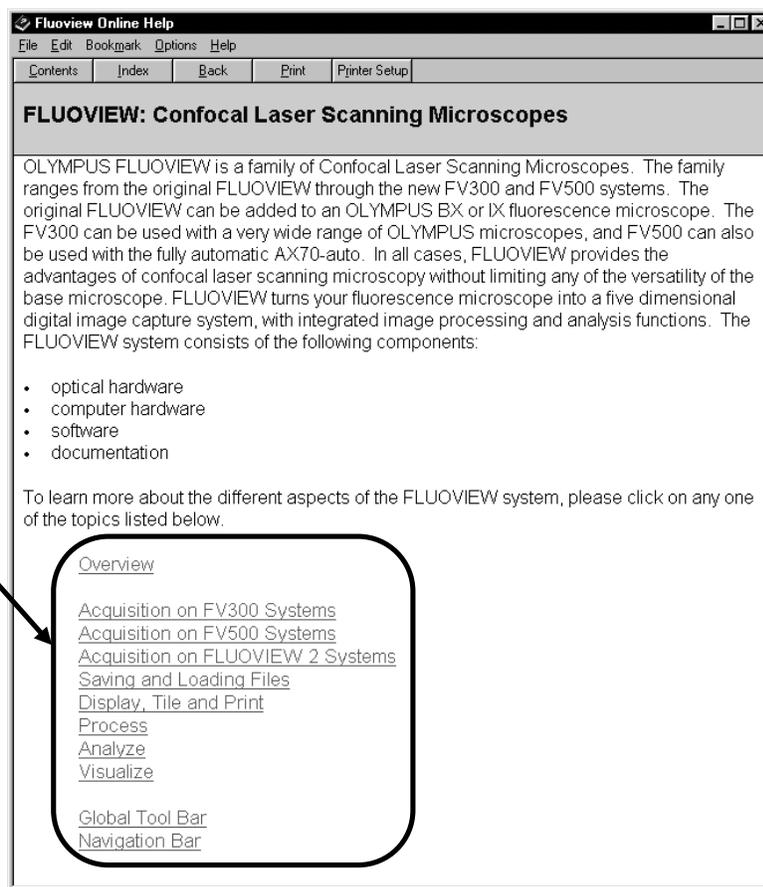


Fig. 1-7 Initial Window



One Point!

Select the <Contents> button to the initial display. Select the <Back> button to return to the previous information page.

1-3-2 Setup of Microscope and Scan Unit

The microscope and scan unit can be set up from the FLUOVIEW software, by selecting the observation method and following the displayed guidance information.

• Selecting the Dyeing Method

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Dyes] sub-panel.

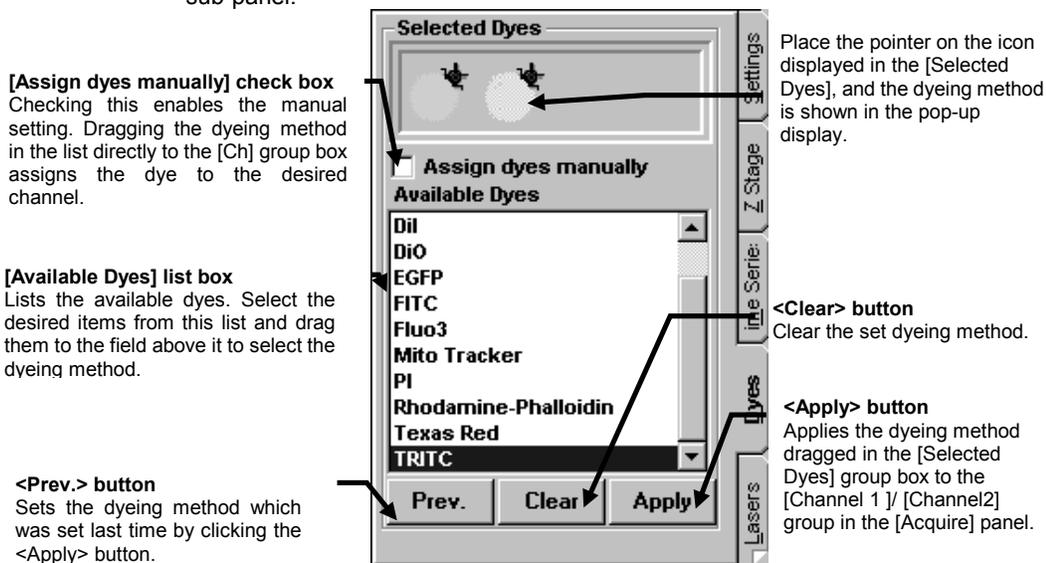


Fig. 1-8 [Dyes] Sub-panel

2. Select the specimen dyeing method by dragging desired dye names in the [Available Dyes] list box in the [Selected Dyes] group box to the field immediately above the list box.
3. Click the <Apply> button to apply the selected dyeing method to the [Channel 1]/[Channel 2] group box on the upper part of the [Acquire] panel.



When the dyeing method is selected from the [Available Dyes] list box and the <Apply> button is clicked, the dyeing method will be set automatically to the optimum channels.

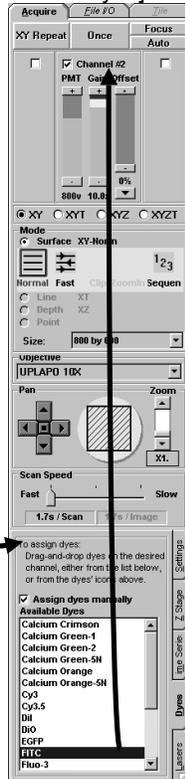
The pinhole diameters are also automatically set to the optimum channels according to the wavelength and the objective selected in the [Acquire] panel.



One Point!

The [Assign dyes manually] check box can also be used to set the dyeing method to the desired channel.

1. Check the [Assign dyes manually] check box in the [Dyes] sub-panel.
2. Select the dyeing method in the [Available Dyes] list box and drag it directly to the field of the [Ch] check box.

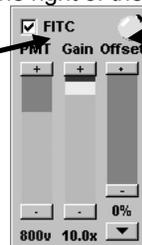


[Assign dyes manually] check box

3. After dragging, the icon appears on the right of the [Ch] check box and the dyeing method is set.

The dyeing method is set

Icon

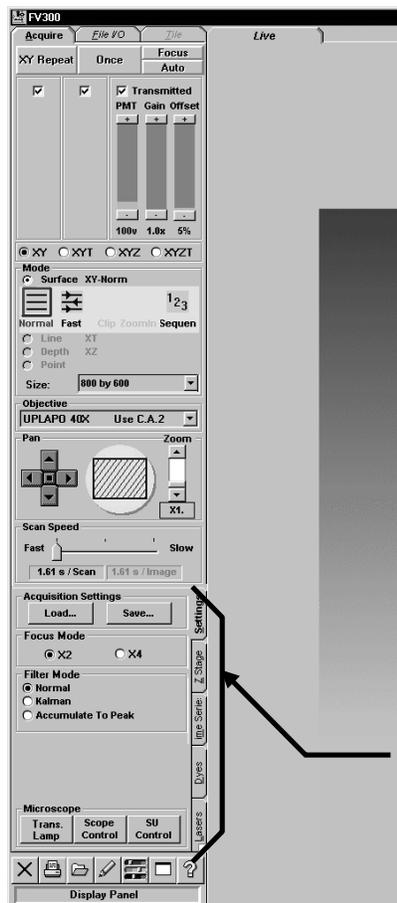


Dragging the icon to the out of the [Ch] check box field cancels the setting of the dyeing method.



1-3-2-1 Configuring the Microscope

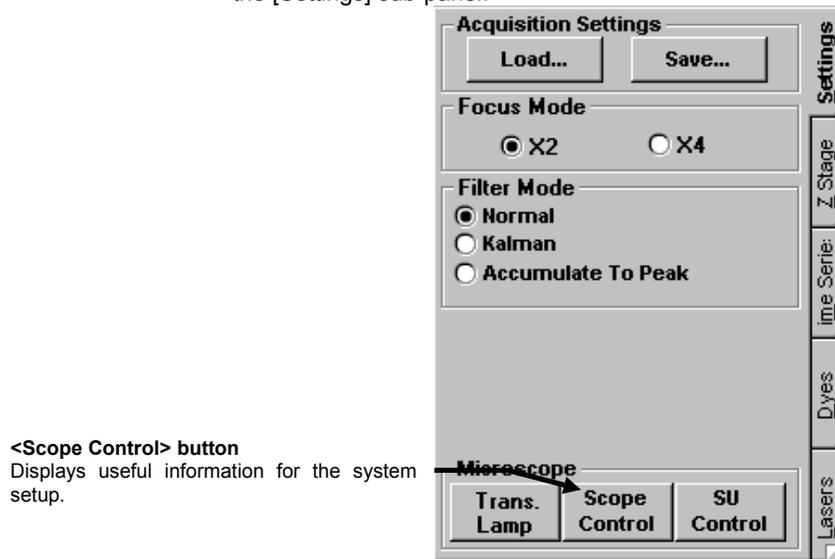
1. Display the [Acquire] panel.



[Settings]/[Z Stage]/[Time Series]/[Dyes]/ [Lasers] sub-panels
These are used to set the information required for image acquisition.



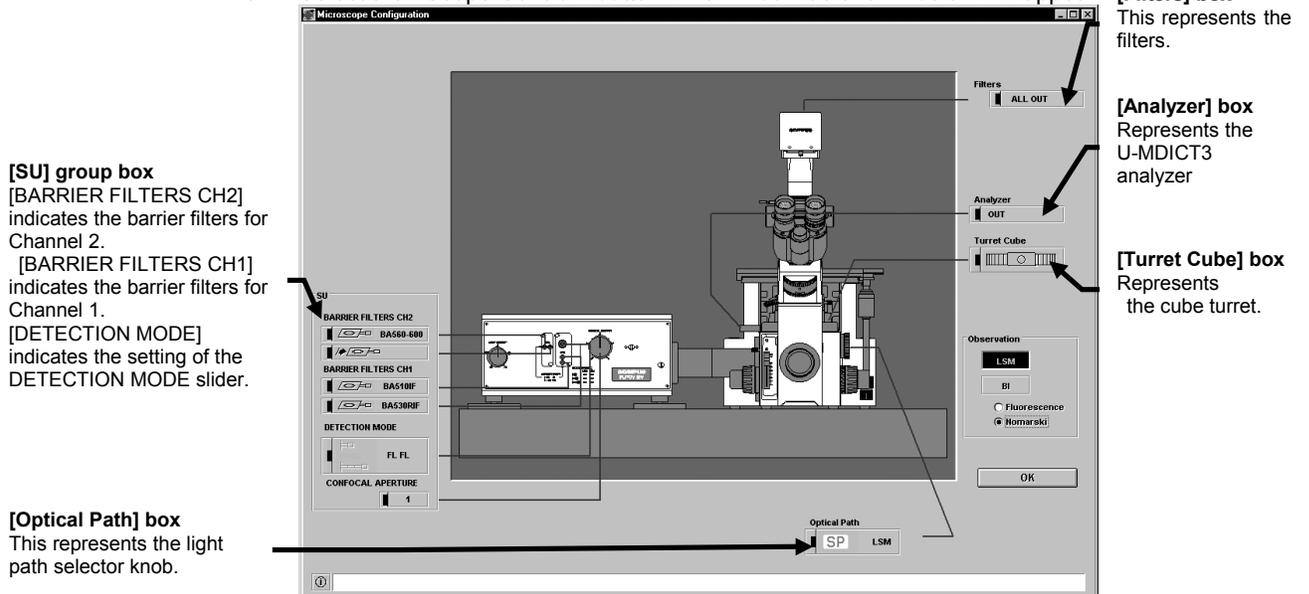
- From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Settings] sub-panel.



<Scope Control> button
Displays useful information for the system setup.

Fig. 1-9 [Settings] Subpanel

- Select the <Scope Control> button. The window as shown below will appear.



[SU] group box
[BARRIER FILTERS CH2]
indicates the barrier filters for Channel 2.
[BARRIER FILTERS CH1]
indicates the barrier filters for Channel 1.
[DETECTION MODE]
indicates the setting of the DETECTION MODE slider.

[Optical Path] box
This represents the light path selector knob.

[Filters] box
This represents the filters.

[Analyzer] box
Represents the U-MDICT3 analyzer

[Turret Cube] box
Represents the cube turret.

Fig. 1-10 [Microscope Configuration] Window

BI

<BI> button

4. Select the <BI> button in the [Observation] group box, and select the microscopy from the option buttons below it. The system setting points to be changed for microscope observation will blink in red.

Optical Path**BI**

5. Change the system configuration (setup of light path selector, etc.) by following the guidance given by the red blinking light.

(For the operation of the light path selector lever, see section 1-1-2, "Microscope" in this volume.)

NOTE

The Red blinking indicates where can be changed. The blinking does not stop even after the indicated configuration setting has been changed.

6. While looking into the microscope, move the stage and check the observed image.

1-3-2-2 Configuring the Scan Unit**LSM**

<LSM> button

1. In the [Observation] group box in the [Microscope Configuration] window, select the <LSM> button.

Optical Path**LSM**

2. The system setting points to be changed for LSM observation will blink in red. Change the system configuration (setup of light path selector, barrier filters, etc.) by following the guidance given by the red blinking light.

NOTE

The Red blinking indicates where can be changed. The blinking does not stop even after the indicated configuration setting has been changed.

3. After completing the system configuration, select the <OK> button.

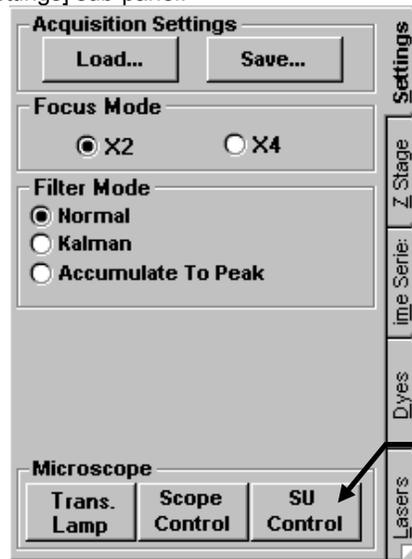


1-3-2-3 Configuring the Filters

The barrier filters, excitation filter and beam splitter are set automatically to the light path according to the dyeing method selected for the specimen.

Use the following procedure to change these filters.

1. From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Settings] sub-panel.



<SU Control> button
Click to display the filter and laser types to be set.
(Combination with the laser combiner sets up the laser automatically.)

Fig. 1-11 [Settings] Sub-panel

- Click the <SU Control> button at the bottom of the panel. The window as shown below will appear.

[Laser Unit] group box

Shows the type of laser to be used.

(With the laser combiner operation, the laser type is set and displayed automatically.)

When the <On> button is pressed-in, the laser is oscillating the beam.

When not using laser for a long time, in order to suppress useless electric-power consumption, we recommend you making <Stby> mode.

When the <Stby> button is pressed-in, the laser is not oscillating.

When the [Auto standby] check box is checked, the [After] text box appears below it.

The laser oscillation stops when the time shown in this box has elapsed after the end of laser scanning. The laser is suspended as standby mode (using Ar or Kr-laser) or the laser oscillation stops (using UV-Ar laser)

When the time shown in this box has elapsed after the end of laser scanning.

[Manual Scan Unit] group box

Shows the type of filters suitable for the dyeing method.

[TD Unit] group box

Shows the transmitted light detection.

[Dyes]

Shows the dyeing method set for each channel.

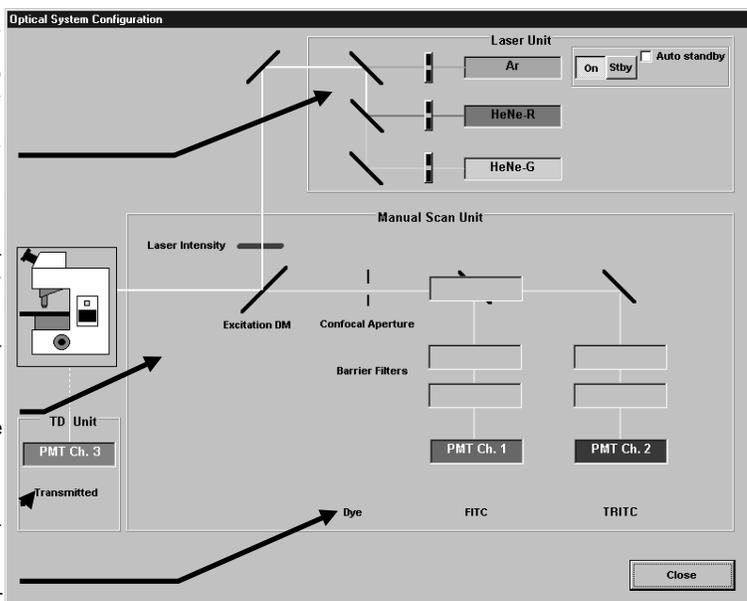


Fig. 1-12 [Optical System Configuration] Window

TIP

Virtual channels can be used. For the virtual channels, see section 2-2-8-1, "Virtual Channel".

- The filter of the showed kind type is set up in the scan unit.

NOTE

The [Microscope Configuration] window is designed to give guidance on the system configuration. The red blinking light does not stop even after the indicated configuration point has been changed.

- Click the <Close> button to click the window.



1-3-2-4 Configuring the Microscope (Combination with BX51, BX61, IX81)

When the combination with BX51, BX61, or IX81 is in use, the microscope and scan unit (FV500) can be configured on the FLUOVIEW software.

Use the following procedure to configure the microscope.

1. From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Settings] sub-panel.

<Scope Control> button
Sets the system.

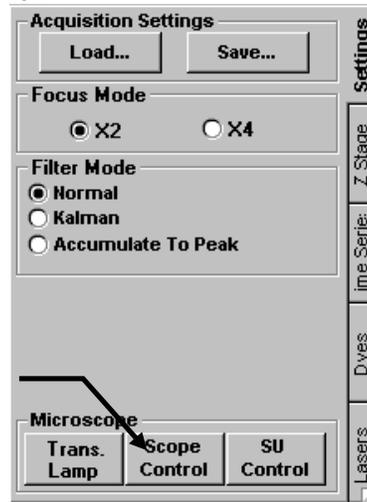
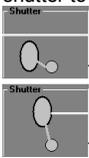


Fig. 1-13 [Settings] Sub-panel

- Select the <Scope Control> button on the bottom of the [Settings] sub-panel. The [Microscope Control panel] window as shown below appears.

[Shutter] group box
Clicking inside the box switches the EPI shutter to be closed/opened,



The shutter is opened.

The shutter is closed.

[EPI lamp]
Indicates the EPI lamp.

[Filter Turret] group box
Clicking the filter for reflected light observation to be set switches the turret automatically.

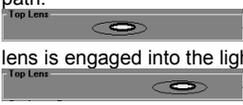
[Light Path] group box
Selects the light path. <BI> is for direct observation, <LSM> is for LSM observation and <TV> is for TV observation.

[Filter Turret] group box
Clicking the filter for visual observation to be set switches the turret automatically.

[Mirror Unit] group box
Clicking the cube automatically switches the turret.

[Nosepiece] group box
Click to change the objective.

[Top Lens] group box
Clicking inside the box switches the top lens to be engaged into the light path.



The top lens is engaged into the light path.



The top lens is disengaged from the light path.

[Condenser Turret] group box
Clicking the universal condenser automatically switches the turret.

[Aperture Iris] group box
Changes the AS value.

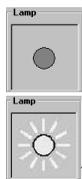
[Filter Turret] group box
Clicking the filter for transmitted light observation to be set switches the turret automatically.

[Options] group box
Used for optional BX settings.

<Link Setting> button
Selects the function to change settings corresponding to the change of BX settings.

<Focus Setting> button
Enables parfocal corrections and adjustments of jogging sensitivity for the objectives.

[Lamp] group box
Clicking inside the box switches the TD lamp ON/OFF.



The TD lamp is set to OFF.



The TD lamp is set to ON.

(Combination with BX)



[Lamp] group box

Clicking inside the box switches the TD lamp ON/OFF.



The TD lamp is set to OFF.



The TD lamp is set to ON.

[Options] group box

Used for optional IX settings.

<Link Setting> button

Selects the function to change settings corresponding to the change of IX settings.

<Focus Setting> button

Enables parfocal corrections and adjustments of jogging sensitivity for the objectives.

[Light Path] group box

Selects the light path. <BI> is for direct observation, <LSM> is for LSM observation.

[Condenser Turret] group box

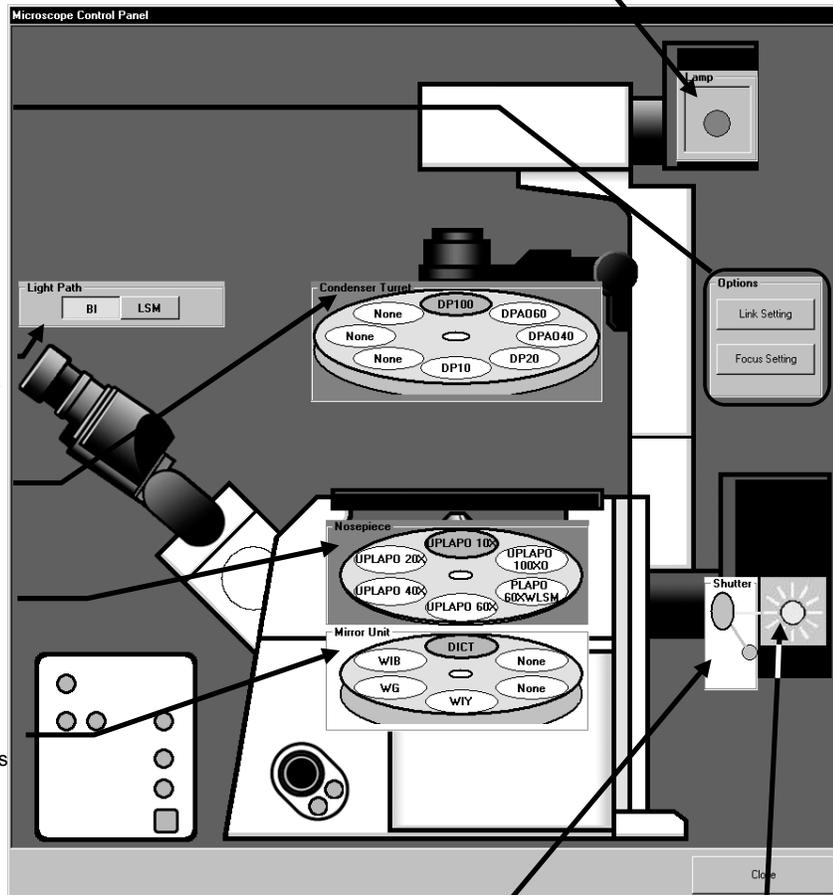
Clicking the universal condenser automatically switches the turret.

[Nosepiece] group box

Click to change the objective.

[Mirror Unit] group box

Clicking the cube automatically switches the turret.



[Shutter] group box

Clicking inside the box switches the EPI shutter to be closed/opened,



The shutter is opened.



The shutter is closed.

[EPI lamp]

Indicates the EPI lamp.

(Combination with IX)

- Clicking the <Link Setting> button displays the [Link Setting] dialog box as shown below.

Checking here links the objective in the [Nosepiece] group box with the condenser turret.

Checking here escapes the stage or revolving nosepiece when the objective is selected and changed in the [Nosepiece] group box.

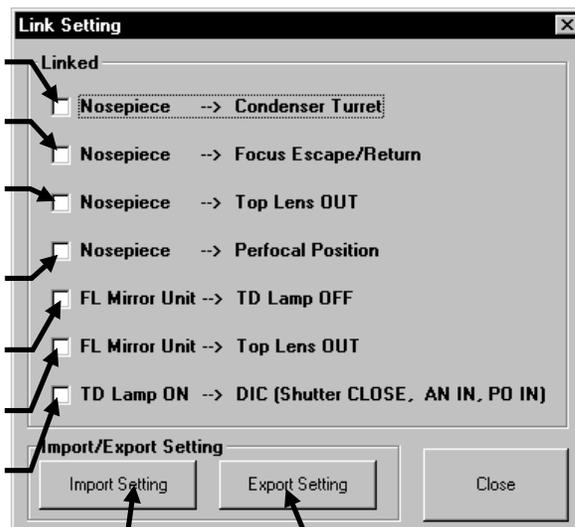
Checking here disengages the top lens from the light path when the objective of $\times 4$ or lower magnification is selected in the [Nosepiece] group box. (BX only)

Checking here enables parfocality correction when the objective is selected and changed in the [NosePiece] group box.

Checking here sets the TD lamp to OFF when the fluorescent cube is selected in the [Mirror Unit].

Checking here disengages the top lens from the light path when the fluorescent cube is selected in the [Mirror Unit].

Checking here closes the FL shutter and engages Analyzer and Polarizer into the light path when the TD lamp is set to ON in the [Lamp] group box.



<Import Setting> button
Loads the microscope settings already registered to reflect it.

<Export Setting> button
Saves the current

Click the desired check box to be checked.

Select the <Close> button to close the dialog box.

The microscope settings are automatically saved and read out when the software is started up next time.



Open the setting

Open the file to which the microscope settings were exported with the <Export Setting> button.

The Settings configured by another user or configured for another combination can be applied.

Selecting the <Import Setting> button of the [Link Setting] dialog box displays the [Open] dialog box as shown below.



When the setting file which you want to read is not displayed in the list box, use the [Look in:] drop-down list and select the drive or directory where the file is saved.

Select "BX Setting File (*.ini)" in the [Files of type:] drop-down list.

In the list box, select the setting file which you want to read.

Select the <Open> button to close the dialog box.

Save the setting

Settings in the [Link Setting] dialog box can be applied to other users or other combinations.

Selecting the <Export Setting> button of the [Link Setting] dialog box displays the [Save As] dialog box as shown below.





To change the save destination drive or directory, use the [Save in:] drop-down list.

Enter the setting file name into the [File name] text box.

Select the <Save> button to close the dialog box.

- The <Focus Setting> button enables parfocal corrections and adjustments of jogging sensitivity for the objectives.

See section 1-3-2-5, "Parfocality Correction and Jog Sensitivity Adjustment (When the BX or IX is used)".

Select the <Exit> button to close the dialog box.

3. After completing the system setup, click the <Close> button to close the window.

1-3-2-5 Parfocality Correction and Jog Sensitivity Adjustment (When the BX or IX is used)

When the system use the BX or IX microscope, the parfocality correction and jog sensitivity can be set per objective.

1. Open the [Microscope Control Panel] window.
For the method of displaying this window, see steps 1 and 2 in section 1-3-2-4, "Configuring the Microscope (Combination with BX51, BX61, IX81)" in this section.

2. Click the <Focus Setting> button in the [Microscope Control Panel] window.
The [Focus Setting] dialog box as shown below opens.

[Objectives] group box
Click the button for the objective subjected to parfocality correction so that the button looks pushed in.

Objectives	Pfcl	Jog Fine
UPLAPO 10X	0.00	200um/rot
PLAPO 40X	0.00	100um/rot
PLAPO 40XWLSM	0.00	100um/rot
PLAPO 60X	0.00	100um/rot
PLAPO 60XWLSM	0.00	100um/rot
PLAPO 100X	0.00	100um/rot

[Pfcl Setup Wizard] group box
The Wizard for parfocality correction.

<Next> button
Click to execute the Parfocality Setup (correction) Wizard.

<Import> button
Click to import the previously saved parfocality correction and jog sensitivity values for each objective.

<Pfcl> button
Depress to activate the parfocality correction. To deactivate, depress this button once again.

[Jog Fine] group box
Select the jog sensitivity of each objective from the drop-down list.

Display of the parfocality correction value of each objective.

<Exit> button
Click to close the [Focus Setting] dialog box.

3. When the [Focus Setting] dialog box is displayed, the objective mounted on the microscope is selected.
To use the setup wizard to begin the parfocality correction from the value of the objective with maximum magnification in descending order, click the <Next> button in the [Pfcl Setup Wizard] group box.

4. The following option button menu is displayed in the [Pfc] Setup Wizard] group box. If you want to begin the parfocality correction value setting with the objective with the maximum magnification, click the [Switch to...] option button then click the <Next> button.

Click to set the objective with the maximum magnification in the list as the reference for correction of other objectives

Click to set the objective being selected in the [Objectives] group box as the reference for correction.

Focus Setting		
Focus Setting		
Objectives	Pfcl	Jog Fine
UPLAPO 10X	4.00	200um/rot
PLAPO 40X	3.00	100um/rot
PLAPO 40XWLSM	4.00	100um/rot
PLAPO 60XD	1.00	100um/rot
PLAPO 60XWLSM	2.00	100um/rot
PLAPO 100XD	0.00	100um/rot

Pfc] Setup Wizard
Select one of the following and Click Next button.

Switch to PLAPO 100XD and Make it as the reference.

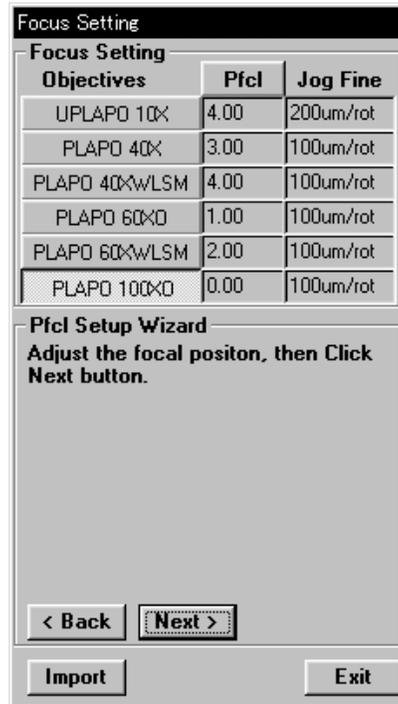
Make the current Objective :UPLAPO 10X as the reference.

< Back Next >

Import Exit



5. The objective set as the specified in the [Objectives] group box is selected, and the [Pfcl Setup Wizard] group box displays the message shown below. Bring the specimen in focus by observing it visually or on the scanned image, and then click the <Next> button.



6. The [Pfc1 Setup Wizard:] group box displays the option button menu shown below. If you want to set the parfocality correction values of objectives with other power values, simply click the <Next> button.

Click to proceed to the setting of the objective with next higher power to the current objective.

Click to select the desired objective from the [Objectives] group box and set the parfocality correction value for it.

Click to save the currently set parfocality correction values and close the dialog box.

Objectives	Pfc1	Jog Fine
UPLAPO 10X	4.00	200um/rot
PLAPO 40X	3.00	100um/rot
PLAPO 40XwLSM	4.00	100um/rot
PLAPO 60XD	1.00	100um/rot
PLAPO 60XwLSM	2.00	100um/rot
PLAPO 100XD	0.00	100um/rot

Pfc1 Setup Wizard
Select one of the following and Click Next button.

- Switch to PLAPO 60XD and Set parfocal value for it.
- Set Parfocal value for the current Objective :PLAPO 100XD.
- Save each Parfocal value and Jog Setting, then Exit.

< Back Next >

Import Exit

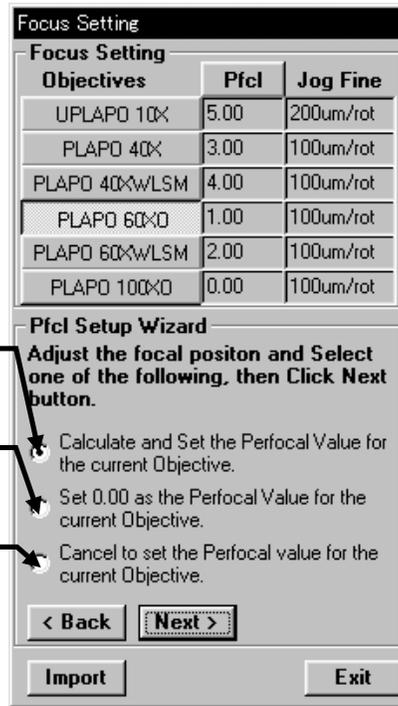


7. The [PfcI Setup Wizard] group box displays the option button menu shown below. Adjust the focal position and click the <Next> button.

Click to set the current focal position as the parfocality correction value for the current objective and proceed to the setting of the next objective.

Click to set the parfocality correction value of the current objective to "0.00" and proceed to the setting of the next objective.

Click to proceed to the parfocality correction setting of the next objective without changing the setting for the current objective.



8. Set the parfocality correction values of objectives by repeating steps 6 and 7 for each.

The [Pfc] Setup Wizard] group box displays the option button menu shown below.

Click the <Pfc> button to activate the parfocality correction.

Objectives	Pfc]	Jog Fine
UPLAPO 10X	5.00	200um/rot
PLAPO 40X	3.00	100um/rot
PLAPO 40XwLSM	4.00	100um/rot
PLAPO 60X	1.00	100um/rot
PLAPO 60XwLSM	2.00	100um/rot
PLAPO 100X	0.00	100um/rot

[Jog Fine] group box
Select the jog sensitivity of each objective from the drop-down list. The * mark represents the value recommended.

Click to save the currently set parfocality correction values and close the dialog box.

Pfc] Setup Wizard
Select one of the following and Click Next button.

- Switch to LAPD 60X and Set perfocal value for it.
- Set Perfocal value for the current Objective :LAPD 100X.
- Save each Perfocal value and Jog Setting, then Exit.

Import Exit

9. Set the jog sensitivity values of the objectives.

Select the jog sensitivity of each objective from the [Jog Fine] drop-down list.

The drop-down list appears when clicking each value in the [Jog Fine].

10. After completing the settings, click the <Exit> button to close the dialog box.

When a dialog box is displayed to ask where or not you want to save the settings, click <Yes> to save them, <No> to not to save them or the <Cancel> button to return to the parfocality correction and jog sensitivity value setting.

2 APPLIED OPERATIONS

2-1 General Operation Procedure

This section describes the general image acquisition procedure with the aim to get accustomed with the operation.

Begin using the FLUOVIEW system by acquiring an image or opening an image from a file. The procedures for the subsequent operations such as image processing are not in question here. For the detailed operation method of each item in the procedure, see the section specified in parentheses (()).

**NOTE**

The following is the general operation procedure of FLUOVIEW. Many other functions that are not shown in the following are also available. Please also study their description.



Turn power ON and start the FLUOVIEW software.
(Sections 1-2-1 & 1-2-2)

(A) Acquire an image.
(According to the selected observation mode)

For detailed operation procedures for image acquisition, see section 2-1-1, "Image Acquisition Procedure (Section A)".

Open an image in a file.
(Section 2-3-2)

Process the image.
Filtering (Section 2-6-1) Contrast conversion (Section 2-6-2)
Inter-image operation (Section 2-6-3)

Observe the image.

- **Observation of image shape**
Image display in simulated colors (Section 2-5-1)
LUT change (Section 2-5-2)
Simultaneous display of multi-channel images (Section 2-5-4)
Side-by-side image display (Section 2-5-7)
Magnified/reduced image display (Section 2-5-9)
Stereo 3D image display (Section 2-9-3)
Color-eyeglass 3D image display (Section 2-9-4)
Animation display (Section 2-9-2)
Continuous display (Frame-by-frame display) (Section 2-9-1)
- **Observation of image change over time**
Side-by-side image display (Section 2-10-1)
Continuous display (Frame-by-frame display) (Section 2-10-2)

Measure the image.

- **Measurement of image shape**
Length measurement (Section 2-7-3-1)
Area measurement (Section 2-7-3-2)
- **Measurement of image intensity value and distribution**
Intensity on a line (Line profile) (Section 2-7-1-1)
Intensity on a plane (Bird's eye view) (Section 2-7-1-2)
Intensity distribution (Section 2-7-2)

Save the image.
(Section 2-3-1)

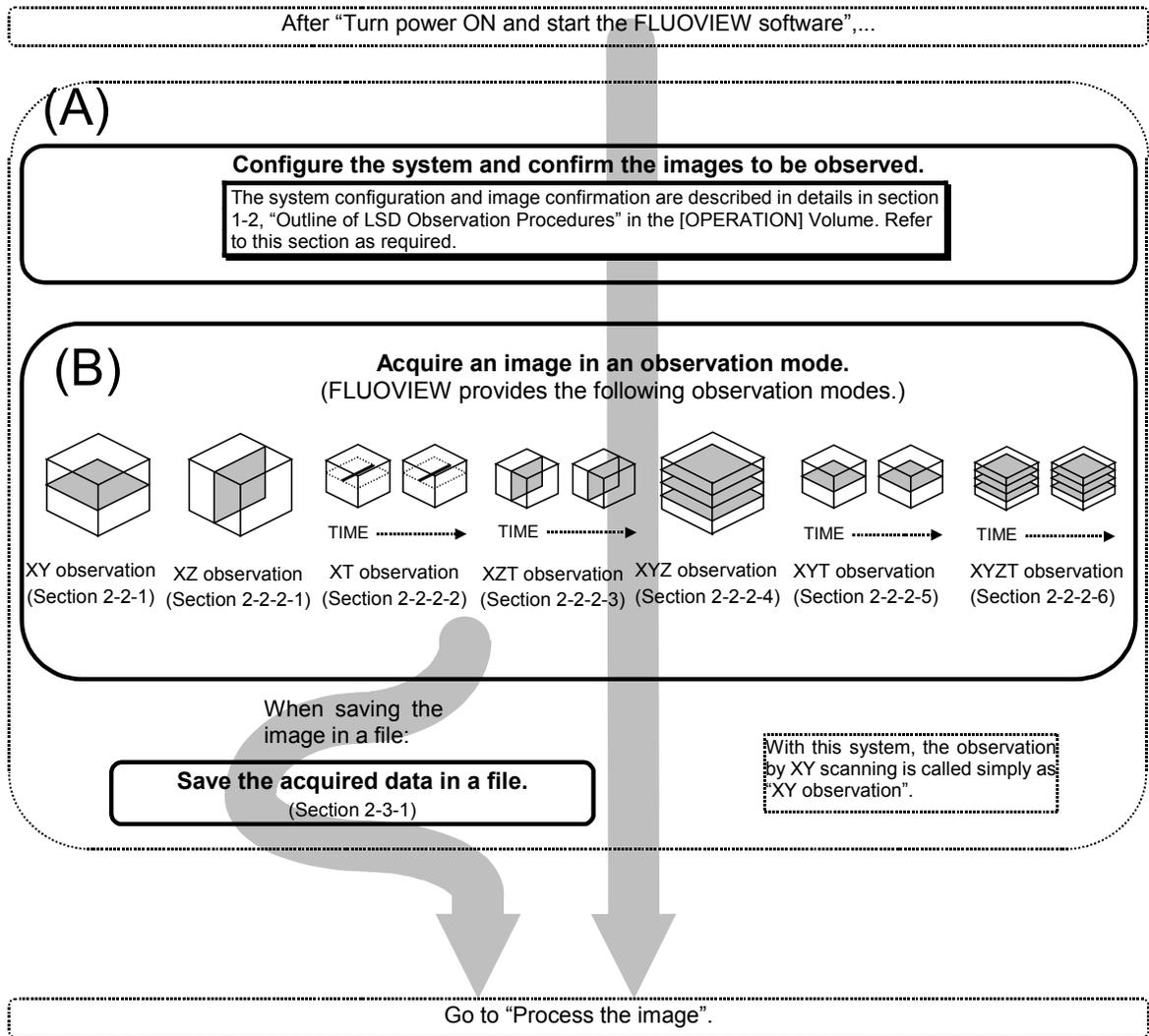
Compile the presentation data.
Drawing characters on image (Section 2-12-1)
Drawing pictures on image (Section 2-12-2)
Drawing scales on image (Section 2-12-3)

Output the image at the printer.
(Section 2-13)

Exit from the FLUOVIEW software and turn power OFF.
(Sections 1-2-14 & 1-2-15)

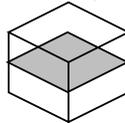
2-1-1 Image Acquisition Procedure (Section (A))

This section describes the procedure for acquiring images. See sections 2-2 and after for the actual operation methods. The detailed operation methods of each item in the procedure are described in the section specified in parentheses (()).



2-1-2 Image Acquisition Procedure in an Observation Mode (Section (B))

As an example of “Acquire an image in an observation mode”, this section describes the procedure in the XY observation mode. For the procedures in other observation modes, see section 2-2-2, “Image Acquisition in Other Observation Modes” as well as the following procedure.



XY observation
(Section 2-2-1)

After “Configure the system and confirm the images to be observed”...

(B)

Set the acquisition parameters.
(Section 2-2-1-3)

- Observation mode
- Scanning speed
- Image brightness
- Cross-section to be observed
- Area to be observed

Acquire an image in the XY observation mode.
(Section 2-2-1-4)

When noise is noticeable:

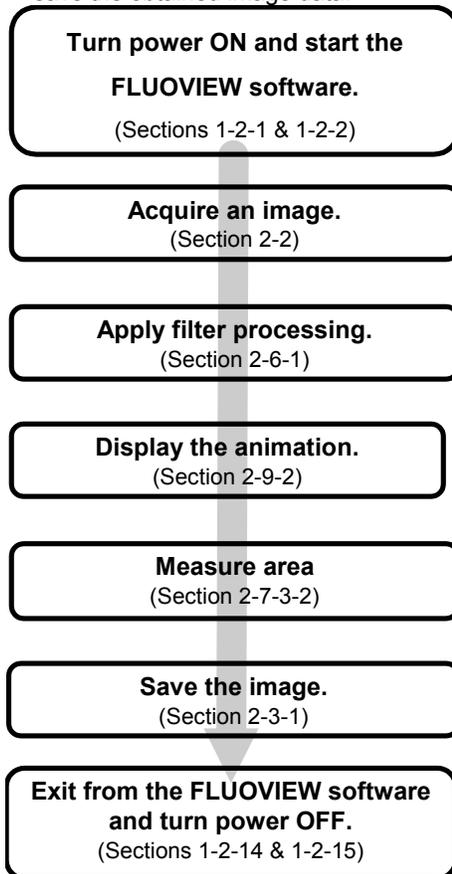
Perform accumulation.
(Section 2-2-1-5)

Go to “Save the acquired data in a file.” or “Process the image”.

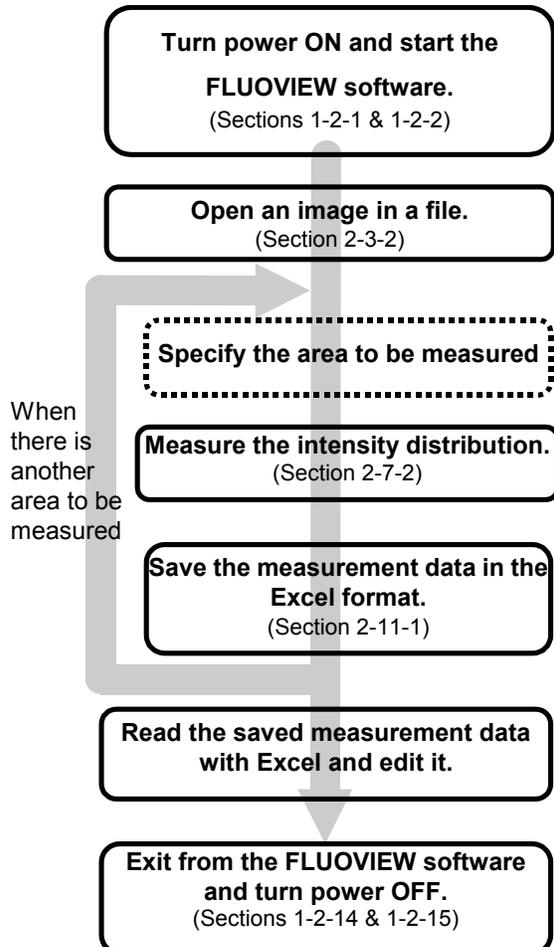
2-1-3 Examples of Operation Procedures

Begin using the FLUOVIEW system by acquiring an image or opening an image in a file. The procedures for the subsequent operations such as image processing are not in question here. For the detailed operation method of each item in the procedure, see the section specified in parentheses (()).

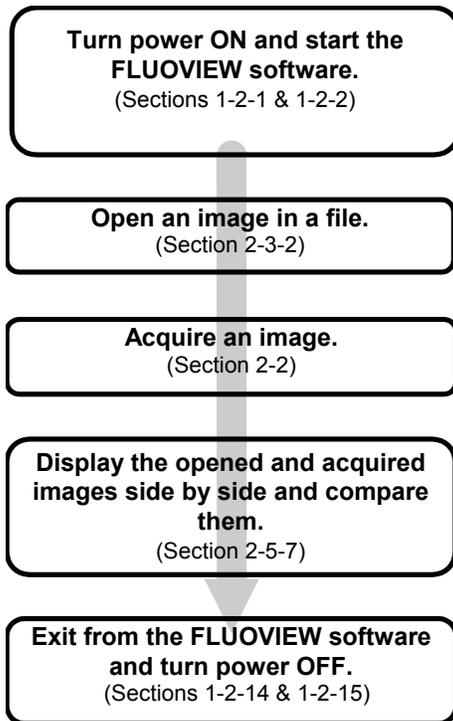
Example 1) To perform XYZ observation of a cell, apply filter processing to the image, display animation to identify the cell shape, then calculate the area, and save the obtained image data:



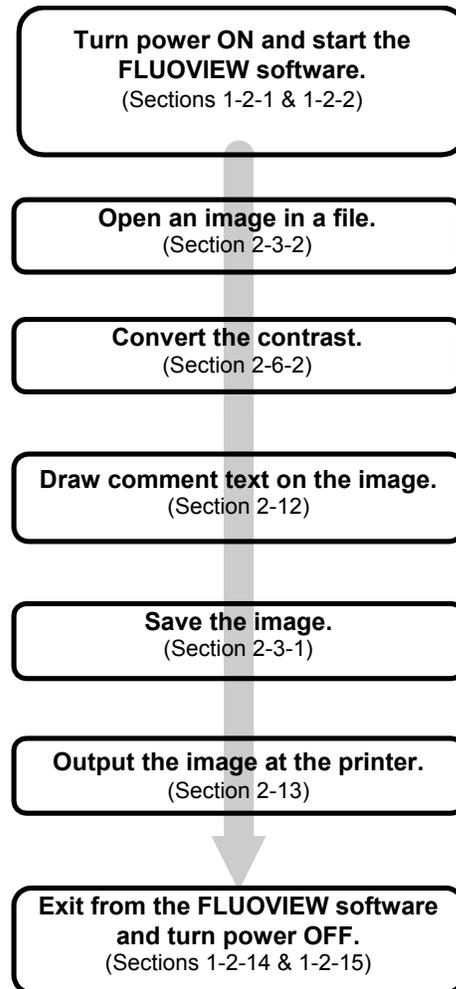
Example 2) To open a previously acquired image in a file, measure the intensity distribution and edit data with Excel:



Example 3) To acquire an image and compared it with a previously acquired image:



Example 4) To open an image in a file, improve its contrast and create a presentation image by entering comment, etc.



2-2 Image Acquisition

Confirm the image to be acquired using the microscope, and acquire its image using FLUOVIEW. The image can be saved in a file as required.

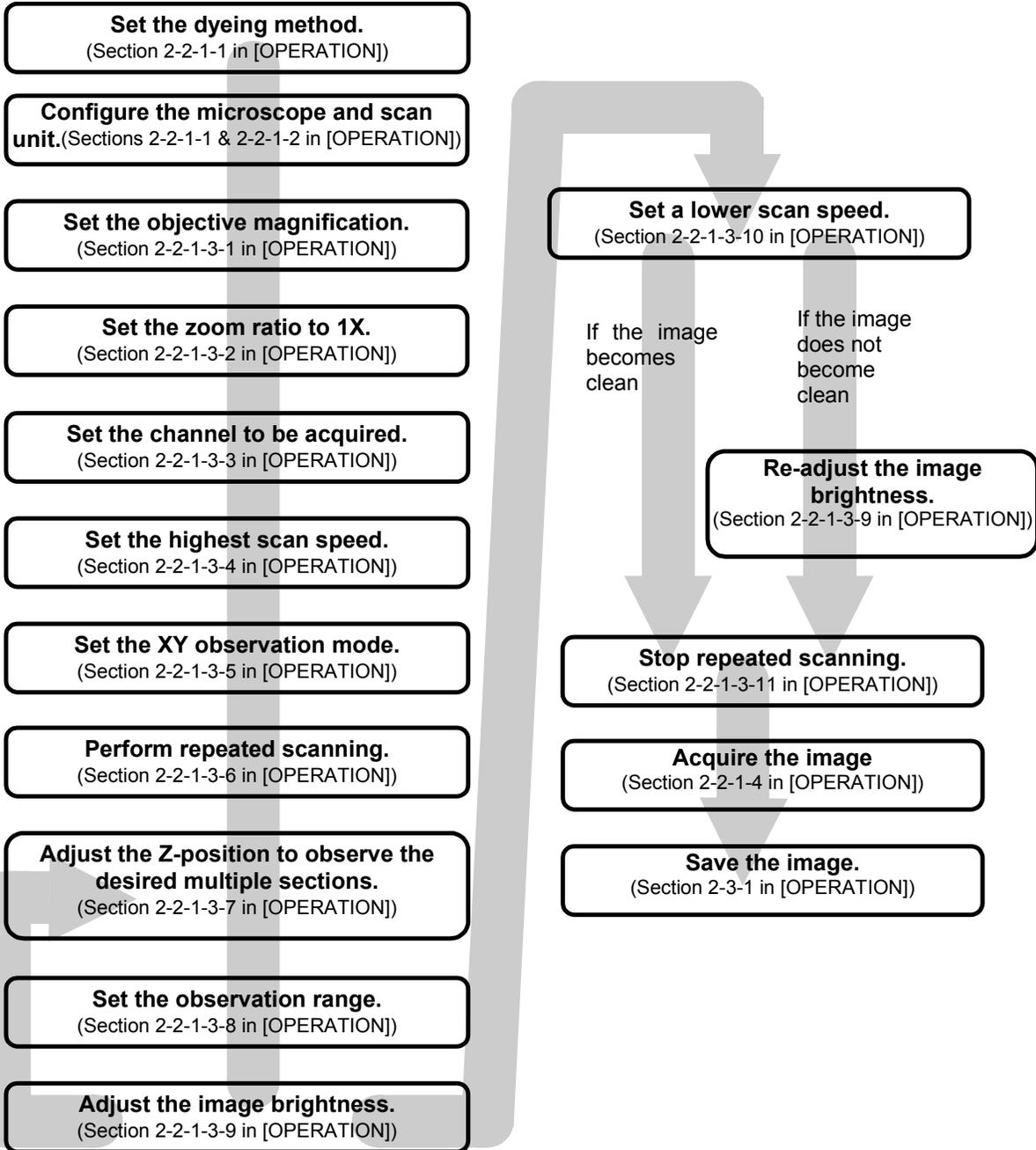
NOTE

Do not move FLOUVIEW FV300 Window while acquiring an image.

2-2-1 Image Acquisition in XY Observation Mode



This section describes the basic operation procedure from the system configuration to the image acquisition in the XY observation mode and image saving in a file as shown in the following chart. The details of each operation will be described in the subsequent sections.



2-2-1-1 Configuring the Microscope

Set the light path so that the image can be observed through the microscope.

1. Display the [Acquire] panel.

<Once> button
Acquires an image in the currently selected observation mode and display the image in the [Live] panel.

<XY Repeat> button
Repeats XY scanning to display images successively in the [Live] panel.

[Ch1], [Ch2] and [Ch3] check boxes
Check to select the image acquisition channel.

Option buttons
Check a button to select one of the displayed observation modes.

[Scan Speed] group box
Sets the scan speed. Sliding to the left of the scale increases the scan speed and to the right decreases it.

<Load> button
Loads the observation conditions at image acquisition.

<Save> button
Saves the observation conditions at image acquisition.

<Focus> button
The repetition images can be acquired at a high speed. Use this button to find an optimum image.

The acquired image is displayed.

[PMT], [Gain] and [Offset] LED sliders
These can be adjusted independently. Increasing the PMT voltage improves the sensitivity. Increasing the Gain brightens the image.

Select the magnification of the objective on the microscope.

[Settings], [Z Stage], [Time Series], [Dyes] and [Lasers] sub-panels
These panels are used to set the information required for image acquisition.

Fig. 2-1 [Acquire] Panel

2. Setting the dyeing method

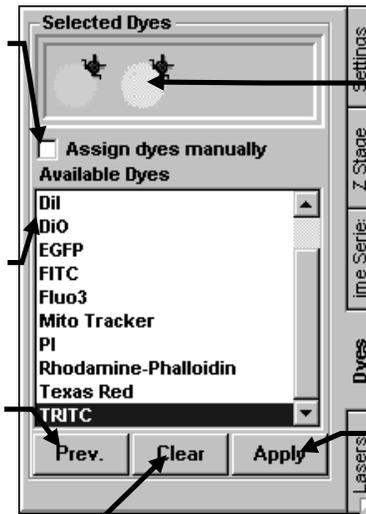
- 1) From the page tabs on the bottom right of the [Acquire] panel, select the [Dyes] sub-panel.

[Assign dyes manually] check box
 Checking this enables the manual setting. Dragging the dyeing method in the list directly to the [Ch] group box assigns the dye to the desired channel.

[Available Dyes] list box
 Lists the available dyes. Select the desired items from this list and drag them to the field above it to select the dyeing method.

<Prev.> button
 Sets the dyeing method which was set last time by clicking the <Apply> button.

<Clear> button
 Clear the set dyeing method.



Place the pointer on the icon displayed in the [Selected Dyes], and the dyeing method is shown in the pop-up display.

<Apply> button
 Applies the dyeing method dragged in the [Selected Dyes] group box to the [Channel 1]/[Channel 2] group box in the [Acquire] panel.

Fig. 2-2 [Dyes] Sub-panel

- 2) Select the specimen dyeing method by dragging desired dye names in the [Available Dyes] list box in the [Selected Dyes] group box to the field immediately above the list box.

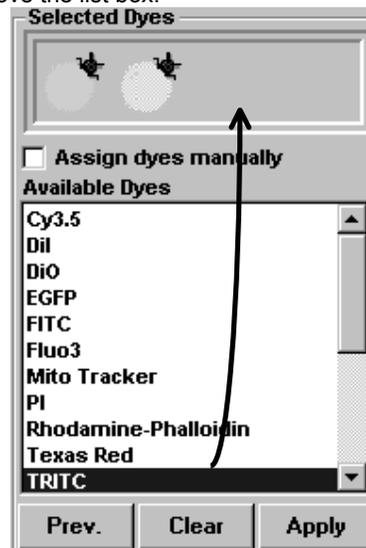


Fig. 2-3 [Dyes] Sub-panel

- 3) Click the <Apply> button to apply the selected dyeing method to the [Channel1]/[Channel 2] group box on the upper part of the [Acquire] panel.

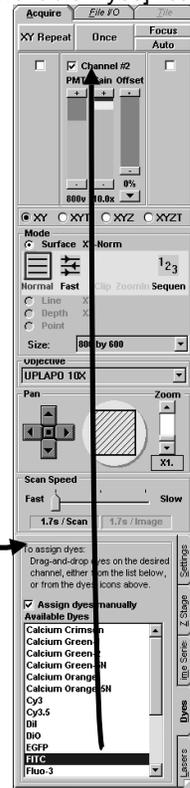
TIP

When a dyeing method is selected from the [Available Dyes] list box and the <Apply> button is clicked to apply it in the image acquisition channel, the dyeing method emitting a shorter light wavelength than 570 nm is set to Ch1 and that emitting a longer light wavelength is set to Ch2 automatically.

One Point!

The [Assign dyes manually] check box can also be used to set the dyeing method to the desired channel.

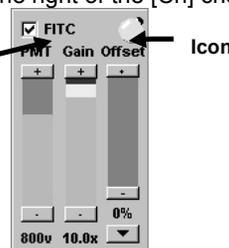
1. Check the [Assign dyes manually] check box in the [Dyes] sub-panel.
2. Select the dyeing method in the [Available Dyes] list box and drag it directly to the field of the [Ch] check box.



[Assign dyes manually] check box

3. After dragging, the icon appears on the right of the [Ch] check box and the dyeing method is set.

The dyeing method is set

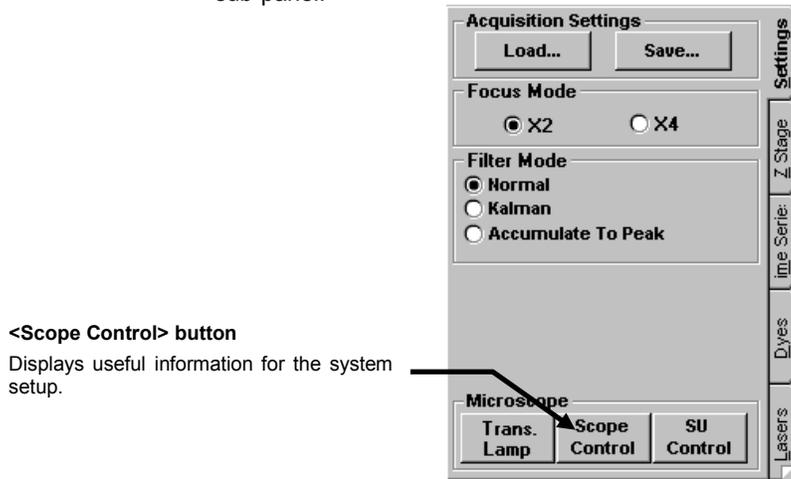


Icon

Dragging the icon to the out of the [Ch] check box field cancels the setting of the dyeing method.



- From the page tabs on the bottom right of the [Acquire] panel, select the [Settings] sub-panel.



<Scope Control> button
 Displays useful information for the system setup.

Fig. 2-4 [Settings] Sub-panel

- Select the <Scope Control> button. The window as shown below will appear.

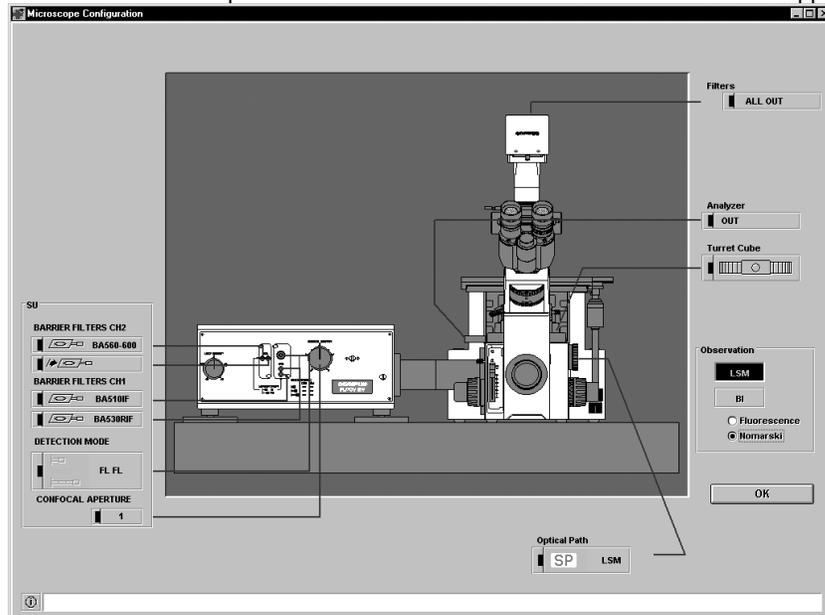
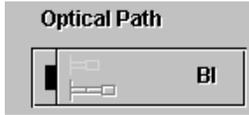


Fig. 2-5 [Microscope Configuration] Window



<BI> button



5. Select the <BI> button in the [Observation] group box, and select the microscopy from the option buttons below it. The system setting points to be changed for microscope observation will blink in red.
6. Change the system configuration (setup of light path selector, etc.) by following the guidance given by the red blinking light.



For the operation of the light path selector lever, see section 1-1-2, "Microscope" in this volume.



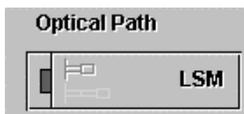
The Red blinking indicates where can be changed. The blinking does not stop even after the indicated configuration setting has been changed.

7. While looking into the microscope, move the stage and check the observed image.

2-2-1-2 Configuring the Scan Unit



<LSM> button



1. In the [Observation] group box in the [Microscope Configuration] window, select the <LSM> button.
2. The system setting points to be changed for LSM observation will blink in red. Change the system configuration (setup of light path selector, barrier filters, etc.) by following the guidance given by the red blinking light.



The Red blinking indicates where can be changed. The blinking does not stop even after the indicated configuration setting has been changed.

3. After completing the system configuration, select the <OK> button.

2-2-1-3 Setting the Observation Condition

1 Setting the Objective Magnification

1. From the drop-down list on the center of the [Acquire] panel, select the objective being used with the microscope.



If the magnification of the objective in use and that set here do not match, the measurement results will be inaccurate.

2 Setting the Zoom Ratio to 1X

1. Use the [Zoom] scale to set the zoom ratio to "X1".

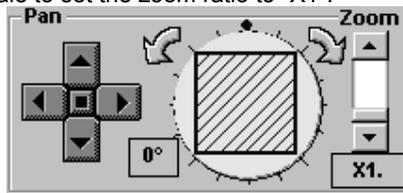
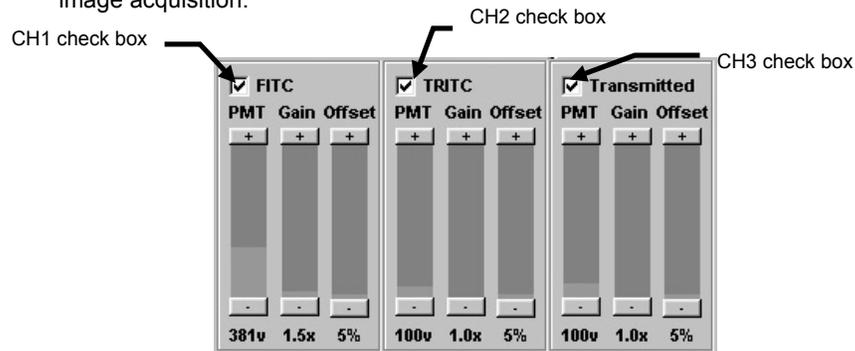


Fig. 2-6 [Pan]/[Zoom] Group Box

3 Setting the Channels

1. In the Channel 1 group box, check the check box showing the applicable dyeing method to make the image acquisition ready.
2. In the Channel 2 group box, check the check box showing the applicable dyeing method to ready the image acquisition.
3. In case of transmitted light observation, also make sure that the check box showing the Ch3 dyeing method is check-marked to indicate that Ch3 is ready for image acquisition.





To display the information on all channels simultaneously, right-click the boundary between channel display boxes.
Click the boundary again to return to the original display.

4 Setting the Highest Scan Speed

Set the scan speed to the fastest speed by using the scale in the [Scan Speed] group box in the [Acquire] panel

[Scan Speed] group box

Set the scan speed by clicking a point on the scale line.



The focus mode makes it possible to increase the line skipped scan speed. From the page tabs on the bottom right of the [Acquire] panel, select the [Settings] sub-panel.

Select either option button in the [Focus Mode] group box.

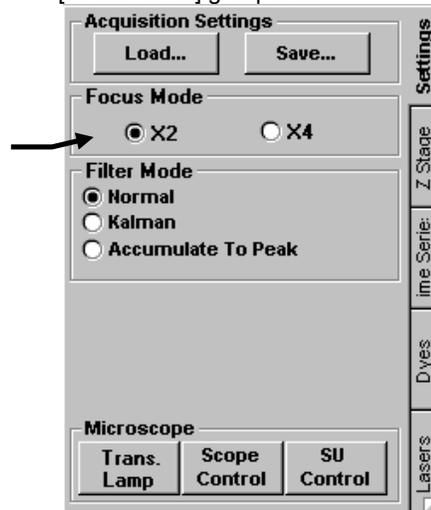
[Focus Mode] group box

[X2] option button

Acquires image at twice the highest speed.

[X4] option button

Acquires image at 4 times the highest speed. Increasing the number of divided images in the [Display] panel, line skipped scan at 4 times (Focus) cannot be done.



The focus mode is enabled when acquiring images using the <Focus> button.



The Focus function reduces the scanning time by line skipped scan. As a result, the acquired images become coarse.

5 Setting the XY Observation Mode

1. In the [Mode] group box, select the [Surface] option button.
2. In the [Acquire] panel, select the XY observation mode option button.

6 Repeated Scanning Operation



<XY Repeat> button



<Focus> button

1. Select the <XY Repeat> button. The acquired image will be displayed in the [Live] panel.



Use the <FOCUS> button to acquire image at an even higher speed. If the specimen is already being scanned, stop scanning with the <STOP SCAN> button before selecting the <XY Repeat> button.

The Focus function reduces the scanning time by line skipped scan. As a result, the acquired images become coarse.

7 Setting the Multiple Sections to be Observed

While acquiring image, move the Z stage to select the multiple sections to be observed.

From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.

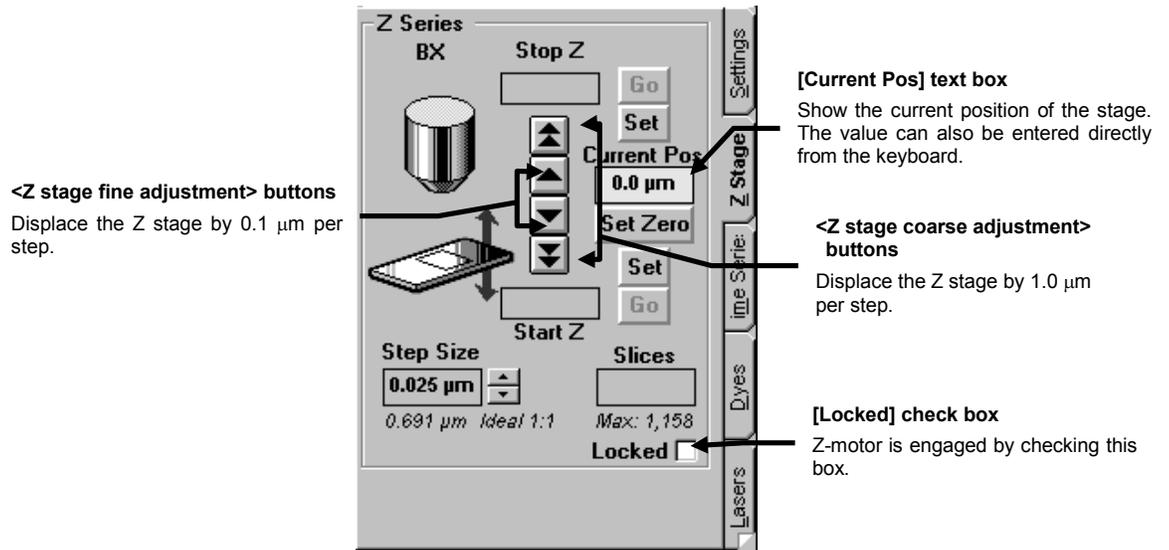


Fig. 2-7 [Z Stage] Sub-panel



The moving amount assigned to the <Z stage fine adjustment> and <Z stage coarse adjustment> buttons can be changed. See section 1-3 in MAINTENANCE, “Setting the System Configuration” for detailed operations.

1. Check the [Locked] check box in the [Z Stage] sub-panel.



Do not turn the fine focus adjustment knob while the [Locked] check box is checked, for this may damage the Z motor.

2. While observing the image in the [Live] panel, locate the plane to be observed by displacing the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

8 Setting the Area to be Observed

When the observation targets are concentrated in a narrow area or when it is necessary to observe the detail of a specific area, the image of a limited area can be acquired selectively.

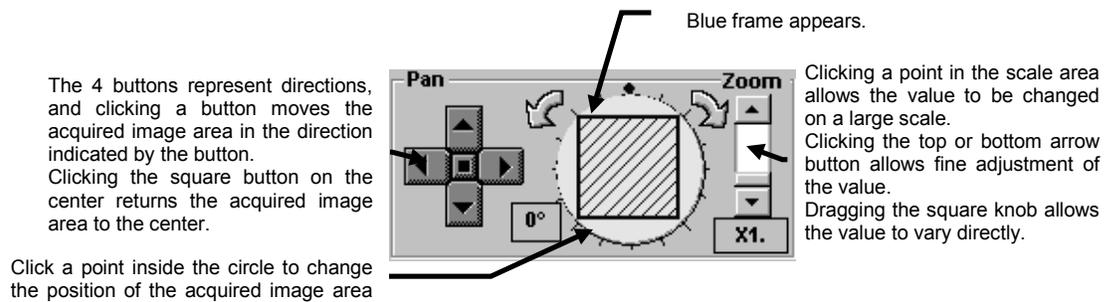


Fig. 2-8 [Pan] / [Zoom] Group Box

For instance, let us assume that the observation target is deviated at the top left of the acquired image.



With this example, the area containing the observation target can be observed using the following procedure.

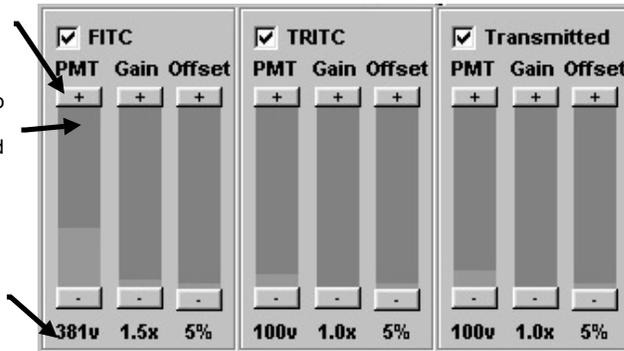
1. Increase the zoom ratio using the [Zoom] scale in the [Acquire] panel.
2. The light-blue circle to the left of the [Zoom] scale represents the field visible through the microscope, and the blue frame indicates the acquired image area. Move the blue frame inside the circle using the <Pan> buttons so that the desired observation targets are displayed in the [Live] panel.

9 Adjusting the image brightness

Clicking this button allows fine adjustment of the value.

Clicking this field allows the value to be changed on a large scale. The ND value which are usually used are displayed in green.

Clicking the <+> or <-> button or the field displays the set value. The set value can be varied by direct input from the keyboard.



1. While observing the image in the [Live] panel, change the setting values of the **PMT voltage**[PMT], **Offset**[Offset] and **Gain**[Gain] in the [Acquire] panel. The functions of these parameters are as described below.

PMT Voltage	Increasing this value improves the sensitivity. However, too high a sensitivity makes image noise noticeable. If sufficient brightness cannot be obtained by setting the PMT voltage to 800 V, leave it as it is and increase Gain. This will usually provide a better result than using a PMT voltage over 800 V.
Offset	Darkens the image at the ratio set during image acquisition. (This value can be set independently from the Gain.)
Gain	Brightens the image at the ratio set during image acquisition. (This value can be set independently from the Offset.)

For example...

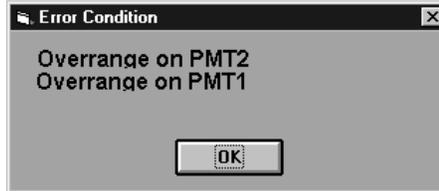
When the acquired image is dark or the observation targets are hardly visible, increase **PMT voltage** to improve the sensitivity. If the image resulting from this becomes too bright, first try decreasing **PMT voltage** slightly. If the image background is still too bright, increase **Offset** to darken the background. As this also reduces the brightness of the observation target, increase **Gain** as required so that the observation target is clearly visible.

Gamma Correction provides you more brightened image even if it was acquired with darkness.

Refer to "2-5-2-2 LUT Graph Editing by Gamma Correction " for details in this operation.



When the light incident to photomultiplier tube is too bright or PMT voltage is set to a high voltage, PMT Over warning may be displayed as shown below.



This warning is displayed to protect photomultiplier tube when the light incident to it exceeds a certain level. When it is displayed, decrease PMT voltage setting.



<LUT> button



[Hi-Lo]LUT



[Hi-Lo] LUT can be utilized to adjust image intensity easily.

1. Click <LUT> button from tool bar located at bottom left of screen. Dialog box – [Color Tool] will appear.
2. Click [Hi-Lo] LUT from group box of [Standard Color LUTs].
3. The intensity value 0 is colored with Blue, and the maximum intensity is colored with Red.
4. When there is noises in the image, different colors may appear in its background. When the intensity is saturated, the portion will be colored with Red. Based on this coloring, you may adjust the image intensity.



[Hi-Lo] LUT switching can be done by use of Hot key even if during image acquisition.

The image channel can be assigned in [Acquire] panel

The LUT of the assigned channel can be switched to [Hi-Lo] LUT by pressing **Ctrl** + **H**. By pressing **Ctrl** + **H** again, LUT returns to the previous. The LUT of all channels can be switched to [Hi-Lo] LUT by pressing **Ctrl** + **Shift** + **H**.

10 Setting a Lower Scan Speed

1. The scan speed can be decreased using the scale in the [Scan Speed] group box on the center of the [Acquire] panel.



In general, setting a lower scan speed allows the acquired image quality to be improved.
However, a low scan speed also lengthens the time required for image acquisition.



When the scan speed is decreased during fluorescence observation, the saturation of fluorescence may darken the image of certain types of specimens. In this case, increase the scan speed and increase the PMT Voltage or use accumulation in scanning.

[Scan Speed] group box

Set the scan speed by clicking a point on the scale line.



11 Stopping Repeated Scanning

1. After the brightness and gain have been adjusted, select the <STOP SCAN> button in the [Acquire] panel to stop scanning temporarily.



2-2-1-4 Acquiring Image



<Once> button

1. Select the <Once> button. The acquired image will be displayed in the [Live] panel.

2-2-1-5 Acquiring Image in Accumulation Mode

When the image is dark or noisy, use an accumulation mode in image acquisition to improve the image quality.

Kalman Accumulation and Peak Accumulation

- The Kalman accumulation acquires images for the specified number of times while averaging the images. This operation is effective for reduction of noise.
- The Peak accumulation acquires images for the specified number of times while adding the images, and stops image acquisition when any intensity value on the image reaches the peak (4095). This operation is effective for with the dark lower part in the XYZ observation and observation of an extremely dark image.

Kalman Accumulation Algorithm

Every time an image is acquired, the pixel values are rewritten based on the following formulae, where it is assumed that;

n: number of image acquisitions;

I(n): Result of n-times of Kalman accumulations (Intensity values of pixels);

I(new): New intensity value obtained after every image acquisition.

The result of the first Kalman accumulation is identical to the result of ordinary image acquisition:

$$I(1) = I(\text{new})$$

The result of the n-th ($n > 1$) Kalman accumulation is:

$$I(n) = (I(n-1) * (n+1) + I(\text{new})) / n$$

1 Acquiring Image in Accumulation Mode (Frame mode)

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Settings] sub-panel.

[Filter Mode] group box

Select the accumulation mode.

Two accumulation modes, [Kalman] and [Accumulate To Peak] are available.

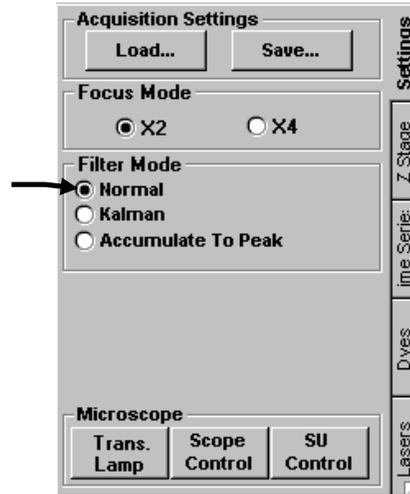
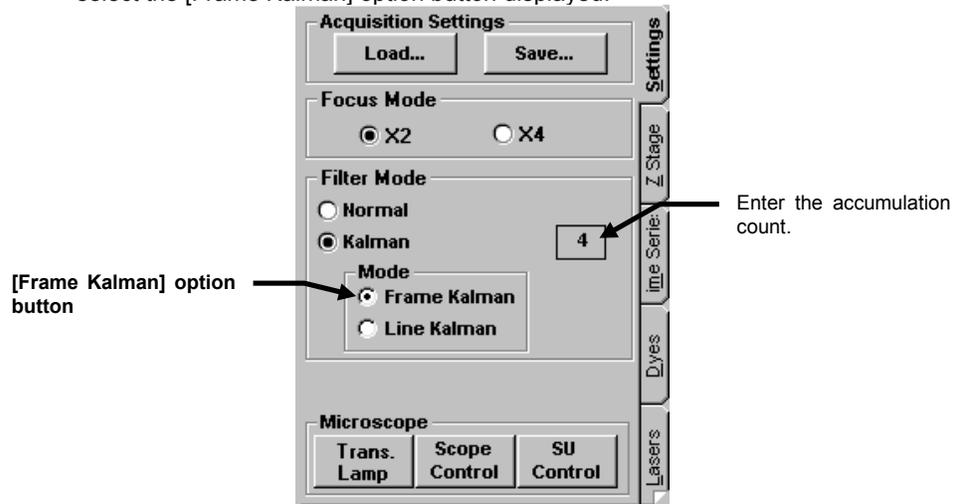


Fig 2-9 [Settings] sub-panel

2. In the [Filter Mode] group box, select the [Kalman] or [Accumulate To Peak] option button.



- When [Kalman] is selected, enter the accumulation count in the text box. And select the [Frame Kalman] option button displayed.



TIP The accumulation count can be set up a maximum of 63 times. When 0 is set as the number of times of accumulation, an ordinary image acquisition is to be performed.

- When [Accumulate To Peak] is selected, enter the addition count in the text box.
- Click the <Once> button in the [Acquire] panel. The acquired image will be displayed in the [Live] panel.

2 Acquiring Image in Accumulation Mode (Line mode)



Image acquisition in the line mode can be performed when you use the FV300 system with AOTF (FV5-COMBA).

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Settings] sub-panel.

[Filter Mode] group box

Select the accumulation mode.

Two accumulation modes, [Kalman] and [Accumulate To Peak] are available.

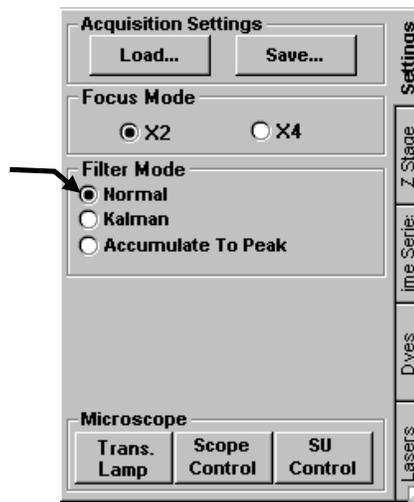
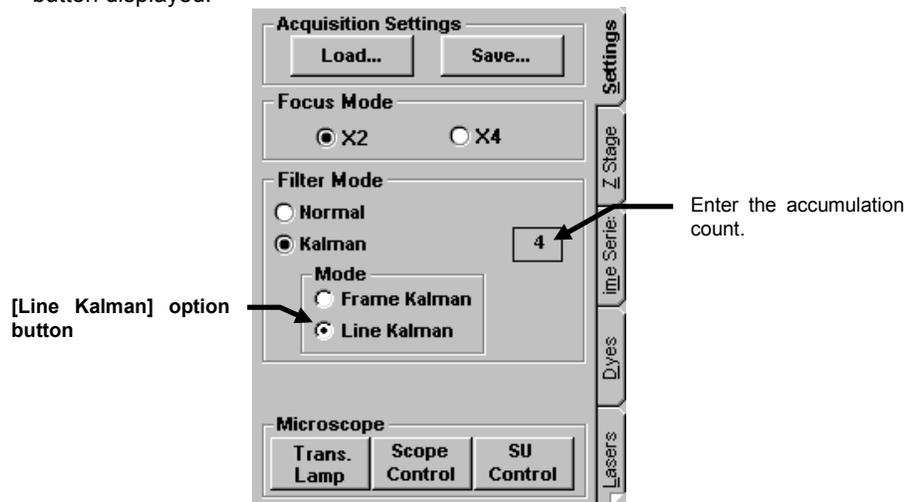


Fig. 2-10 [Settings] sub-panel

2. In the [Filter Mode] group box, select the [Kalman] option button.
3. Enter the accumulation count in the text box and select the [Line Kalman] option button displayed.





The accumulation count can be set up a maximum of 63 times.
When 0 is set as the number of times of accumulation, an ordinary image acquisition is to be performed.

4. Click the <Once> button in the [Acquire] panel.
The acquired image will be displayed in the [Live] panel.
And the accumulated image can be displayed in the [Display] panel without being displayed in full.

The difference between the Frame and Line modes of Kalman Accumulation

In frame mode, the image is accumulated every one frame during acquisition.
In line mode, accumulation is done every one line during acquisition.
The frame mode is intended to utilize for fixed specimen observation, and the line mode is for living specimen observation. Line mode makes it possible to shorten sampling interval so that it is suitable for live cell observation.

2-2-1-6 Saving the Acquired Image in File

Experiment

<Experiment> button

1. Display the [File I/O] panel.
2. Click the page tab of the [Live] panel showing the image to be saved, so that the image is displayed at the front.
3. Click the <Experiment> button in the [Save] group box in the [File I/O] panel.
For details, see section 2-3-1, "Saving Image".

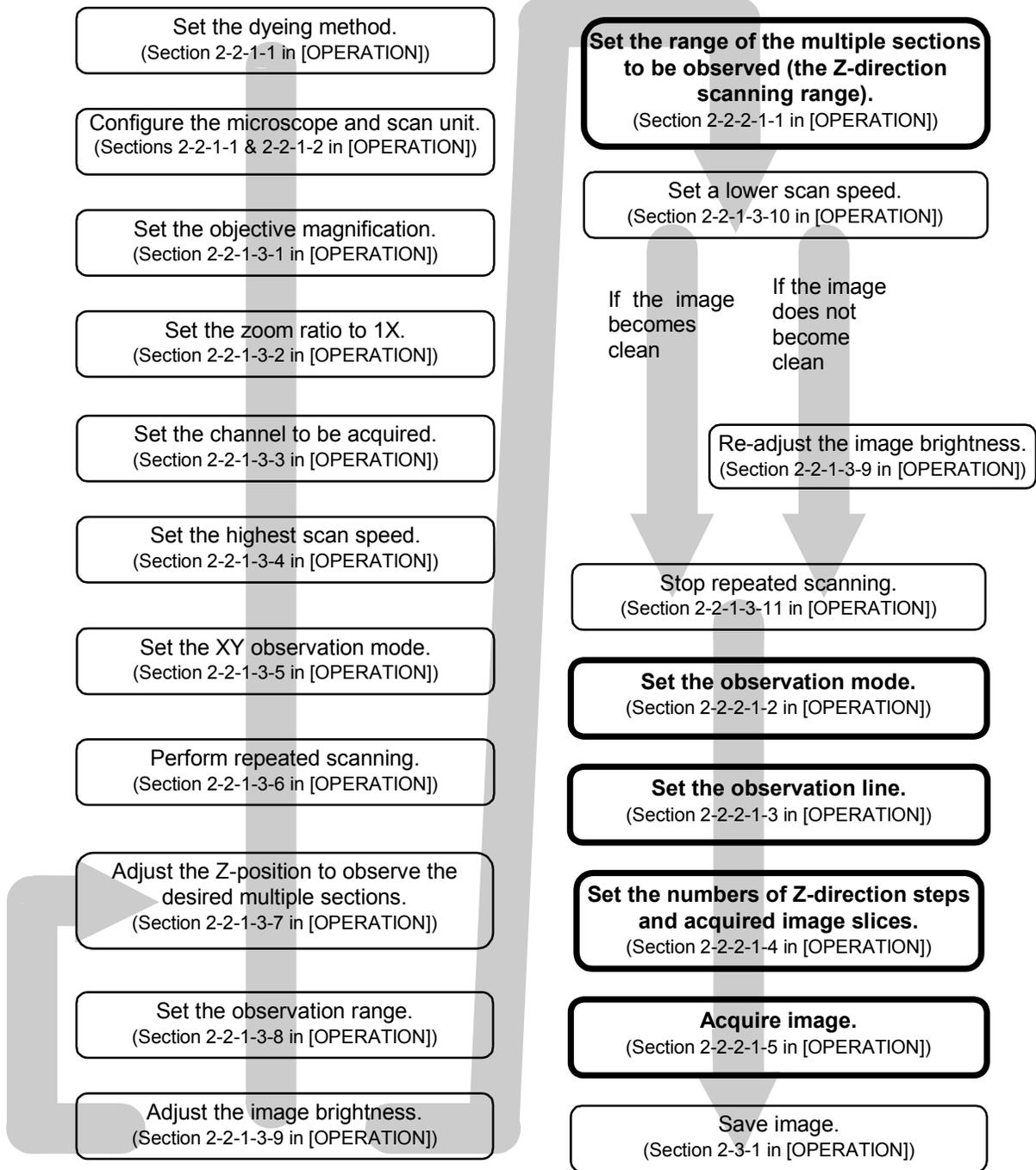


2-2-2 Image Acquisition in Other Observation Modes

2-2-2-1 XZ Observation Mode



The description in this section will be focused on the image acquisition operations in the XZ observation mode that are not used in the XY observation modes (which are the operations enclosed in  in the chart on the next page). For other operations, see section 2-2-1, "Image Acquisition in XY Observation Mode". The details of each operation will be described in the subsequent sections.



1 Setting the Z-direction scanning range

While acquiring image, move the Z stage according to the range of the multiple sections to be observed (i.e. the Z-direction scanning range).

From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.

[Stop Z] text box
Shows the scan stop position in the range of the observed cross-section (Z-direction scanning range).

<Z stage coarse adjustment> buttons
Displaces the Z stage on a large scale.

<Z stage fine adjustment> buttons
Displaces the Z stage on a fine scale.

[Start Z] text box
Shows the scan start position in the range of the observed cross-section (Z-direction scanning range).

[Step Size] text box
Set the number of steps using the <▲> or <▼> button. This number can also be input directly from the keyboard.

Recommended step size
Shows the number of steps calculated by the system so that the scale of depth in the Z-direction of the acquired image is identical to the scale of the plane in the X- and Y-directions.

[Slices] text box
Shows the number of images acquired. This number can also be input directly from the keyboard. Under text box, it displays max. number of slices that can be acquired with use of physical memory only available at that time.

<Go> button
Moves to the set scanning stop position. Use this button to check the scanning stop position.

<Set> button
Sets the current stage position as the scanning stop position of the range of the observed cross-section (Z-direction scanning range).

[Current Pos] text box
Shows the current position of the stage.

<Set Zero> button
Sets the current stage position as the home position. Pressing this button also clears the [Stop Z] and [Start Z] values. When stage movement takes time, it changes to <Stop> button. By pressing <Stop> button, the move stops.

<Set> button
Sets the current stage position as the scanning start position of the range of the observed cross-section (Z-direction scanning range).

<Go> button
Moves to the set scan start position. Use this button to check the scan start position.

[Locked] check box
Z-motor is engaged by checking this box.

Fig. 2-11 [Z Stage] Sub-panel



The moving amount assigned to the <Z stage fine adjustment> and <Z stage coarse adjustment> buttons can be changed. See section 1-3 in MAINTENANCE, "Setting the System Configuration" for detailed operations.

1. Check the [Locked] check box in the [Z Stage] sub-panel.



Do not turn the fine focus adjustment knob while the [Locked] check box is checked, for this may damage the Z motor.

2. While observing the image in the [Live] panel, locate the upper edge of the range to be observed by moving down the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

When using the FLUOVIEW system with an inverted microscope, locate the bottom edge of the range to be observed by moving down the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

3. When the upper edge position is located, click the <Set> button. The [Start Z] text box will show the scan start position of the range of the multiple sections to be observed (Z-direction scanning range).
4. While observing the image in the [Live] panel, locate the bottom edge of the range to be observed by moving up the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

When using the FLUOVIEW system with an inverted microscope, locate the upper edge of the range to be observed by moving up the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

5. When the position is located, click the <Set> button. The [Stop Z] text box will show the scanning stop position of the range of the multiple sections to be observed (Z-direction scanning range).

2 Setting the observation mode

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Settings] sub-panel.
2. Click the [Depth] option button in the [Mode] group box.
3. In the [Acquire] panel, select the XZ observation mode option button.

3 Setting the observation line

1. A line is displayed on the image in the [Live] panel. Place the mouse pointer arrow on the line and drag it to the position you want to observe.

4 Setting the numbers of steps and acquired image slices



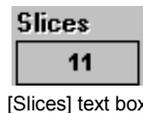
1. From the page tabs on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.
2. The number of steps has already been set and displayed in the [Step Size] text box. This number can be changed using the <▲> or <▼> button in the [Step Size] text box.



The number of steps in the [Step Size] text box has been calculated by the system so that the depth scale of the acquired image is identical to the horizontal scale.



The number of steps calculated by the system may be erroneous unless the XZ observation mode has been set previously.



The number of acquired images shown in the [Slices] text box can also be input from the keyboard. After setting [Start Z] (Z-direction scan start position) and [Stop Z] (Z-direction scanning stop position), input the desired number of images in the [Slices] text box. This automatically sets [Step Size] (number of steps).



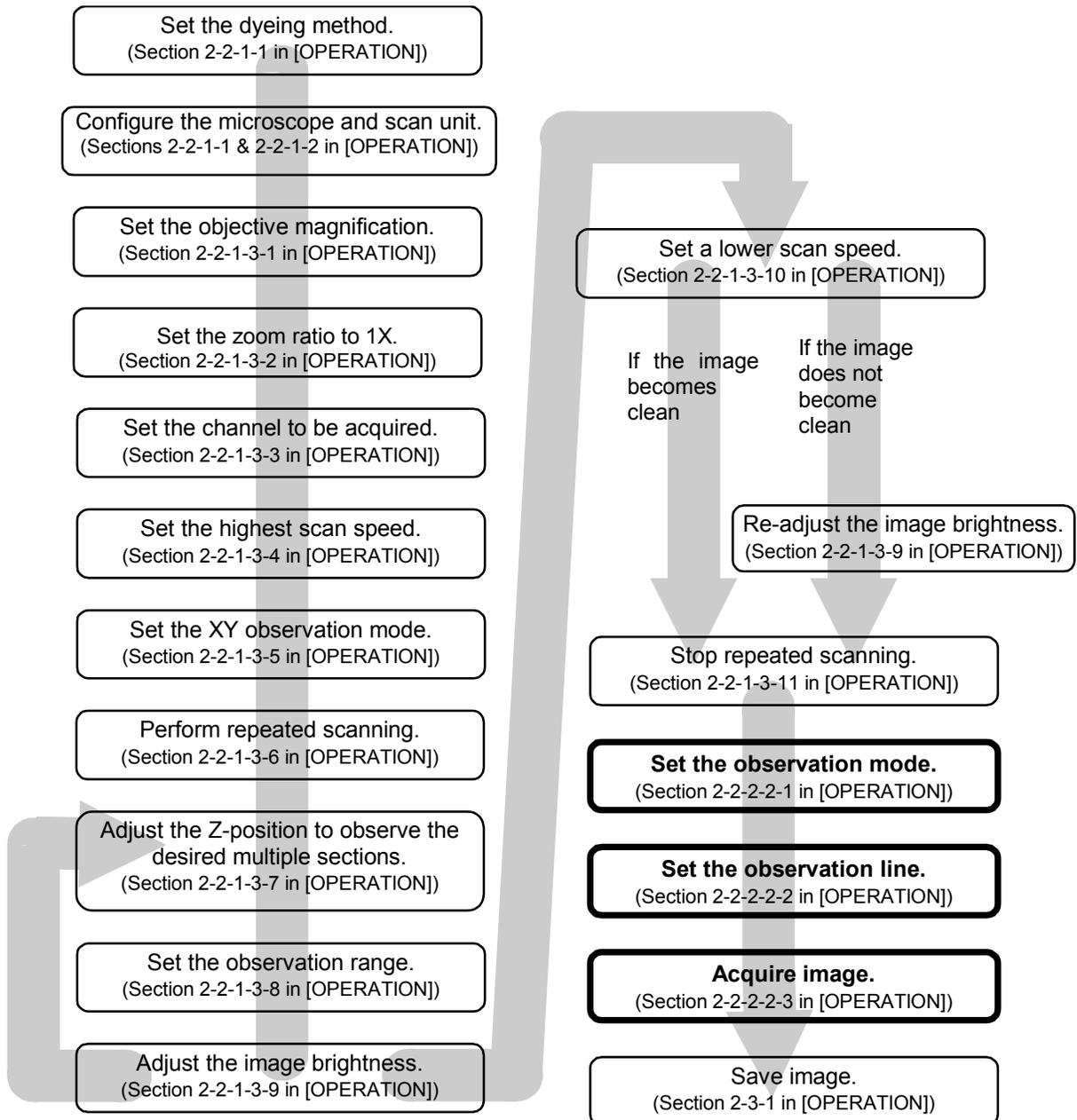
5 Acquiring image

1. Click the <XZ> button in the [Acquire] panel.
The acquired image will be displayed in the [Live] panel.

2-2-2-2 XT Observation Mode



The description in this section will be focused on the image acquisition operations in the XT observation mode that are not used in the XY observation modes (which are the operations enclosed in  in the chart on the next page). For other operations, see section 2-2-1, "Image Acquisition in XY Observation Mode". The details of each operation will be described in the subsequent sections.



1 Setting the observation mode

1. Click the [Line] option button in the [Mode] group box.
2. In the [Acquire] panel, select the XT observation mode option button.

2 Setting the observation line

1. A line is displayed on the image in the [Live] panel. Place the mouse pointer arrow on the line and drag it to the position you want to observe.

3 Acquiring image

Click the <XT> button in the [Acquire] panel.

The acquired image will be displayed in the [Live] panel.

The XT observation acquires the images of the same position (line) for **2000** times successively at the set scanning speed. For reference, the time required for the image acquisitions is shown in the frame on the bottom left of the [Scan Speed] group box.



The number of image acquisitions can be changed by changing the value in the [N] text box in the [Time Series] sub-panel.

While acquiring an image in the XT observation mode, clicking the <STOP SCAN> button changes the buttons at the upper part of the [Acquire] panel as shown below. The <Resume> button restarts image acquisition at the frame next to the frame where the acquisition is suspended.



<Resume> button
 Restarts image acquisition at the frame next to the frame where the acquisition is suspended.

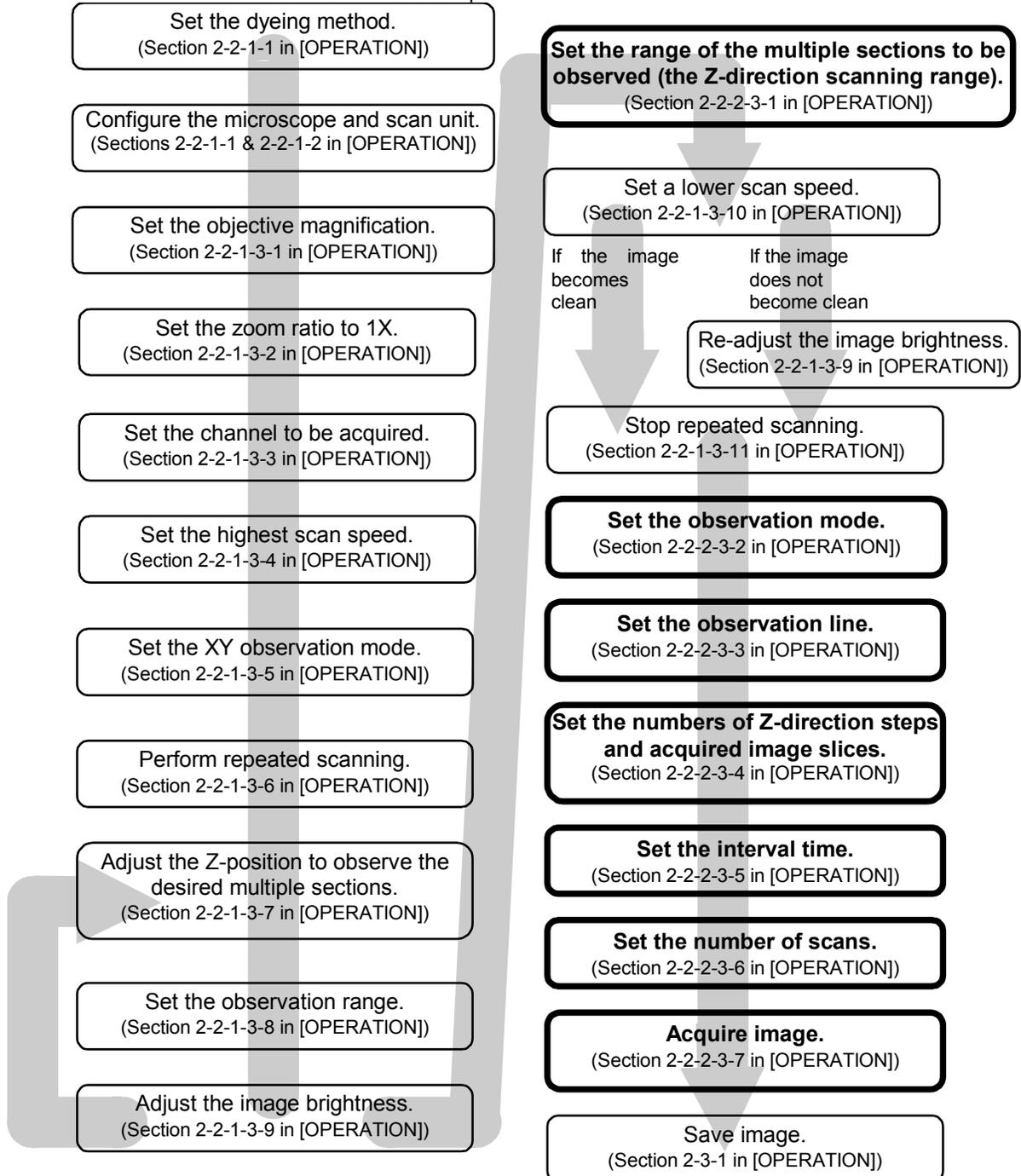


<Series Done> button
 Determines the acquired images. Once this button is clicked, it is not possible to append an image.

2-2-2-3 XZT Observation Mode



The description in this section will be focused on the image acquisition operations in the XZT observation mode that are not used in the XY observation modes (which are the operations enclosed in  in the chart on the next page). For other operations, see section 2-2-1, "Image Acquisition in XY Observation Mode". The details of each operation will be described in the subsequent sections.



1 Setting the Z-direction scanning range

While acquiring image, move the Z stage according to the range of the multiple sections to be observed (Z-direction scanning range).

From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.

[Stop Z] text box
Shows the scan stop position in the range of the observed cross-section (Z-direction scanning range).

<Z stage coarse adjustment> buttons
Displaces the Z stage on a large scale.

<Z stage fine adjustment> buttons
Displaces the Z stage on a fine scale.

[Start Z] text box
Shows the scan start position in the range of the observed cross-section (Z-direction scanning range).

[Step Size] text box
Set the number of steps using the <▲> or <▼> button. This number can also be input directly from the keyboard.

Recommended step size
Shows the number of steps calculated by the system so that the scale of depth in the Z-direction of the acquired image is identical to the scale of the plane in the X- and Y-directions.

[Slices] text box
Shows the number of images acquired. This number can also be input directly from the keyboard. Under text box, it displays max. number of slices that can be acquired with use of physical memory only available at that time.

<Go> button
Moves to the set scanning stop position. Use this button to check the scanning stop position.

<Set> button
Sets the current stage position as the scanning stop position of the range of the observed cross-section (Z-direction scanning range).

[Current Pos] text box
Shows the current position of the stage.

<Set Zero> button
Sets the current stage position as the home position. Pressing this button also clears the [Stop Z] and [Start Z] values. When stage movement takes time, it changes to <Stop> button. By pressing <Stop> button, the move stops.

<Set> button
Sets the current stage position as the scanning start position of the range of the observed cross-section (Z-direction scanning range).

<Go> button
Moves to the set scan start position. Use this button to check the scan start position.

[Locked] check box
Z-motor is engaged by checking this box.

Fig. 2-12 [Z Stage] Sub-panel



The moving amount assigned to the <Z stage fine adjustment> and <Z stage coarse adjustment> buttons can be changed. See section 1-3 in MAINTENANCE, "Setting the System Configuration" for detailed operations.

1. Check the [Locked] check box in the [Z Stage] sub-panel.



Do not turn the fine focus adjustment knob while the [Locked] check box is checked, for this may damage the Z motor.

2. While observing the image in the [Live] panel, locate the upper edge of the range to be observed by moving down the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

When using the FLUOVIEW system with an inverted microscope, locate the bottom edge of the range to be observed by moving down the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

3. When the upper edge position is located, click the <Set> button. The [Start Z] text box will show the scan start position of the range of the multiple sections to be observed (Z-direction scanning range).
4. While observing the image in the [Live] panel, locate the bottom edge of the range to be observed by moving up the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

When using the FLUOVIEW system with an inverted microscope, locate the upper edge of the range to be observed by moving up the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

5. When the position is located, click the <Set> button. The [Stop Z] text box will show the scanning stop position of the range of the multiple sections to be observed (Z-direction scanning range).

2 Setting the observation mode

1. Click the [Depth] option button in the [Mode] group box.
2. In the [Acquire] panel, select the XZT observation mode option button.

3 Setting the observation line

1. A line is displayed on the image in the [Live] panel. Place the mouse pointer arrow on the line and drag it to the position you want to observe.

4 Setting the numbers of steps and acquired image slices

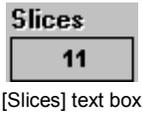
1. From the page tabs on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.
2. A number of steps is displayed in the [Step Size] text box. This number can be changed using the <▲> or <▼> button in the [Step Size] text box.



The number of steps shown in the [Step Size] text box has been calculated by the system so that the depth scale of the acquired image is identical to the horizontal scale.



The number of steps calculated by the system may be erroneous unless the XZT observation mode has been set previously.



The number of acquired images shown in the [Slices] text box can also be input from the keyboard.
After setting [Start Z] (Z-direction scan start position) and [Stop Z] (Z-direction scanning stop position), input the desired number of images in the [Slices] text box. This automatically sets [Step Size] (number of steps).

5 Setting the interval time

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Time Series] sub-panel.
2. Set the interval time using the <▲> or <▼> button in the [Interval] text box.

[Interval] text box

Set the interval time using the <▲> or <▼> button or by input from the keyboard.

[N] text box

Set the number of scans using the <▲> or <▼> button or by input from the keyboard. Under text box, it displays max. number of slices that can be acquired with use of physical memory only available at that time.

[Rest Time] text box

Shows the time after end of acquisition of an image until the start of next acquisition.

[Total Time] text box

Shows the total time required for image acquisitions.

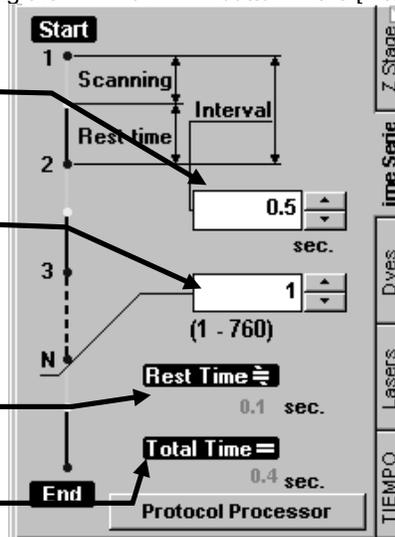


Fig. 2-13 [Time Series] Sub-panel

TIP

An image with no interval can be acquired by entering [0] in the [Interval] text box.

In this case, the [Interval] text box shows "Free Run" message.

Using <▼> button also sets the [Interval] text box to "Free Run".

6 Setting the number of scans

1. Set the number of scans using the <▲> or <▼> button in the [N] text box in the [Time Series] sub-panel.

7 Acquiring image

1. Click the <XZT> button in the [Acquire] panel.

The acquired image will be displayed in the [Live] panel.

While acquiring an image in the XZT observation mode, clicking the <STOP SCAN> button changes the buttons at the upper part of the [Acquire] panel as shown below. The <Resume> button restarts image acquisition at the frame next to the frame where the acquisition is suspended.



<Resume> button

Restarts image acquisition at the frame next to the frame where the acquisition is suspended.



<Series Done> button

Determines the acquired images. Once this button is clicked, it is not possible to append an image.

8 Appending image

XZT image can be added after the image acquisition.

Immediately after acquisition of an image in the XZT observation mode, the buttons at the upper part of the [Acquire] panel changes as shown below.

<Append Next> button

Acquires another image and appends it to the image acquired immediately before.



<Series Done> button

Determines the acquired images. Once this button is clicked, it is not possible to append an image.

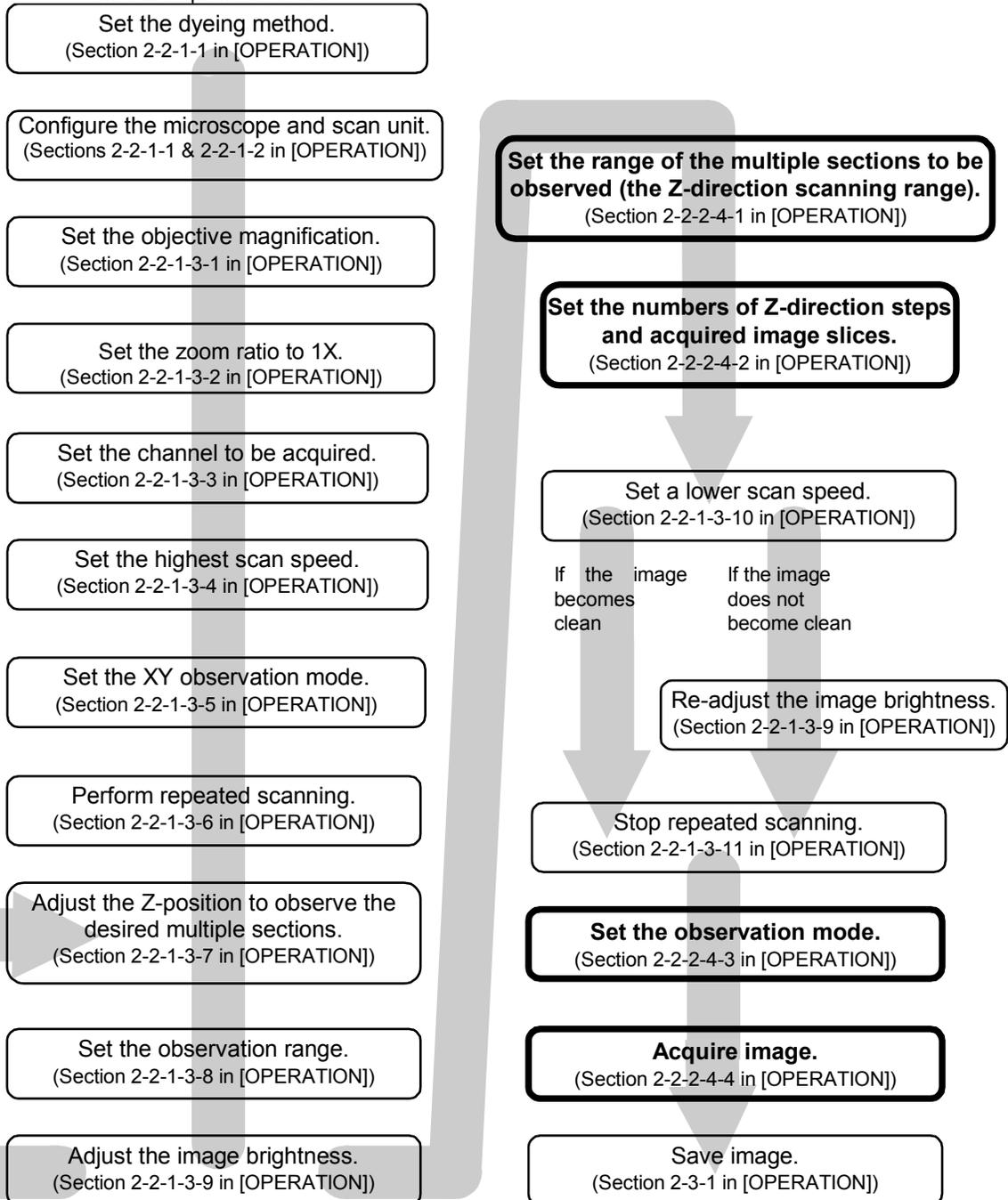
Click the <Append Next> button to append an image. An image will be acquired with the same number of steps as the image acquired immediately before and appended to it.

Click the <Series Done> button when it is not required to append an image.

2-2-2-4 XYZ Observation Mode



The description in this section will be focused on the image acquisition operations in the XYZ observation mode that are not used in the XY observation modes (which are the operations enclosed in  in the chart on the next page). For other operations, see section 2-2-1, "Image Acquisition in XY Observation Mode". The details of each operation will be described in the subsequent sections.



1 Setting the Z-direction scanning range

While acquiring image, move the Z stage according to the range of the multiple sections to be observed (Z-direction scanning range).

From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.

[Stop Z] text box
Shows the scan stop position in the range of the observed cross-section (Z-direction scanning range).

<Z stage coarse adjustment> buttons
Displaces the Z stage on a large scale.

<Z stage fine adjustment> buttons
Displaces the Z stage on a fine scale.

[Start Z] text box
Shows the scan start position in the range of the observed cross-section (Z-direction scanning range).

[Step Size] text box
Set the number of steps using the <▲> or <▼> button. This number can also be input directly from the keyboard.

Recommended step size
Shows the number of steps calculated by the system so that the scale of depth in the Z-direction of the acquired image is identical to the scale of the plane in the X- and Y-directions.

[Slices] text box
Shows the number of images acquired. This number can also be input directly from the keyboard. Under text box, it displays max. number of slices that can be acquired with use of physical memory only available at that time.

<Go> button
Moves to the set scanning stop position. Use this button to check the scanning stop position.

<Set> button
Sets the current stage position as the scanning stop position of the range of the observed cross-section (Z-direction scanning range).

[Current Pos] text box
Shows the current position of the stage.

<Set Zero> button
Sets the current stage position as the home position. Pressing this button also clears the [Stop Z] and [Start Z] values. When stage movement takes time, it changes to <Stop> button. By pressing <Stop> button, the move stops.

<Set> button
Sets the current stage position as the scanning start position of the range of the observed cross-section (Z-direction scanning range).

<Go> button
Moves to the set scan start position. Use this button to check the scan start position.

[Locked] check box
Z-motor is engaged by checking this box.

Fig. 2-14 [Z Stage] Sub-panel



The moving amount assigned to the <Z stage fine adjustment> and <Z stage coarse adjustment> buttons can be changed.
See section 1-3 in MAINTENANCE, “Setting the System Configuration” for detailed operations.

1. Check the [Locked] check box in the [Z Stage] sub-panel.



Do not turn the fine focus adjustment knob while the [Locked] check box is checked, for this may damage the Z motor.

2. While observing the image in the [Live] panel, locate the upper edge of the range to be observed by moving down the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

When using the FLUOVIEW system with an inverted microscope, locate the bottom edge of the range to be observed by moving down the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

3. When the upper edge position is located, click the <Set> button. The [Start Z] text box will show the scan start position of the range of the multiple sections to be observed (Z-direction scanning range).
4. While observing the image in the [Live] panel, locate the bottom edge of the range to be observed by moving up the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

When using the FLUOVIEW system with an inverted microscope, locate the upper edge of the range to be observed by moving up the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

5. When the position is located, click the <Set> button. The [Stop Z] text box will show the scanning stop position of the range of the multiple sections to be observed (Z-direction scanning range).

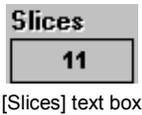
2 Setting the numbers of steps and acquired image slices



1. Set the number of steps using the <▲> or <▼> button in the [Step Size] text box.



The number of steps shown in the [Step Size] text box has been calculated by the system so that the depth scale of the acquired image is identical to the horizontal scale.



The number of acquired images shown in the [Slices] text box can also be input from the keyboard.

After setting [Start Z] (Z-direction scan start position) and [Stop Z] (Z-direction scanning stop position), input the desired number of images in the [Slices] text box. This automatically sets [Step Size] (number of steps).

3 Setting the observation mode

1. Click the [Surface] option button in the [Mode] group box in the [Acquire] panel.
2. In the [Acquire] panel, select the XZT observation mode option button.

4 Acquiring image

1. Click the <XYZ> button in the [Acquire] panel.

The acquired image will be displayed in the [Live] panel.

While acquiring an image in the XYZ observation mode, clicking the <STOP SCAN> button changes the buttons at the upper part of the [Acquire] panel as shown below. The <Resume> button restarts image acquisition at the frame next to the frame where the acquisition is suspended.



<Resume> button

Restarts image acquisition at the frame next to the frame where the acquisition is suspended.



<Series Done> button

Determines the acquired images. Once this button is clicked, it is not possible to append an image.

5 Appending image

XZT image can be added after the image acquisition.

Immediately after acquisition of an image in the XYZ observation mode, the buttons at the upper part of the [Acquire] panel changes as shown below.



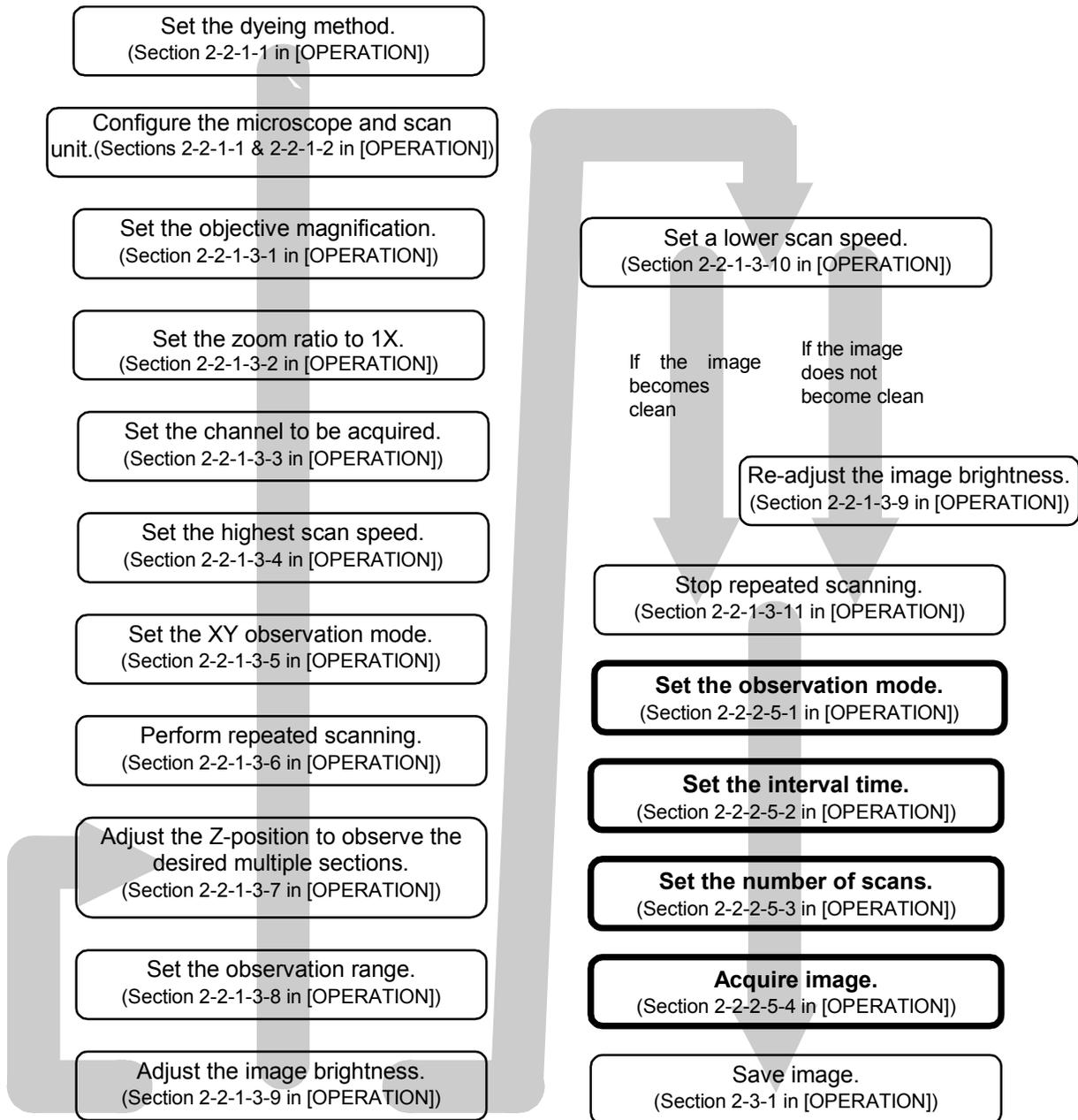
Click the <Append Next> button to append an image. An image will be acquired with the same number of steps as the image acquired immediately before and appended to it.

Click the <Series Done> button when it is not required to append an image.

2-2-2-5 XYT Observation Mode



The description in this section will be focused on the image acquisition operations in the XYT observation mode that are not used in the XY observation modes (which are the operations enclosed in  in the chart on the next page). For other operations, see section 2-2-1, "Image Acquisition in XY Observation Mode". The details of each operation will be described in the subsequent sections.



1 Setting the observation mode

1. Click the [Surface] option button in the [Mode] group box in the [Acquire] panel.
2. In the [Acquire] panel, select the XYT observation mode option button.

2 Setting the interval time

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Time Series] sub-panel. A panel as shown below will be displayed.

[Interval] text box
Set the interval time using the <▲> or <▼> button or by input from the keyboard.

[N] text box
Set the number of scans using the <▲> or <▼> button or by input from the keyboard.
Under text box, it displays max. number of slices that can be acquired with use of physical memory only available at that time.

[Rest Time] text box
Shows the time after end of acquisition of an image until the start of next acquisition.

[Total Time] text box
Shows the total time required for image acquisitions.

Fig. 2-15 [Time Series] Sub-panel

1. Set the interval time using the <▲> or <▼> button in the [Interval] text box.

TIP An image with no interval can be acquired by entering [0] in the [Interval] text box.
In this case, the [Interval] text box shows "Free Run" message.
Using <▼> button also sets the [Interval] text box to "Free Run".

3 Setting the number of scans

1. Set the number of scans using the <▲> or <▼> button in the [N] text box in the [Time Series] sub-panel.

4 Acquiring image

Click the <XYT> button in the [Acquire] panel.

The acquired image will be displayed in the [Live] panel.

When the built-in power supply for transmitted light illumination is used for a long period in the XYT mode, the metallic parts may be expanded by heat, causing the focusing to be deviated. To acquire precise image data, it is recommended to set the power switch of the transmitted light illumination to "O" (OFF).

While acquiring an image in the XYT observation mode, clicking the <STOP SCAN> button changes the buttons at the upper part of the [Acquire] panel as shown below. The <Resume> button restarts image acquisition at the frame next to the frame where the acquisition is suspended.



<Resume> button

Restarts image acquisition at the frame next to the frame where the acquisition is suspended.

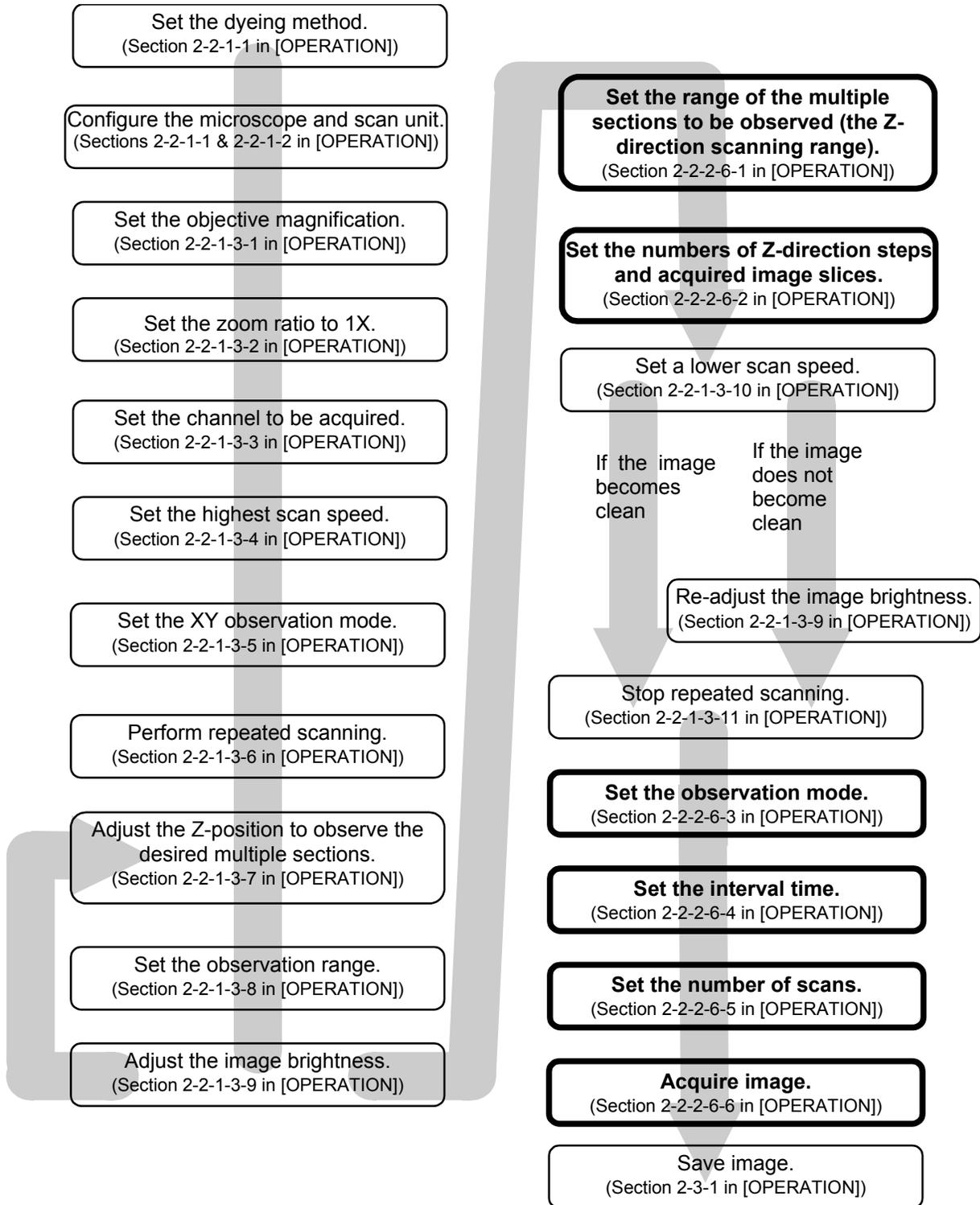


<Series Done> button

Determines the acquired images. Once this button is clicked, it is not possible to append an image.

2-2-2-6 XYZT Observation Mode

The description in this section will be focused on the image acquisition operations in the XYZT observation mode that are not used in the XY observation modes (which are the operations enclosed in  in the chart on the next page). For other operations, see section 2-2-1, "Image Acquisition in XY Observation Mode". The details of each operation will be described in the subsequent sections.



1 Setting the Z-direction scanning range

While acquiring image, move the Z stage according to the range of the multiple sections to be observed (Z-direction scanning range).

From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.

[Stop Z] text box
Shows the scan stop position in the range of the observed cross-section (Z-direction scanning range).

<Z stage coarse adjustment> buttons
Displaces the Z stage on a large scale.

<Z stage fine adjustment> buttons
Displaces the Z stage on a fine scale.

[Start Z] text box
Shows the scan start position in the range of the observed cross-section (Z-direction scanning range).

[Step Size] text box
Set the number of steps using the <▲> or <▼> button. This number can also be input directly from the keyboard.

Recommended step size
Shows the number of steps calculated by the system so that the scale of depth in the Z-direction of the acquired image is identical to the scale of the plane in the X- and Y-directions.

[Slices] text box
Shows the number of images acquired. This number can also be input directly from the keyboard. Under text box, it displays max. number of slices that can be acquired with use of physical memory only available at that time.

<Go> button
Moves to the set scanning stop position. Use this button to check the scanning stop position.

<Set> button
Sets the current stage position as the scanning stop position of the range of the observed cross-section (Z-direction scanning range).

[Current Pos] text box
Shows the current position of the stage.

<Set Zero> button
Sets the current stage position as the home position. Pressing this button also clears the [Stop Z] and [Start Z] values. When stage movement takes time, it changes to <Stop> button. By pressing <Stop> button, the move stops.

<Set> button
Sets the current stage position as the scanning start position of the range of the observed cross-section (Z-direction scanning range).

<Go> button
Moves to the set scan start position. Use this button to check the scan start position.

[Locked] check box
Z-motor is engaged by checking this box.

Fig. 2-16 [Z Stage] Sub-panel



The moving amount assigned to the <Z stage fine adjustment> and <Z stage coarse adjustment> buttons can be changed. See section 1-3 in MAINTENANCE, "Setting the System Configuration" for detailed operations.

1. Check the [Locked] check box in the [Z Stage] sub-panel.



Do not turn the fine focus adjustment knob while the [Locked] check box is checked, for this may damage the Z motor.

2. While observing the image in the [Live] panel, locate the upper edge of the range to be observed by moving down the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

When using the FLUOVIEW system with an inverted microscope, locate the bottom edge of the range to be observed by moving down the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

3. When the upper edge position is located, click the <Set> button. The [Start Z] text box will show the scan start position of the range of the multiple sections to be observed (Z-direction scanning range).
4. While observing the image in the [Live] panel, locate the bottom edge of the range to be observed by moving up the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

When using the FLUOVIEW system with an inverted microscope, locate the upper edge of the range to be observed by moving up the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

5. When the position is located, click the <Set> button. The [Stop Z] text box will show the scanning stop position of the range of the multiple sections to be observed (Z-direction scanning range).

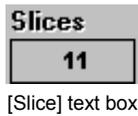
2 Setting the numbers of steps and acquired image slices



1. Set the number of steps using the <▲> or <▼> button in the [Step Size] text box.



The number of steps shown in the [Step Size] text box has been calculated by the system so that the depth scale of the acquired image is identical to the horizontal scale.



The number of acquired images shown in the [Slices] text box can also be input from the keyboard.

After setting [Start Z] (Z-direction scan start position) and [Stop Z] (Z-direction scanning stop position), input the desired number of images in the [Slices] text box. This automatically sets [Step Size] (number of steps).

3 Setting the observation mode

1. Click the [Surface] option button in the [Mode] group box in the [Acquire] panel.
2. In the [Acquire] panel, select the XYZT observation mode option button.

4 Setting the interval time

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Time Series] sub-panel. A panel as shown below will be displayed.

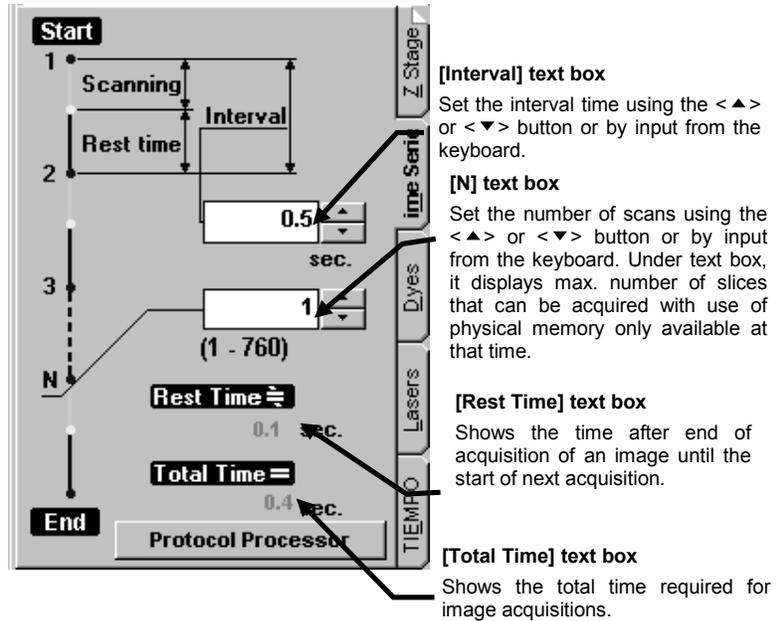


Fig. 2-17 [Time Series] Sub-panel

2. Set the interval time using the <▲> or <▼> button in the [Interval] text box.

TIP An image with no interval can be acquired by entering [0] in the [Interval] text box.
In this case, the [Interval] text box shows "Free Run" message.
Using <▼> button also sets the [Interval] text box to "Free Run".

5 Setting the number of scans

1. Set the number of scans using the <▲> or <▼> button in the [N] text box in the [Time Series] sub-panel.

6 Acquiring image

1. Click the <XYZT> button in the [Acquire] panel.

The acquired image will be displayed in the [Live] panel.

When the built-in power supply for transmitted light illumination is used for a long period in the XYZT mode, the metallic parts may be expanded by heat, causing the focusing to be deviated. To acquire precise image data, it is recommended to turn off the power of the transmitted light.

While acquiring an image in the XYZT observation mode, clicking the <STOP SCAN> button changes the buttons at the upper part of the [Acquire] panel as shown below. The <Resume> button restarts image acquisition at the frame next to the frame where the acquisition is suspended.



<Resume> button

Restarts image acquisition at the frame next to the frame where the acquisition is suspended.

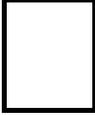


<Series Done> button

Determines the acquired images. Once this button is clicked, it is not possible to append an image.

2-2-3 Differences in Image Acquisition Method Between Fluorescent and Transmitted Images

2-2-3-1 Monochrome Image



The wavelength obtained by monochrome dyeing can be acquired and observed as an image of a channel (either Ch1 or Ch2).

The subsequent description deals with the differences in operation between the monochrome dyeing and dual-fluorochrome dyeing.

When the specimen is dyed so that the wavelength of the fluorescence emission from it is **below 570 nm**, the image is acquired in **channel 1 (Ch1)**.

When the specimen is dyed so that the wavelength of fluorescence emission from it is **above 570 nm**, the image is acquired in **channel 2 (Ch2)**.

- Set the [DETECTION MODE] lever of the scan unit to “FL -” or “- FL”.
FL -: When acquiring the image in Ch1.
- FL: When acquiring the image in Ch2.
- Engage the filter by setting the [BARRIER FILTERS] lever of the scan unit. (Setting the lever to “IN” engages the filter.)
CH1: When observing a specimen dyed to generate fluorescence wavelength below 570 nm.
CH2: When observing a specimen dyed to generate fluorescence wavelength above 570 nm.

Also see section 2-2-1-2, “Configuring the Scan Unit” and follow the instructions given by the [Microscope Configuration] window.

- Set the dyeing method
 1. From the page tabs on the bottom right of the [Acquire] panel, select the [Dyes] sub-panel.

[Assign dyes manually] check box

Checking this enables the manual setting. Dragging the dyeing method in the list directly to the [Ch] group box assigns the dye to the desired channel.

[Available Dyes] list box

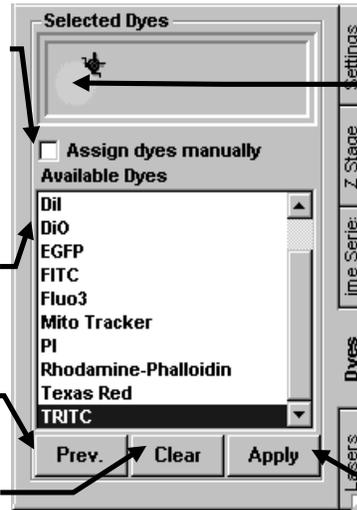
Lists the available dyes. Select the desired items from this list and drag them to the field above it to select the dyeing method.

<Prev.> button

Sets the dyeing method which was set last time by clicking the <Apply> button.

<Clear> button

Clear the set dyeing method.



Place the pointer on the icon displayed in the [Selected Dyes], and the dyeing method is shown in the pop-up display.

<Apply> button

Applies the dyeing method dragged in the [Selected Dyes] group box to the [Channel 1]/[Channel 2] group box in the [Acquire] panel.

Fig. 2-18 [Dyes] Sub-panel

2. Select the specimen dyeing method in the [Available Dyes] list box in the [Selected Dyes] group box, and drag the selected method into the field immediately above the list box.
3. Click the <Apply> button to apply the selected dyeing method to the [Channel1]/[Channel 2] group box on the upper part of the [Acquire] panel.



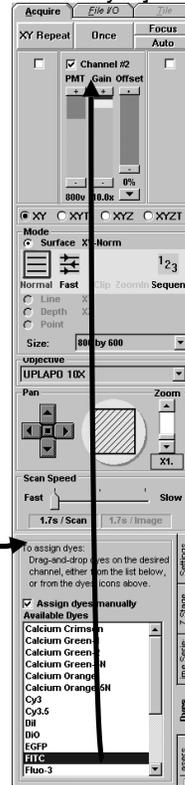
When a dyeing method is selected from the [Available Dyes] list box and the <Apply> button is clicked to apply it in the image acquisition channel, the dyeing method emitting a shorter light wavelength than 570 nm is set to Ch1 and that emitting a longer light wavelength is set to Ch2 automatically.



One Point!

The [Assign dyes manually] check box can also be used to set the dyeing method to the desired channel.

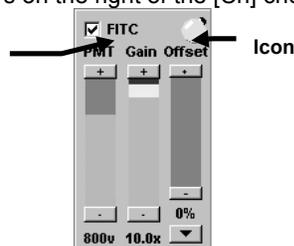
1. Check the [Assign dyes manually] check box in the [Dyes] sub-panel.
2. Select the dyeing method in the [Available Dyes] list box and drag it directly to the field of the [Ch] check box.



[Assign dyes manually] check box

3. After dragging, the icon appears on the right of the [Ch] check box and the dyeing method is set.

The dyeing method is set



Icon

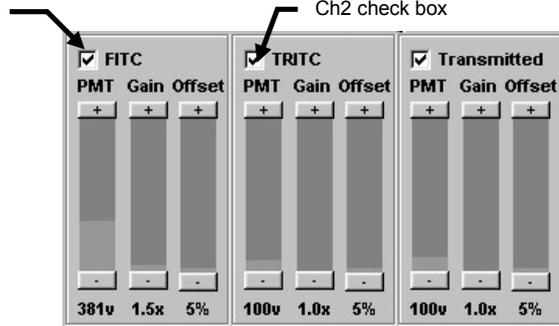
Dragging the icon to the out of the [Ch] check box field cancels the setting of the dyeing method.



- Make the channel ready for image acquisition.

In the [Acquire] panel, make sure that the check box in the [Ch1] or [Ch2] group box that shows the dyeing method is check-marked to indicate that the corresponding channel is ready for image acquisition.

Ch1 check box



Ch2 check box

- Set the image brightness.

Adjust the brightness of the acquisition channel by using the [PMT], [Offset] and [Gain] LED sliders in the [Acquire] panel. For details, refer to section 2-2-1-3-9, “Adjusting the Image Brightness” in the [OPERATION] volume.

2-2-3-2 Dual-Fluorochrome Image

The wavelength obtained by dual-fluorochrome dyeing can be acquired and observed as images of the channels (Ch1 or Ch2).

The subsequent description deals with the differences in operation between the monochrome dyeing and dual-fluorochrome dyeing.

When the specimen dyed by dual-fluorochrome dyeing is observed, the image of wavelengths **below 570 nm** is acquired in **channel 1 (Ch1)** and the image of wavelengths **above 570 nm** is acquired in **channel 2 (Ch2)**.

- Set the [DETECTION MODE] lever of the scan unit to “FL FL”.
- Set the two dyeing methods.
 1. From the page tabs on the bottom right of the [Acquire] panel, select the [Dyes] sub-panel.

[Assign dyes manually] check box

Checking this enables the manual setting. Dragging the dyeing method in the list directly to the [Ch] group box assigns the dye to the desired channel.

[Available Dyes] list box

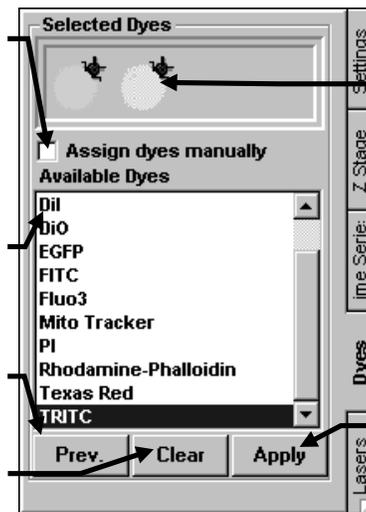
Lists the available dyes. Select the desired items from this list and drag them to the field above it to select the dyeing method.

<Prev.> button

Sets the dyeing method which was set last time by clicking the <Apply> button.

<Clear> button

Clear the set dyeing method.



Place the pointer on the icon displayed in the [Selected Dyes], and the dyeing method is shown in the pop-up display.

<Apply> button

Applies the dyeing method dragged in the [Selected Dyes] group box to the [Channel 1]/[Channel 2] group box in the [Acquire] panel

Fig. 2-19 [Dyes] Sub-panel

2. Select each of the desired specimen dyeing methods in the [Available Dyes] list box in the [Selected Dyes] group box, and drag the selected method into the field immediately above the list box.
3. Click the <Apply> button to apply the selected dyeing method to the [Channel1]/[Channel 2] group box on the upper part of the [Acquire] panel.

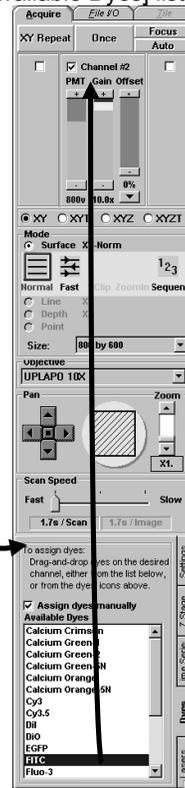
TIP

When dyeing methods are selected from the [Available Dyes] list box and the <Apply> button is clicked to apply them in the image acquisition channel, the dyeing methods emitting a shorter light wavelength than 570 nm are set to Ch1 and those emitting a longer light wavelength are set to Ch2 automatically.

One Point!

The [Assign dyes manually] check box can also be used to set the dyeing method to the desired channel.

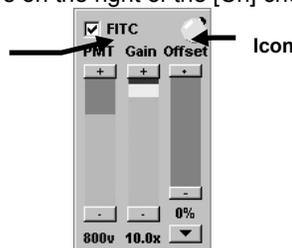
1. Check the [Assign dyes manually] check box in the [Dyes] sub-panel.
2. Select the dyeing method in the [Available Dyes] list box and drag it directly to the field of the [Ch] check box.



[Assign dyes manually] check box

3. After dragging, the icon appears on the right of the [Ch] check box and the dyeing method is set.

The dyeing method is set



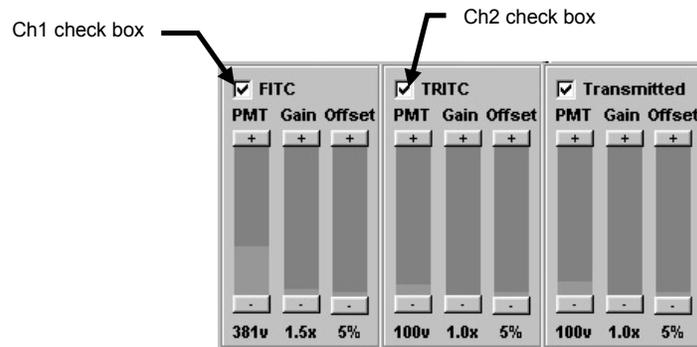
Icon

Dragging the icon to the out of the [Ch] check box field cancels the setting of the dyeing method.



- Engage the filter by setting the [BARRIER FILTERS] lever of the scan unit. (Setting the lever to “IN” engages the filter.)
Also see section 2-2-1-2, “Configuring the Scan Unit” and follow the instructions given by the [Microscope Configuration] window.

- Make the two channels ready for image acquisition.
In the [Acquire] panel, make sure that the check box in the [Ch1] and [Ch2] group boxes that show the dyeing methods are check-marked to indicate that the corresponding channels are ready for image acquisition.



- Set the image brightness of each channel.
Adjust the brightness of the image of each channel by using the [PMT], [Offset] and [Gain] LED sliders in the [Acquire] panel. For details, refer to section 2-2-1-3-9, “Adjusting the Image Brightness”.

2-2-3-3 Transmitted Image

Images obtained by transmitted light observation can also be acquired or observed simultaneously with images obtained by fluorescence observation.

- Set the [DETECTION MODE] lever of the scan unit as shown below.
 - FL -: Fluorescence observation with monochrome or dual-fluorochrome dyeing (Ch1), and transmitted light observation (Ch3).
 - FL: Fluorescence observation with monochrome or dual-fluorochrome dyeing (Ch2), and transmitted light observation (Ch3).
 - FL FL: Fluorescence observations with dual-fluorochrome (Ch1, Ch2), and transmitted light observation (Ch3).

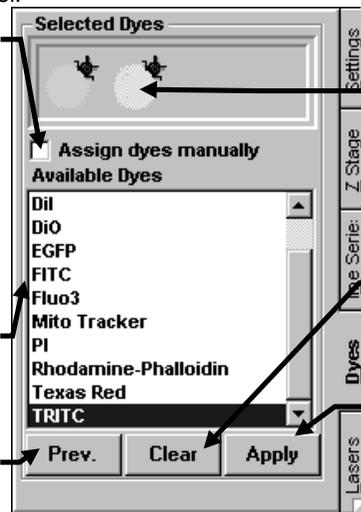
TIP For the difference between Ch1 and Ch2, see sections 2-2-3-1, "Monochrome Image" and 2-2-3-2, "Dual-Fluorochrome Image".

- When observing fluorescence images simultaneously, set the dyeing method.
 1. From the page tabs on the bottom right of the [Acquire] panel, select the [Dyes] sub-panel.

[Assign dyes manually] check box
 Checking this enables the manual setting. Dragging the dyeing method in the list directly to the [Ch] group box assigns the dye to the desired channel.

[Available Dyes] list box
 Lists the available dyes. Select the desired items from this list and drag them to the field above it to select the dyeing method.

<Prev.> button
 Sets the dyeing method which was set last time by clicking the <Apply> button.



Place the pointer on the icon displayed in the [Selected Dyes], and the dyeing method is shown in the pop-up display.

<Clear> button
 Clear the set dyeing method.

<Apply> button
 Applies the dyeing method selected in the [Selected Dyes] group box to [Ch1] or [Ch2] in the [Acquire] panel.

Fig. 2-20 [Dyes] Sub-panel

2. Select each of the desired specimen dyeing methods in the [Available Dyes] list box in the [Selected Dyes] group box, and drag the selected method into the field immediately above the list box.
3. Click the <Apply> button to apply the selected dyeing methods to the [Ch1] and [Ch2] group boxes on the upper part of the [Acquire] panel.



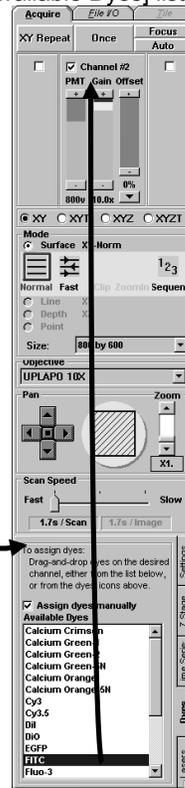
TIP When dyeing methods are selected from the [Available Dyes] list box and the <Apply> button is clicked to apply them in the image acquisition channel, the dyeing methods emitting a shorter light wavelength than 570 nm are set to Ch1 and those emitting a longer light wavelength are set to Ch2 automatically.

One Point!

The [Assign dyes manually] check box can also be used to set the dyeing method to the desired channel.

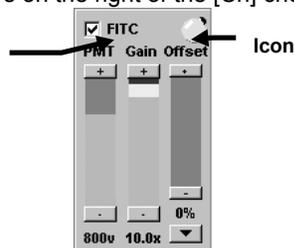
1. Check the [Assign dyes manually] check box in the [Dyes] sub-panel.
2. Select the dyeing method in the [Available Dyes] list box and drag it directly to the field of the [Ch] check box.

[Assign dyes manually] check box



3. After dragging, the icon appears on the right of the [Ch] check box and the dyeing method is set.

The dyeing method is set



Dragging the icon to the out of the [Ch] check box field cancels the setting of the dyeing method.



- Engage the filter by setting the [BARRIER FILTERS] lever of the scan unit. (Setting the lever to “IN” engages the filter.)
Also see section 2-2-1-2, “Configuring the Scan Unit” and follow the instructions given by the [Microscope Configuration] window.
- Turn off the transmitted light bulb of the microscope.
 1. From the page tabs on the bottom right of the [Acquire] panel, select the [Settings] sub-panel.

<Trans. Lamp> button
Turns off the transmitted light bulb of the microscope.
(The lamp lights when the button is in the pressed status.)

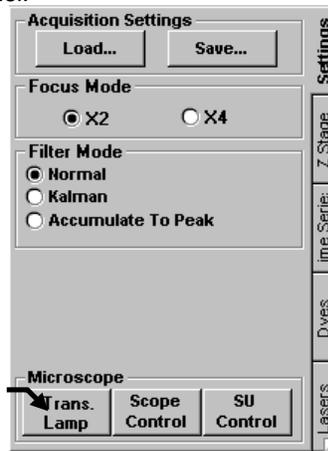
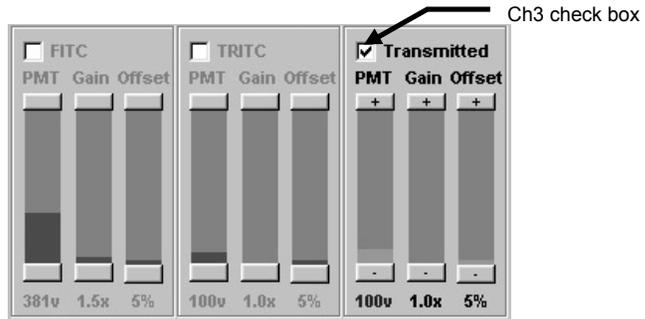


Fig. 2-21 [Settings] Sub-panel

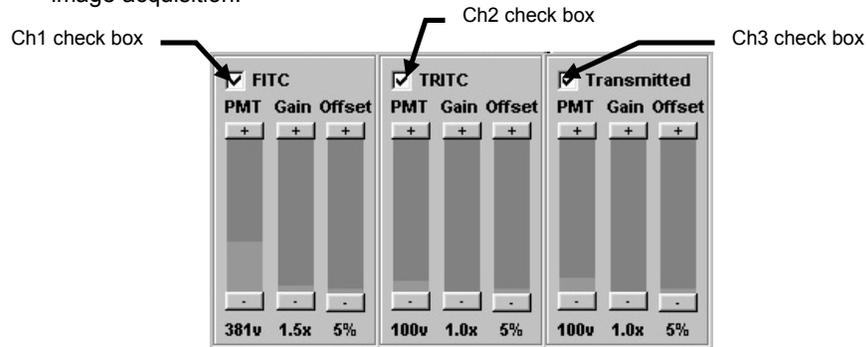
2. Click the <Trans. Lamp> button to turn the lamp off (set the button to the non-pressed status).



- Make channel ready for transmitted image acquisition.
In the [Acquire] panel, make sure that the [Transmitted] check box in the [Ch3] group box is check-marked to indicate that Ch3 is ready for image acquisition.



- When observing a fluorescence image simultaneously, set the required channel ready for acquisition of fluorescence image.
Make sure that the check box showing the dyeing method in the [Ch1] or [Ch2] group box is check-marked to indicate that the corresponding channel is ready for image acquisition.



- Set the brightness of the transmitted image.
Adjust the brightness of the image of Ch3 image by using the [PMT], [Offset] and [Gain] LED sliders in the [Ch3] group box in the [Acquire] panel. For details, refer to section 2-2-1-3-9, "Adjusting the Image Brightness" in the [OPERATION] volume.
- When observing a fluorescence image(s) simultaneously, also set the brightness of the fluorescence image(s).
Adjust the brightness of the image of the transmitted image acquisition channel(s) by using the [PMT], [Offset] and [Gain] LED sliders in its group box in the [Acquire] panel. For details, refer to section 2-2-1-3-9, "Adjusting the Image Brightness" in the [OPERATION] volume.

2-2-4 Image Acquisition by Rotating It (Rotation Scan)

Rotation scan enables image acquisition with tilting the field of viewed.



NOTE

Rotation Scan can not be used with Free Line Scan.



NOTE

This function is available when the FV5-IO3 is in use.

If you do not use the FV5-IO3, the [Pan] group box does not display the arrow buttons and angle.

When acquiring an image by rotating it, also see section 2-2-6, "Image Acquisition by Magnifying the Rectangular Position (Zoom-In Scan)".

1. Acquire an image in the XY observation mode. For the operating procedure, see section 2-2-1, "Image Acquisition in XY Observation Mode".
2. Set the image acquisition mode.
For details, see sections 2-2-1, "Image Acquisition in XY Observation Mode", 2-2-2, "Image Acquisition in Other Observation Modes", 2-2-5, "Image Acquisition of Only the Rectangular Position (Clip Scan)", 2-2-6, "Image Acquisition by Magnifying the Rectangular Position (Zoom-In Scan)", 2-2-7, "High-Speed Image Acquisition" and 2-2-8, "Image acquisition to prevent crosstalk between fluorescence (Sequential Scan)".
3. Specify the image rotation angle using the rotation arrow buttons on both sides of the circuit in the [Pan] group box in the [Acquire] panel.

Assuming that the angle at the top of the circle is 90°, tilts the acquisition area by 1° per step in the counterclockwise direction.

Displays the image rotation angle. The angle can also be specified by direct entry.

Dragging the blue handle along the circuit allows the acquisition area to be tilted.

Assuming that the angle at the top of the circle is 90°, tilts the acquisition area by 1° per step in the counterclockwise direction.

4. Acquire the image by clicking the <XYZ>, <XYT> or <XYZT> button.

2-2-5 Image Acquisition of Only the Rectangular Position (Clip Scan)

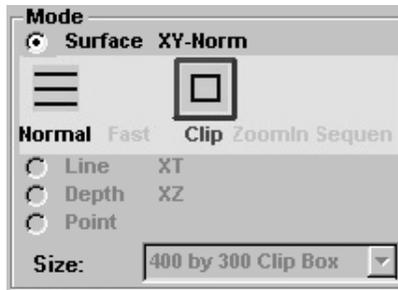
The Clip Scan mode limits the image acquisition range to the range to be observed and acquires the image of only that range.

Applying this mode in an image acquisition mode involving large amount of data, such as the XYZ, XYT and XYZT observation modes, makes it possible to acquire data of a long period of time in a small file size.

For example, let us assume that an 800 x 600 pixel image is displayed in the [Live] panel and the image acquisition area is limited to an area with 400 x 300 pixels (halved in both X and Y directions).

When the image is acquired in this mode, the size per pixel on the specimen is identical to that before start of this mode.

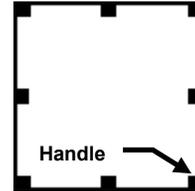
1. Acquire an image in the XY observation mode. For the detailed operation method, see section 2-2-1, "Image Acquisition in XY Observation Mode".
2. Select the [Surface XY-Norm] option button in the [Mode] group box in the [Acquire] panel, then select <Clip> from the list displayed below it.
A frame indicating the scanning range will be displayed in the [Live] panel.
In the initial setting, the image is displayed with both the X and Y sizes set to half the original sizes.



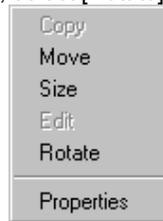
3. Move the frame around the range to be observed. The frame can be moved by placing the mouse pointer inside it and dragging.



4. Change the frame size. To change the frame size, click a point inside the frame with the mouse pointer. When square handles are displayed on the frame edges, place the mouse pointer on one of them and drag it.



5. Change the inclination angle of the line. Right-click the mouse on the line and, when a pop-up menu appears, select [Rotate] from it.



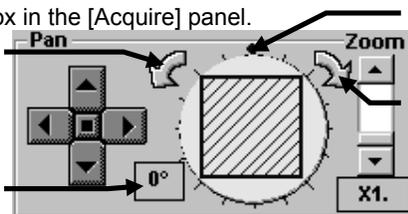
6. The inclination angle of the line can be varied according to the mouse pointer movement.

Place the mouse pointer on an inclining position and click the mouse left button to fix the frame inclination angle.

7. When using hardware that is compatible with rotation scan (FV5-IO3), specify the image rotation angle using the rotation arrow buttons on both sides of the circuit in the [Pan] group box in the [Acquire] panel.

Assuming that the angle at the top of the circle is 90°, tilts the acquisition area by 1° per step in the counterclockwise direction.

Displays the image rotation angle. The angle can also be specified by direct entry.



Dragging the blue handle along the circuit allows the acquisition area to be tilted.

Assuming that the angle at the top of the circle is 90°, tilts the acquisition area by 1° per step in the counterclockwise direction.

8. Acquire images by clicking the <XYZ>, <XYT> or <XYZT> button.
9. After acquiring images, select <Normal> from the list displayed below the [Surface XY-Norm] option button in the [Mode] group box to set the scanning range to the original setting.



It is not permitted to change the scanning range after having acquired images. If you want to change the scanning range again, select <Normal> from the list displayed below the [Surface XY-Norm] option button in the [Mode] group box to set the scanning range to the original setting, acquire an image in the XY observation mode, then select <Clip> in the [Mode] group box and reselect the scanning range.

2-2-6 Image Acquisition by Magnifying the Rectangular Position (Zoom-In Scan)

The Zoom-In Scan mode limits the scanning area to the range to be observed and acquires the image of that area by magnifying it.

For example, let us assume that an 800 x 600 pixel image is displayed in the [Live] panel and the image acquisition area is limited to an area with 400 x 300 pixels (halved in both X and Y directions).

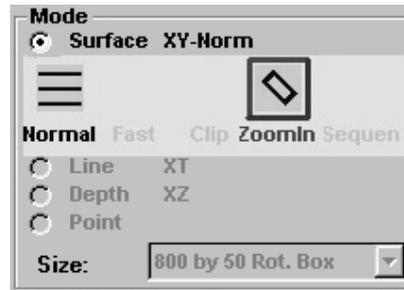
When the image is acquired in this mode, the number of pixels in the X direction becomes identical to the image size before start of this mode, and the magnification applied is determined according to the size of the limited range. High-speed image acquisition can be made possible by decreasing the number of pixels in the Y direction.



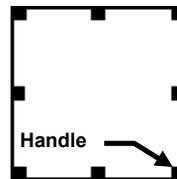
Please prepare hardware that is compatible with rotation scan (FV5-IO3). If you use the FV5-IO2, the sizes of the acquirable images are fixed as follows.

256x80, 320x81, 512x64, 640x58, 800x50, 1024x42, 2048x25

1. Acquire an image in the XY observation mode. For the operation procedure, see section 2-2-1, "Image Acquisition with XY observation".
2. Select <ZoomIn> under the [Surface XY] option button in the [Mode] group box in the [Acquire] panel. A frame indicating the control range appears in the [Live] panel.

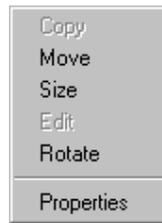


3. Move the frame to the area you want to observe. To move the frame, place the mouse pointer inside it and drag the mouse.
4. Change the frame size. Place the mouse pointer inside the frame and click to display square handles around the frame. Then place the mouse pointer on one of the handles and drag it to change the frame size. (Here the X:Y ratio of the frame is not changed.)

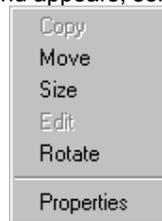


5. Change the number of pixels in the Y direction. Right click the mouse on the frame to display a pop-up menu and select [Size] in it to display square handles around the frame. Place the mouse pointer on one of these handles and drag it to change the number of pixels. Note that the X:Y ratio of the frame is not changed during this.

If high-speed image acquisition is required, decrease the number of pixels in the Y direction.



- Change the inclination angle of the frame. Click the mouse right button inside the frame and, when a pop-up menu appears, select [Rotate] from it.



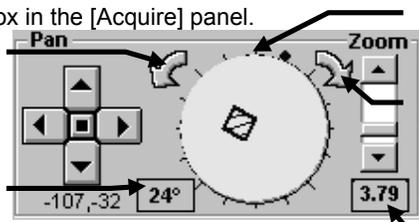
- The inclination angle of the frame can be varied according to the mouse pointer movement.

Place the mouse pointer on an inclined position and click the mouse left button to fix the frame inclination angle.

- When using hardware that is compatible with rotation scan (FV5-IO3), specify the image rotation angle using the rotation arrow buttons on both sides of the circuit in the [Pan] group box in the [Acquire] panel.

Assuming that the angle at the top of the circle is 90°, tilts the acquisition area by 1° per step in the counterclockwise direction.

Displays the image rotation angle. The angle can also be specified by direct entry.



Dragging the blue handle along the circuit allows the acquisition area to be tilted.

Assuming that the angle at the top of the circle is 90°, tilts the acquisition area by 1° per step in the counterclockwise direction.

Actual zoom value appears.

- Acquire the image by clicking <XYZ>, <XYT>, <XYZT> etc.
- After image acquisition, select <Normal> under the [Surface XY] option button in the [Mode] group box in the [Acquire] panel to return from the image acquisition mode.

2-2-7 High-Speed Image Acquisition

An image can be acquired in 0.25 second.

This image acquisition mode is valid under the following condition:

- Max. 2 channels
 - Image size: 512 x 512 pixels
 - Zoom ratio of 2X or more, with setting in 2X steps
1. Check one or both of the [Ch] check boxes in the [Ch] group box in the [Acquire] panel.
 2. Select the [Surface XY-Norm] option button in the [Mode] group box in the [Acquire] panel and select <Fast> from the list displayed below it.



[512 by 512] is set automatically in the [Size] drop-down list in the [Mode] group box.

"X2" is set and shown in gray-out display automatically in the [Zoom] scale in the [Pan] group box.

3. To change the zoom ratio, use the [Zoom] scale in the [Pan] group box.
4. Click an image acquisition button such as the <XY Repeat> button.



<XY Repeat> button



High-speed image acquisition is possible only in XY, XYT and XT observations.

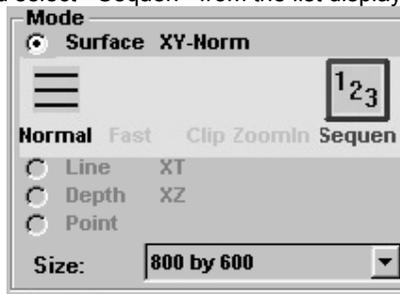
5. After image acquisition, select <Normal> under the [Surface XY] option button in the [Mode] group box in the [Acquire] panel to return from the image acquisition mode.

2-2-8 Image acquisition to prevent crosstalk between fluorescence (Sequential Scan)

An image which is suppressed crosstalk can be acquired sequentially by combination of excitation laser and those image acquisition channel.

With this image capturing method, the image of a multiple-dyed specimen can be obtained by sequentially acquiring image slice of each type of fluorescence.

1. Select the [Surface XY-Norm] option button in the [Mode] group box in the [Acquire] panel and select <Sequen> from the list displayed below it.



Selecting <Sq Clip>, <Sq Rct>, or <Line Sq> enables the image acquisition of the desired rectangle area, that of the rectangle area at desired angle, or a line sequential scan.

Line Sequential Scan

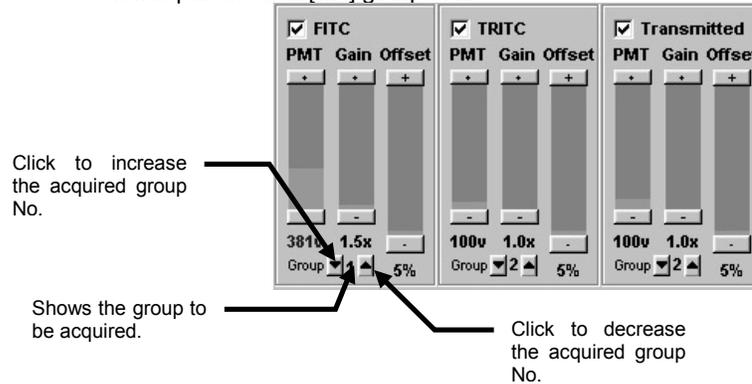
Ordinary sequential scan is performed for every one frame for every group, whereas line sequential scan is performed for every one line for every group. Therefore, line sequential scan is of advantage in minimizing the time lag between wavelength and acquiring a fewer cross-talk image.



Image acquisition in the line mode can be performed when you use the FV300 system with AOTF (FV5-COMBA).

2. Select the observation mode with the option buttons in the [Acquire] panel.

- [Group] (which means each laser) and the <▲> and <▼> buttons appear on the lower part of each [CH] group box.



- Set the group number according to the reagent in use with the <▲> and <▼> buttons. This setting is not required if the desired number is already displayed.



Image acquisition starts with the smallest group number set.



To prevent unwanted color fading, it is recommended to acquire the image from the reagents with longer wavelengths.

- Click the <Seq. Once> button to acquire the image.
- When transmitted light observation is required, the [FLUOVIEW] dialog box and [Lasers] sub-panel are displayed to allow you select the laser type which has not been specified yet.

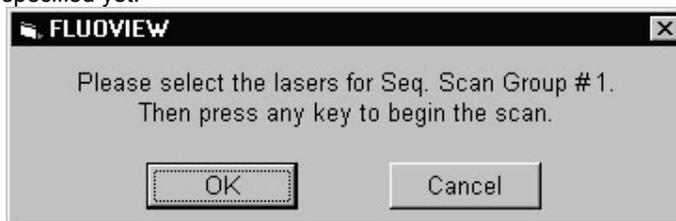


Fig. 2-22 [FLUOVIEW] Dialog Box

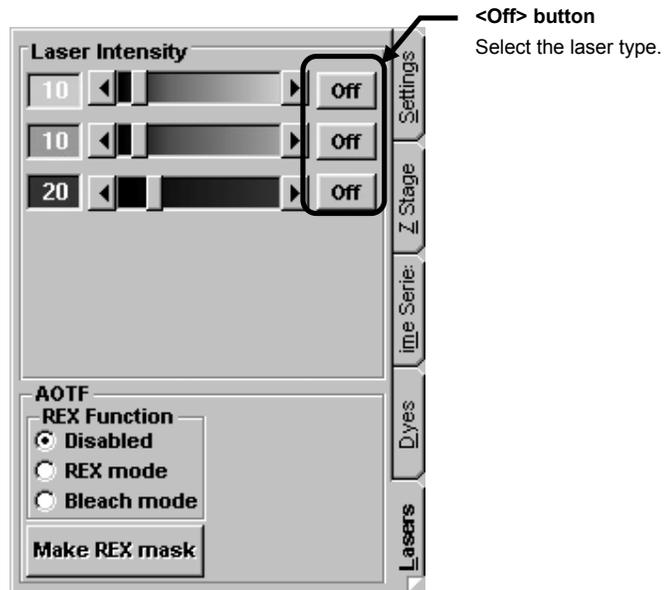


Fig. 2-23 [Lasers] Sub-panel

7. Among the laser <Off> buttons on the right of the [Laser Intensity] scales in the [Lasers] group box, click the <Off> button of the laser you want to use in transmitted observation. This should change the <Off> button to the <Rdy> button (displayed in the pressed-in condition).
8. Click the <OK> button in the [FLUOVIEW] dialog box.
Image acquisition starts.
9. Adjust the brightness with the [PMT], [Offset] and [Gain] LED sliders in the [Ch] group box in the [Acquire] panel. See section 2-2-1-3-9, "Adjusting the Image Brightness" for details.
10. After image acquisition, select <Normal> under the [Surface XY] button in the [Mode] group box in the [Acquire] panel to return from the image acquisition mode.

2-2-8-1 Virtual Channel Function

The virtual channels refer to the simulated detection channels produced when a single detector switched in sequential scan. The virtual channels make it possible to perform sequential scan by switching the lasers and filters according to the preset condition by increasing the number of detection channels. (With the FV300, they should be switched manually.)

Operation Example

The following procedure deals with the case of observing FITC in CH1, PI in CH2 and Cy5 in a virtual channel.

1. In the [Dyes] sub-panel of the [Acquire] panel, select the dyeing methods and click the <Apply> button. (Example: FITC and PI are selected.)

For details, see step 2 in the procedure in section 2-2-1-1, "Configuring the Microscope".

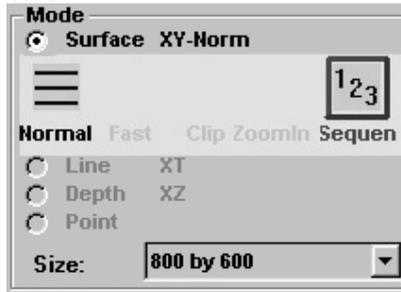


<Once> button

2. Click the <Once> button to acquire an image.



- In the [Mode] group box in the [Acquire] panel, click the [Surface XY-Norm] option button, select <Sequen> from the list below it and select the scan mode to be used from the displayed icons.



Do not select <Line Aq> in the [Mode] group box in the [Acquire] panel because the virtual channels are not compatible with Line Sequential Scan.



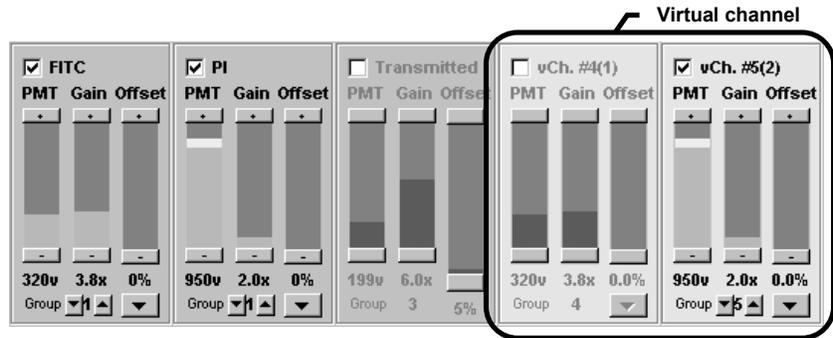
If the observation mode is set to Sequential Scan, the [Dyes] sub-panel in the [Acquire] panel cannot be used.

- Using the <▲> and <▼> buttons in [Group], set the group according to the reagent in use. This operation is not required if the current setting needs not be changed. (Example: CH1 (FITC) and CH2 (PI) are set to Group 1.)

- Right-click the mouse on the [Ch] group box on the upper part of the [Acquire] panel. When a pop-up menu appears, select the channel to be added.



A virtual channel will be added in the [Ch] group box. (Example: When [Using PMT 1] and [Using PMT 2] are selected from the pop-up menus.)



NOTE It is not permitted to set the physically identical channels (an ordinary channel and virtual channel) in the same group. (For example, ordinary channel 1 and virtual channel 1 cannot be set in the same group.)



TIP Sequential Group means a set of image channel which is acquired in a sequential image acquisition. Channel 1 and Virtual channel 1 needs to assign in deferent Sequential Group since those channel can not acquire at the same time.

6. Set the laser and filter types condition.

Select <SU Control> button of the [Settings] sub-panel in the [Acquire] panel to display the [Optical System Configuration] window.

In the [Optical System Configuration] window, set the dyeing method of virtual channel, then set the displayed optimum filter, etc. of the window in the scanning unit.

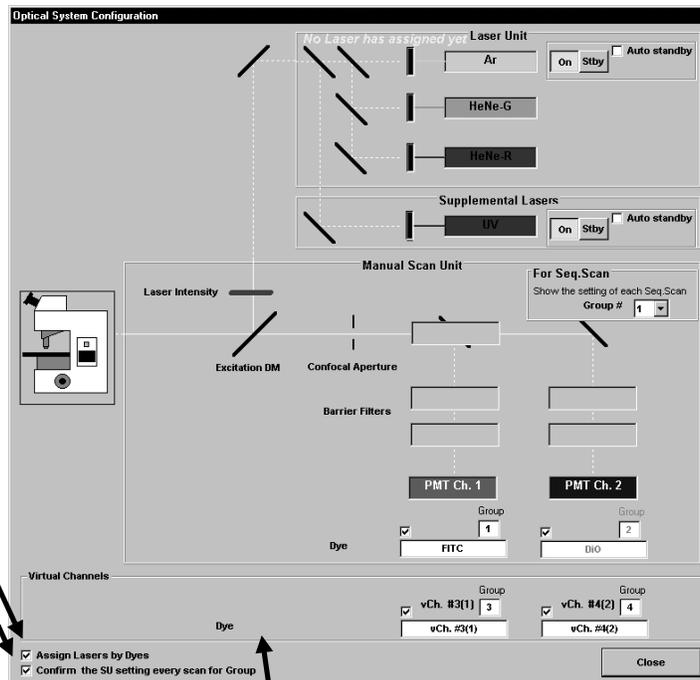
For details on the [Optical System Configuration] window, see section 1-3-2-3, "Configuring the Filters".

(Example: Set the group No. to [2] in the [Group #] drop-down list of [For Seq. Scan]. Then open the [Group] drop-down list of vCh (2) in the [Virtual Channel] group box and select [2].

In the [Dye] drop-down list of vCH (2), select [CY5].

Displayed each optimum filters, etc. in [Excitation DM], [Beam Splitter], [Barrier Filter] and [Laser Unit] group box.

After completing the setup, select the <Close> button to close window.



[Assign Laser by Dye] check box

When checked, settings of filter and laser will be automatically done according to selection of dye method.

[Confirm the SU setting every scan for Group] check box

When check is removed, dialog does not appear each time group is changed during scan; and it is possible to execute scanning consecutively.

[Virtual Channels] group box

Show the virtual channels.

Click a channel checkbox to check it makes the virtual channel available. Select the dyeing method from the drop-down list and select the group No. in the [Group] drop-down list.



NOTE

Filters cannot be set if you are using the FV300. Please switch manually.



NOTE

Setting will disappear if <Normal> is selected at [Mode] group box of [Acquire] panel after setting virtual channel. It is useful that the setting is saved with use of <Save> button at [Acquisition Settings] group box on [Settings] sub panel of [Acquire] panel before selecting <Normal>.



TIP

When the laser to be used with a group has not been set, the [Laser Unit] dialog box shows “No Laser has assigned yet”. In this case, set the laser in the [Laser Unit] group box.

- Adjust the image brightness for every group.

Select the group to adjust the image brightness in the [Group] drop-down list displayed on the upper part of the [Acquire] panel.



[Group] drop-down list
Select the sequential scan group.



TIP

Select a group before repeated scanning. The group selection cannot be changed during repeated scanning.



<XY Repeat> button



<Focus> button

- Click the <XY Repeat> or <Focus> button to perform repeated scanning in order to adjust the brightness of the channels.



TIP

Select a group before repeated scanning. The group selection cannot be changed during repeated scanning.

- Adjust the brightness using the [PMT], [Offset] and [Gain] LED sliders in the [Ch] group box in the [Acquire] panel. For details, see section 2-2-1-3-9, “Adjusting the image brightness”.



- Click the <STOP SCAN> button to stop repeated scanning.



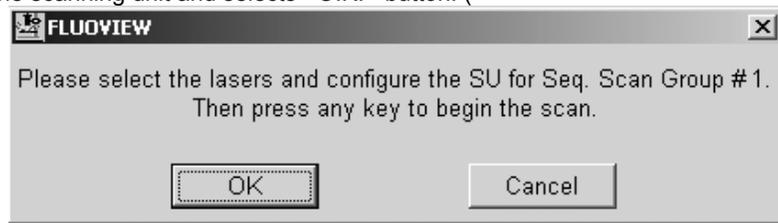
- Repeat steps 7 and 10 for each of the groups to be configured.



<Seq Once> button

- Click the <Seq. Once> button to start sequential scan.

- When a group switches, the following dialog box is displayed. Set filter types etc. in the scanning unit and selects <O.K.> button. (



One Point!

When change of setting for filter that meets with scan changeover is not required, uncheck the check box of [Confirm the SU setting every scan for Group] at [Optical System Configuration] window so that this dialog does not appear and scanning can be executed consecutively.

- After image acquisition, click <Normal> below the [Surface XY] option button in the [Mode] group box in the [Acquire] panel to return from the image acquisition mode. This hides the virtual channels from the display.

TIP Only the image(s) of the current Sequential Group is updated when image is acquired by pressing <XY Repeat> button or <Focus>. The other Sequential Group image stays in [Live] panel.

2-2-9 Image Acquisition of a Line at Desired Angle

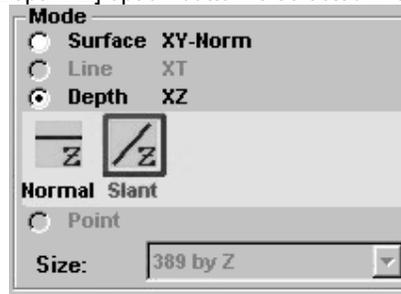
When the specimen is not standing upright, the image of a line can be acquired by changing the angle.

A line can be specified on each display.

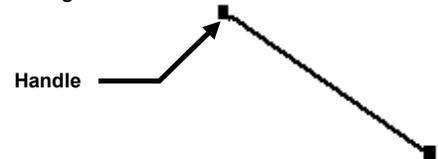


1. Acquire an image in the XY observation mode. For the operation procedure, see section 2-2-1, "Image Acquisition with XY observation".
2. Select [Line XT] option button or <Slant> under the [Depth XZ] option button in the [Mode] group box in the [Acquire] panel. A line indicating the control range appears in the [Live] panel.

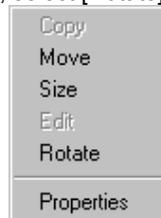
(<Slant > under the [Depth XZ] option button is selected in the following Fig.)



3. Move the line to the area you want to observe. To move the line, place the mouse pointer on it and drag the mouse.
4. Change the line length. Place the mouse pointer on the line and click to display square handles on the two extremities of the line. Then place the mouse pointer on either handle and drag it to change the line length.



5. Change the inclination angle of the line. Right-click the mouse on the line and, when a pop-up menu appears, select [Rotate] from it.





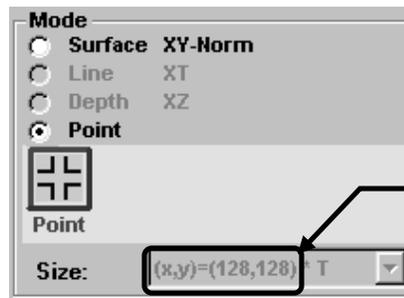
6. The inclination angle of the line can be varied according to the mouse pointer movement.
Place the mouse pointer on an inclined position and click the mouse left button to fix the line inclination angle.
7. Set the interval time or the number of scans.
The operation procedure is same as that in the XY observation mode. See section 2-2-2, "XT Observation Mode".
8. Acquire the image by clicking <XYZ>, <XYT>, <XYZT> etc.
9. After image acquisition, select <Normal> under the [Surface XY] option button in the [Mode] group box in the [Acquire] panel to return from the image acquisition mode.

2-2-10 Display the change of image intensity (Point Scan)

The change of fluorescence intensity according to time-lapse can be displayed graphically by irradiating the laser to certain point on the image.

If you have FLUOVIEW TIME COURSE software, image acquisition can be started with buttons, keys or input of external trigger signals.

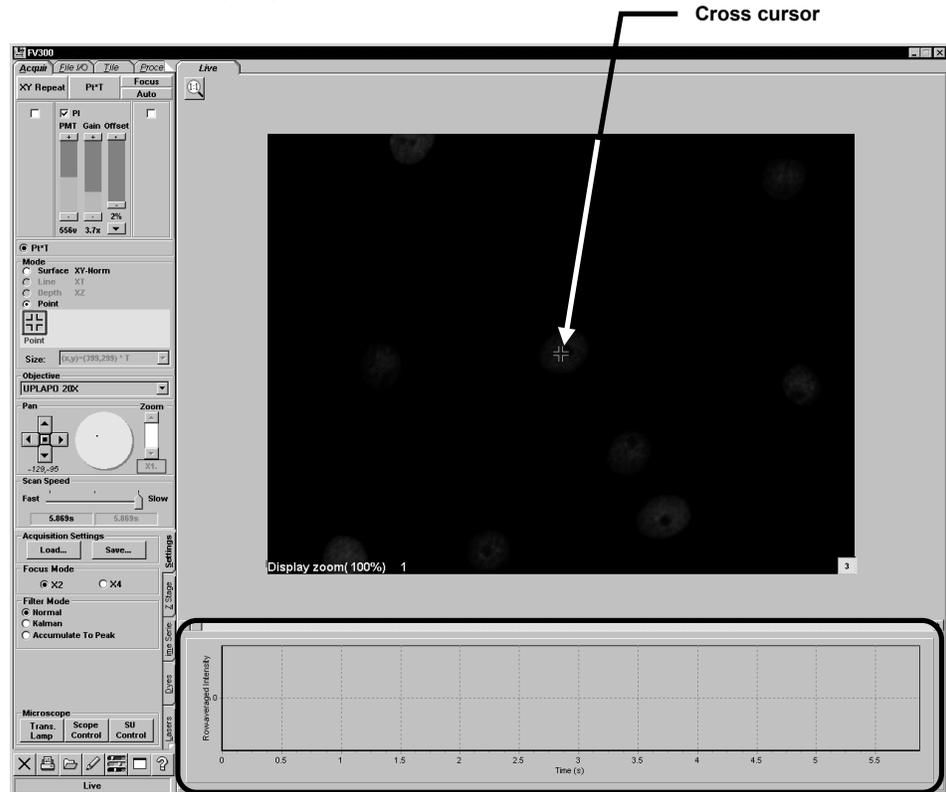
1. Acquire an image in the XY observation mode. For the operation procedure, see section 2-2-1, "Image Acquisition with XY observation".
2. Select <Point> under the [Point] option button in the [Mode] group box in the [Acquire] panel.



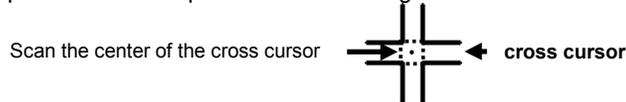
Shows the X, Y coordinates on the image.



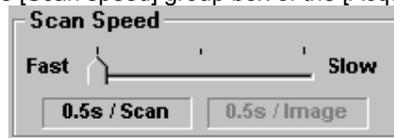
The graph window showing the cross cursor for scanning and the intensity values appears in the [Live] panel.



3. Move the cross cursor to the area you want to observe. To move the cross cursor, place the mouse pointer on it and drag the mouse.



4. Set the scan speed for image acquisition.
Set the speed in the [Scan speed] group box of the [Acquire] panel.



TIP

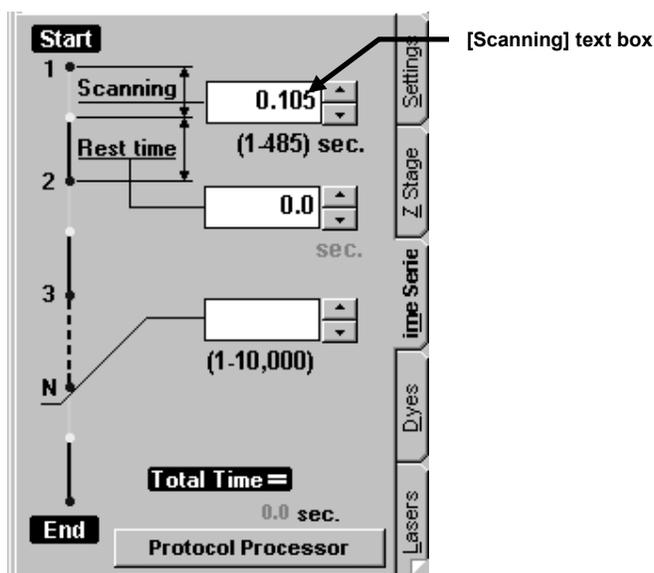
The speed recommended for point scan are as follows.

2 μ s(The scroll bar indicates “Fast” position. It is useful for the specimen changes rapidly.), 10 μ s, 100 μ s(The scroll bar indicates “Slow” position. It is useful for the specimen changes slowly.)

- Set the time for measurement.

From the page tabs on the bottom right of the [Acuire] panel, select the [Time Series] sub-panel.

Set the time for measurement with the <▲> or <▼> button in the [Scanning] text box.



- Set the starting method for image acquisition.

(If you have FLUOVIEW TIME COURSE software)

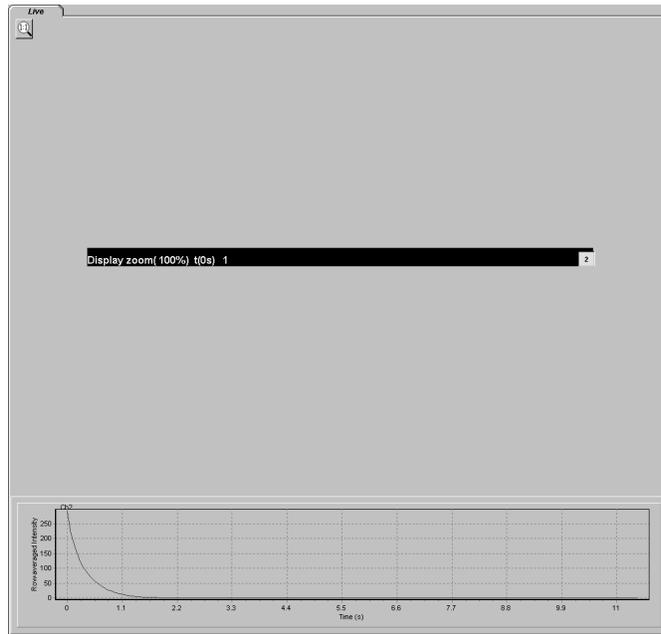
For setting procedure, see “FLUOVIEW TIME COURSE software user’s manual”.

- Click the <PtT> button to acquire the image.

The image at the coordinates set in the [Live] panel appears in the image display area and the graph showing the fluorescence intensity also appears in the graph window.



TIP The image shown in the [Live] panel after image acquisition is the image that acquired at the coordinates specified in the [Live] panel and arranged in the X direction, from the top left to the bottom right.
The image shown after image acquisition is the same a width as that in the X direction specified in the [Live] panel.



TIP The size of the graph window can be modified by double-clicking the mouse button on it.

TIP When a certain area is specified by dragging the mouse from the top left to the bottom right on the graph while pressing down its left button, the specified area can be magnified.

TIP When the right button of the mouse is dragged on the graph, the graph can be scrolled.

TIP The magnification or scrolling of the graph can be canceled by dragging the left button of the mouse on the magnified graph from the bottom left to the top right, the top right to the bottom left or the bottom right to the top left.

TIP

When click the mouse on the X or Y Axis on the graph window, the [Editing] dialog box appears and the graph parameters or the graph display method can be modified.

- After image acquisition, select <Normal> under the [Surface XY] option button in the [Mode] group box in the [Acquire] panel to return from the image acquisition mode.

2-2-11 Image Acquisition on Desired Line (XZ, XT or XZT Observation)

When the specimen is in curved form, the image can be acquired by drawing a desired line on it.

- Acquire an image in the XY observation mode. For detailed operation, see section 2-2-1, "Image Acquisition with XY observation".
- Select [Line XT] option button in the [Mode] group box in the [Acquire] panel or <Free> under the [Depth XZ] option button. A line indicating the scanning range appears in the [Live] panel.

(<Free> under the [Line XT] option button is selected in the following Fig.)

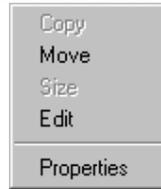
**TIP**

A line can be specified for one display.

- Move the curved line to the area you want to observe. To move the curve, place the mouse pointer in the box and drag the mouse.



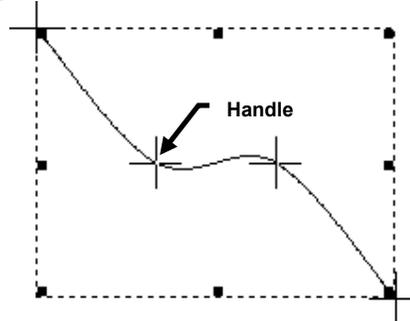
- Change the curved line shape. Place the mouse pointer within the box and right click to display the pop-up menu. Then select <Edit>.



The cross shaped handles appear around the curve. Place the mouse pointer on either handle and drag it to change the line shape. The color of the mouse pointer is changed on the handle.

The handle can be add by clicking the mouse on desired coordinates.

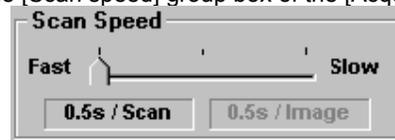
To delete the handle, click the mouse on it.



Right click the mouse on the coordinates but the handles to fix the shape after the change.

- Set the scan speed for image acquisition.

Set the speed in the [Scan speed] group box of the [Acquire] panel.



The speed recommended for this scan mode are as follows.

2 μ s: The scroll bar indicates "Fast" position. It is useful for the specimen changes rapidly.

4 μ s, 8 μ s : The scroll bar indicates "Slow" position. It is useful for the specimen changes slowly.

6. Set the interval time or the number of scans.

The operating procedure is same as that in the XY observation mode. See section 2-2-2, "XT Observation Mode" for details.



Up to 8000 times, images can be acquired. Set the number of scans in the [N] text box in the [Time Series] sub-panel.

7. Click <XZ>, <XT>, or <XZT> etc. to acquire the image.



The image shown in the [Live] panel after image acquisition is the image that acquired at the curved line specified on the image and arranged uprightly in the X direction, from the top to bottom.

The image shown after image acquisition is the same a width as that in the X direction specified in the [Live] panel. (Same as that in the XT observation mode).

8. After image acquisition, select <Normal> under the [Surface XY] option button in the [Mode] group box in the [Acquire] panel to return from the image acquisition mode.

2-2-12 Image Acquisition in the Laser Excitation Mode

When you use the FV system with AOTF (FV5-COMBA), the function described in this section is available.

In the FV system with AOTF, it is possible to cut excitation of laser except the region where scanning is performed. Moreover, it is also possible to set up a region of laser excitation to excite laser only to the target part.

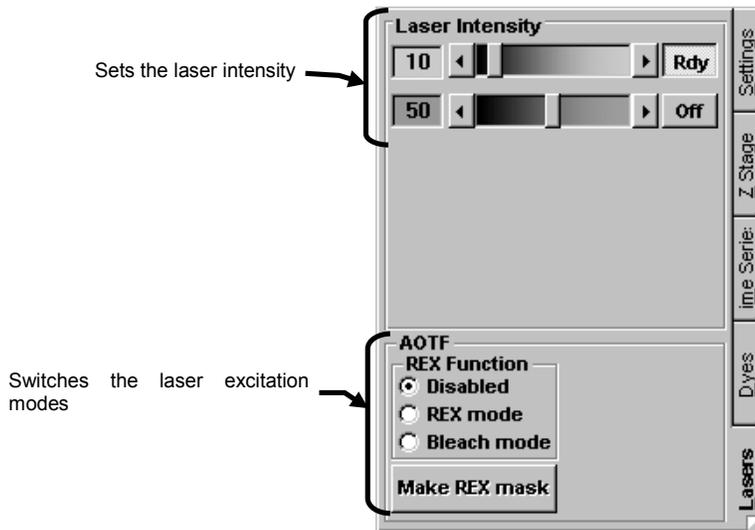
TIP REX represents the “region of laser excitation” in this section.

Three kinds of the laser excitation mode, Disable, REX, and Bleach, are selectable according to your purpose. And each mode can be switched in the XYT observation. In use of the REX or Bleach mode, a setup of REX mask file is required.

Disable mode: An ordinary mode to acquire an image of specified size.

REX mode: The mode which sets the REX mask file to excite laser only to the target part and acquire image. The same laser setup as Disable mode is applied.

Bleach mode: The REX mask file other than that of the REX mode can be set. And each laser setup can also be obtained apart from the Disable/REX mode.





NOTE

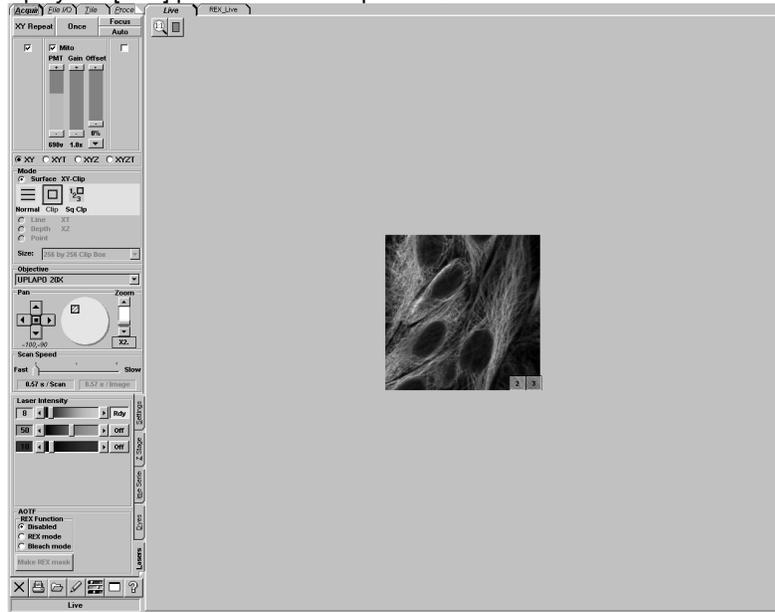
In the REX and Bleach mode, image acquisition in the Focus mode, fast scan, and image acquisition in the Line mode (Normal, Slant, and Free) can not be performed.

NOTE

The specimen in the images carried in this section is not for FRAP, but for the user's manual.

2-2-12-1 Making REX Mask File

1. Display the [Live] panel at the front position.

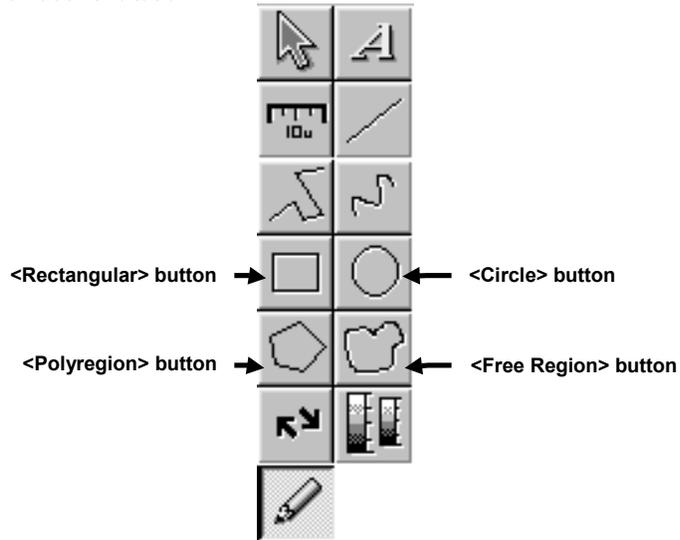


<Annotate> button

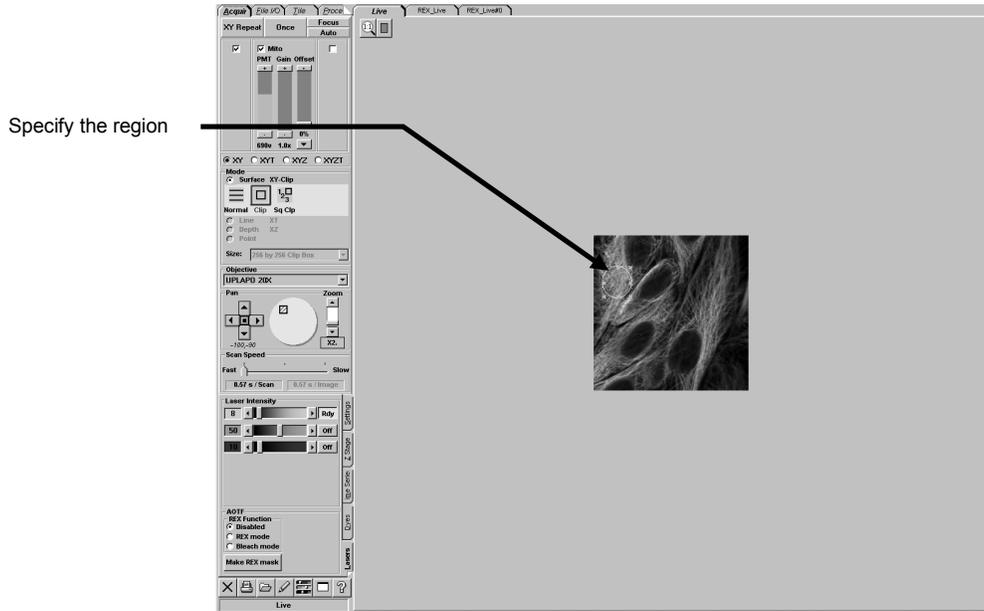
2. Select the <Annotate> button in the tool bar at the bottom left of the screen.



- In the list of buttons displayed as shown below, select the <Rectangular>, <Circle>, <Polyregion>, or <Free region> button. And drag on the image to specify the region of laser excitation.



TIP In order to specify two or more regions, click the mouse on or inside the region with holding down the **SHIFT** key after a region is specified.



- From the page tabs on the bottom right of the [Acquire] panel, select the [Lasers] sub-panel.

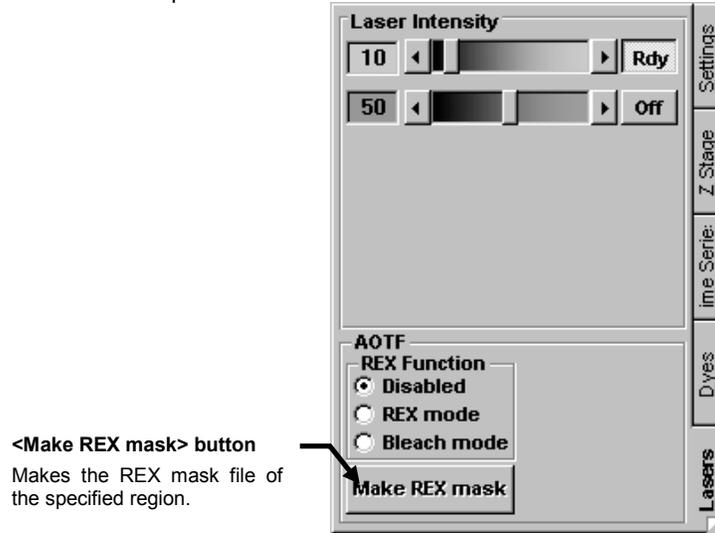
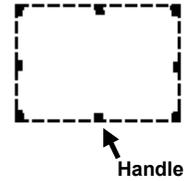


Fig. 2-24 [Lasers] sub-panel



When the <Make REX mask> button is displayed in gray, click and select the specified region on the [Live] panel. When two or more regions are specified, select all the regions to be masked and let the handles be displayed around each region.





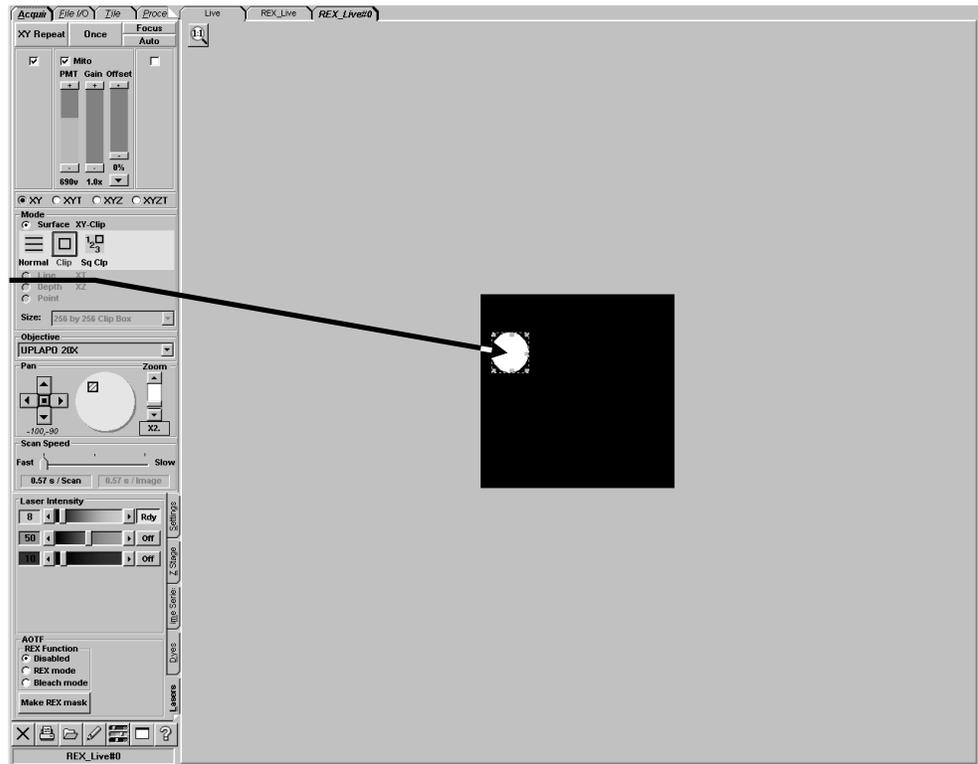
Make REX mask

<Make REX mask> button

5. Select the <Make REX mask> button.

The [REX Live] panel (the REX mask file) is made in the [Display] panel.

Make the REX mask file which masks the region outside of the specified region.

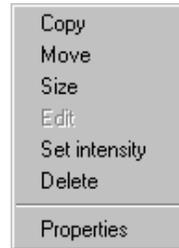


TIP When the [REX Live] panel has already been created on the FV300 software, A panel is continuously created after [REX_Live0#].

TIP The REX mask file can be saved. The saving procedure is completely the same as that of a display. For details, refer to section 2-3-1-2, "Saving a Display" in this manual.



- In order to adjust the laser intensity for every region, right-click the mouse on the white region on the [Display] panel where the REX mask file is displayed. The pop-up menu as shown below appears.

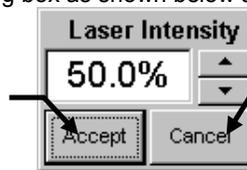


- Select [Set intensity] in the menu.

The [Mask Intensity] dialog box as shown below appears.

<Accept> button

Accepts the value set in the [Laser Intensity] text box and closes the dialog box.



<Cancel> button

Cancels the value set in the [Laser Intensity] dialog box and closes the dialog box.

- Set the value for the laser intensity on percentage and click the <Accept> button.

The brightness of the region is changed according to the value set in the [Mask Intensity] dialog box.



The laser intensity of the REX mask file is setup on percentage to 100%.



The region becomes brighter as the value is close to "100".



In case of using REX function, setting of [Laser Intensity] in [Laser] sub-panel is recommended to set 100%. Otherwise, the actual laser intensity may not be reproducible since the [Laser Intensity] setting affects as followings.

$$\text{Actual laser intensity} = (\text{laser intensity in [Laser Intensity] group box}) \times (\text{Percentage in the [Laser Intensity] dialog box})$$

2-2-12-2 Example of FRAP experiment

The example of procedure of FRAP experiment using AOTF is described in this section.

In order to perform photobleaching to a specimen, the value of laser intensity is raised to 100% to acquire an image since strong laser intensity is temporarily required. When 100% or more of laser intensity value is required, zoom magnification is gathered to acquire an image. (It is because the square power of zoom magnification per time can be secured.) Moreover, using the objective of larger NA concentrates the laser irradiation spot in Z direction, and stronger laser can be irradiated.

Therefore, a preliminary experiment is required to set up the laser intensity, the objective, the zoom ration of the REX mask file according to a specimen.

1 Preliminary Experiment

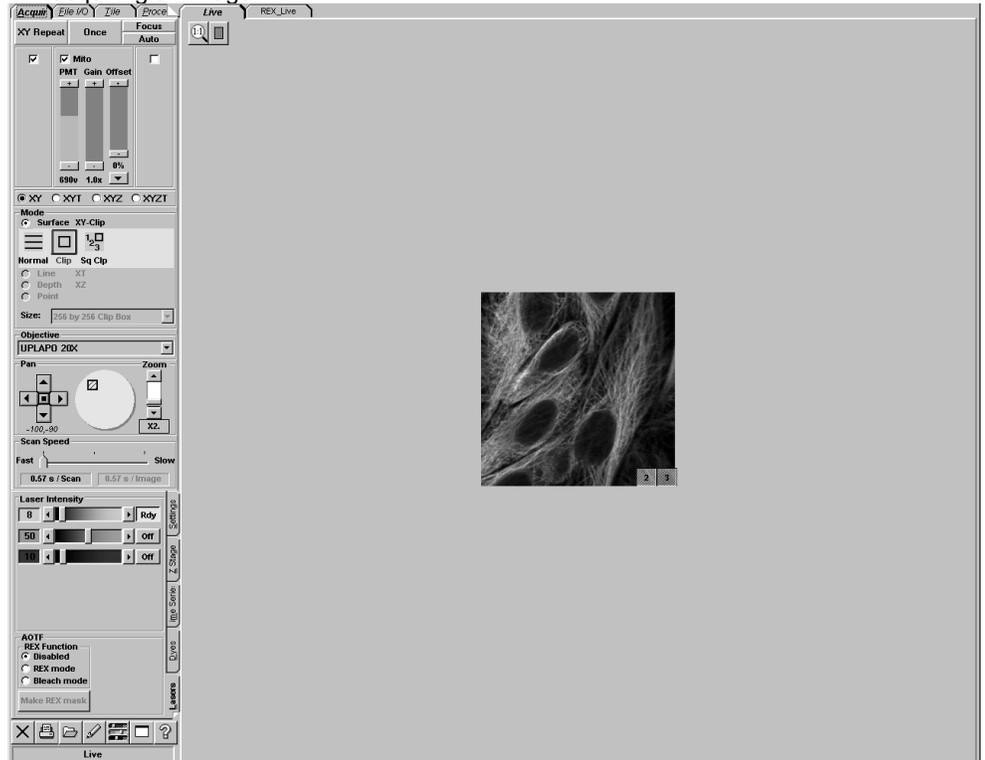
1. Set the laser intensity, the objective, and the zoom ration in the [Acquire] panel so that they are suitable for a specimen.

2 Acquiring Image in XY mode

1. Confirm that [XY-Norm] is displayed in the [Mode] group box in the [Acquire] panel.
2. Select the [XY] option button in the [Acquire] panel.

3. Select the <Once> button in the [Acquire] panel and acquire an image.

If necessary, set the range for image acquisition with clip scan, for example, after acquiring an image.



3 Making REX Mask File

Refer to section 2-2-12-1, “Making REX Mask File” for the procedure to make the REX mask file.

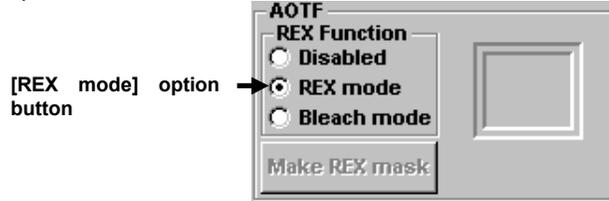
If the setup of the region of laser excitation remains after making the REX mask file, the intensity change of the region can be observed in the TIME COURSE software (optional).

Refer to the “FV-TIEMPO TIME COURSE software User’s manual” for details of the TIME COURSE software.

4 Selecting REX Mask File and Setting the Laser ON/OFF

Set the REX mask file in the REX mode and Bleach mode, and the laser ON/OFF respectively.

1. Select the [REX mode] option button in the [AOTF] group box in the [Lasers] sub-panel.



2. A frame to specify the REX mask file appears on the right side of the [REX mode] option button. Right-click the mouse inside the frame to display the pop-up menu as shown below.

Select the image to be masked in the menu.



In order to use the already saved file as a REX mask file, open the image beforehand.

The opening method of the REX mask file is completely the same as that of an image. For details, refer to section 2-3-2, "Opening Previously Saved Images" in this manual.

3. The icon of the selected REX mask file is displayed inside the frame.



The icon of the selected REX mask file is displayed inside the frame. And the file name and the observation mode are also displayed above and under the frame.

4. Select the [Bleach mode] option button in the [AOTF] group box of the [Laser] sub-panel.



5. A frame to specify the REX mask file appears on the right side of the [Bleach mode] option button. Right-click the mouse inside the frame to display the pop-up menu as shown below.

Select the image to be masked in the menu.



The REX mask file displayed in the [Display] panel is pop-up displayed.



In order to use the already saved file as a REX mask file, open the image beforehand.

The opening method of the REX mask file is completely the same as that of an image. For details, refer to section 2-3-2, "Opening Previously Saved Images" in this manual.

6. The icon of the selected REX mask file is displayed inside the frame.



The icon of the selected REX mask file is displayed inside the frame. And the file name and the observation mode are also displayed above and under the frame.

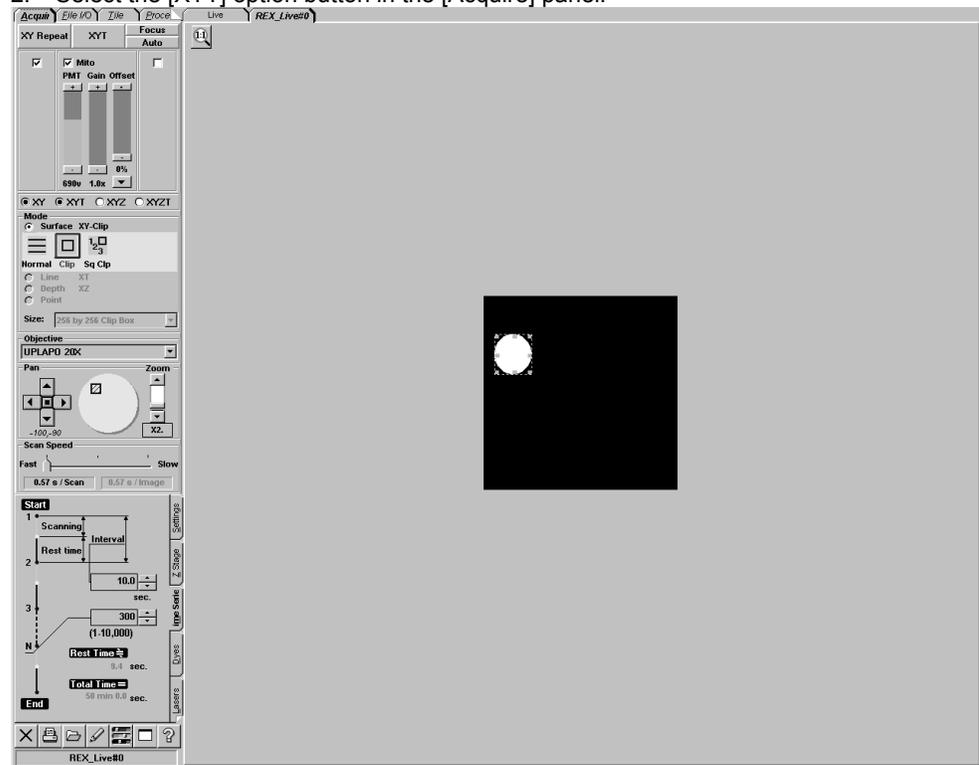
7. Set each laser ON/OFF, and their intensity.

If necessary, use the [Laser Intensity] dialog box in the [Lasers] sub-panel to set the laser ON/OFF.

And the value set in the [Laser Intensity] dialog box can be obtained apart from the value set in the Disable/REX mode.

5 Setting the XYT Observation mode

1. Confirm that [XY-Norm] is displayed in the [Mode] group box in the [Acquire] panel.
2. Select the [XYT] option button in the [Acquire] panel.



- From the page tabs on the bottom right of the [Acquire] panel, select the [Time Series] sub-panel. The panel as shown below appears.

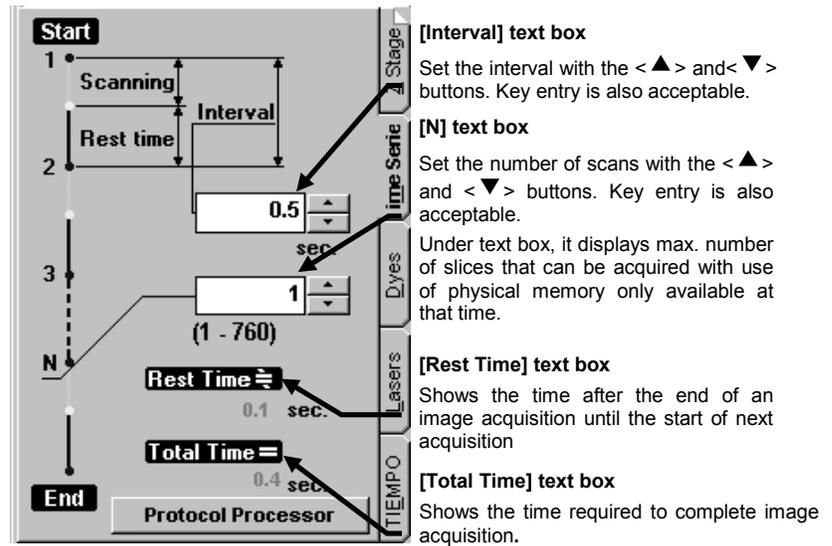


Fig. 2-25 [Time Series] sub-panel

- Set the interval time to "10" using the <▲> and <▼> buttons in the [Interval] text box. Change the interval time corresponding to the specimen.
- Set the number of scans to "30" using the <▲> and <▼> buttons in the [N] text box in the [Time Series] sub-panel. Change the number of scans corresponding to the specimen.
- Confirm that the time for image acquisition is set to 5 minutes (or 300 seconds) in the [Total Time] text box. The time for image acquisition differs according to the interval and the number of scans.

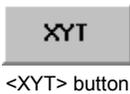
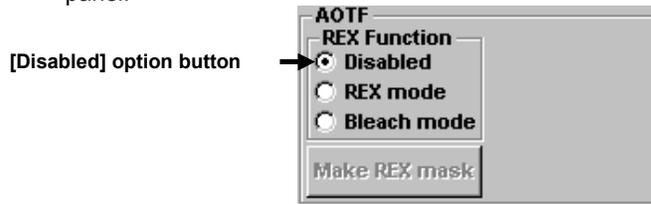
6 Setting the TIME COURSE Software (optional)

Setting of the real time graph is required when you use the TIME COURSE software. Refer to the "FV-TIEMPO TIME COURSE software User's manual" for details of the TIME COURSE software.

7 Acquiring Data

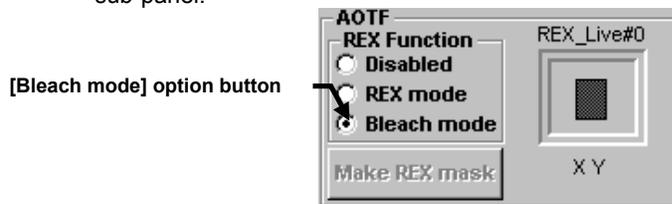
The experiment data before and after photobleaching of certain region can be obtained after performing an ordinary image acquisition in the first scan, an image acquisition in the Bleach mode in the second scan, and an ordinary image acquisition in the third scan. When the REX mode is selected in the third scan, the data of the region other than the specified region can not be obtained since the laser is irradiated only to the specified region. However, the REX mode is effective to prevent fading of the obtained data.

1. Select the [Disabled] option button in the [AOTF] group box in the [Lasers] sub-panel.



2. Click the <XYT> button to start image acquisition.

3. Select the [Bleach mode] option button in the [AOTF] group box in the [Lasers] sub-panel.

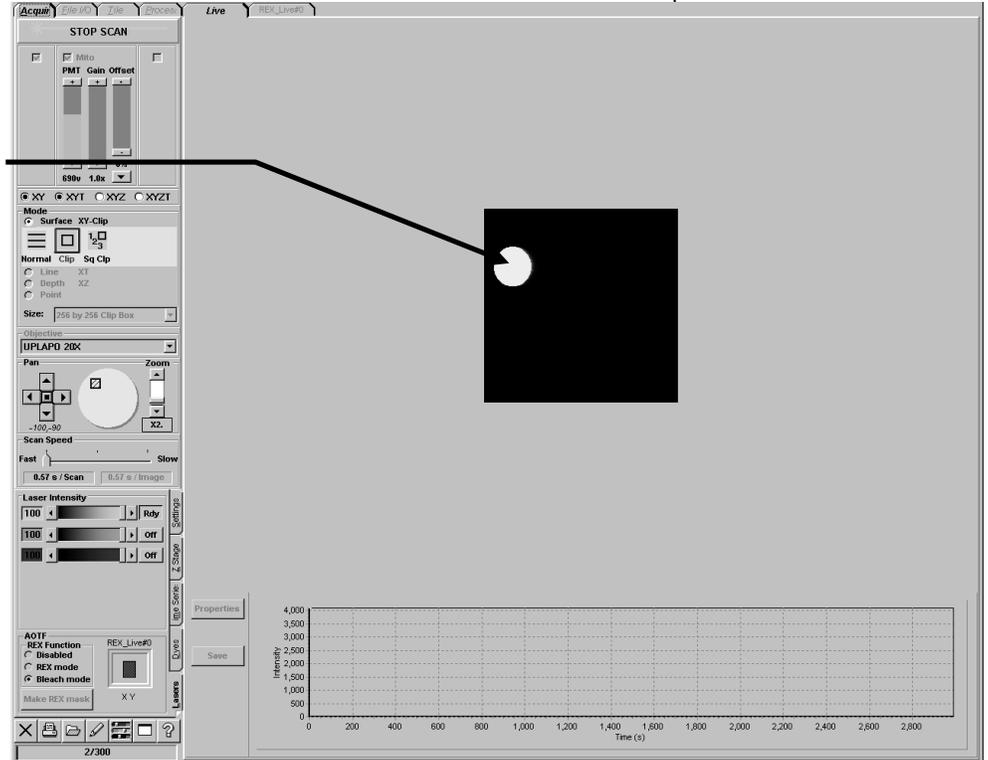


NOTE Select the [Bleach mode] option button within 10 seconds before the next scanning is started.

NOTE In the TIME COURSE software (optional), when the REX mask file in the REX or Bleach mode is changed while image scanning is performed obtaining the real time graph, the laser is irradiated while the pop-up menu is displayed. however, the real time graph is not plotted. Please be aware this before using.

- Start the second scan in 10 seconds after the first scan is performed.

The laser of strong intensity is irradiated to the specified region.



- Select the [Disabled] or [REX mode] option button in the [AOTF] group box in the [Lasers] sub-panel.



(The [Disabled] option button is selected.)



NOTE Select the [Disabled] or [REX mode] option button within 10 seconds before the next scanning is started.

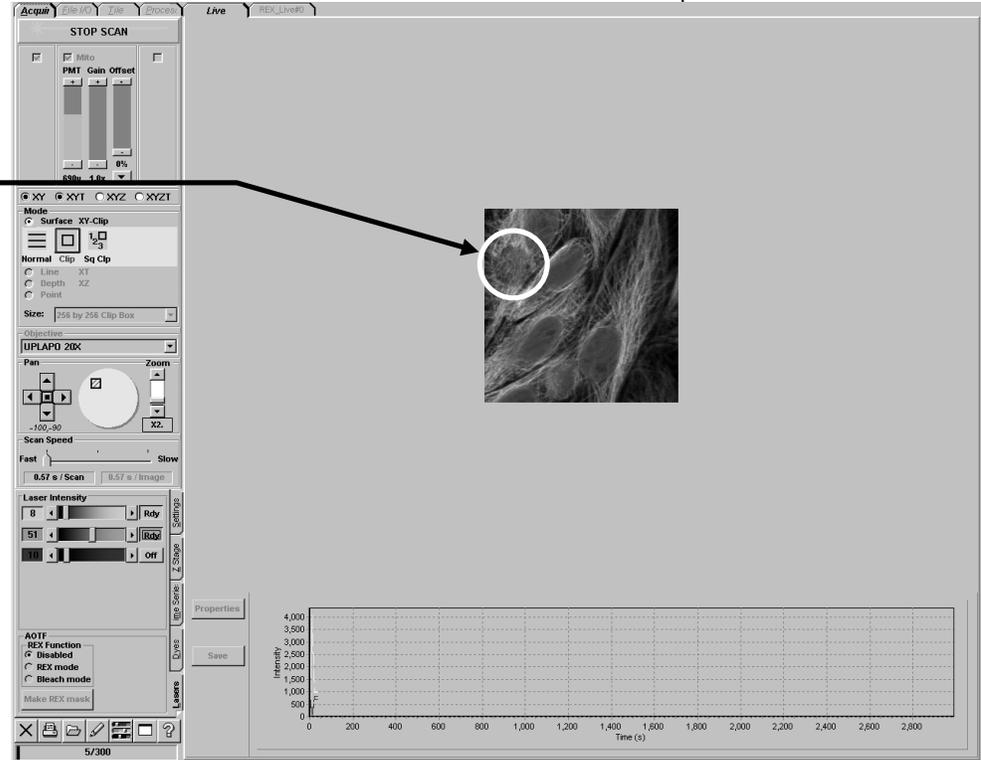


NOTE In the TIME COURSE software (optional), when the REX mask file in the REX or Bleach mode is changed while image scanning is performed obtaining the real time graph, the laser is irradiated while the pop-up menu is displayed, however, the real time graph is not plotted. Please be aware this before using.



6. Start the third scan in 10 seconds after the second scan is performed.

The image of the specimen is fading only where the laser is irradiated.



(The [Disabled] option button is selected.)

7. Start the forth and subsequent scan in 10 seconds after the third scan is performed.

The image of the photobleached specimen is acquired.



When performing the forth or subsequent scan, be sure to switch the [REX mode] and [Bleach mode] between scans.



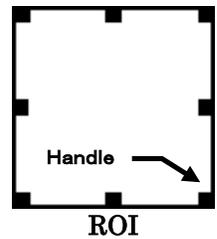
In the TIME COURSE software (optional), when the REX mask file in the REX or Bleach mode is changed while image scanning is performed obtaining the real time graph, the laser is irradiated while the pop-up menu is displayed, however, the real time graph is not plotted. Please be aware this before using.

2-2-13 Notes for image acquisition

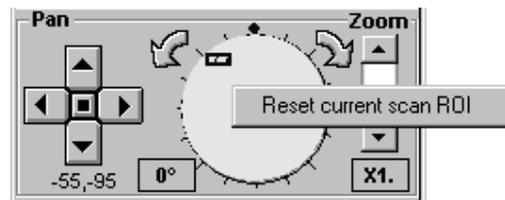
2-2-13-1 Memory of setting information for scanning region

Frame with handle described in 2-2-5 Clip Scan, Frame with hand described in 2-2-6 Zoom-in Scan, Straight line with handle described in 2-2-9, Cross line cursor described in 2-2-10 Point Scan are all called Scanning Region Setting - ROI (Region Of Interest). This Scanning Region Setting – ROI holds the setting information described below when scanning is executed; and the information appears in the same state when each scan mode is selected next time.

- Angle
- Profile
- Position



When default setting information is desired,



click (mouse right) over circle region in [Pan Zoom] group box.

As [Reset current scan ROI] menu appears, select it.

The ROI selected at this time is returned to default setting information.



When size of image acquisition is changed, all ROI setting information would be initialized.

2-2-13-2 Limits for image acquisition

Depending upon the system used, some scanning modes cannot be used. It depends upon kind of I/O board and kind of combiner, etc. Table for combination is shown below.



Observation	Acquiring mode	Kind of I/O board	
		I/O2	I/O3
XY	Normal scan	0	0
	Clip scan	0	0
	Zoom-in scan	0	0
	Fast normal scan	0	0
	Fast clip scan	0	0
	Sequential normal scan	0	0
	Sequential clip scan	0	0
	Sequential zoom-in scan	0	0
	Line sequential normal scan	0*	0*
	Line sequential clip scan	0*	0*
	Line sequential zoom-in scan	X	0*
XYT	Normal scan	0	0
	Clip scan	0	0
	Zoom-in scan	0	0
	Fast normal scan	0	0
	Fast clip scan	0	0
	Sequential normal scan	0	0
	Sequential clip scan	0	0
	Sequential zoom-in scan	0	0
	Line sequential normal scan	0*	0*
	Line sequential clip scan	0*	0*
	Line sequential zoom-in scan	X	0*
XYZ	Normal scan	0	0
	Clip scan	0	0
	Zoom-in scan	0	0
	Sequential normal scan	0	0
	Sequential clip scan	0	0
	Sequential zoom-in scan	0	0
	Line sequential normal scan	0*	0*
	Line sequential clip scan	0*	0*
Line sequential zoom-in scan	X	0*	

0 Image acquisition possible

X Image acquisition not possible

0* Image acquisition is possible when combiner FV5-CMBA is used

Mode	Sub Mode	Kind of I/O board	
		I/O2	I/O3
XYZT	Normal scan	O	O
	Clip scan	O	O
	Zoom-in scan	O	O
	Sequential normal scan	O	O
	Sequential clip scan	O	O
	Sequential zoom-in scan	O	O
	Line sequential normal scan	O*	O*
	Line sequential clip scan	O*	O*
	Line sequential zoom-in scan	X	O*
XT	Normal scan	O	O
	Clip scan	X	O
	Zoom-in scan	O	O
	Free line scan	O	O
	Fast normal scan	O	O
	Fast clip scan	X	O
	Line sequential normal scan	O*	O*
	Line sequential clip scan	X	O*
	Line sequential zoom-in scan	X	O*
XZ	Normal scan	O	O
	Clip scan	X	O
	Zoom-in scan	O	O
	Free line scan	O	O
	Line sequential normal scan	O*	O*
	Line sequential clip scan	X	O*
XZT	Normal scan	O	O
	Clip scan	X	O
	Zoom-in scan	O	O
	Free line scan	O	O
	Line sequential normal scan	O*	O*
	Line sequential clip scan	X	O*
	Line sequential zoom-in scan	X	O*
Pt*T	Normal scan	O	O

O Image acquisition possible

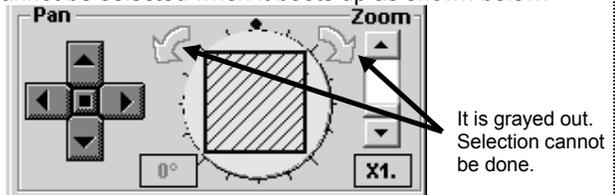
X Image acquisition not possible

O* Image acquisition is possible when combiner FV5-CMBA is used

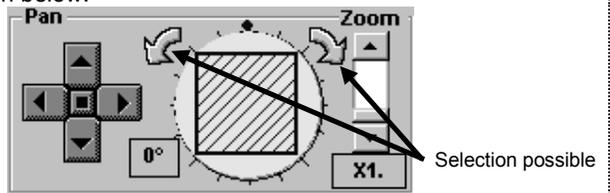


TIP

It is possible to identify which I/O board is used with the following method. In case of I/O2, the arrow of [Pan][Zoom] group box of [Acquire] panel is grayed out and cannot be selected when it boots up as shown below.



In case of I/O3, the arrow of [Pan][Zoom] group box is alive and can be selected as shown below.



2-3 Saving, Opening and Shredding Images

Use the [File I/O] panel to save, open or shred an image.

Display the [File I/O] panel at the front.

[Open]

To open a file, select the desired file in the [Files] list box and drag the file here.

[File Type] drop-down list

Selects the type of the files to be displayed in the [Files] list box.

[Files] list box

Lists the image files of the type selected with the [File Type] drop-down list and their

[Directory] list box

Shows the hierarchical list of disks and directories. Double-click the required disk and directory. If the required directory is not displayed, double-click the hierarchical level above the current level.

<Experiment> button

Click to open the selected image file.

[Display] panel

Displays the image. The image file name is shown in the panel page tab.

<List> and <Details> buttons

These buttons switch the [Files] list box display format.

Clicking the <List> button displays only the file names. Clicking the <Details> button displays the file names together with the information (number of channels, comment, etc.) of each file.

<Up> button

Click to move to the directory above the current directory in the [Files] list box.

<Experiment> button

Saves the image displayed in the [Display] panel together with its file name.

<Display> button

Saves the image displayed in the [Display panel] as if a hardcopied image.

<Animation> button

Saves the animation image displayed in the [Display panel].

<Auto Numbering> check box

Enables automatic image file name setting when this box is checked.

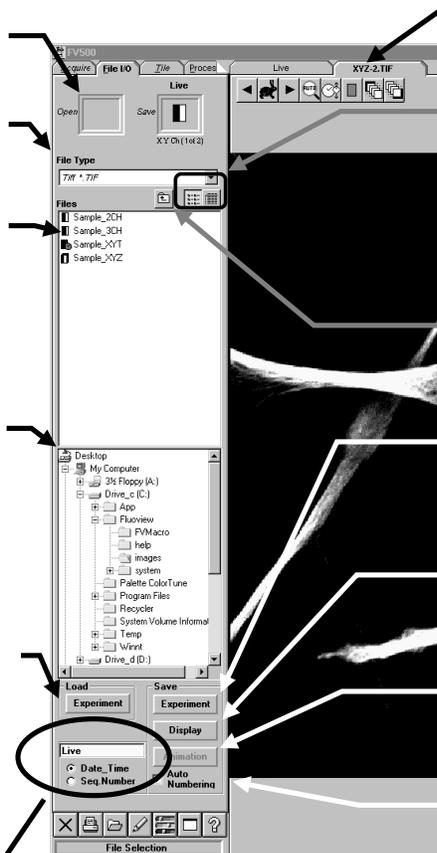


Fig. 2-26 [File I/O] Panel



< File Name Header> text box

Enter the prefix for automatic image file

< Date_Time> option button

Date and time is added with the image file name for auto numbering.

< Seq. Number> option button

Sequential number is added with the image file name for auto numbering.



<Image Icons>

Images are represented by icons which can also identify the observation modes used when acquiring them.

The icon of the selected image (image in the [Display] panel) is displayed in the frame at the top of the function panel such as the [File I/O] panel.

In the [File I/O] panel, image icons are also displayed in the [Icon] field in the [Files] list box.

TIP In all observation modes, the icons for 3 or more channels are identical.

Image Icon	Significance
	XZ observation
	XZ observation, 2-channel mode
	Xt observation
	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
	Point Scan
	Animation image
	Stereo 3D image: Image to be viewed with color eyeglasses.
	3 or more channels



<[Files] List Box>



<Details> button



<List> button

The information displayed in the list box can be changed.

To display all information, click the <Details> button above the [Files] list box to broaden it.

To return to the display of icons and file names only, click the <List> button.

Icon:

Icons showing the image observation modes.

Name:

Image file names.

X:

Image sizes in the X-direction.

Y:

Image sizes in the Y-direction.

T:

Number of images acquired over time.

Name	X	Y	Z	T	Ch	An	St	Size	Date	Bits	User Comments
Sample_2CH	800	600	1	1	2	1	1	1.853 MB	Fri Sep 14 16:04:40 2001	12 bit	
Sample_3CH	512	512	1	1	3	1	1	1.529 MB	Fri Sep 14 16:04:42 2001	12 bit	
Sample_XYT	256	256	1	15	1	1	1	1.917 MB	Fri Sep 14 16:04:46 2001	12 bit	
Sample_XYZ	214	247	16	1	2	1	1	3.309 MB	Fri Sep 14 16:04:52 2001	12 bit	
SAMPLE_XYZ2	214	247	16	1	2	1	1	3.309 MB	Tue Dec 11 18:52:57 2001	12 bit	OLYMPUS FLUOVUEW

Ch:

Number of image channels.

An:

Number of images created when constructing a stereo image using the Visualize function.

Stx

"2" is displayed when stereo images (stereo 3D image, or stereo image to be viewed through color eyeglasses) are constructed and saved using the Visualize function.

Size:

Image file sizes.

Date:

Image saving dates.

Bits:

Shows the number of image pixels.

User Comment:

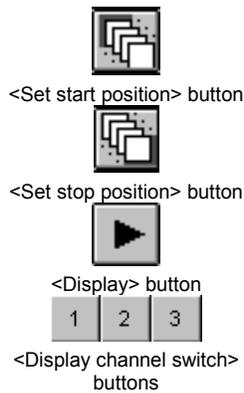
Part of the comment saved with each image.

2-3-1 Saving Images

Either a series of images or single image being displayed can be saved.

2-3-1-1 Saving Images As a Series

The displayed images can be saved in a disk as a series image file.



1. Display the [Display] panel showing the image to be saved at the front.
2. When there is more than one image to be saved, the <Set start position> and <Set end position> buttons will be displayed on the upper part of the image. Display the image with which the saving should start using the <Display> buttons, then click the <Set start position> button.

Also set the save end position in the similar way.

3. When the images to be saved are acquired from more than one channel, select whether saving images from more than one channel simultaneously or only a single image from a single channel.

Use the <Display channel switch> buttons for this selection. The images will be saved according to the conditions of the selected channels.

Example) When only the image of Channel 1 is displayed, only the image of Channel 1 will be saved.

TIP

For the channel switching, see section 2-5-3, "Switching the Displayed Channels".

4. Click the <Experiment> button in the [Save] group box. The [Save Experiment As] dialog box appears as shown below.

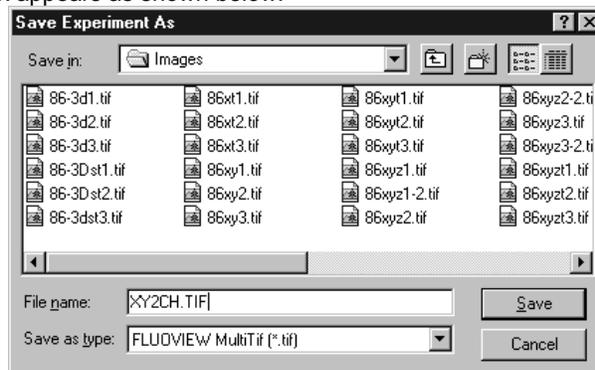


Fig. 2-27 [Save Experiment As] Dialog Box



5. When it is required to change the save destination drive or directory, use the [Save in:] drop-down list.
6. When it is required to change the saved file type, use the [Save as Type:] drop-down list. See section 2-3-1-5, "File Types Available for Save" for details.
7. Enter the file name in the [File Name:] text box.



[Auto Numbering] is enabled image file name appears in [File Name] text box. Then, the image file can store with the numbering just pressing <Save> button.

8. Click the <Save> button.

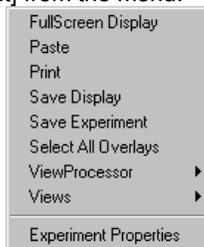


If a file with the same name as the entered file name already exists, a dialog box is displayed to ask if you want to overwrite the existing file. If you do not want to overwrite it, click the <NO> button and enter another file name.

One Point!

The [Save Experiment As] dialog box can also be displayed by a mouse operation.

1. Display the image to be saved at the front of the [Display] panel, and right-click a point in the image.
2. A pop-up menu as shown below is displayed.
3. Select [Save Experiment] from the menu.





One Point!

Live image file is stored to the assigned folder with consecutive numbered file name when [Seq. Number] is selected and [Auto Numbering] is enabled.



NOTE

[Seq. Number] function is enabled when [Save Experiment As] dialog box appears for not stored Live image is existing.

Seq. Numbering function is disabled once after the [Save Experiment As] setting is changed.

2-3-1-2 Saving a Display

The displayed images can be hardcopied and saved in a disk. This method is used when it is required to use a FLUOVIEW image in another application.

1. Display the [Display] panel showing the image to be saved at the front.
2. When there is more than one image to be saved, display the image to be saved using the <Display> buttons.
3. Click the <Display> button in the [Save] group box. The [Save Display] dialog box appears as shown below.



Selects [All Views] when you want to save Tiled Image.

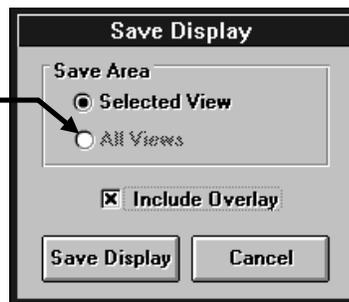


Fig. 2-28 [Save Display] Dialog Box

4. When it is required to save the comment drawn on the image together with the image, check the [Include Overlay] check box.
5. Click the < Display> button in the [Save] group box. The [Save Experiment As] dialog box appears as shown below.

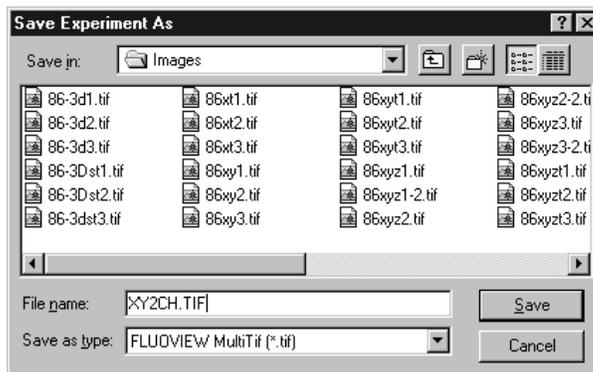


Fig. 2-29 [Save Experiment As] Dialog Box



6. When it is required to change the save destination drive or directory, use the [Save in:] drop-down list.
7. When it is required to change the saved file type, use the [Save as Type:] drop-down list. See section 2-3-1-5, "File Types Available for Save" for details.
8. Enter the file name in the [File Name:] text box.
9. Click the <Save> button.

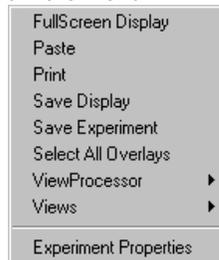


NOTE If a file with the same name as the entered file name already exists, a dialog box is displayed to ask if you want to overwrite the existing file. If you do not want to overwrite it, click the <NO> button and enter another file name.

One Point!

The [Save Display] dialog box can also be displayed by a mouse operation.

1. Display the image to be saved at the front of the [Display] panel, and right-click a point in the image.
2. A pop-up menu as shown below is displayed.
3. Select [Save Display] from the menu.



2-3-1-3 Saving Specified Area of Image

Only the specified part of an image can be saved as an image in a disk.

1. Display the [Display] panel of the image containing the part to be saved in the front.
2. When the image was acquired through multiple channels, you can select whether the image slices for multiple channels are saved together or the image slice of only 1 channel is saved.

Use the <display switching> button for the selection. The image will be saved in the channel condition as displayed.

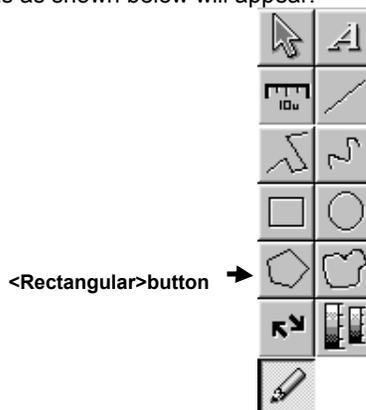
TIP For the channel switching, see section 2-5-3, "Switching the Displayed Channels".



<Annotate> button

3. Specify a desired area in the image. This operation is not necessary if an area has already been specified.

Click the <Annotate> button in the toolbar at the bottom of the [Acquire] panel. A list of buttons as shown below will appear.



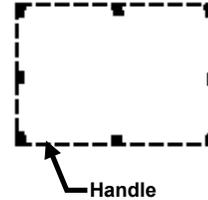
4. Click the <Rectangular> button in this displayed buttons.



- Specify the area to be saved in the image in the [Display] panel.

The area is displayed on the image with the handles on its frame.

The area becomes the save target while these handles are displayed.



- Click the <Annotate> button so that the list of buttons disappear.

- Click the <Experiment> button in the [Save] group box.

The [Save Experiment As] dialog box appears.

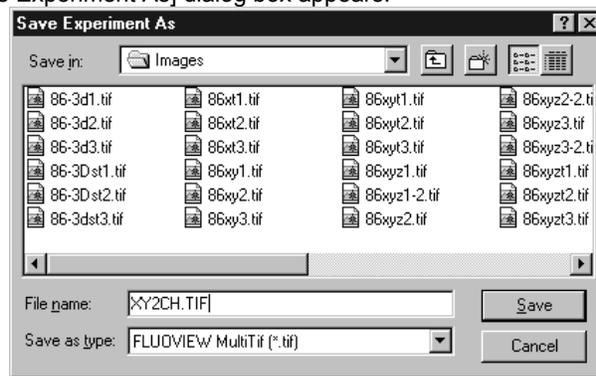


Fig. 2-30 [Save Experiment As] Dialog Box

- Use the [Save In:] drop-down list if you want to change the save destination drive and directory.

- Select "FLUOVIEW Multi Tiff" in the [Save as Type:] drop-down list.



It is not possible to save only the specified area of image in the Single TIF, BMP or microVoxel format.

- Enter the file name in the [File Name:] text box.

- Click the <Save> button.

12. A dialog box as shown below appears.

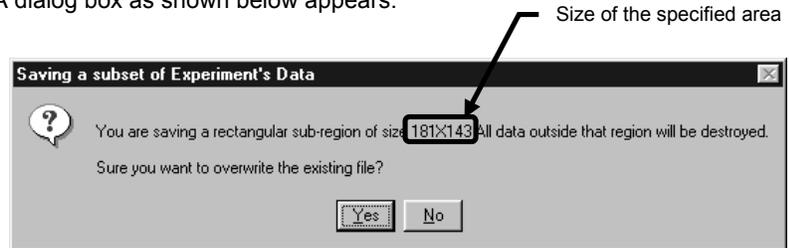


Fig. 2-31 [Saving a subset of Experiment's Data]Dialog Box



The [Saving a subset of Experiment's Data] dialog box does not appear if no area is specified in the image on the [Display] panel.

13. Click the <Yes> button.

2-3-1-4 Saving Animation images

Transform a created animation image into the AVI file format and save it in the file type which can display the animation image without this software.

1. Create an animation image.
Refer to “2-9-2 Animation” for details.
2. Display the [Display] panel of the created animation image at the front.

TIP

The AVI file is created to save the animation at the display speed mode selected here. For varying the animation display speed, see section 2-9-1-1, “Changing the Successive Display Speed”.

One Point !

When saving image slices acquired in Z observation, it is recommended to lower the animation display mode by clicking the <Turtle> button. (If image slices are saved with the <Rabbit> button selected, the display speed of the animation speed would become too high.)

To change the animation image display speed, see section 2-9-1-1, “Changing the successive display speed”.

3. Select whether saving images from multiple channels simultaneously or only an image from a channel if the images to be saved are acquired from multiple channels.
Use the <Display channel switch> buttons for this selection. The image is saved according to the condition of the selected channel.

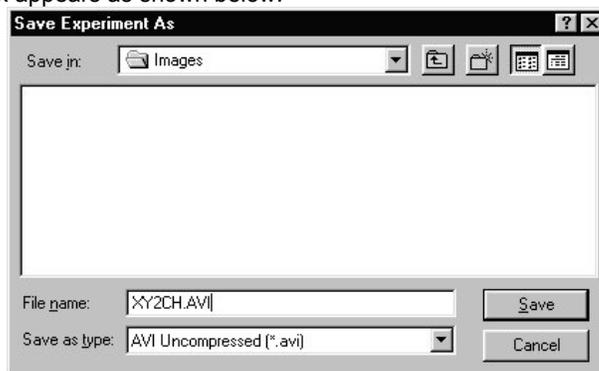
TIP

For the channel switching, see section 2-5-3, “Switching the Displayed Channels”.

Animation

<Save Animation> button

- Click the <Animation> button in the [Save] group box. The [Save Experiment As] dialog box appears as shown below.

**Fig. 2-32 [Save Experiment As] Dialog Box**

- When it is required to change the save destination drive or directory, use the [Save in:] drop-down list.
- When it is required to change the saved file type, use the [Save as Type:] drop-down list.

TIP

Two saved file types can be selected.

AVI Compressed: Saves the AVI file being compressed.

AVI Uncompressed: Saves the AVI file not being compressed.

- Enter the file name in the [File Name:] text box.
- Click the <Save> button.

NOTE

If a file with the same name as the entered file name already exists, the dialog box appears to ask if you want to overwrite the existing file. If you do not want to do so, click the <NO> button and enter another file name.

2-3-1-5 File Types Available for Save

The file type used for saving image in a file can be selected by the user. See section 2-3-1-1, "Saving Images As a Series" for the operation method.

1. Click the <Experiment> button in the [Save] group box.
2. Select the file type from the [Save as Type:] drop-down list in the [Save Experiment As] dialog box. Three file types are available as detailed below.
 - Fluoview Multi Tiff(*.tif): TIFF format designed for use with FLUOVIEW.
Used for image analysis, processing, etc., on FLUOVIEW.
 - Single TIF(s) 8-bit(*.tif):
 - Single TIF(s) 16-bit(*.tif):
 - Single TIF(s) 24-bit(*.tif):

} TIFF (Tagged-Image File Format) is used for image exchange between applications or computers.
(Three types including the 8-bit, 16-bit and 24-bit types are available.)

 - Bitmap 8-bit(*.bmp):
 - Bitmap 24-bit(*.bmp):

} The BMP format is the standard raster format of MS-Windows.
(Two types including the 8-bit and 24-bit types are available.)

- mVx Files (*.img, *.ani): File format for microVoxel.
Used for exchanging images with microVoxel.

TIP In the file types above, characters inside () indicate the extension when a file is saved.

NOTE When saving a display, the file format can be selected from Single TIF(s) 8-bit (*.tif), Single TIF(s) 16-bit (.tif), Single TIF(s) 24-bit (*.tif), Bitmap 8-bit (*.bmp) and Bitmap 24-bit (*.bmp).

NOTE When saving an image obtained by merging more than one channel, use a 24-bit file type.



If you want to save the image with the comment drawn on it as an image, select the file type according to the usage of the image as described below.

Fluoview Multi Tiff: Select when the image will be analyzed or processed after save. Opening the image also opens the comment, which can be changed or measured as required.

Single TIF(s): Select when the image will be handed to another application.

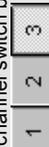
The following table shows the list of image save status by taking the below-mentioned acquisition parameters as an example.

Observation mode:	XYZ observation
Number of channels:	3 channels
Number of acquired images:	4 slices
Save file name:	abcdefgh

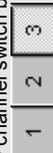
APPLIED OPERATIONS/Saving, Opening and Shredding Images

	<p>Saving a channel under displayed condition (Example: When only Ch3 is displayed)</p> <p>Display channel switch buttons</p> 	<p>Saving merged images (Example: When Ch1, Ch2 and Ch3 are displayed)</p> <p>Display channel switch buttons</p> 	<p>Saving side-by-side Over and Under images</p> <p>Display channel switch buttons</p> 	<p>Saving extended image</p> <p>Z/T series switch button</p> 	<p>Comment drawn on images</p>
<p>Fluoview Multi Tiff</p>	<p>The image of only Ch3 is saved. Save file name: abcdefgh.tif</p>	<p>The images of Ch1, Ch2 and Ch3 are saved. Save file name: abcdefgh.tif</p>	<p>The images of Ch1, Ch2 and Ch3 are saved. Save file name: abcdefgh.tif</p>	<p>The image is saved in the selected channel condition. Note that a file does not always contain the corresponding number of channels. (See "Saving a channel under displayed condition", "Side-by-side images" and "Merged images" on the left.) The image is saved in the original, non-extended status.</p>	<p>To be saved.</p>

APPLIED OPERATIONS/Saving, Opening and Shredding Images

	Saving a channel under displayed condition (Example: When only Ch3 is displayed) Display channel switch buttons 	Saving merged images (Example: When Ch1, Ch2 and Ch3 are displayed) Display channel switch buttons 	Saving side-by-side Over and Under images Display channel switch buttons  or  images	Saving extended image Z/T series switch button 	Comment drawn on images
Single TIF(s) 8-bit	12 images obtained by merging Ch1, Ch2 and Ch3 images are saved. Save file names: abcdefgh0.tif (Ch1: No. 0) abcdefgh3.tif (Ch1: No. 3) abcdefgh4.tif (Ch2: No. 0) abcdefgh7.tif (Ch2: No. 3) abcdefgh8.tif (Ch3: No. 0) abcdefgh11.tif (Ch3: No. 3)	4 images are saved only from Ch3. Save file names: abcdefgh0.tif (Ch3: No. 0) abcdefgh3.tif (Ch3: No. 3)	12 images are saved from each of Ch1, Ch2 and Ch3. Save file names: abcdefgh0.tif (Ch1: No. 0) abcdefgh3.tif (Ch1: No. 3) abcdefgh4.tif (Ch2: No. 0) abcdefgh7.tif (Ch2: No. 3) abcdefgh8.tif (Ch3: No. 0) abcdefgh11.tif (Ch3: No. 3)	The image is saved in the selected channel condition. Note that a file does not always contain the corresponding number of channels. (See "Saving a channel under displayed condition", "Side-by-side images" and "Merged images" on the left.) The image is saved in the original, non-extended status.	Not to be saved.

APPLIED OPERATIONS/Saving, Opening and Shredding Images

	Saving a channel under displayed condition (Example: When only Ch3 is displayed) Display channel switch buttons 	Saving merged images (Example: When Ch1, Ch2 and Ch3 are displayed) Display channel switch buttons 	Saving side-by-side Over and Under images Display channel switch buttons 	Saving extended image Z/T series switch button 	Comment drawn on images
Single TIF(s)	16-bit images obtained by merging Ch1, Ch2 and Ch3 images are saved. Save file names: abcdefgh0.tif (Ch1: No. 0) abcdefgh3.tif (Ch1: No. 3) abcdefgh4.tif (Ch2: No. 0) abcdefgh7.tif (Ch2: No. 3) abcdefgh8.tif (Ch3: No. 0) abcdefgh11.tif (Ch3: No. 3)	4 images are saved only from Ch3. Save file names: abcdefgh0.tif (Ch3: No. 0) abcdefgh3.tif (Ch3: No. 3)	12 images are saved from each of Ch1, Ch2 and Ch3. Save file names: abcdefgh0.tif (Ch1: No. 0) abcdefgh3.tif (Ch1: No. 3) abcdefgh4.tif (Ch2: No. 0) abcdefgh7.tif (Ch2: No. 3) abcdefgh8.tif (Ch3: No. 0) abcdefgh11.tif (Ch3: No. 3)	The image is saved in the selected channel condition. Note that a file does not always contain the corresponding number of channels. (See "Saving a channel under displayed condition", "Side-by-side images" and "Merged images" on the left.) The image is saved in the original, non-extended status.	Not to be saved.



NOTE All single TIF(s) 16-bit (*.tif) data is saved as gray scale images.

APPLIED OPERATIONS/Saving, Opening and Shredding Images

	Saving a channel under displayed condition (Example: When only Ch3 is displayed) Display channel switch buttons 	Saving merged images (Example: When Ch1, Ch2 and Ch3 are displayed) Display channel switch buttons 	Saving side-by-side Over and Under images Display channel switch buttons 	Saving extended image Z/T series switch button 	Comment drawn on images
Single TIF(s)	24-bit 4 images obtained by merging Ch1, Ch2 and Ch3 images are saved. Save file names: abcdefgh0.tif (Ch1 + Ch2 + Ch3: No. 0) abcdefgh3.tif (Ch1 + Ch2 + Ch3: No. 3)	4 images are saved from each of Ch1, Ch2 and Ch3. Save file names: abcdefghV0.tif (Ch1: No. 0) abcdefghV2.tif (Ch3: No. 0) abcdefghV3.tif (Ch1: No. 1) abcdefghV5.tif (Ch3: No. 1) abcdefghV6.tif (Ch1: No. 2) abcdefghV8.tif (Ch3: No. 2) abcdefghV9.tif (Ch1: No. 3) abcdefghV11.tif (Ch3: No. 3)	4 images are saved only from Ch3. Save file names: abcdefgh0V0.tif (Ch1: No. 0) abcdefgh0V3.tif (Ch1: No. 1) abcdefgh0V6.tif (Ch1: No. 2) abcdefgh0V9.tif (Ch1: No. 3) abcdefgh1V1.tif (Ch2: No. 0) abcdefgh1V4.tif (Ch2: No. 1) abcdefgh1V7.tif (Ch2: No. 2) abcdefgh1V11.tif (Ch2: No. 3) abcdefgh2V2.tif (Ch3: No. 0) abcdefgh2V5.tif (Ch3: No. 1) abcdefgh2V8.tif (Ch3: No. 2) abcdefgh2V11.tif (Ch3: No. 3)	The relationship between the saved channels and files are variable depending on the condition of the displayed channels. (See "Saving a channel under displayed condition", "Side-by-side images" and "Merged images" on the left.) File No. 0 stores image No. 0, file No. 1 stores the image obtained y accumulating images Nos. 0 and 1,..... The image in the last file is the image obtained by accumulating all of the images. Save file names: abcdefg0.tif (Image No. 0) abcdefg3.tif (Image Nos. 0 + 1 + 2 + 3)	To be saved.

APPLIED OPERATIONS/Saving, Opening and Shredding Images

	Saving a channel under displayed condition (Example: When only Ch3 is displayed) Display channel switch buttons 	Saving merged images (Example: When Ch1, Ch2 and Ch3 are displayed) Display channel switch buttons 	Saving side-by-side Over and Under images Display channel switch buttons 	Saving extended image Z/T series switch button 	Comment drawn on images
Bitmap	8-bit 12 images obtained by merging Ch1, Ch2 and Ch3 images are saved. Save file names: abcdefgh0.bmp (Ch1: No. 0) abcdefgh3.bmp (Ch1: No. 3) abcdefgh4.bmp (Ch2: No. 0) abcdefgh7.bmp (Ch2: No. 3) abcdefgh8.bmp (Ch3: No. 0) abcdefgh11.bmp (Ch3: No. 3)	12 images are saved from each of Ch1, Ch2 and Ch3. Save file names: abcdefgh0.bmp (Ch1: No. 0) abcdefgh3.bmp (Ch1: No. 3) abcdefgh4.bmp (Ch2: No. 0) abcdefgh7.bmp (Ch2: No. 3) abcdefgh8.bmp (Ch3: No. 0) abcdefgh11.bmp (Ch3: No. 3)	4 images are saved only from Ch3. Save file names: abcdefgh0.bmp (Ch3: No. 0) abcdefgh3.bmp (Ch3: No. 3)	The image is saved in the selected channel condition. Note that a file does not always contain the corresponding number of channels. (See "Saving a channel under displayed condition", "Side-by-side images" and "Merged images" on the left.) The image is saved in the original, non-extended status.	Not to be saved.

APPLIED OPERATIONS/Saving, Opening and Shredding Images

	Saving a channel under displayed condition (Example: When only Ch3 is displayed) Display channel switch buttons 	Saving merged images (Example: When Ch1, Ch2 and Ch3 are displayed) Display channel switch buttons 	Saving side-by-side Over and Under images Display channel switch buttons 	Saving extended image Z/T series switch button 	Comment drawn on images
Bitmap	<p>24-bit</p> <p>4 images obtained by merging Ch1, Ch2 and Ch3 images are saved. Save file names: abcdefgh0.bmp (Ch1 + Ch2 + Ch3: No. 0) abcdefgh3.bmp (Ch1 + Ch2 + Ch3: No. 3)</p>	<p>4 images are saved from each of Ch1, Ch2 and Ch3. Save file names: abcdefghV0.bmp (Ch1: No. 0) abcdefghV2.bmp (Ch3: No. 0) abcdefghV3.bmp (Ch1: No. 1) abcdefghV5.bmp (Ch3: No. 1) abcdefghV6.bmp (Ch1: No. 2) abcdefghV8.bmp (Ch3: No. 2) abcdefghV9.bmp (Ch1: No.3) abcdefghV11.bmp (Ch3: No.3)</p>	<p>4 images are saved only from Ch3. Save file names: abcdefgh0V0.bmp (Ch1; No. 0) abcdefgh0V3.bmp (Ch1; No. 1) abcdefgh0V6.bmp (Ch1; No. 2) abcdefgh0V9.bmp (Ch1; No. 3) abcdefgh1V1.bmp (Ch2; No.0) abcdefgh1V4.bmp (Ch2; No.1) abcdefgh1V7.bmp (Ch2; No. 2) abcdefgh1V11.bmp (Ch2; No.3) abcdefgh2V2.bmp (Ch3; No. 0) abcdefgh2V5.bmp (Ch3; No. 1) abcdefgh2V8.bmp (Ch3; No. 2) abcdefgh2V11.bmp (Ch3; No. 3)</p>	<p>The relationship between the saved channels and files are variable depending on the condition of the displayed channels. (See "Saving a channel under displayed condition", "Side-by-side images" and "Merged images" on the left.) File No. 0 stores image No. 0, file No. 1 stores the image obtained by accumulating images Nos. 0 and 1,..... The image in the last file is the image obtained by accumulating all of the images. Save file names: abcdefgh0.bmp (Image No. 0) abcdefgh3.bmp (Image Nos. 0 + 1 + 2 + 3)</p>	To be saved.

2-3-2 Opening Previously Saved Images

Image files saved in the disk can be opened as follows.

1. If the image file name that you want to open is not displayed in the [Files] list box, change the drive and/or directory to those containing the desired file using the [Drive] drop-down list and/or [Directory] list box.
2. From the [File Type] drop-down list, select the file type of the files to be listed in the [Files] list box.
3. Click the <Experiment> button in the [Load] group box.

TIP

Other methods are also available for opening a file:

Perform the same operations as steps 1 and 2 above before the following.

- Place the mouse pointer on the desired image file name in the [Files] list box and drag the file name to the [Open] frame on the upper part of the [File I/O] panel.
(The mouse pointer transforms to an image icon during dragging.)
- Place the mouse pointer on the desired image file name in the [Files] list box and double-click it.

2-3-3 Shredding Images

Shredding an image refers to removing it from the objects of processing including display. **Shredding does not actually deletes the image saved in the disk.**



<Experiment List> button

1. Click the <Experiment List> button in the toolbar at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box appears as shown below.



Fig. 2-33 [Experiments in Memory] Dialog Box



<Shredder> button

2. In the [Experiments in Memory] dialog box, select the file name of the image to be shredded and click the <Shredder> button.

The file can also be shredded by placing the mouse pointer on it and dragging it to the <Shredder> button.



The mouse pointer transforms to an image icon during dragging.

3. Click the <Done> button in the [Experiments in Memory] dialog box to close it.



TIP

Multiple Images displayed can be shredded simultaneously.

1. Display the [Experiments in Memory] dialog box while showing multiple images in the [Display] panel.
2. Pressing down the Shift key, select multiple image files to be shredded.
3. Click the <Shredder> button.



<Shredder> button



4. Click the <Done> button in the [Experiments in Memory] dialog box to close.

2-3-4 Saving Comment Together with Image



<Experiment List> button

1. Click the <Experiment List> button in the toolbar at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box appears as shown below.



Fig. 2-34 [Experiments in Memory] Dialog Box

2. In the [Experiments in Memory] dialog box, select the file name of the image to be saved with comment and click the <Comments> button. The [Image Comments] dialog box appears as shown below.

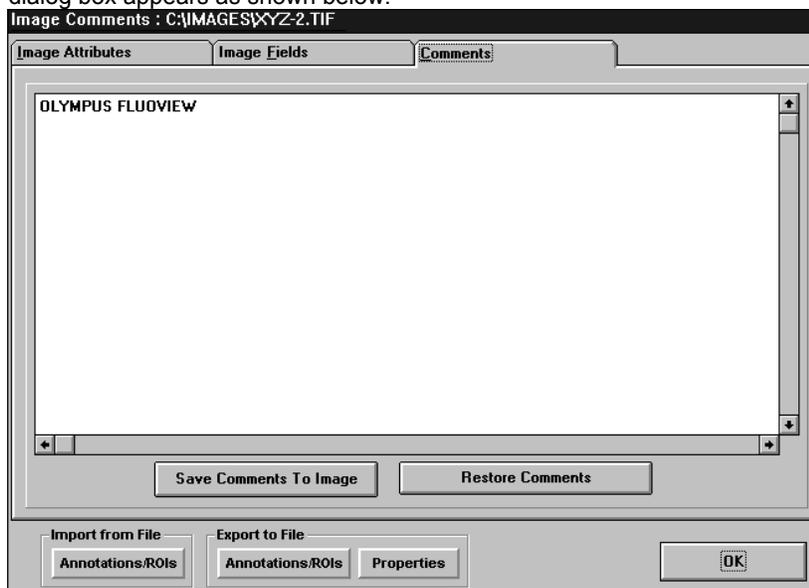


Fig. 2-35 [Image Comments] Dialog Box

3. Display the [Comments] panel at the front.



4. Enter comment from the keyboard.



NOTE

During modification of previously entered comment, if it is required to restore the originally entered comment, click the <Restore Comments> button. However, once the <Save Comments To Image> button is pressed, the original comment cannot be restored.

5. Click the <Save Comments To Image> button.



NOTE

At the moment the <Save Comments To Image> button is clicked, the comment in the [Comments] panel is saved simply, but it is not yet saved in the image file. To save the comment in the image file, use the <Save> button in the [File I/O] panel.

6. Click the <OK> button.
7. Click the <Done> button in the [Experiments in Memory] dialog box to close it.
8. Display the [File I/O] panel at the front.
9. Click the <Experiment> button in the [Save] group box.



TIP

See section 2-3-1, "Saving Images" for details.

One Point!

The [Image Comments] dialog box can also be displayed by a mouse operation.

1. Display the image to be saved at the front of the [Display] panel, and right-click a point in the image.
2. A pop-up menu as shown below is displayed.
3. Select [Experiment Properties] from the menu.



2-3-5 Checking the Image Information/Acquisition Parameters



<Experiment List> button

1. Click the <Experiment List> button in the toolbar at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box appears as shown below.



Fig. 2-36 [Experiments in Memory] Dialog Box

2. In the [Experiments in Memory] dialog box, select the file name of the image to check the image information and acquisition parameters and click the <Comments> button.
3. Display the [Image Attributes] panel at the front. The panel as shown below appears, where the information on the selected image is shown.

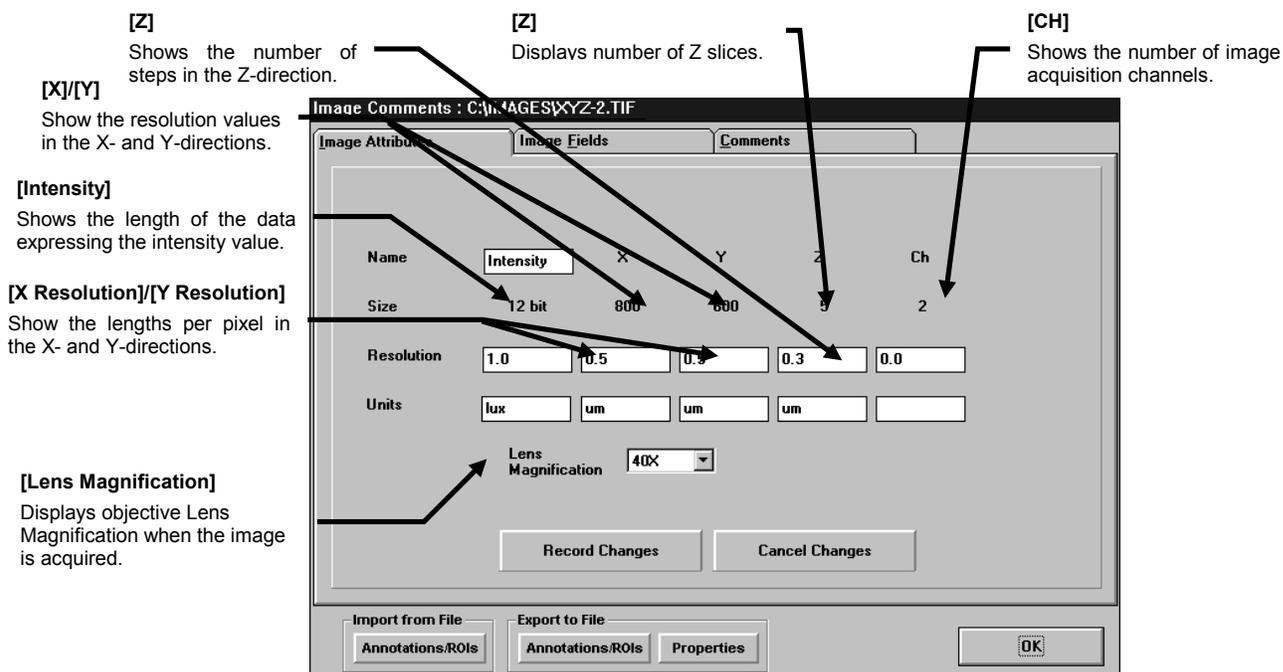


Fig. 2-37 [Image Attributes] Panel



The [X Size], [Y Size] and [Z Size] text boxes can also be used to change the lengths per image pixel or the number of steps in the Z-direction. These values are used in the scale display and many other analysis operations. When opening and analyzing an image file created with another application on FLUOVIEW, enter the lengths per image pixel and the number of steps in the Z-direction if these values are known, then click the <Set Attributes> button.



During modification of the lengths per image pixel, the number of steps in the Z-direction or the objective setting, if it is required to restore the previous setting, click the <Restore Attributes> button. However, once the <Set Attributes> button is pressed, the original setting cannot be restored.



The objective setting can be changed even after having acquired the image. If the objective is not set with the [Acquire] panel before image acquisition, the analysis and measurement results of the image may be erroneous. In such a case, set the objective again.



When the objective setting is changed, the lengths per image pixel will be re-calculated automatically and changed.

4. Display the [Image Fields] panel at the front. The panel as shown below appears, where the information on the selected image is shown.

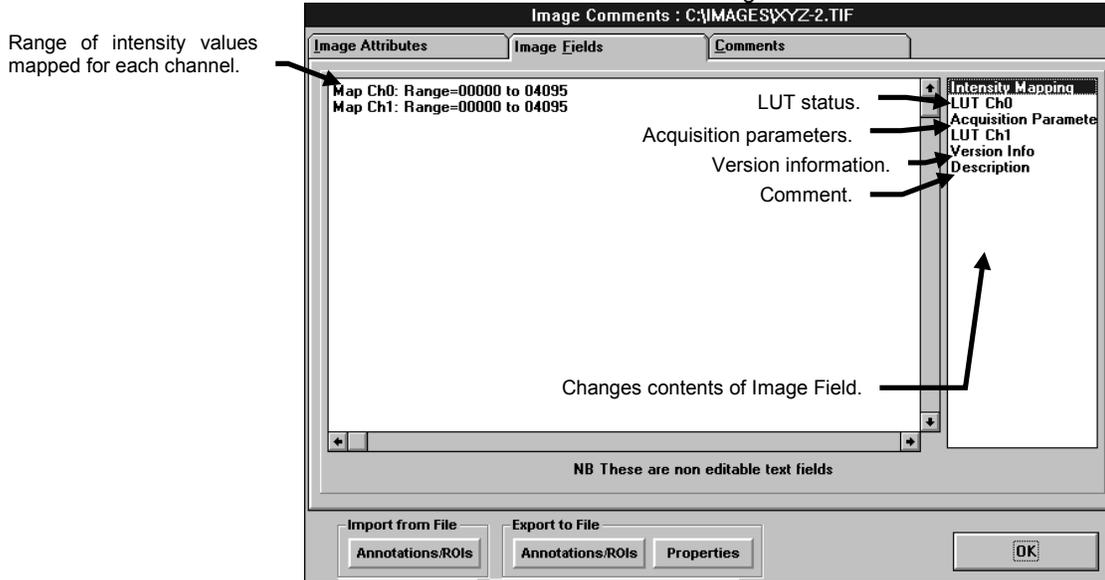
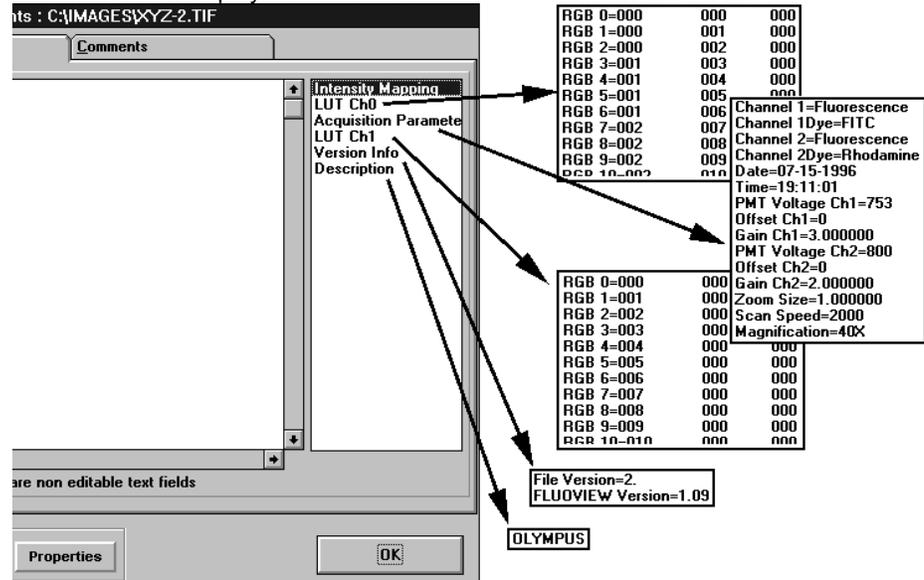


Fig. 2-38 [Image Fields] Panel

5. Select the information items to be checked from the list box on the right. The kind of information displayed when each item is selected is shown below.



6. After checking the information, click the <OK> button.
7. Click the <Done> button in the [Experiments in Memory] dialog box to close it.



One Point!

The [Image Comments] dialog box can also be displayed by a mouse operation.

1. Display the image to be saved at the front of the [Display] panel, and right-click a point in the image.
2. A pop-up menu as shown below is displayed.
3. Select [Experiment Properties] from the menu.



2-3-6 Saving the Image Information/Observation Condition

The image information and observation condition can be saved as ASCII text file in a disk.

1. Display the [Display] panel of the image to be saved at the front position.
2. Select the <Experiment List> button in the tool bar displayed at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box as shown below appears.



Fig. 2-39 [Experiments in Memory] dialog box

- In the list in the [Experiments in Memory] dialog box, select the file name including the image whose image information and observation condition are to be saved. And click the <Comments> button. The [Image Comments] dialog box as shown below appears.

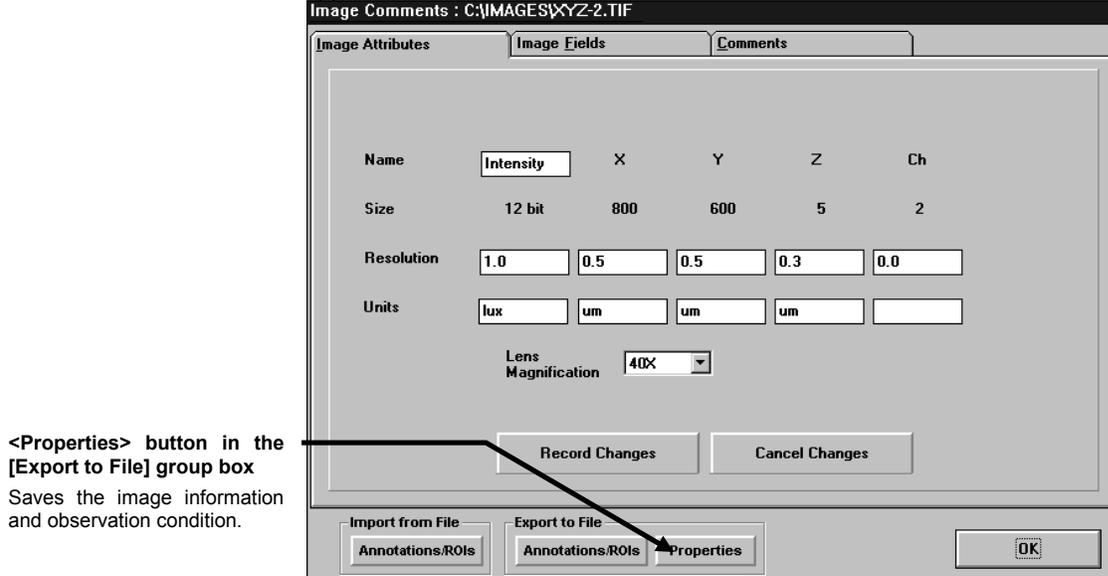


Fig. 2-40 [Image Comments] dialog box

- Select the <Properties> button in the [Export to File] group box. The [Save to txt] dialog box as shown below appears.

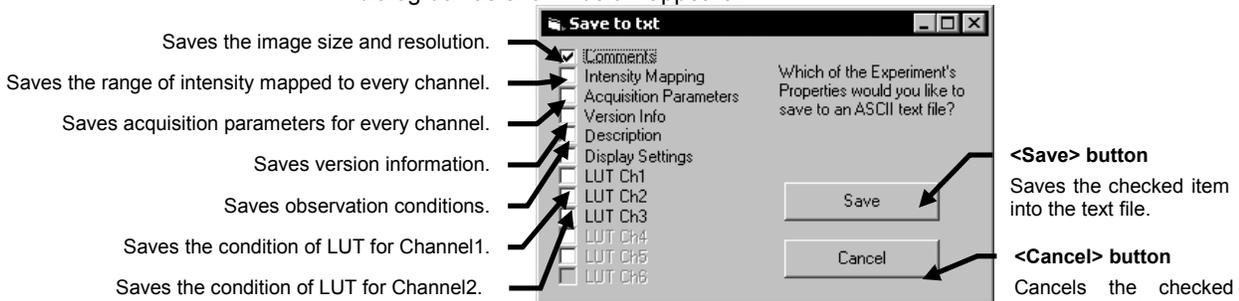


Fig. 2-41 [Save to txt] dialog box

TIP The [LUT Ch1] to [LUT Ch6] check boxes are displayed according to the channel used for image acquisition.

- Check the check box of the item to be saved.

6. Select the <Save> button. The [Save As ASCII Text] dialog box as shown below appears.

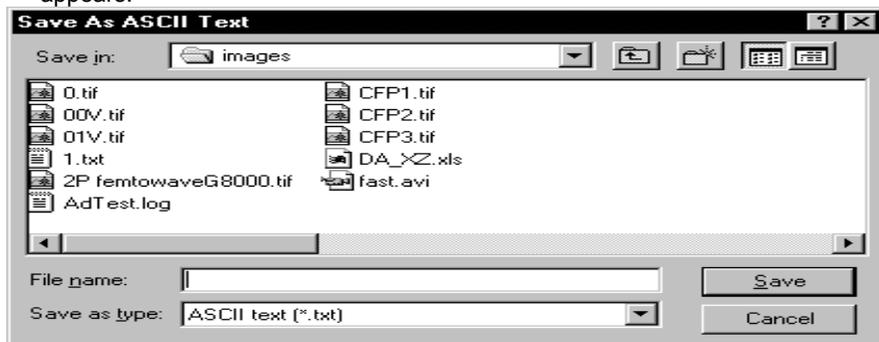


Fig. 2-42 [Save As ASCII Text] dialog box

7. In order to change the save destination drive or directory, use the [Save in:] drop-down list.
8. Confirm that "ASCII text (*.txt)" is selected in the [Save as type:] drop-down list.
9. Enter a file name into the [File Name:] text box.
10. Click the <Save> button.

NOTE

When the text file having the same name as the entered name is already exists, the dialog box appears to ask you to overwrite the existing file or not. When overwriting is not required, click the <NO> button and enter another name to save the file.

TIP

When the image to be saved was acquired through multiple channels, the observation conditions for all channels are saved.

2-3-7 Saving/Reading the Region File

The information on the shape, position, and color of certain region can be saved in a file and they can also be read.

2-3-7-1 Saving the Region File

1. Display the [Display] panel of the image including the region to be saved at the front position.



<Experiment List> button

2. Select the <Experiment List> button in the tool bar at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box as shown below appears.



Fig. 2-43 [Experiments in Memory] dialog box

3. Select the image file name including the region to be saved in the [Experiments in Memory] dialog box and click the <Comments> button.

The [Image Comments] dialog box as shown below appears.

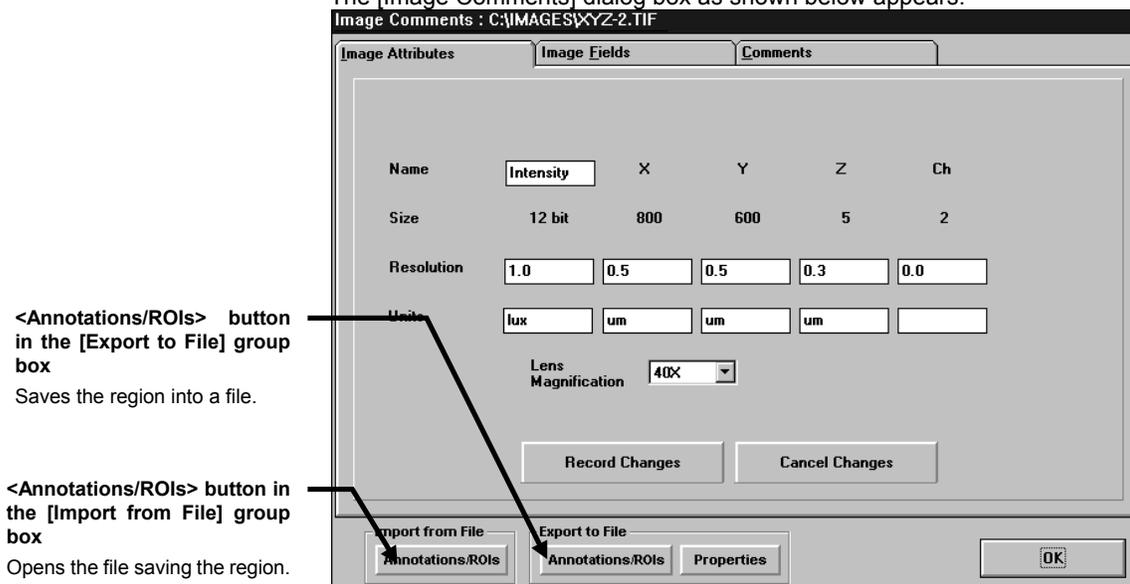


Fig. 2-44 [Image Comments] dialog box

4. Select the <Annotations/ROIs> button in the [Export to File] group box. The [Save Experiment As] dialog box as shown below appears.

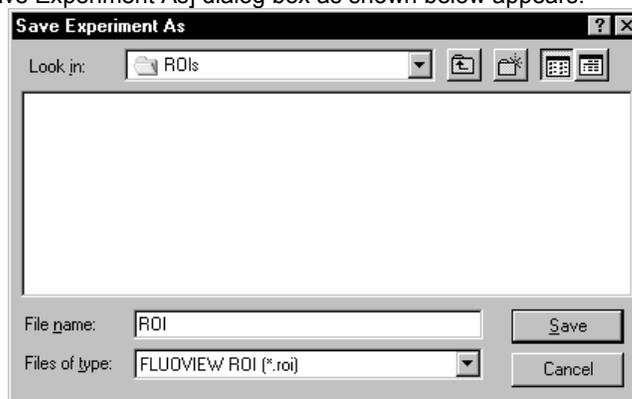


Fig. 2-45 [Save Experiment As] dialog box

5. Select the save destination drive or folder in the [Look in] drop-down list.
6. Select "FLUOVIEW ROI (*.roi)" in the [Files of type] drop-down list.



7. Enter the file name into the [File name] text box and click the <Save> button.



When the text file having the same name as the entered name is already exists, the dialog box appears to ask you to overwrite the existing file or not. When overwriting is not required, click the <NO> button and enter another name to save the file.

2-3-7-2 Reading the Region File

1. Display the [Display] panel of the image including the region to be read at the front position.



<Experiment List> button

2. Select the < Experiment List> button in the tool bar at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box as shown below appears.

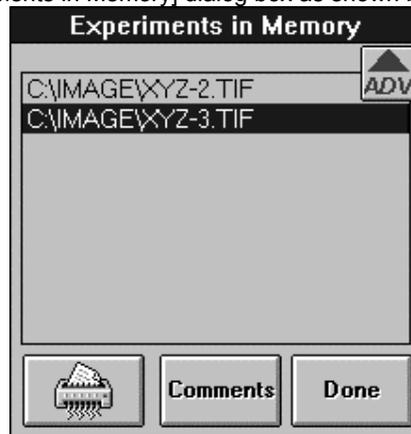


Fig. 2-46 [Experiments in Memory] dialog box

3. In the list in the [Experiments in Memory] dialog box, select the image file name including the region to be read and click the <Comments> button. The [Image Comments] dialog box as shown below appears.

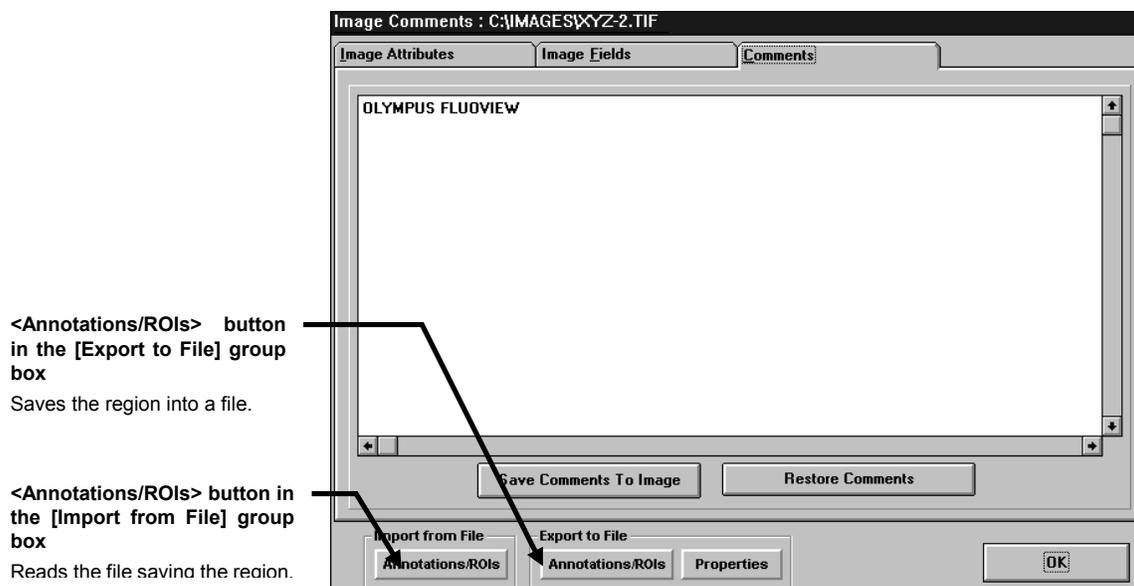


Fig. 2-47 [Image Comments] dialog box

4. Select the <Annotations/ROIs> button in the [Import from File] group box. The [Save Experiment As] dialog box as shown below appears.

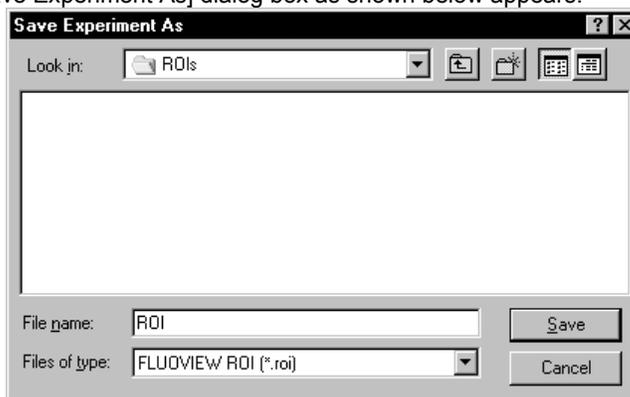


Fig. 2-48 [Save Experiment As] dialog box

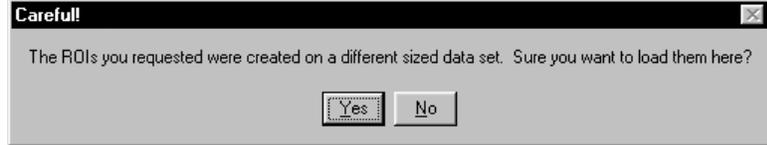
5. Select the drive or folder where the file to be read is saved in the [Look in] drop-down list.
6. In the list below the [Look in] drop-down list, double-click the folder or sub-folder where the data to be read is saved to open.
7. Select "FLUOVIEW ROI (*.roi)" in the [Files of type] drop-down list.



8. Select the file to be read and click the <Open> button.



When the image size of the file including the region to be read and that of the specified file differ, the [Careful] dialog box as shown below appears.



Clicking the <OK> button reads the file in different image size, whereas clicking the <No> button cancels reading the region file.

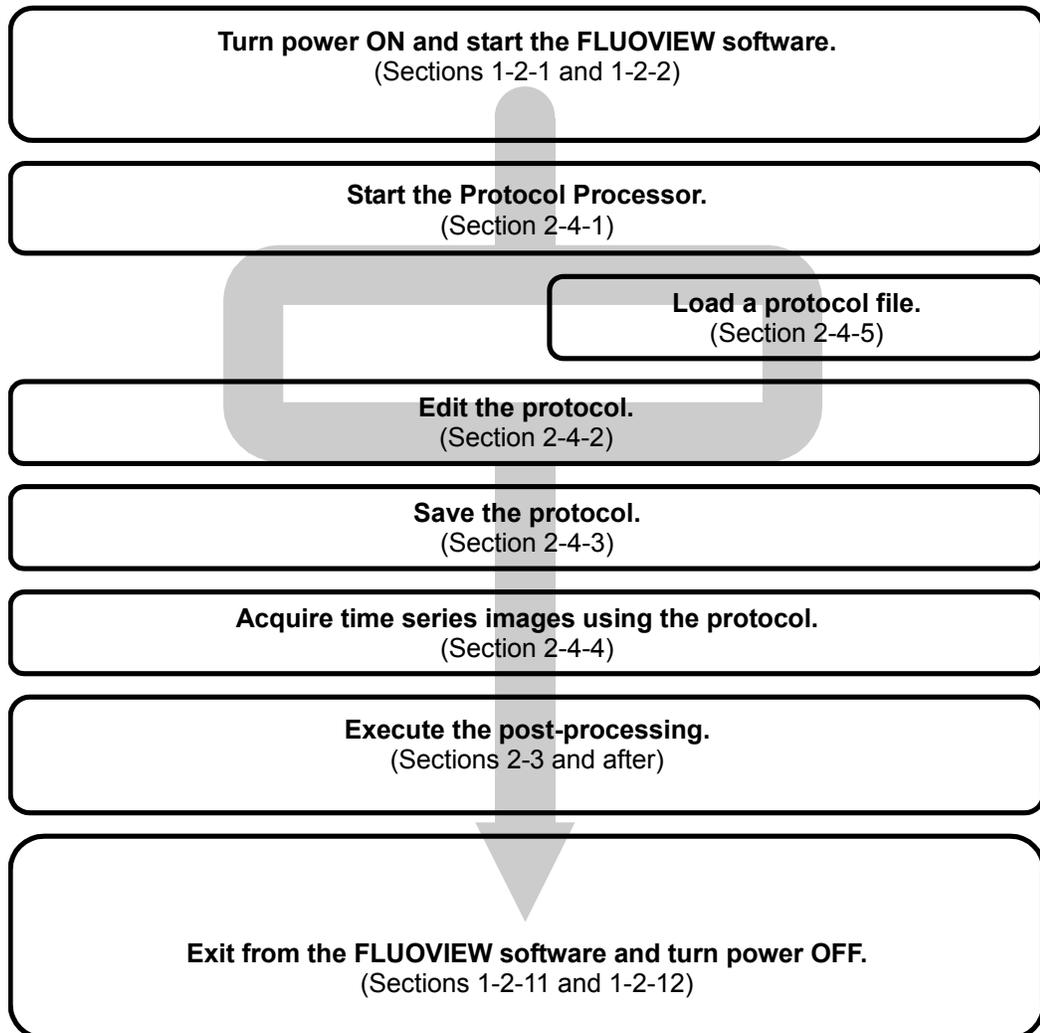
When the region file is read in different image size, the upper left of the image is assumed as reference.

2-4 Protocol processor

The Protocol Processor makes FLUOVIEW image acquisition flexible, especially time series image acquisition. The function enables to create and modify the experiment procedure such as image acquisition condition with interval setting, laser excitation area and its power setting.

The protocol can save and modify to be suitable to new experiment.

Refer to the reference sections written in the following diagram for detail operation.

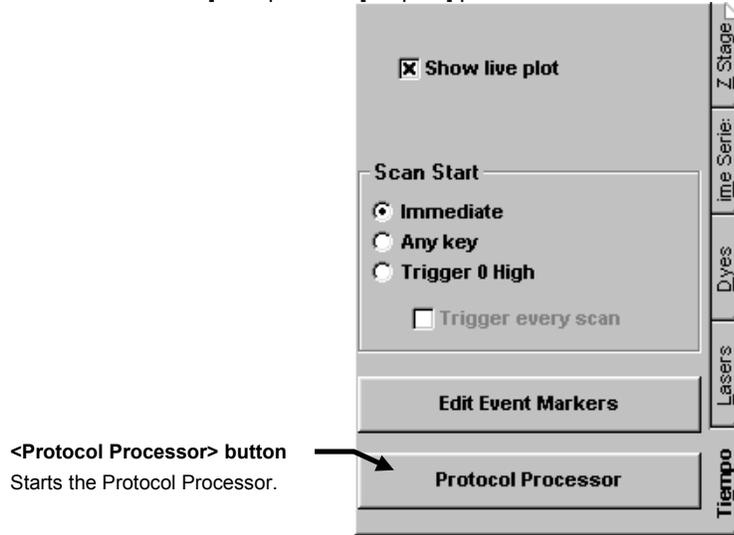




The protocol can be edited by Protocol Processor software that comes with the FLUOVIEW software.

2-4-1 Starting the Protocol Processor

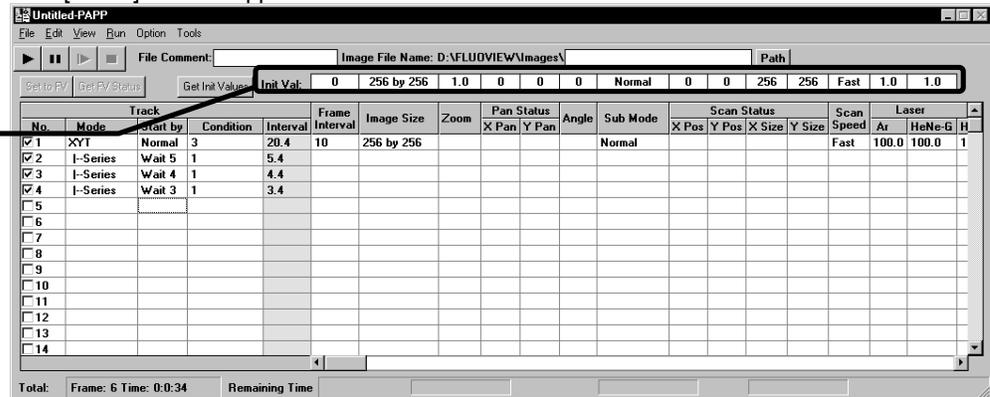
1. Starts the Protocol Processor by clicking <Protocol Processor> button in [Time Series] sub-panel of [Acquire] panel.



The [Programmable Acquisition Protocol Processor] window appears as shown below. (It is referred to as the [PAPP] window in this manual.)

[PAPP] window appears where is the last time in use.

[Init Val] box
Shows the default values of each parameters.



- : <Start> button : Starts the protocol.
- : <Break> button : Temporally stops the protocol, it can re-start by pressing <Start> button.
- : <Skip> button : Skips to the next Track of the protocol.
- : <End> button : Stops the protocol.
- : <Get Init Values> button: Sets [Acquire] panel parameter to the default value.

1 Description of Setting Items

The [PAPP] window has the setting items as described in the following.

<Get Init Values> button
Click to acquire the current settings in the [Acquire] panel as the initial values of the settings.

[Init Val] box
Shows the initial values of the settings.

When each item on each column is mouse-right clicked, pop up menu as shown below appears. It is possible to select Display/Not to display of each column.

[Image File Name] text box
Enter the file name in which the image acquired will be saved.

<Path> button
Specify the folder for saving the image acquired.

[Remaining Time]
Shows the time till the end of protocol by means of figures and indicators. When [Alarm in Interval remaining time] in the [Option] menu is checked, beep tones can be generated at 30 seconds before the end of protocol (single beep), 20 seconds before it (two beeps), between 10 seconds and 5 seconds before it (a beep every second) and between 5 seconds before it and the actual end of protocol (successively).

No.	Mode	Start by	Condition	Interval	Frame Interval	Image Size	Zoom	Pan Status	Angle	Sub Mode	Scan Status	Scan Speed	Laser
1	XYT	Normal	3	20.4	10	256 by 256		X Pan	Y Pan	Normal	X Pos	Y Pos	X Size
2	I-Series	Wait 5	1	5.4							Fast	100.0	100.0
3	I-Series	Wait 4	1	4.4									
4	I-Series	Wait 3	1	3.4									
5													
6													
7													
8													
9													
10													
11													
12													
13													
14													

Gain context menu:

- Frame Interval
- ImageSize
- Zoom
- Pan Status
- Angle
- Sub Mode
- Scan Status
- Scan Speed
- Laser Intensity
- PMT Voltage
- Gain
 - Offset
 - Z Status
 - Save File Name
 - Trigger Out
 - Comment
- Show
 - Ch1
 - Ch2
 - Ch3
 - Ch4
 - Ch5
 - Ch6

Setting items, particularly each laser of [Laser Intensity], [PMT Voltage], [Gain] and [Offset] may be different, depending upon the system; and usable laser and channel on the system are displayed in each column of laser and channel.

Value in each column is set in FLUOVIEW software right after protocol execution.

When [Init] is selected in each column, the default value set in [Init Val] box is used.

Blank cell refers to the setting of one previous track.

Width of each row can be adjusted by mouse drag action on border line of row. By double click action over the border, the width can automatically be adjusted according to the length of characters.



Only available Laser type and PMT channel column is used with [Laser Intensity] and [PMT Voltage].

The values in each cell are applied to the [Acquire] panel at the beginning of the protocol.

When "Init" is specified in a cell, the value set in the [Init Val] box is applied.

The blank cell applies the value set to the track immediately above.

The width and height of PAPP window is adjustable. Width of each row is also adjustable.

The columns are used to set the following data.

No.: Track number. The Track is available if it is checked.

Mode: Select the mode of the track.

New mode : Assign primary image acquisition mode. The scan mode listed below can be assignable.

Series mode: This mode can set the previous scan mode to acquire consecutive XYT, XYZT, or XZT image with the acquisition parameter modified.

Sub-menu of New mode	Description	Series
XY	Acquires XY image.	X
XYT	Acquires XYT image.	O
XYZ	Acquires XYZ image.	X
XYZT	Acquires XYZT image.	O
XT	Acquires XT image.	X
XZ	Acquires XZ image.	X
XZT	Acquires XZT image.	O
Pt*T	Acquires Point-T image.	X

O Series mode (track for linkage) can be connected continuously.

X Series mode (track for linkage) cannot be connected continuously.



When [Mode] column is changed and initializations for all subsequent columns are required, put a check in [Refresh when changing Mode]. When information registered is not changed, uncheck [Refresh when changing Mode] at [Option] menu.

Command : The MACRO command mode.

For : Start of repetition (repetition processing using variables)

Next : End of repetition (repetition processing using variables)

Start by: Select the image acquisition start method.



Normal : Starts image acquisition when protocol comes the Track.

Trigger : Starts image acquisition after receiving external trigger input.

Wait : Starts image acquisition after wait time describes the track, the unit is second.

Condition: See the following table in case that [New mode] or [Series mode] is selected in [Mode].

When “Command” is selected in column [Mode], the macro command can be enter to the track. For the macro commands detail, see section 2, “Command List”.

When “For” is selected in column [Mode], enter the repetition condition using a variable. For the description of the repetition processing, see section 3 , “Protocol Repetition Processing”.

Sub-menu of New mode	Condition column of New Mode		Condition column of the following Series Mode
	Change	Input details	
XY	X	Set 1 automatically	
XYT	O	Set number of images to be acquired	Enter number of slices to acquire
XYZ	X	Set 1 automatically	
XYZT	O	Set number of images to be acquired	Enter number of slices to acquire
XT	O	Set number of lines	
XZ	X	Set 1 automatically	
XZT	O	Set number of lines	Enter number of lines to acquire
Pt*T	X	Set 1 automatically	

Interval: When “Frame” is selected in column [Mode], time required to execute the track is displayed. Change of [Interval] column cannot be done in case of [Series mode].

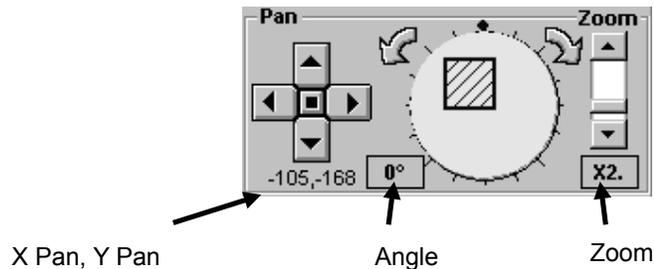
Frame Interval: Enter the interval time between image acquisitions in number of seconds.

A number between 0.1 and 60000.0 can be entered.

Image Size: Select acquisition image pixels. Select either [256 by 256], [320 by 240], [512 by 512], [640 by 640], [800 by 600], [1024 by 768], [1024 by 1024] or [2048 by 2048] at [X-size by Y-size] list box.

Zoom: Enter the zoom ratio in the range between 1.0 and 10.0.

Pan Status: Enter Pan value, refer to [Pan] [Zoom]group box in [Acquire] panel.



FLUOVIEW software [Pan][Zoom] Group Box

TIP

Pan value can not enter the following condition.

- Mode is set to any of XY, XYT, XYZ or XYZT.
- SubMode is Normal.
- Zoom is set to 1 (1.0 x).

TIP

Variables are available in [For] statement for X Pan and Y Pan.
In addition, four-rule calculation is possible.
For details about how to use variables, see One Point of 2-4-8 “2 Example of Setting Procedure of XYT Observation for hours (Protocol using repetition processing)”.

Angle: Assign rotated image scan angle of field of view. 0 through 359 can be entered.

Angle can not set at a certain sub scan mode, and a certain hardware. Please see 2-4-10 [Restrictions at [PAPP] window input time]. for entered angle value restriction.

TIP

Variables are available in [For] statement for Angle setting.
In addition, four-rule calculation is possible.
For details about how to use variables, see One Point of 2-4-8 “2 Example of Setting Procedure of XYT Observation for hours (Protocol using repetition processing)”.

Sub Mode: Sub Mode is for scan mode setting of New mode appears by click on Mode area. A certain scan mode may not set because of hardware configuration. See 2-4-10 “Restrictions at [PAPP] window input time” for restriction of scan



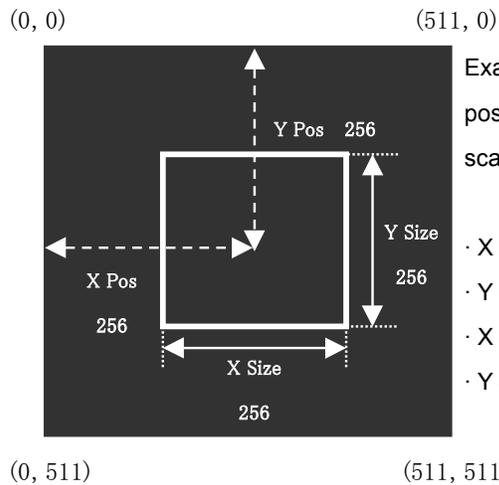
mode setting.

In this case of Normal scan, there might be scanned before the scan mode changes.

Scan Status: Change scanning position and its size with the selected scan mode. See 2-4-10 “Restrictions at [PAPP] window input time” for restriction of Scan status input.



Variables are available in [For] statement for X Pos, Y Pos, X Size, Y Size. In addition, four rule calculation is possible. For details about how to use variables, see One Point of 2-4-8 “2 Example of Setting Procedure of XYT Observation for hours (Protocol using repetition processing)”.



Examples of parameters: Default clip scan position setting which appears when the clip scan is selected with 512 x 512 pixel.

- X Pos : 256
- Y Pos : 256
- X Size : 256
- Y Size : 256

Scan speed: Sets image acquisition speed from “Fast”, “Middle”, “Slow” and “Init” in the pull-down menu.

The above speed is the same with those of [Scan Speed] group box in [Acquire] panel. In case that [Fast] or [Fast Clip] is selected in [Sub mode], [Scan Speed] is automatically set to [High Speed].

Laser Intensity: Enter the intensity setting for each laser. The setting is 0 to 100, and the unit is %. When it sets to 0, a laser shutter is closed.

REX Mask: Select the REX mask file from the pull-down list whether to use the REX

mask which is displayed on FLUOVIEW software or to disable the mask by selecting "None".

[REX Mask] appears only when AOTF (FV5-COMBA) is equipped.

PMT Voltage: Enter the PMT voltage of each channel. The setting is 1 to 1100, and the unit is V.

Gain: Enter the gain of each channel. The setting is 1.0 to 10.0.

Offset: Enter the offset of each channel. The setting is 0 to 100, the unit is %.

XY Stage Track No: When multi-point time lapse software is used, track number of multi-point time lapse software that meets to this track will be displayed. When multi-point time lapse software is not used, the display does not appear.

Zstatus: Enter Z stage information.

Start Pos: Enter the Z-scanning start position of cross section of which observation is required.

Stop Pos: Enter the Z-scanning stop position of cross section of which observation is required.

Step Size: Enter the number of steps in Z direction.

Slices: Enter the number of image slices to be acquired Save FileName: Input file name for acquired image. Image would be saved under path of [Image File Name:].



One Point!

It is possible to designate folder in [Save File Name] column. For example, if [R001 \Image001] is entered, folder – [R001] is created under path of [Image File Name:] and image file is saved under the name of [Image001].

Trigger Out: Select the trigger output from “00”, “01”, “10” and “11” in the pull-down menu.

Comment: Enter any comment as desired.

In case of the range of acceptable values are specified for a cell, it is checked according to the specified range. If a value out of the specified range is entered, it is corrected in the vicinity value.



Variables will be created as defined with For statement for input value. Also, four operations are possible. Regarding the use of variables, see [One Point] in 2-4-8 Example of Setting Procedure of XYT Observation for hours (Protocol using repetition processing).

2 Command List

The list of macro commands is shown below.

When “Command” is selected in column [Mode] in the [PAPP] window, enter the macro command to be carried out for the track. The macro command to be entered can be selected from the drop-down list. And enter each value directly using keyboard.

Command	Entry form	Description
Append	Append [FolderName] [,AppendFileName] [,T/Z/AN] [,Date/Name]	It appends images that exist in the same folder into one file. [FolderName]: Specify the folder that holds a file to be appended. The default is [C:FLUOVIEW\Images]. [AppendFileName]: Specify the file name which use after append. The default is [Append_Year/Month/Day]. [T/Z/AN]: Specify the appended image data type from T series image, Z series image or animation image. The default is T series. [Date/Name]: Specify the order of append, date or name. The default is date.
CopyROI	CopyROI	Copies active ROI of FLUOVIEW image into memory in the track is in use.
Dye	Dye [,string1] [,string2][,string3] [,string4]	It selects dye methods that use String 1 to 4. In case of FV300, max. 2 kinds can be set and, in case of FV500, 4 kinds – 1 to 4 - can be set. Dye colors that can be selected are of the ones displayed in dyeing method when [Dyes] sub panel of [Acquire] panel on FV300/500 is opened. Fluorescent reagents are set in sequence of reagent set in [string]. <E.g> In case that FITC and Cy5 are selected as dye method, enter [Dye FITC, Cy5].
EnCh	EnCh Ch , [ON/OFF]	It sets Valid/Invalid of each channel. In Ch, channel number is designated. Depending upon system configuration, max. 5 channels can be selected. When the channel designated is to be Valid, select ON and, in



		<p>case that Invalid is to be set, select OFF.</p> <p><E.g> In case that channel 1 is to be Valid, enter [EnCh1, ON].</p>
Filter	<p>Filter┐[NORMAL /KALMAN/PEAK]</p> <p>[,value]</p>	<p>[NORMAL/KALMAN/PEAK]: It defines type of integration.</p> <p>NORMAL: No integration</p> <p>KALMAN: KALMAN filter</p> <p>PEAK: Peak detecting integration</p> <p>value: In case that KALMAN is selected, integration cycle of KALMAN filter is set and, in case of PEAK, max. addition cycle at PEAK integration is set. In case that NORMAL is selected, no setting is required.</p> <p><E.g> In case that integration is required 4 times, using KALMAN filter, enter [Filter KALMAN, 4].</p>
Gain	<p>Gain┐ch,┐value</p>	<p>Sets the Gain value of channel.</p> <p><E.g.> Enter "Gain 2, 50" to set 50 to Gain of channel 2.</p>
LivePlot	<p>LivePlot┐</p> <p>[ON/OFF]</p>	<p>[LivePlot ON] is selected, LivePlot is displayed.</p> <p>In case of [LivePlot OFF], LivePlot is not displayed. (This is TIEMPO option.)</p>
Merge	<p>Merge┐</p> <p>MergeFile1,</p> <p>MergeFile2</p> <p>[,Ch(s)][,Ch(s)]</p>	<p>Merges image data that is located under FLUOVIEW software.</p> <p>The following example is to merge 2ch of File1 and 3ch of File2.</p> <p><E.g></p> <p>[Merge C:¥FLUOVIEW¥Images ¥File1,C:¥FLUOVIEW¥Images¥File2,2,3]</p>
Message	<p>Message┐message</p>	<p>Displays a message to suspend executing a protocol.</p>



Offset	Offset┐ch┐value	Sets the Offset value of channel. <E.g.> Enter "Offset 2, 5" to set 5 to the offset value of channel 2.
PasteROI	PasteROI	Pastes ROI to the next track. Note: Before the paste, Normal scan is executed one time.
Pause	Pause	Suspends executing a protocol.
ReceiveCOM	ReceiveCOM┐ message	Receives RS-232C command from other PC. The following data returns after command is executed. Completes normally: "OK" + Received characters Ends with error: "NG" + Received characters. For further details, see 2-4-7.
Save	Save	Saves whole series of images.
SendCOM	SendCOM┐ message┐[,0/1]	Sends data through RS-232C to other PC. The following data has to be returned in synchronous mode., Completed normally: "OK" + sent characters Ends with error: "NG" + sent characters For further details, see 2-4-7.
Size	Size┐X, ┐Y	Sets the image size in pixel. <E.g.> Enter "Size 512, 512" to set 512X512 to the image size. Note: The images acquired before and after executing the Size command cannot be saved together. In the previous or succeeding track, execute the Save, Store, or Stop&Save command to save the images acquired. Note) Image size can be set at the following combination. Be careful that other combination than the following cannot be entered. (256, 256) (320, 240) (512, 512) (640, 480) (800, 600) (1024, 768)



		(1024, 1024) (2048, 2048).
Stop&Save	Stop&Save	Saves all the images acquired before the Stop&Save command is executed. Note: The images are saved separately from the images acquired in succeeding tracks.
Store	Store	Opens a new [Display] panel to display the image.
Zlock	Zlock <input type="checkbox"/> [ON/OFF]	It sets Valid/Invalid of Z motor lock. Note that fine tuning handle and coarse tuning handle cannot be manually operated when Z motor is locked. In case that manual operation is required by releasing Z motor lock, difference of Z stage position displayed on [Zstage] sub panel of [Acquire] panel of FV300/500 may occur. Use the handle after confirming the moving range adequately. In case that Z stage is controlled with use of command, lock Z motor. Do not turn fine tuning handle of microscope in the state that Zlock command is turned ON as it may cause failure. <E.g> In case that Z motor lock is required, enter [Zlock ON].
Zoom	Zoom <input type="checkbox"/> value	Sets the zoom ratio. <E.g.> Enter "Zoom 5" to set 5 to the zoom ratio.



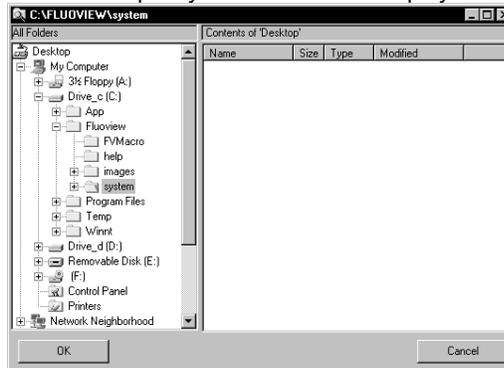
The images acquired in different sizes cannot be saved together. Execute the Stop&Save command to save the image acquired before changing the image size.

One Point!

Multiple image files saved using the Stop&Save command can be combined into a single time-lapse image file.
Combine the file by referring to section 2-6-6-1, "Appending two images"

TIP

The destination of file save using the Save and Stop&Save commands is the path displayed on the left of the [Image File Name] text box. If it is required to change the file save destination, click the <Path> button on the right of the text box and specify the folder in the displayed dialog box.



3 Protocol Repetition Processing

A protocol can be executed repeatedly for the specified number of times using a variable.

Use the For and Next commands to execute repetition.

The track enclosed between For and Next can be repeated for the number of times specified using a variable.

Create the For track immediately before and the Next track immediately after the track to be repeated by specifying a variable. After entering [For] in column [Mode], enter the variable name and repetition condition in column [Condition].

Command	Condition	Description
For	Variables = start To end (Step n)	Define a variable name in <Variables>, the start value in <start>, end value in <End> and an integer representing the number of steps in <n>. The range to which the variable is specified will be executed repeatedly. The number of steps can be entered as required. If it is not entered, the default repetition count of 1 will be applied. <Ex.> To repeat processing from the next track to [For] to the previous track to [Next] until variable i becomes from 1 to 5 (5 times), enter "i = 1 To 5".
Next	None (It is not required to enter [Condition].)	Specify the turnover point of repetition. The processing will be executed until the track immediately before [Next], returns to the track immediately after [For] and repeats from there.

Processing is possible only when [For] and [Next] are entered as a set. Their combination allows nesting for up to 16 times.



One Point!

It is possible to use variables in [For] statement in other cell of protocol processor.

<E.g>

It is possible to change laser intensity value every image acquiring time. When laser intensity of argon laser is defined as variable N, it creates protocols that acquire image with interval of step 10 by N equal to 10 to 100.

No.	Track				Scan Speed	Laser Intensity		
	Mode	Start by	Condition	Interval		Ar	HeNe-G	HeNe-R
<input checked="" type="checkbox"/> 1	For	Normal	N = 10 To 100					
<input checked="" type="checkbox"/> 2	XY	Normal	1	0.4		N		
<input checked="" type="checkbox"/> 3	Next	Normal						

1. Enter [N = 10 To 100 Step 10] for Condition column in No.1 track.
2. Define laser intensity of argon laser as variable N for No.2 track.
3. Define return of repetition for No.3 track.
4. Select <Start> button.

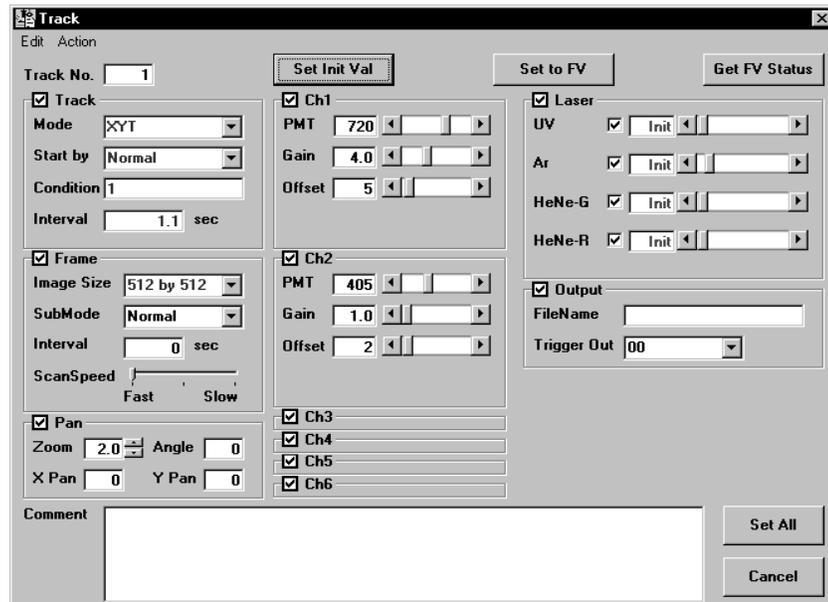
Image will be acquired by changing laser intensity of argon laser by 10% as shown below.

	1st	2nd	3rd	4th	5th	...	10th
Laser (%)	10	20	30	40	50	...	100

4 Input supporting function

[Track] window is available as input supporting function for each track.

[Track] window appears by double-clicking No of [PAPP] window.





- All of parameters that can be set for the Track can modify.
- Parameter that is deferent with the previous Track is colored with Red.
- Parameters that is modified on [Track] windows is colored with purple.
- When **Set Init Val** button is clicked, the setting of item checked at check box of group box name is changed to the value in [Init Val] of [PAPP] window and [init] is displayed.
- When **Get FV Status** button is clicked, the setting of item checked at check box of group box name is acquired from FLUOVIEW software.
- When **Set to FV** button is clicked, the setting of item checked at check box of group box name is set to FLUOVIEW software.
- **Set Init Val** <Set Init Val> button loads [Init Val] box in [PAPP] window into [Track] parameters.

One Point!

It is possible to select [Set Init Val] and [Get FV Status] from [Edit] menu.

When [Select All] is selected from [Edit] menu, checks will enter all check boxes of group boxes.

When [Invert Selection] is selected from [Edit] menu, state of checks in all check boxes of group boxes will be inverted. When check is entered, the check will be removed and, when check is removed, the check is entered.

It is possible to select [Set to FV] from [Action] menu.

- **Get FV Status** <Get FV Status> button loads parameters which FLUOVIEW software has.
- By pressing **Set All** <Set> button, [Track] window setting is registered to [PAPP] window.
- **Cancel** <Cancel> button is for close [Track] window.



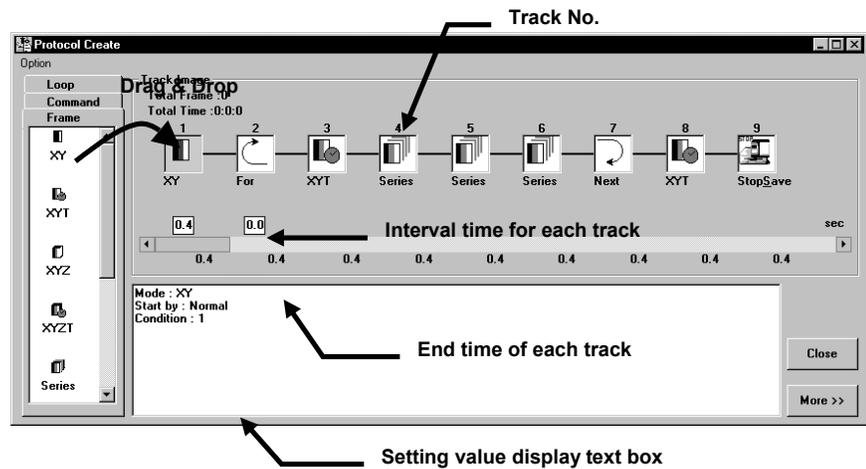
Parameter changes in [Track] does not affect to FLUOVIEW software setting.



[Track] parameter can not modify nor register during the Track is running.

5 Protocol procedure supporting function

[Protocol Creator] window helps to make procedure, especially time-series, easily..



When [Protocol Creator] that exists in [Tool] menu bar of [PAPP] window is selected, [Protocol Create] window appears. [Protocol Create] window is displayed at the position and in width when the window was previously closed.

- Left side of [Protocol Creator] window has [Frame] tab, [Command] tab and [Loop] tab.
Track can be made by drag and drop those icon(s) into [Track Image].
[Track] window appears and setting value can be entered.
- [Track] window does not appear with every drag and drop if [Show detail (when the drop)] in [Option] menu of [Protocol Creator] is not checked.
- A pair of [For] and [Next] icon is added after drag and dropped [Loop] icon in [Loop] panel into [Track Image]. [For] and [Next] icon can move to the Track by dragging the appropriate position.



- [Track] window opens by double-clicking icon on [Track Image] group for detail setting.
- Details of parameters are displayed in setting value display text box by clicking icon.
- Interval time of each Track can be entered into the text box.
- The parameters affect to the [PAPP] window by pressing  <Close> button.

TIP By pressing  <More> button, the detail information is displayed.
By pressing  <Less> button, close the information display..

During [Protocol Creator] window is displayed, [PAPP] window can not be operated.

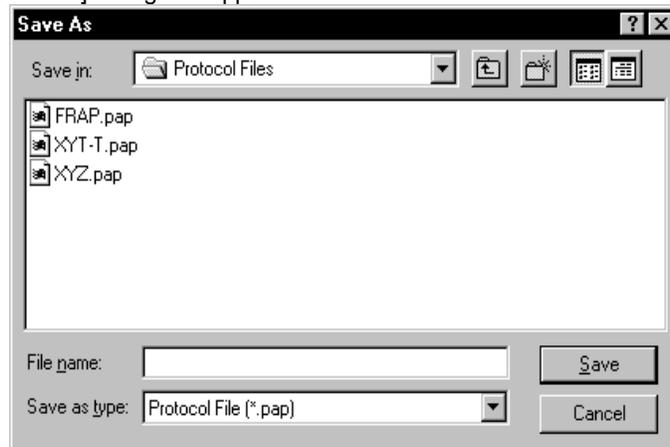
2-4-3 Saving the Protocol

The Protocol Processor creates and saves protocol files in the CSV file format.

TIP The CSV file format applies the text format delimiting the items with comma in a database. Protocol files can be saved only in the CSV format.

1. In the [File] menu, select [Save] or [Save As].

The [Save As] dialog box appears as shown below.



2. To change the save destination drive and/or directory, use the [Save in:] drop-down

list.

3. Enter the file name in the [File Name:] text box.
4. Select file type, .pap or .csv, with [Save as type:] dropdown list.
5. Click the <Save> button.

**NOTE**

If a CSV file having the same file name as the file name entered above already exists, a dialog box appears to ask if you want to overwrite the existing file. If you do not want to overwrite it, click the <No> button and use another file name for saving.

2-4-4 Executing the Protocol

The created protocol can be executed to acquire time series images.



1. In the [PAPP] window, click the <Start> button.

The number of rows in the [No.] list in the [PAPP] window becomes 2, and the window display is reduced. The row being executed is shown in reverse display and the reverse-displayed row changes in synchronism with the change in the executed track.

One Point!

The [PAPP] dialog box can be arranged as follows;

- **Always display the [PAPP] dialog box at the front.**
Check "On Top" in the [View] menu in the [PAPP] dialog box.
- **Display the [PAPP] dialog box in full screen.**
Check "Full Screen" in the [View] menu in the [PAPP] dialog box.
- **Shrink the [PAPP] dialog box to the bottom of the screen while executing a protocol.**
Before executing a protocol, check the "To Bottom on Running" in the [View] menu in the [PAPP] dialog box.
- **Shrink the [PAPP] dialog box at any position in the screen while executing a protocol.**
Before executing a protocol, check "Shrink on Running" in the [View] menu in the [PAPP] dialog box and move the [PAPP] dialog box to anywhere in the screen.
- **[PAPP] window and [Display] panel are separated and displayed.**
Prior to executing protocol, select "Share the screen with FV" from [View] menu of [PAPP] window and make it checked state. When it is done so, "To Bottom on Running" and "Shrink on Running" are simultaneously checked. Further, when check of either "To Bottom on Running" or "Shrink on Running" is removed, the check of "Share the screen with FV" is also removed.

2. When stopping the protocol, click the <Break> button.

When the protocol is executed again, click the <Start> button.



When clicked while the protocol is executed, the <Break> button performs the same function as the <Stop Scan> button in the [Acquire] panel.



<Break> button



<End> button

- To stop the protocol without executing it till the end, click the <End> button.



When clicked while the protocol is executed, the <End> button performs the same function as the <Series Done> button in the [Acquire] panel.

One Point!

The protocol can be processed using the items under the [Run] menu in the [PAPP] window.

- **Start executing the protocol.**
Select “Start” in the [Run] menu in the [PAPP] window.
- **Suspend executing the protocol.**
Select “Break” in the [Run] menu in the [PAPP] window.
- **Fast forward the protocol.**
Select “Skip” in the [Run] menu in the [PAPP] window.
- **Stop executing the protocol.**
Select “End” in the [Run] menu in the [PAPP] window.

One Point!

Option	
Stop when an error occurs	Ctrl+F5
<input checked="" type="checkbox"/> Alarm an Interval remaining time	Ctrl+F6
Stop an interval remaining timer during pause	Ctrl+F7
Use RS232-C command in protocol	Ctrl+F8
COM Control	Ctrl+F9
<input checked="" type="checkbox"/> Start up with XYStage	Ctrl+F11
Refresh When Changing Mode	Ctrl+F12

- ⊙ When [Stop when error occurs] is selected – checked from [Open] menu, process is stopped in case that error occurs during protocol processing at the track where the error occurred. In case that the check is removed, the process goes to next track when error occurred.
- ⊙ When [Stop an interval remaining timer during pause] is selected – checked from [Option] menu, the time at pause is not counted. In case that the check is removed, the time at pause is counted and, if the time counted exceeds the time set for track, the process will be immediately started at next track when protocol is executed again.

2-4-5 Loading a Protocol

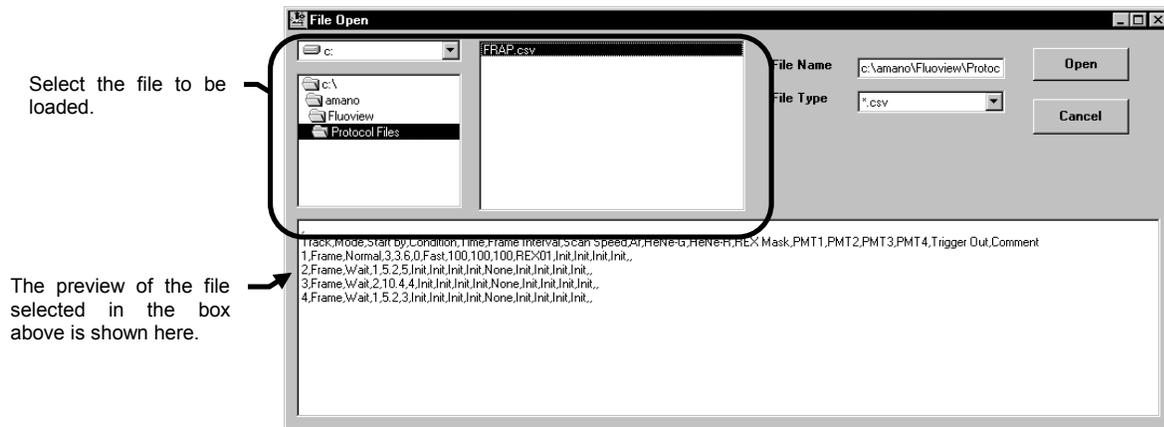
The Protocol Processor creates and saves protocol files in the CSV file format.

The following procedure loads a CSV file (extension .pap or .csv) for editing the protocol in it.

1. Start the Protocol Processor software and display the [PAPP] window.
For details, see section 2-4-1, "Starting the Protocol Processor".

2. In the [File] menu, select [Open].

The [File Open] dialog box appears as shown below.



3. Specify the file to be loaded in the box at the top left of the dialog box.

4. Click the <Open> button.

The loaded track data will be displayed in the [PAPP] window.

2-4-6 Loading a protocol of previous format

FLUOVIEW software Ver4.2 inform the protocol data conversion when the software reads previous version of the data.

The rule of the conversion is explained below.

Rule A

- Before Ver4.2, Frame = XYT or XYZT is converted to XYT or XYZT in count of Z slice parameter.
 - If Z Slices = 1, the Frame becomes XYT.
 - If Z Slices is more than 1, the Frame becomes XYZT.
 - If the Frame is continuous one, Frame becomes Series.

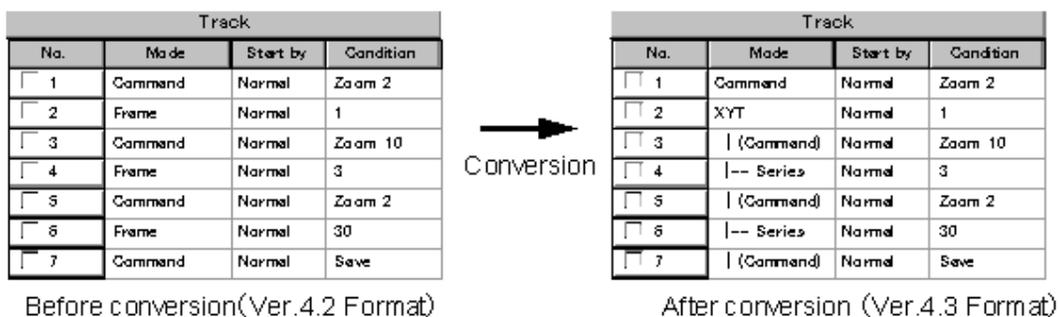


Fig. 2-51 Sample Program (FRAP)

Rule B

- Before Ver4.2, Frame (Separate) is converted with condition of Track and Z slices.
 - If Condition = 1, and Z slice =1, Frame becomes XY.
 - If Condition is more than 1, and Z slice = 1, Frame becomes XYT.
 - If Condition = 1, and Z slice is more than 1, Frame becomes XYZ.
 - If Condition and Z slice are more than 1, Frame becomes XYZT.

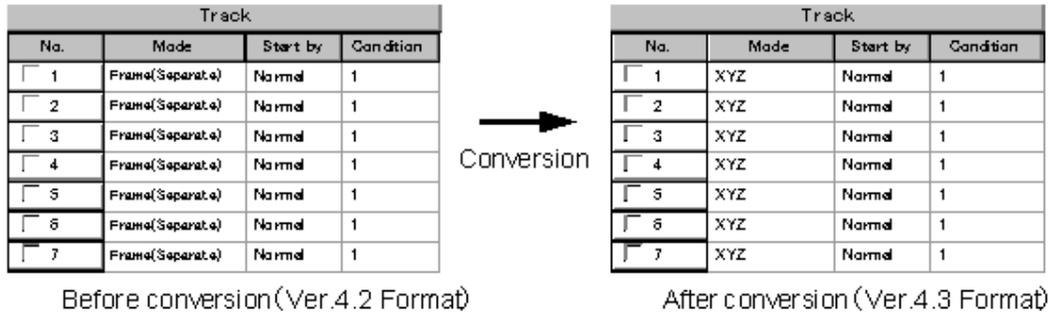


Fig. 2-52 Multi-Point Time Lapse Protocol

Rule C

- For and Command in Series appears with indented + ().
- Combined For – Next structure becomes single layer.

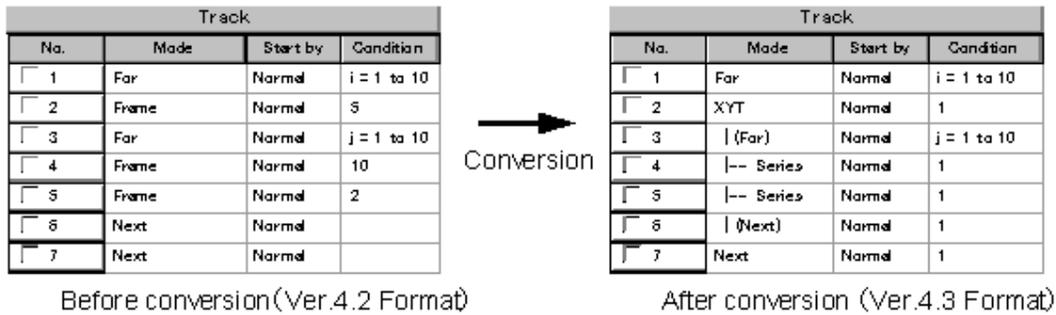


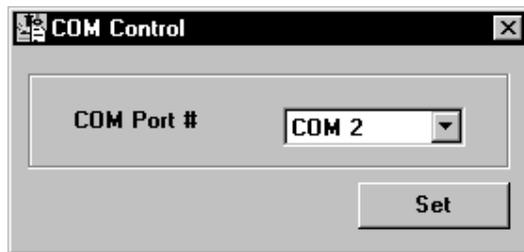
Fig. 2-53 Protocol Using [For] Statement

2-4-7 COM Communication function

PAPP can communicate with RS-232C to the other PC. The data for sending and receiving should be described with Macro commands. For detail of Macro command, see 2-4-2 [2 Table of commands].

Select COM port of PC.

- Click [Option] menu in [PAPP] window, then click [COM Control].
[COM Control] dialog appears.
- Select COM port # from [COM Port#] dropdown list.
- By clicking <Set> button to be able to use the port.



[COM Control] dialog box

- “SendCOM” and “ReceiveCOM” commands are available when [Use RS232-C command in protocol] in [Option] menu is checked.
- In case of using “SendCOM” command with synchronous mode, the following data should be returned from the other PC.

When the command completes normally

'OK' + Transmitted command

When the command ends with error

'NG' + Transmitted command

- In case of receiving “ReceiveCOM” command, the following data will be returned.

When the command completed normally

'OK' + Received character strings

When the command ends with error

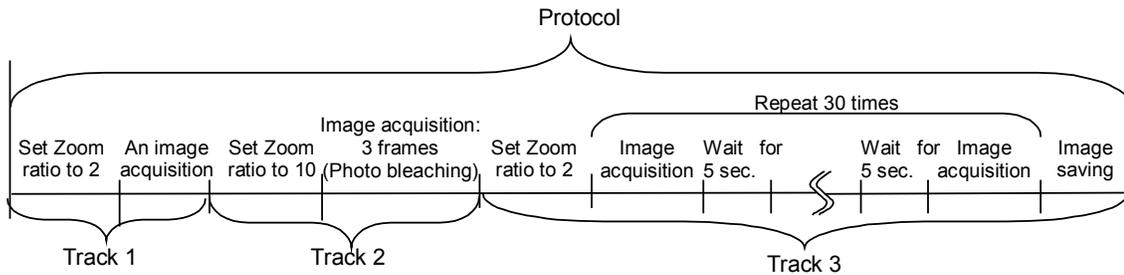
'NG' + Received character strings

2-4-8 Example of assembling a protocol

This section describes an example of assembling a protocol.

1 Example of Setting Procedure of FRAP Observation

An example of editing a protocol is described below assuming the FRAP observation.



Initial image acquisition: Track 1 sets the zoom ratio to 2 x, and acquires an image.

Photobleaching: Track 2 sets the zoom ratio to 10 x, and acquires image 3 times to make photobleach on the specimen.

Recovery measurement: Track 3 sets the zoom ratio to 2 x, and acquire image 3 times with 5 seconds interval to observe the recovery. Then, stores the image.

TIP Execute a XY repeated scanning and adjust the set values for image acquisition before executing the protocol.

1- 1 Setting the observation condition

Before setting and starting the FRAP observation using the Protocol Processor, execute a repeated scan and set the condition for the observation.

Assuming FITC to Channel 2 and image size to 320X240 pixels, the following figure shows an example of image acquisition.

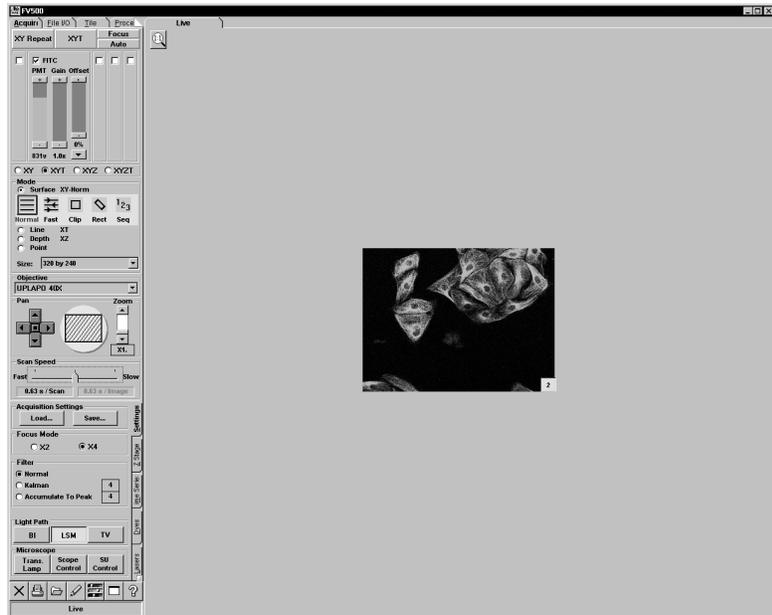


Fig. 2-54 Panel After a Repeated Scan

1-2 Displaying the Real-Time Graph (TIEMPO option software required)

1. Specify the region in the image to display the real-time graph.
For specifying method, refer to section 2-4-3, "Specifying the Regions Where Intensity Graph is Displayed in Real Time".
2. Display the [Tiempo] sub-panel in the [Acquire] panel to check the [Show live plot] check box.

For checking method, refer to section 2-4-4, "Displaying the Real-Time Graph".

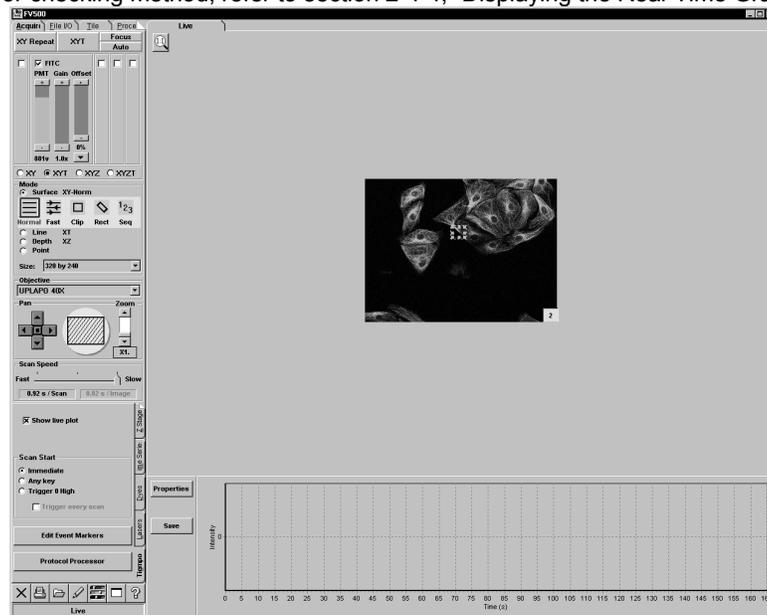


Fig. 2-55 Panel Displaying the Real-time Graph

1-3 Editing the Protocol

Start and edit the protocol after setting of the observation condition has been completed.

For starting method, refer to section 2-4-1, "Starting the Protocol Processor".

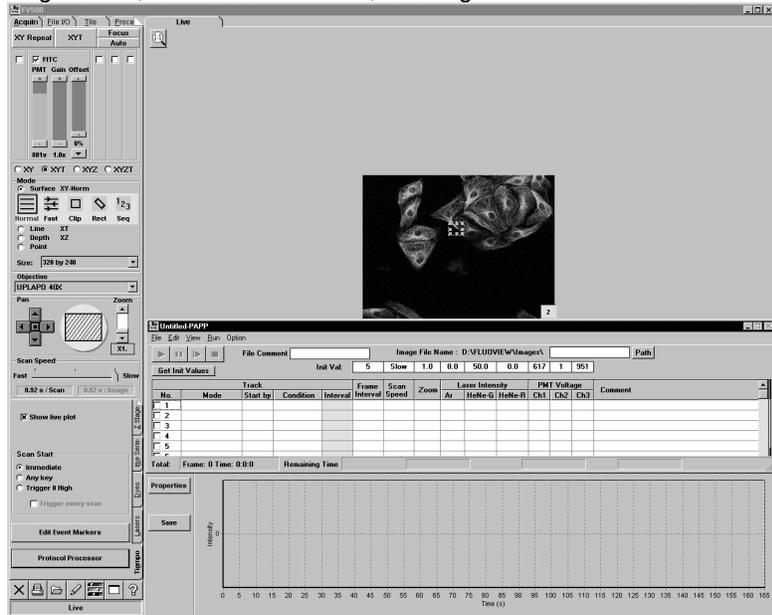


Fig. 2-56 Panel Displaying the [PAPP] window

Set the [PAPP] window as shown below;

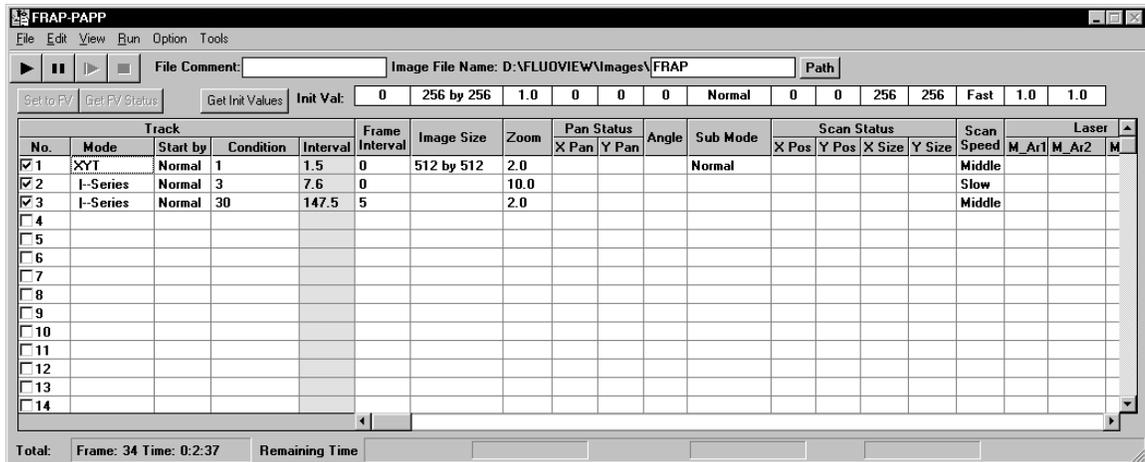


Fig. 2-57 [PAPP] window Setting an Example of Observation Condition

Set each Track as shown below;

- **Setting Track 1**

In XYT observation, set the zoom ratio to 2X and acquire an image before bleaching.

1. Select [New mode]-"XYT" in [Mode] of [Track].
2. Select "Normal" in [Start by] of [Track].
3. Enter "1" in [Condition] of [Track].
4. Enter "0" to [Frame Interval].
5. Enter "2" to [Zoom].
6. Select "Middle" in [Scan Speed].
7. Enter the laser intensity value for image acquisition to [Laser Intensity]
8. Enter the PMT value of each channel to [PMT Voltage].
9. Enter a comment in [Comment] if necessary.

- **Setting Track2**

In XYT observation, set the zoom ratio to 10X and acquire three images. (Irradiate strong laser onto the region to bleach.)

1. Select "Series mode" in [Mode] of [Track].
2. Select "Normal" in [Start by] of [Track].
3. Enter "3" to [Condition] of [Track].
4. Enter "0" to [Frame Interval].
5. Enter "10" to [Zoom].
6. Select "Slow" in [Scan Speed] for fast photo-bleaching.
7. Enter the laser intensity value for image acquisition to [Laser Intensity]
8. Enter the PMT value of each channel to [PMT Voltage].
9. Enter a comment in [Comment] if necessary



To properly irradiate the laser for bleaching, enter a larger value in [Condition] and fast forward the tracks using the <Skip> button in accordance with the grade of bleaching.

- **Setting Track 3**

In XYT observation, return the zoom ratio to 2X and acquire 30 images every 5 seconds. (Observe the change in fluorescence image with time after bleaching.)

1. Select "Series mode" in [Mode] of [Track].
2. Select "Normal" in [Start by] of [Track].
3. Enter "30" to [Condition] of [Track].
4. Enter "5" to [Frame Interval].
5. Enter "2" to [Zoom].
6. Select "Middle" in [Scan Speed]. (Return to the setting before bleaching.)
7. Enter the laser intensity value for image acquisition to [Laser Intensity].
8. Enter the PMT value of each channel to [PMT Voltage].
9. Enter the image file name to be stored to [Save FileName].
10. Enter a comment in [Comment] if necessary.



One Point!

The protocol to perform the following operations can be assembled after setting the observation conditions.

- **Specifying the region for bleaching in the laser irradiation mode using REX mask files.**

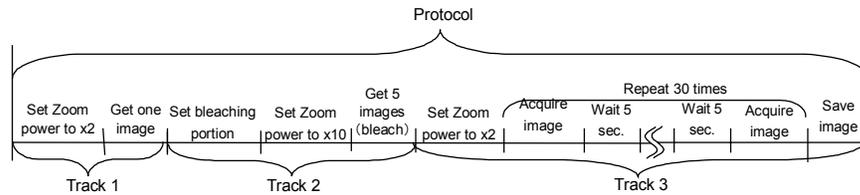
In Track 4, select a REX mask file in [Mask] of [REX]. Then in the laser irradiation mode specified by the REX mask file selected, images can be acquired. (When using the system with AOTF.) To select a REX mask file, display the REX mask file in the [Display] panel in advance.

<The setting example>

No.	Mode	Track			Frame Interval	Image Size	Zoom	Sub Mode	Scan Speed	Laser Intensity			REX Mask	Save FileName	Trigger Out
		Start by	Condition	Interval						Ar	HeNe-G	HeNe-R			
<input checked="" type="checkbox"/> 1	XYT	Normal	1	10.0		512 by 512	2.0	Normal	Middle						
<input checked="" type="checkbox"/> 2	I-Series	Normal	3	30.1			10.0		Slow	100.0	0.0		REX_Live		
<input checked="" type="checkbox"/> 3	I-Series	Normal	30	300.7			2.0		Middle	INIT	INIT		None	BLEACH	
<input type="checkbox"/> 4															

For details, see section 2-2-11, "Image Acquisition in the Laser Excitation Mode".

- **Setting bleaching region by use of rectangular image acquisition (RECT).**



Reference image acquisition:

Acquire an image with the Zoom power 2 x in Track 1 in XYT observation.

Photo-bleaching:

Expose laser to photo-bleach with 5 images of acquisition using ZoomIn mode in Track 2 in XYT observation.

Recovery observation:

Acquire 3 images in XYT observation every 5 seconds with the zoom power 2 x, the field of view is the same with the reference image, and the images are stored in Track 3.

<Example>

No.	Mode	Track			Frame Interval	Image Size	Zoom	Pan Status			Angle	Sub Mode	Scan Status				Scan Speed
		Start by	Condition	Interval				X Pan	Y Pan				X Pos	Y Pos	X Size	Y Size	
<input checked="" type="checkbox"/> 1	XY	Normal	1	1.0	0	512 by 512						Normal					
<input checked="" type="checkbox"/> 2	XYT	Normal	5	1.6	0	512 by 512	2.0	0	0	0		ZoomIn	44	128	256	256	Fast
<input checked="" type="checkbox"/> 3	XYT	Normal	30	146.5	5	512 by 512						Normal					
<input type="checkbox"/> 4																	

1-4 Saving the Protocol

Save the edited protocol data.

The protocol can also be saved after being executed.

1. Select [Save] or [Save as] in the [File] menu in the [PAPP] window.
Save using the dialog box displayed.
For details, see section 0, “

1-5 Executing the Protocol

Executes the protocol.



<Start> button

1. Click the <Start> button in the [PAPP] window.

For details, see section 2-4-4, “Executing the Protocol”.

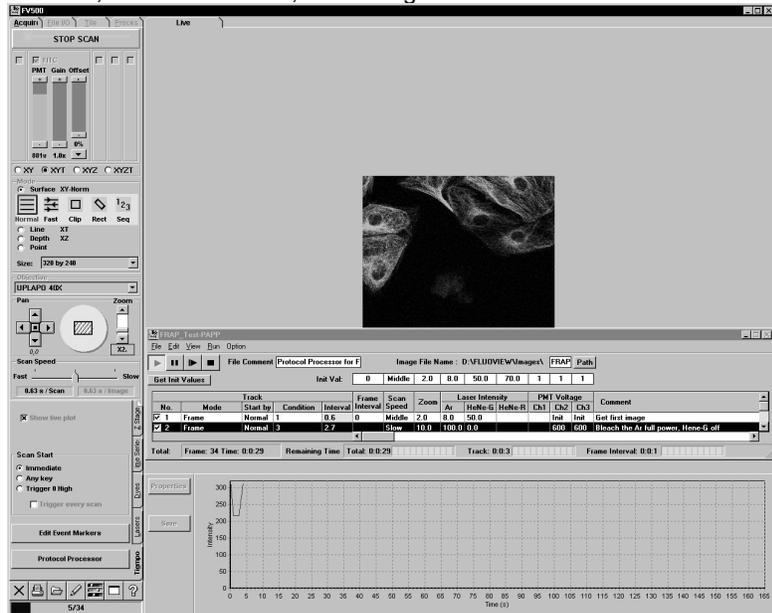


Fig. 2-58 Panel While Executing the Protocol

1-6 Exiting the Protocol

Terminate the protocol after acquiring the images.

The images saved.

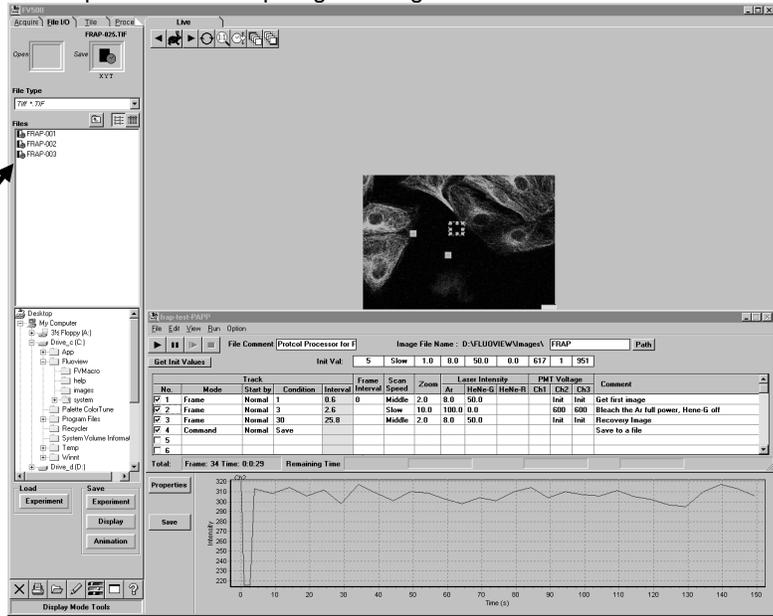
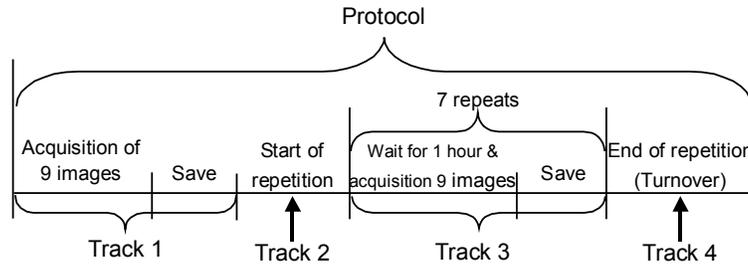


Fig. 2-59 Panel When Exiting the Protocol

2 Example of Setting Procedure of XYT Observation for hours (Protocol using repetition processing)

An example of editing a protocol using the repetition processing is described below assuming the XYT observation including the GFP recovery observation for hours.



Reference image acquisition: Acquire 9 images with the zoom power 2 x, then save the images acquired in Track 1.

Repeated Protocol start : Repeats protocol of Track 3, the repeated times is 7 which is assigned with For statement in Track 2.

Long interval image acquisition: Acquire 9 images every one hour, and the image is stored in Track 3.

Repeated Protocol end: Track 4 defines whether ends or continues Track 3 protocol.

TIP

Specify the Z range and select XYZT mode before starting the XYZT observation. For details, see section 2-2-1-4, "Setting the Observation Condition".



One Point!

The variable in the [For] statement can also be used by another cell in the protocol processor.

<Setting example>

It is also possible to change the laser intensity value at every image acquisition. In this case, assume that the laser intensity of the Ar laser is variable N and create a protocol for acquiring image as N varies from 10 to 100 in steps of 10.

Track					Scan Speed	Laser Intensity		
No.	Mode	Start by	Condition	Interval		Ar	HeNe-G	HeNe-R
<input checked="" type="checkbox"/> 1	For	Normal	N = 10 To 100					
<input checked="" type="checkbox"/> 2	XY	Normal	1	0.4		N		
<input checked="" type="checkbox"/> 3	Next	Normal						

5. In Condition column to track No. 1, enter For statement "N = 10 To 100 Step 10".
6. In track No. 2, specify the laser intensity value of the Ar laser as variable N.
7. In track No. 3, specify the turning point of repetition processing.
8. Click the <Start> button.

Acquire images by varying the laser intensity value of the Ar laser in 10% steps as shown below

	1st	2nd	3rd	4th	5th	...	10th time
Laser intensity %	10	20	30	40	50	...	100



<Start> button

2-1 Setting the Observation Condition

Before setting and starting the XYT observation for hours using the Protocol Processor, execute a repeated scan and set the condition for the observation.

Assuming FITC to Channel 2 and image size to 320X240 pixels, the following figure shows an example of image acquisition.

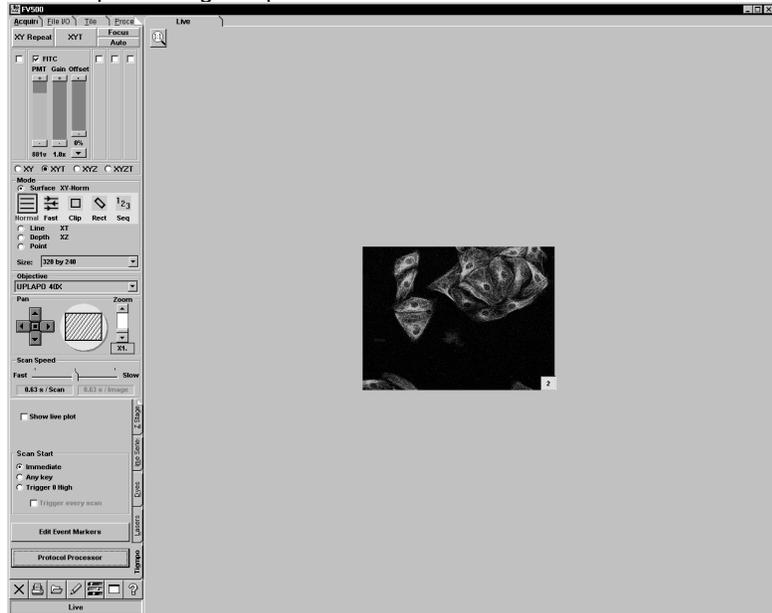


Fig. 2-60 Panel After a Repeated Scan

2-2 Editing the Protocol

Start and edit the protocol after setting of the observation condition has been completed.
 For starting method, refer to section 2-4-1, "Starting the Protocol Processor".

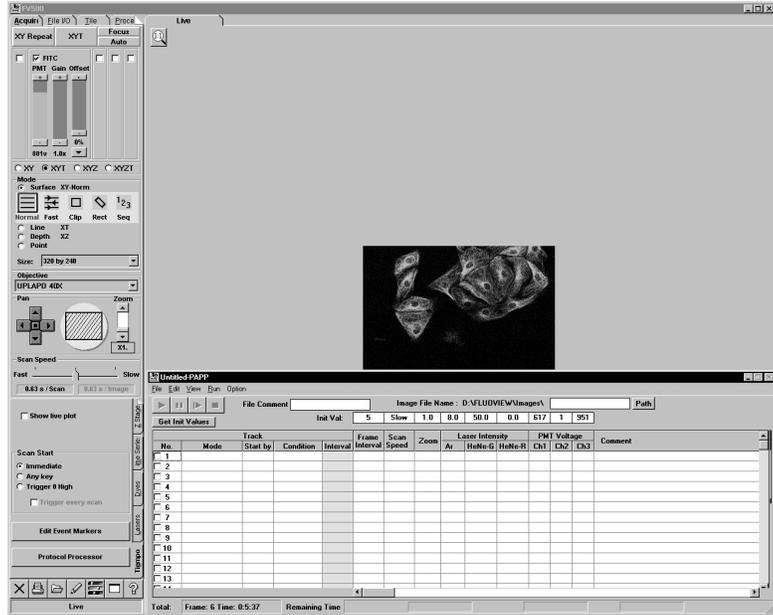


Fig. 2-61 Panel Displaying the [PAPP] window

Set the [PAPP] window as shown below;

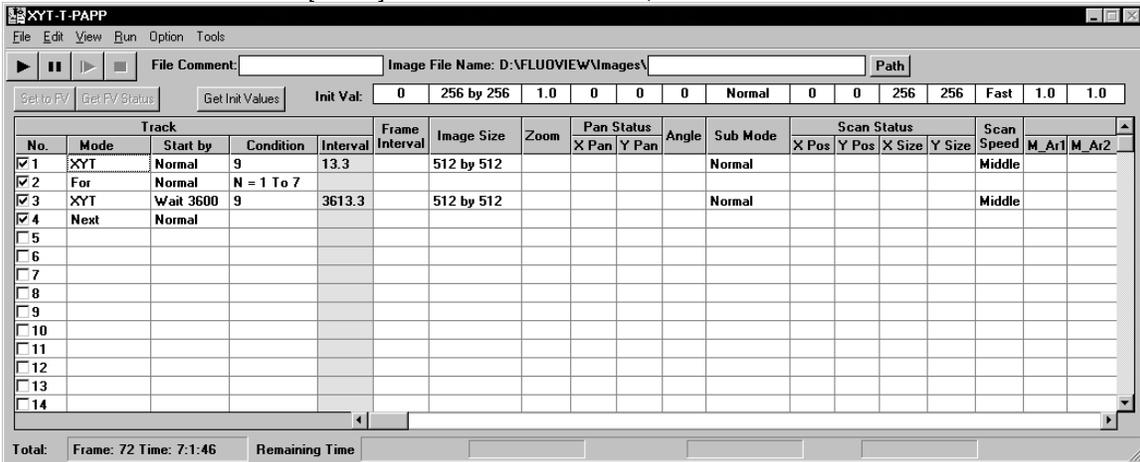


Fig. 2-62 [PAPP] window Setting an Example of Observation Condition

Set each Track as shown below;

- **Setting Track 1**

Acquire nine images in XYT observation.

1. Select "New mode"- "XYT" in [Mode] of [Track].
2. Select "Normal" in [Start by] of [Track].
3. Enter "9" to [Condition] of [Track].
4. Select "Middle" in [Scan Speed].
5. Enter the laser intensity value for image acquisition to [Laser Intensity].
6. Enter the PMT value of each channel to [PMT Voltage].
7. Enter image file name to be stored to [Save FileName].
8. Enter a comment in [Comment] if necessary.



The actual image file name becomes using the name of [Image File Name] that followed by the acquisition order number.
Assign the image file name in the [Image File Name] text box area before protocol starts..

- **Setting Track 2**

Specifies N variable and designate range to be repeated. Protocol continues between the next Track of "For" statement and the Track just before "Loop" statement.

1. Select "For" in [Mode] of [Track].
2. Enter "N = 1 To 7" to [Condition] of [Track].
3. It is not necessary to enter the values in other cells.

- **Setting Track 3**

Acquire nine images at one-hour interval in each Track in XYT observation.

1. Select "New mode"- "XYT" in [Mode] of [Track].
2. Enter "Wait 3600" in [Stand by] of [Track]. (Enter the value by the second.)
3. Enter "9" in [Condition] of [Track].
4. Select "Middle" in [Scan Speed].
5. Enter the laser intensity value for use in image acquisition in the cell for each laser in [Laser Intensity].
Enter "8" in [Ar] and "50" in [HeNe-G].

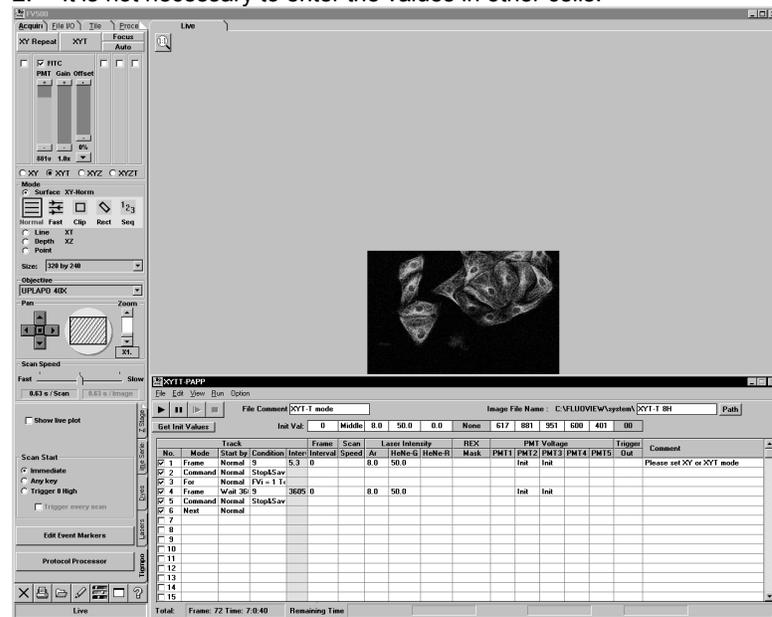


6. Enter the PMT value of each channel in the cell for each channel in [PMT Voltage].
Enter "Init" in [PMT2] and [PMT3].
7. Enter the image file name to be stored to [Save FileName.].

● **Setting Track 4**

Specifies the repeated protocol by use N variable.

1. Select "Next" in [Mode] of [Track].
2. It is not necessary to enter the values in other cells.





One Point!

XYZT observation protocol becomes available when “New mode”-“XYZT” is selected instead of selecting “New mode” – XYT on [Mode] of [Track].

2-3 Saving the Protocol

Save the protocol edited.

The protocol can also be saved after being executed.

1. Select [Save] or [Save as] in the [File] menu in the [PAPP] window.
Save using the dialog box displayed.
For details, see section 2-4-3, “Saving the Protocol”.

2-4 Executing the Protocol

Execute the protocol assembled.



<Start> button

1. Click the <Start> button in the [PAPP] window.

For details, see section 2-4-4, “Executing the Protocol”.

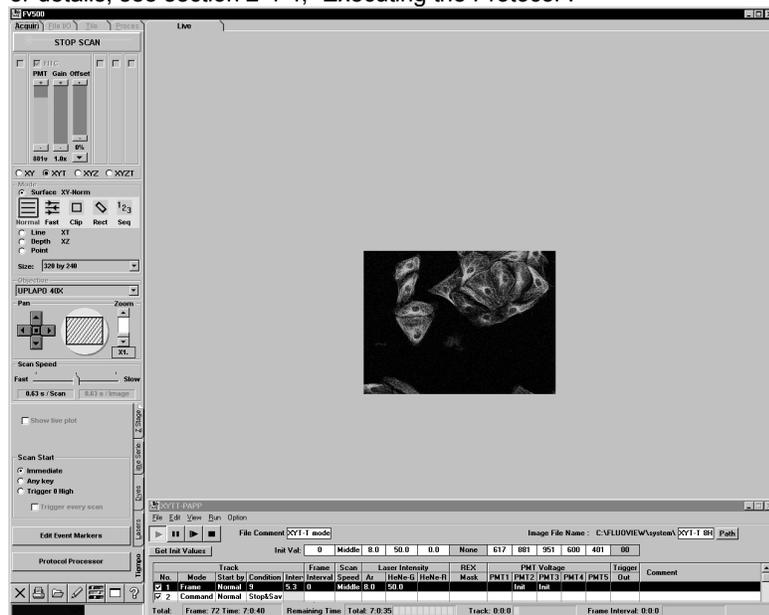


Fig. 2-63 Panel While Executing the Protocol

2-5 Exiting the Protocol

After the initial image has been acquired and saved, “saving the image acquired in Track 4 in Track 5” is repeated for 7 times, after which the protocol terminates.

The images saved

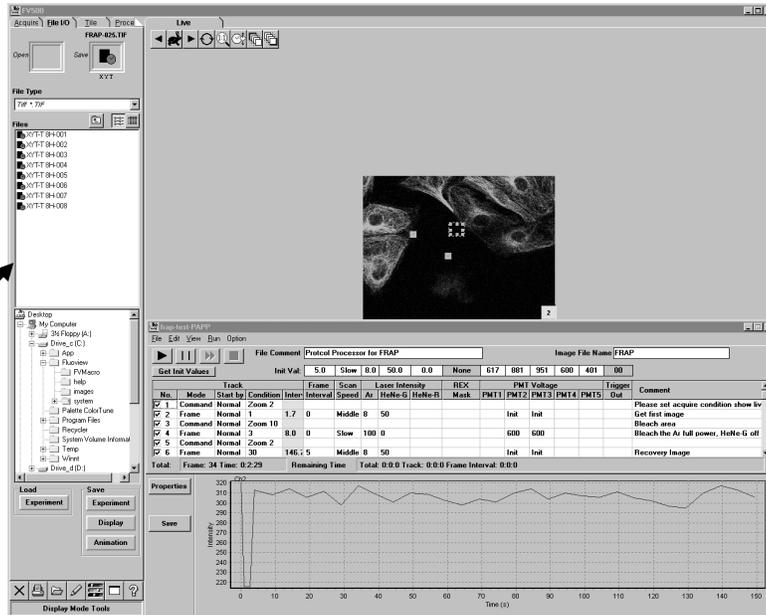


Fig. 2-64 Panel When Exiting the Protocol

2-4-9 Pop-up Menu

Right-clicking the mouse in the cells in the [PAPP] window displays the pop-up menu to edit the protocol as shown below;

<u>U</u> ndo	Ctrl+Z	Removes the effect of the previous operation.
<u>R</u> edo	Ctrl+Y	Repeats the previous operation.
<u>C</u> opy	Ctrl+C	Copies the value in the selected cell.
<u>C</u> ut	Ctrl+X	Cuts the value in the selected cell.
<u>P</u> aste	Ctrl+V	Pastes the value copied or cut into another cell.
<u>I</u> nitial	Ctrl+I	Applies the initial value into cell.
<u>R</u> ead Current Setting	Ctrl+U	Applies the value in the [Acquire] panel into cell.

One Point!

In addition to the pop-up menu, the menu in the [PAPP] window is available to edit the protocol.

- **Remove the effect of the previous operation.**
Select "Undo" in the [Edit] menu in the [PAPP] window.
- **Repeat the previous operation.**
Select "Redo" in the [Edit] menu in the [PAPP] window.
- **Copy the value in cell.**
Select "Copy" in the [Edit] menu in the [PAPP] window.
- **Cut the value in cell.**
Select "Cut" in the [Edit] menu in the [PAPP] window.
- **Paste the value copied.**
Select "Paste" in the [Edit] menu in the [PAPP] window.
- **Insert a cell.**
Select "Insert" in the [Edit] menu in the [PAPP] window.
- **Insert the cell copied.**
Select "Insert Copied Cell" in the [Edit] menu in the [PAPP] window.
- **Delete the value in cell.**
Select "Delete" in the [Edit] menu in the [PAPP] window.

2-4-10 Restrictions of [PAPP] setting

1 Restrictions with [Mode] and I/O card

There are restrictions at Sub Mode and Angle setting in [PAPP] that comes from [Mode] and I/O card in use. The following table shows the restriction.

Mode	Sub Mode	Sub Mode Row		Angle Row	
		I/O2	I/O3	I/O2	I/O3
XY	Normal	O	O	X	O
	Clip	O	O	X	O
	ZoomIn	O	O	O	O
	Fast	O	O	X	O
	FastClip	O	O	X	O
	Seq_Normal	O	O	X	O
	Seq_Clip	O	O	X	O
	Seq_ZoomIn	O	O	O	O
	LineSeq_Normal	O*	O*	X	O*
	LineSeq_Clip	O*	O*	X	O*
	LineSeq_ZoomIn	X	O*	X	O*
XYT	Normal	O	O	X	O
	Clip	O	O	X	O
	ZoomIn	O	O	O	O
	Fast	O	O	X	O
	FastClip	O	O	X	O
	Seq_Normal	O	O	X	O
	Seq_Clip	O	O	X	O
	Seq_ZoomIn	O	O	O	O
	LineSeq_Normal	O*	O*	X	O*
	LineSeq_Clip	O*	O*	X	O*
	LineSeq_ZoomIn	X	O*	X	O*
XYZ	Normal	O	O	X	O
	Clip	O	O	X	O
	ZoomIn	O	O	O	O
	Seq_Normal	O	O	X	O
	Seq_Clip	O	O	X	O
	Seq_ZoomIn	O	O	O	O
	LineSeq_Normal	O*	O*	X	O*
	LineSeq_Clip	O*	O*	X	O*
LineSeq_ZoomIn	X	O*	X	O*	

O means can use the combination.
 X means can not set
 O* Input is possible when combiner is FV5-COMBA only.



XYZT	Normal	O	O	X	O	
	Clip	O	O	X	O	
	ZoomIn	O	O	O	O	
	Seq_Normal	O	O	X	O	
	Seq_Clip	O	O	X	O	
	Seq_ZoomIn	O	O	O	O	
	LineSeq_Normal	O*	O*	X	O*	
	LineSeq_Clip	O*	O*	X	O*	
	LineSeq_ZoomIn	X	O*	X	O*	
	XT	Normal	O	O	X	O
		Clip	O	O	X	O
ZoomIn		O	O	O	O	
FreeLine		O	O	X	O	
Fast		O	O	X	O	
FastClip		X	O	X	O	
LineSeq_Normal		O*	O*	O*	O*	
LineSeq_Clip		X	O*	X	O*	
LineSeq_ZoomIn		X	O*	X	O*	
XZ		Normal	O	O	X	O
	Clip	X	O	X	O	
	ZoomIn	O	O	O	O	
	FreeLine	O	O	X	O	
	LineSeq_Normal	O*	O*	O*	O*	
	LineSeq_Clip	X	O*	X	O*	
	LineSeq_ZoomIn	X	O*	X	O*	
	XZT	Normal	O	O	X	O
Clip		X	O	X	O	
ZoomIn		O	O	O	O	
FreeLine		O	O	X	O	
LineSeq_Normal		O*	O*	O*	O*	
LineSeq_Clip		X	O*	X	O*	
LineSeq_ZoomIn		X	O*	X	O*	
Pt*T		Normal	O	O	X	O

O means can use the combination.

X means can not set

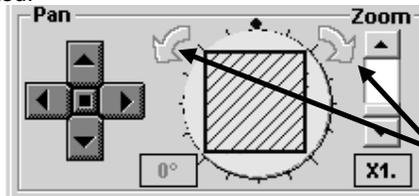
O* Input is possible when combiner is FV5-COMBA only.



TIP

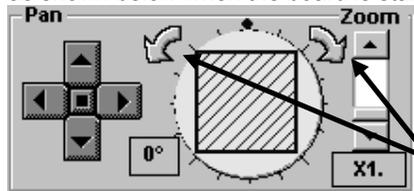
It is possible to check which I/O board is being used by the following method.

In case that I/O2 is used, [Pan][Zoom] group boxes on [Acquire] panel cannot be selected because arrows are grayed out as shown below when the board is started.



It is grayed out. Selection cannot be done.

In case that I/O3 is used, it is possible to select [Pan][Zoom] group boxes as arrows appear as shown below when the board is started.



Selection possible

2 Restrictions with [Mode] and [Sub Mode]

There are restrictions at Mode and its Sub Mode setting in [PAPP].

The following table shows the restriction.

Mode	Sub Mode	Scan Status Row			
		X Pos	Y Pos	X Size	Y Size
XY	Normal	X	X	X	X
	Clip	O	O	O	O
	ZoomIn	O	O	O	O
	Fast	X	X	X	X
	FastClip	O	O	O	O
	Seq_Normal	X	X	X	X
	Seq_Clip	O	O	O	O
	Seq_ZoomIn	O	O	O	O
	LineSeq_Normal	X	X	X	X
	LineSeq_Clip	O*	O*	O*	O*
	LineSeq_ZoomIn	O*	O*	O*	O*
	XYT	Normal	X	X	X
Clip		O	O	O	O
ZoomIn		O	O	O	O
Fast		X	X	X	X
FastClip		O	O	O	O
Seq_Normal		X	X	X	X
Seq_Clip		O	O	O	O
Seq_ZoomIn		O	O	O	O
LineSeq_Normal		X	X	X	X
LineSeq_Clip		O*	O*	O*	O*
LineSeq_ZoomIn		O*	O*	O*	O*
XYZ		Normal	X	X	X
	Clip	O	O	O	O
	ZoomIn	O	O	O	O
	Seq_Normal	X	X	X	X
	Seq_Clip	O	O	O	O
	Seq_ZoomIn	O	O	O	O
	LineSeq_Normal	X	X	X	X
	LineSeq_Clip	O*	O*	O*	O*
	LineSeq_ZoomIn	O*	O*	O*	O*
	XYZT	Normal	X	X	X
Clip		O	O	O	O
ZoomIn		O	O	O	O
Seq_Normal		X	X	X	X
Seq_Clip		O	O	O	O
Seq_ZoomIn		O	O	O	O
LineSeq_Normal		X	X	X	X
LineSeq_Clip		O*	O*	O*	O*
LineSeq_ZoomIn		O*	O*	O*	O*

O means can use the combination.

X means can not set

O* Input is possible when combiner is FV5-COMBA only.



XT	Normal	X	O	X	O
	Clip	O	O	O	O
	ZoomIn	O	O	O	O
	FreeLine	X	X	X	X
	Fast	X	O	X	O
	FastClip	O	O	O	O
	LineSeq_Normal	X	O*	X	O*
	LineSeq_Clip	O*	O*	O*	O*
	LineSeq_ZoomIn	O*	O*	O*	O*
XZ	Normal	X	O	X	O
	Clip	O	O	O	O
	ZoomIn	O	O	O	O
	FreeLine	X	X	X	X
	LineSeq_Normal	X	O*	X	O*
	LineSeq_Clip	O*	O*	O*	O*
	LineSeq_ZoomIn	O*	O*	O*	O*
XZT	Normal	X	O	X	O
	Clip	O	O	O	O
	ZoomIn	O	O	O	O
	FreeLine	X	X	X	X
	LineSeq_Normal	X	O*	X	O*
	LineSeq_Clip	O*	O*	O*	O*
	LineSeq_ZoomIn	O*	O*	O*	O*
Pt*T	Normal	O	O	O	O

O means can use the combination.

X means can not set

O* Input is possible when combiner is FV5-COMBA only.

2-5 Changing the Image Display Method

The method of displaying an image acquired by observation or opened from a file can be changed as described below.

2-5-1 Displaying an Image in Simulated Colors



<LUT> button

1. Display the [Display] panel of the image to be colored at the front.
2. Click the <LUT> button in the toolbar at the bottom left of the screen. The [Color Tool] dialog box appears.

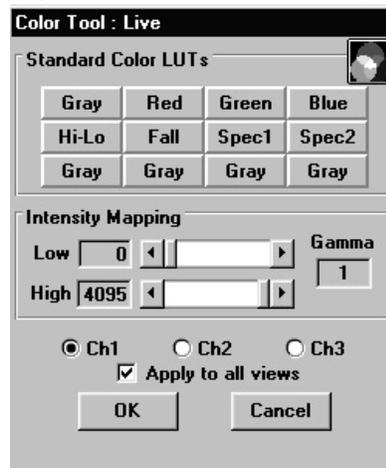


Fig. 2-64 [Color Tool] Dialog Box

3. When the image was acquired from more than one channel, select the channels to be colored using the [Ch1], [Ch2] and/or [Ch3] option buttons. (The [Ch1], [Ch2] and [Ch3] option buttons are displayed only when an image acquired in the 2-channel or 3-channel mode is displayed (selected).
4. From the [Standard Color LUTs] group box, select the desired color button. The selected LUT will be applied immediately to the image in the [Display] panel.
5. Click the <OK> button.

TIP

The [Apply to all views] check box can be selected while the [Display] panel showing multiple images created using the [Tile] panel is displayed.

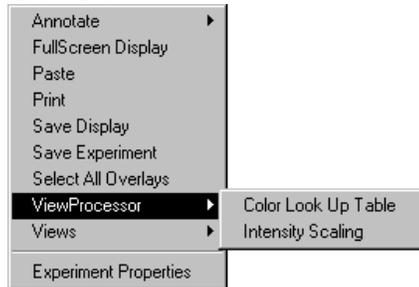
When this check box is checked, all changes are applied to all of the images shown in the [Display] panel.



One Point!

The [Color Tool] dialog box can also be displayed by a mouse operation.

1. Display the image to be colored at the front of the [Display] panel, and right-click a point in the image.
2. A pop-up menu as shown below is displayed.
3. Select [View Processor] from the menu, then select [Intensity Scaling] from the displayed sub-menu.



2-5-2 Editing the LUT (Look Up Table)

An original LUT can be created by editing an existing LUT.

2-5-2-1 LUT Graph Editing According to Colors



<LUT> button



<Graph display> button

1. Display the [Display] panel of the image that you want to edit the LUT.
2. Click the <LUT> button in the toolbar at the bottom of the screen. The [Color Tool] dialog box appears as shown in Fig. 2-64.
3. Click the <Graph display> button on the top right of the [Color Tool] dialog box. The dialog box shows the LUT intensity graph.
4. Select the LUT color to be edited by checking the check boxes below the graph in the [Color LUT Tool] group box.
5. Set the range of intensity graph application using the [Low] and [High] scales in the [Intensity Mapping] group box.

box.

TIP

When the [Low] scale, [High] scale and [Gamma] text box are double-clicked, they are reset to the default values("0" with the [Low] scale, "4095" or "255" with the [High] scale and "1.0" with the [Gamma] text box).

6. The inclination of the graph in the [Color LUT Tool] group box can be changed by dragging an end of the graph line. The selected LUT will immediately be applied to the image in the [Display] panel.
7. If it is required to save the edited LUT in a file, click the <Save LUT> button in the [Color LUT Tool] group box.

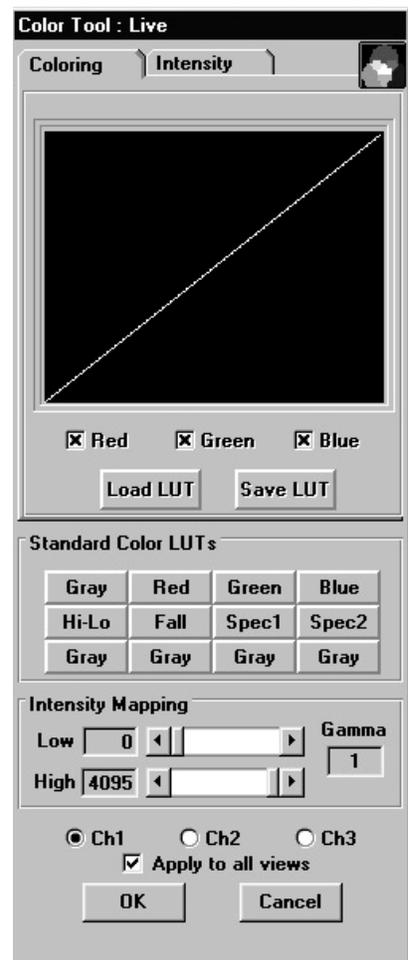


Fig. 2-65 LUT Intensity Graph Display

TIP

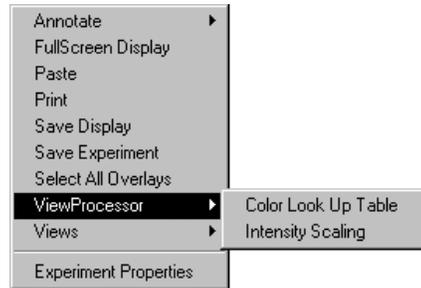
To load a previously saved LUT, click <Load LUT>.

8. Click the <Graph display> button at the top right of the [Color Tool] dialog box again.
9. Finally, click the <OK> button to exit from the LUT editing.

One Point!

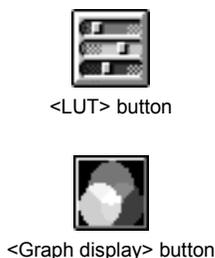
The [Color Tool] dialog box can also be displayed by a mouse operation.

1. Display the image to be colored at the front of the [Display] panel, and right-click a point in the image.
2. A pop-up menu as shown below is displayed.
3. Select [View Processor] from the menu, then select [Intensity Scaling] from the displayed sub-menu.



2-5-2-2 LUT Graph Editing by Gamma Correction

The intensity data of an image can be reallocated to make it easier to view.



1. Display the [Display] panel of the image to be subjected to LUT change.
2. Click the <LUT> button in the toolbar at the bottom left of the screen.
3. Click the <Graph display> button on the top right of the [Color Tool] dialog box, then select the [Intensity] sub-panel. The intensity graph of the LUT appears in the [Color Tool] dialog box.
4. Set the range of intensity graph application using the [Low] and [High] scales in the [Intensity Mapping] group box.

TIP Dragging one end of the graph makes it possible to change the inclination. The set intensity graph is immediately reflected in the image in the [Display] panel.

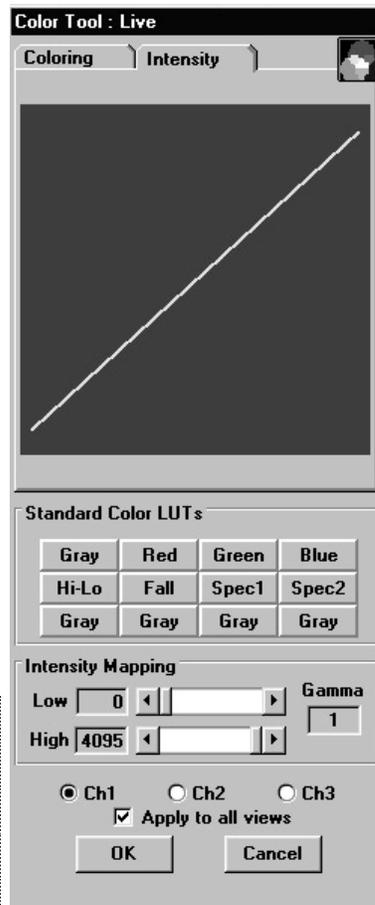


Fig. 2-66 LUT Intensity Graph Display

TIP When the [Low] scale, [High] scale and [Gamma] text box are double-clicked, they are reset to the default values (“0” with the [Low] scale, “4095” or “255” with the [High] scale and “1.0” with the [Gamma] text box).

5. The gamma value can be changed by dragging on the graph. The set intensity graph is immediately reflected in the image in the [Display] panel.

TIP The gamma value can also be changed by entering a gamma value in the [Gamma] text box in the [Intensity Mapping] group box.

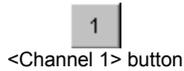
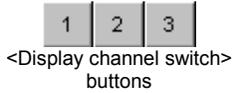
6. If it is required to save the edited LUT in a file, click the <Save LUT> button in the [Color LUT Tool] group box.



To load a previously saved LUT, click <Load LUT>.

2-5-3 Switching the Displayed Channels (Ch1, Ch2, Ch3)

The buttons on the bottom left of the screen can be used to select where the image of a single channel or images of multiple channels are to be displayed. For the simultaneous display of multi-channel images, see section 2-5-4, “Displaying Images of Multiple Channels Simultaneously”.



1. Display the [Display] panel of for the image obtained from multiple channels at the front.
2. Click the image to display the <Display channel switch> buttons on the bottom left of the image.
3. Click the <Channel 1>, <Channel 2> and/or <Channel 3> buttons of the <Display channel switch> buttons.

The [Display] panel shows the images of the channels selected with the <Display channel switch> buttons. At the same time, the clicked <Display channel switch> buttons are displayed in the pressed-in condition.

4. Press the previously pressed <Display channel switch> buttons to let them disappear.

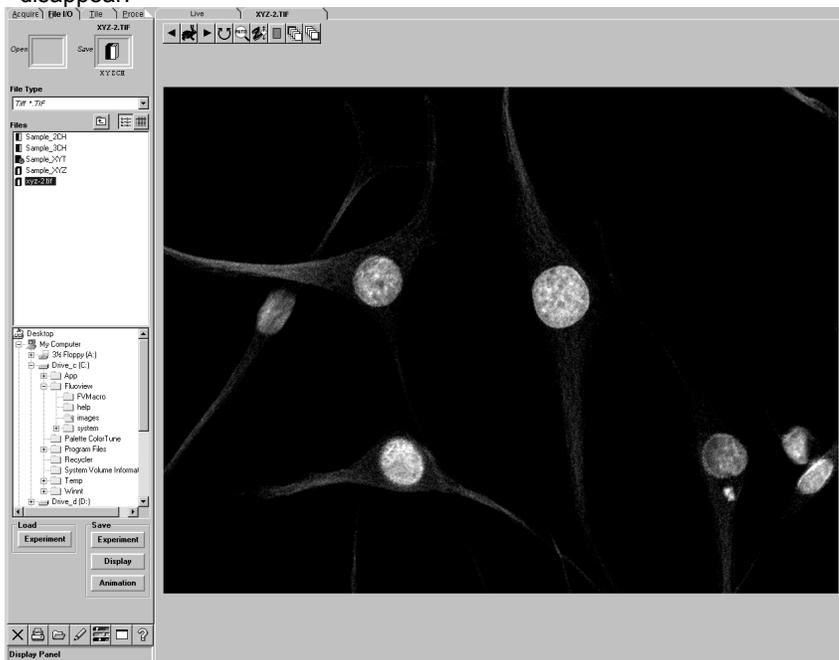


Fig. 2-67 Panel Displaying the Channel 2 Image

2-5-4 Displaying Images of Multiple Channels Simultaneously (Side By Side Views, Over And Under Views. Single View)

Images from multiple channels can be displayed either by merging them or placing them side by side. It is also possible to display the image of only one of these channels.

Use the buttons displayed at the top of the [Display] panel and those on the bottom right which are displayed when the corresponding image is clicked. For the display of the image of only one channel, see section 2-5-3, "Switching the Displayed Channels".

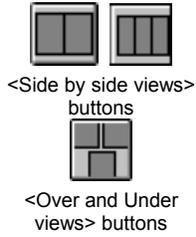
2-5-4-1 Displaying Images Separately Per Channel (Side By Side Views, Over And Under Views)

1. Display the [Display] panel of one of the images to be displayed side by side at the front.
2. Click the <Display switching> button at the top of the [Display] panel. The list of buttons as shown below appears.



<Display switching> button





- From the displayed list of buttons, click one of the <Side by side views> button or <Over And Under views> button. The icon shown in the <Display switching> button will change to the icons of the <Side by side views> button or <Over and under views> button.

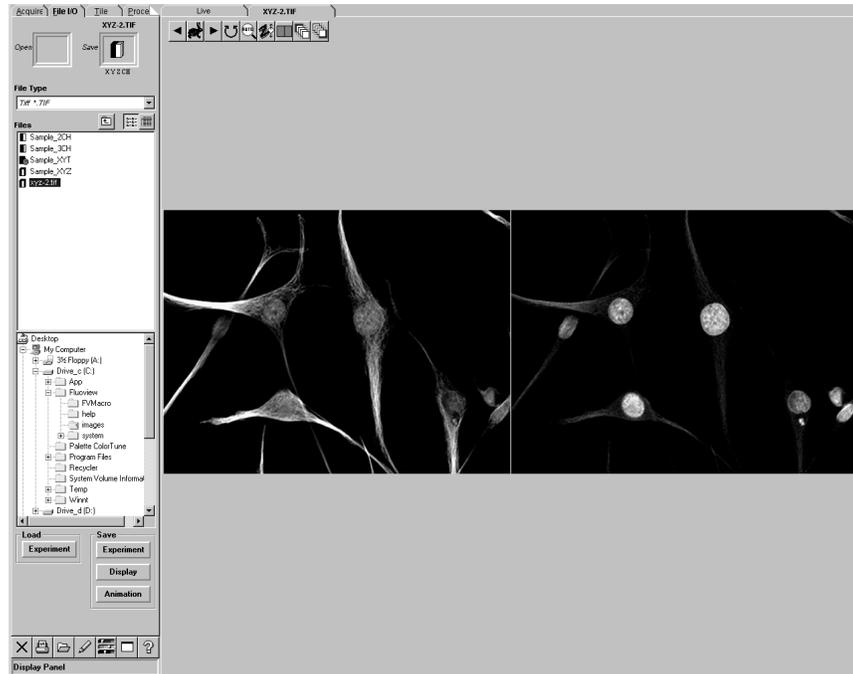


Fig. 2-68 Panel Displaying Images of Two Channels Side By Side

2-5-4-2 Displaying Merged Image of Multiple Channels (Single Views)



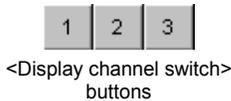
<Display switching> button

1. Display the [Display] panel of the merged image of multiple channels at the front.
2. Click the <Display switching> button at the top of the [Display] panel. The list of buttons as shown below appears.



<Single View>button

3. From the displayed list of buttons, press the <Single View> button. The [Display] panel will show the images of multiple channels side by side. At the same time, the icon shown in the <Display switching> button will change to the icon of the <Single View> button.



<Display channel switch> buttons

4. Click the image to display the <Display channel switch> buttons at the bottom left of the image.



<Channel 1> button

5. Click the <Channel 1>, <Channel 2> and/or <Channel 3> buttons of the <Display channel switch> buttons.



<Channel 2> button

The [Display] panel shows the merged image of the channels selected with the <Display channel switch> buttons. At the same time, the clicked <Display channel switch> buttons are displayed in the pressed-in condition.



<Channel 3> button

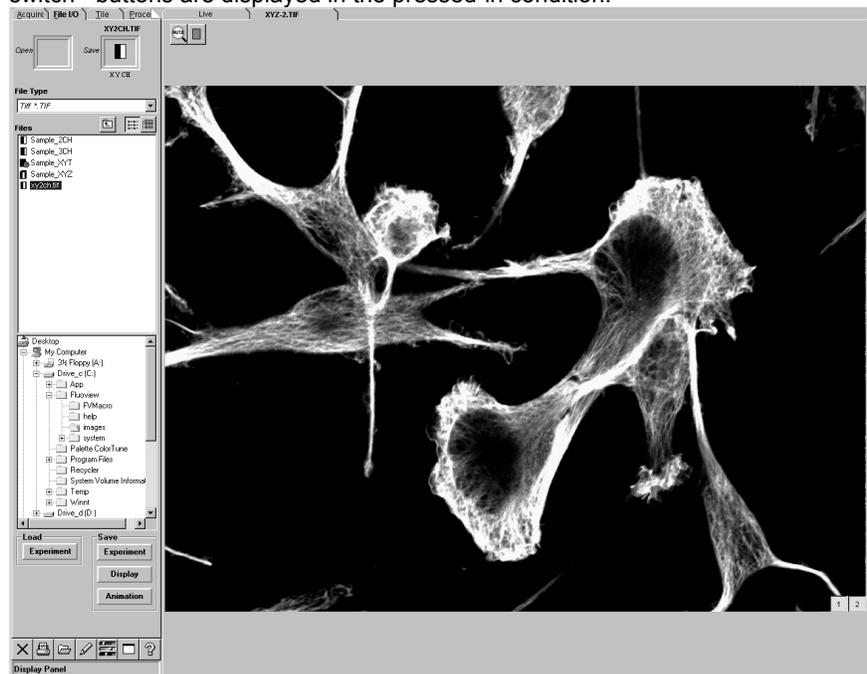


Fig. 2-69 Panel Displayed Merged Image of Two Channels

2-5-5 Changing the Number of Divided Images

The number of images viewed simultaneously can be changed.



Increased image is only to be displayed. The image increased in Add View is not subjected to these operations described below.

2-5-5-1 Increasing the Number of Divided Images

1. Display the [Display] panel of the image to be changed at the front.

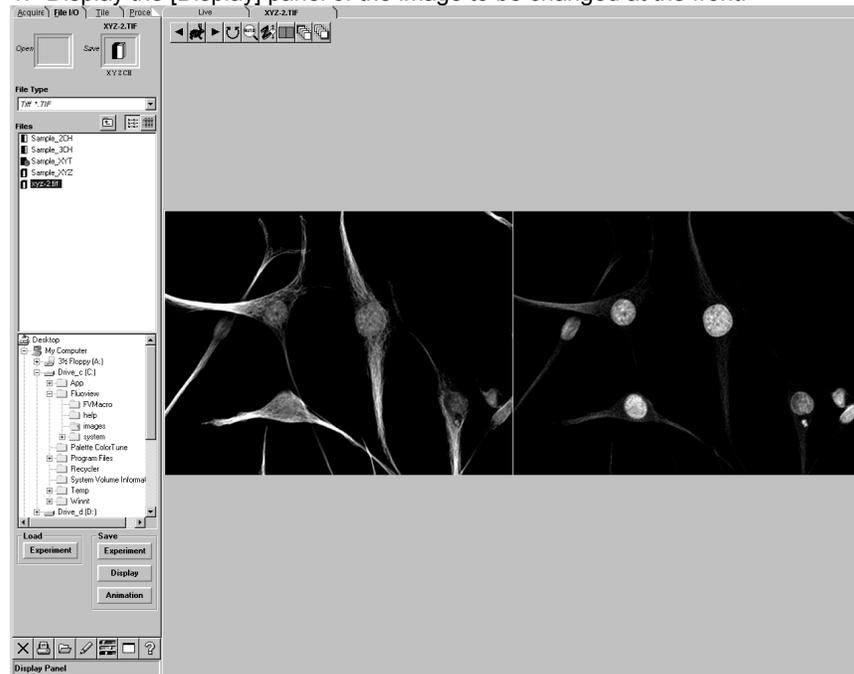
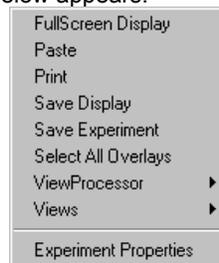


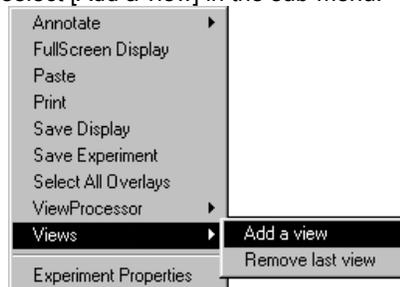
Fig. 2-70 [Display] panel

2. Right-click the image.

A pop-up menu as shown below appears.



3. Select [Views], then select [Add a view] in the sub-menu.



4. A view is added to the rightmost position on the [Display] panel.

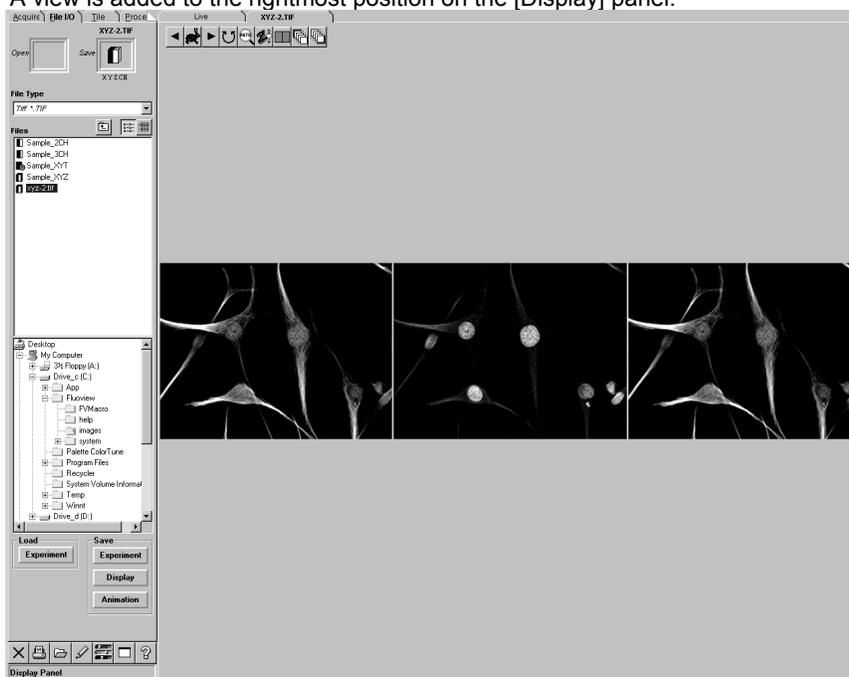


Fig. 2-71 [Display] Panel After View Addition



Up to 6 views can be displayed at once.

2-5-5-2 Decreasing the Number of Divided Images

1. Display the [Display] panel of the image to be changed at the front.

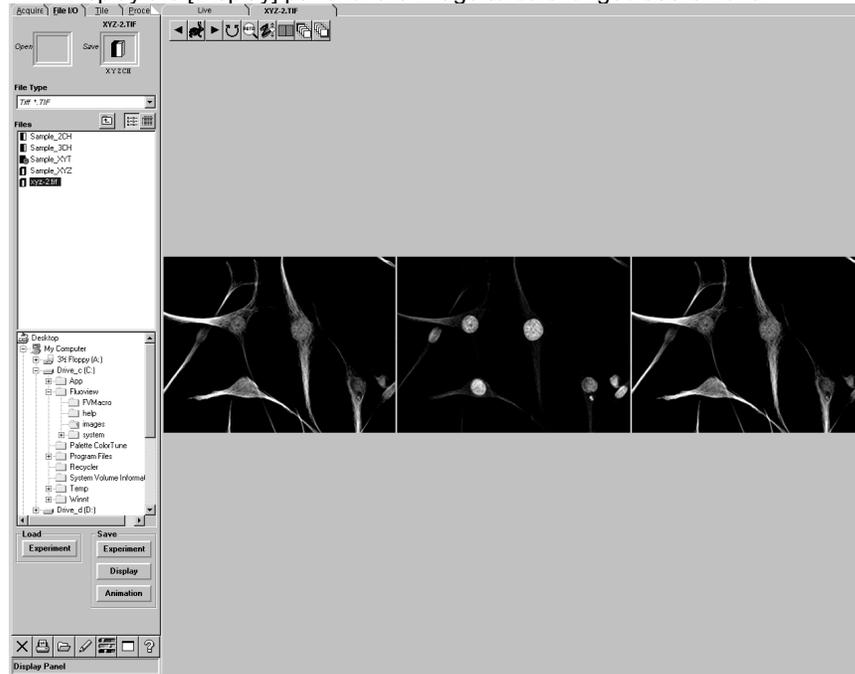
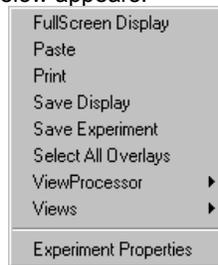
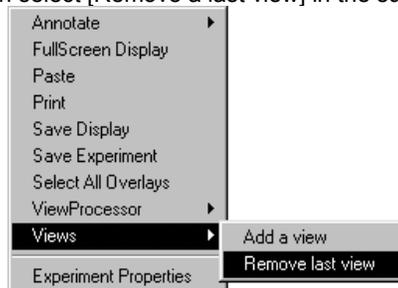


Fig. 2-72 [Display] panel

2. Right-click the image.
A pop-up menu as shown below appears.



3. Select [Views], then select [Remove a last view] in the sub-menu.



4. A view is removed from the [Display] panel.

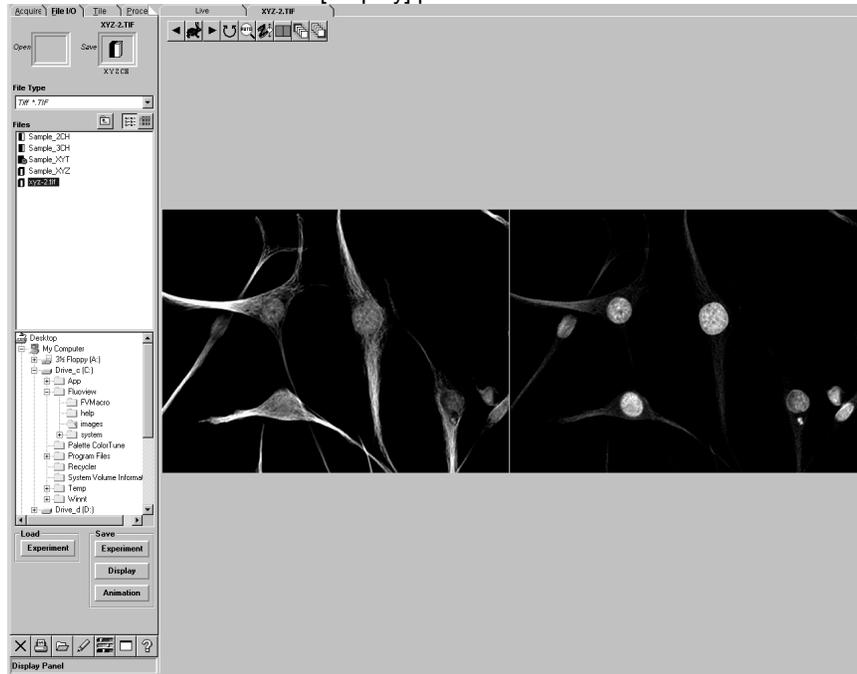
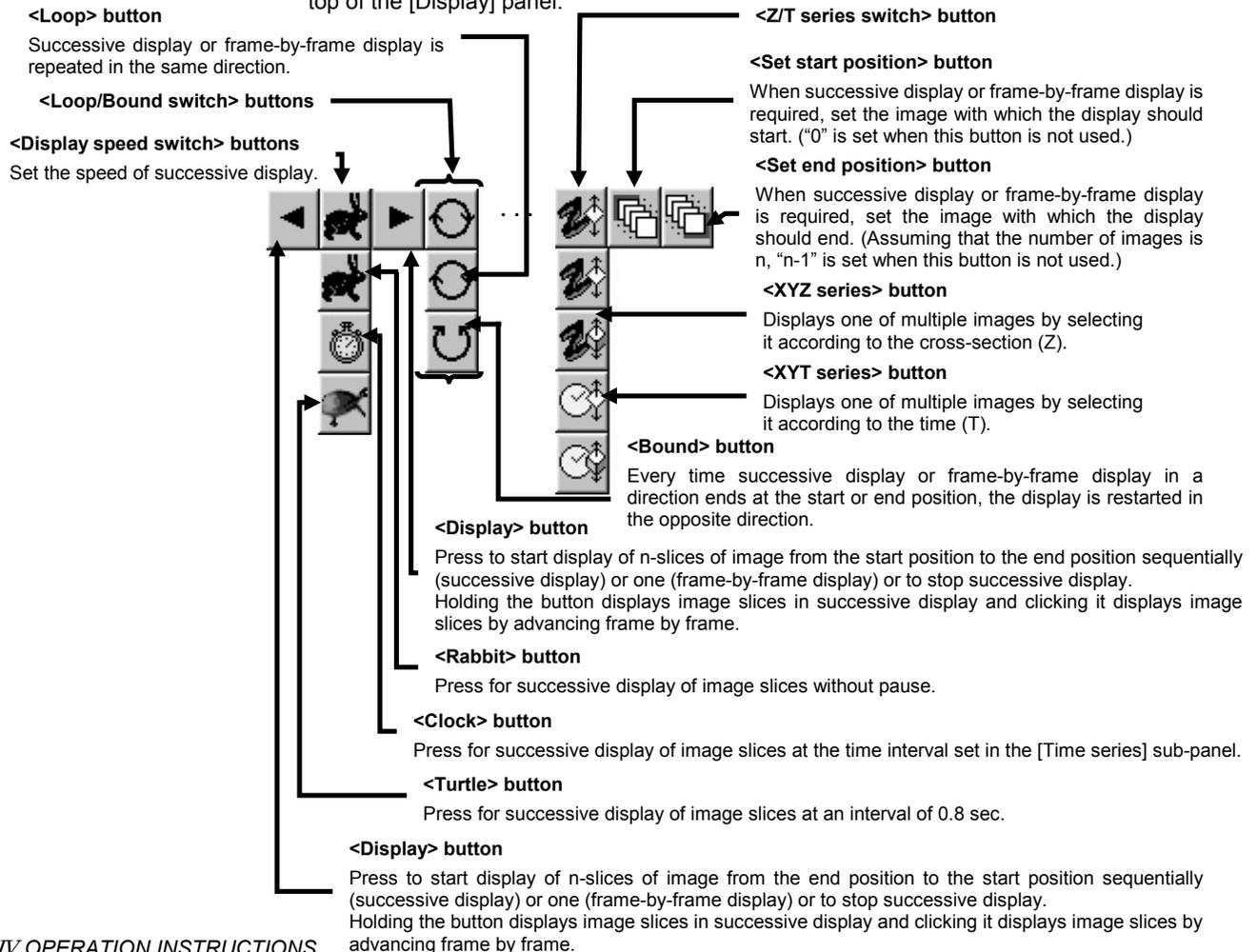


Fig 2-73 [Display] panel after View Removal

2-5-6 Switching the Display Method of Multiple Images

With images composed of multiple slices, such as time-lapse images or images acquired by changing the cross-sections, the image to be displayed at the front position can be switched or the images can be displayed successively.

1. Display the [Display] panel of the multiple images.
The buttons as shown below are displayed at the top of the [Display] panel.
2. To switch the image to the image of another cross-section, click the <Z/T series switch> button then, from the displayed list of buttons, click the <XYZ series> button.
To switch the image to the image of another moment in the elapsed time, click the <Z/T series switch> button then, from the displayed list of buttons, click the <XYT series> button. (The icon in the <Z/T series switch> button will change to the icon of the selected button.)
3. Display the image to be displayed at the front by using the <Display> buttons at the top of the [Display] panel.





Click and hold the <Display> button for successive display. To stop it, click the <Display> button again.
Simply click the <Display> button for frame-by-frame display.

2-5-7 Displaying Multiple Image Slices Together

With images composed of multiple slices, such as time-lapse images or images acquired by changing the cross-sections, the image slices can be displayed together for simultaneous viewing. However, note that the size per image reduces when the number of displayed image slices increases.

Use the [Tile] panel for displaying images together.

Display the [Tile] panel.

Icon of the displayed images (image slices shown together).

[Tiling] group box

Shows how the images are displayed in the [Display] panel.

[Columns] text box

Sets the number of columns, or the number of image slices displayed in a horizontal row.

[Experiment]

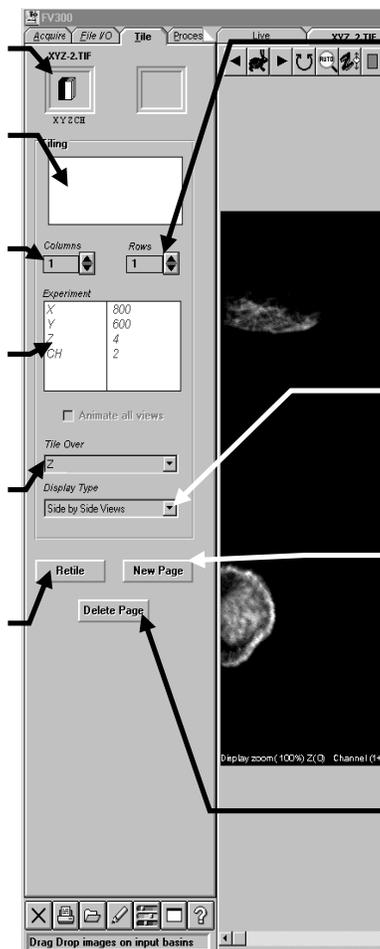
Shows the acquisition parameters used in acquisition of the displayed images.

[Tile Over] drop-down list

Select the acquisition parameter to be based on when arranging the images. [Self], [Z] or [T] can be selected.

<Retile> button

Displays the images by arranging them in the currently displayed [Display] panel.



[Rows] text box

Sets the number of rows, or the number of image slices displayed per vertical column.

[Display Type] drop-down list

Select the display method. [Single View], [Side by Side Views] or [Over and Under Views] can be selected.

[New Page] button

Displays images by arranging them in a new [Display] panel.

[Delete Page] button

Deletes the [Display] panel being displayed.

Fig. 2-74 [Tile] panel

2-5-7-1 Displaying Multiple Images Per Channel

1. Display the [Display] panel of one the images which are to be displayed together. The icon of the image is displayed in the frame at the top left of the [Tile] panel and the acquisition parameters used in image acquisition are displayed in the [Experiment] panel.
2. Set the number of images to be displayed together by using the <▲> or <▼> buttons in the [Columns] and [Rows] text boxes. How the images will be arranged can be confirmed in the gray box at the upper part of the [Tiling] group box.
3. When there are multiple images to be displayed, select the following items in the [Tile Over] drop-down list.
 - Self: The same images as the image being displayed will be displayed.
 - Z: Images are displayed according to change in cross-section.
 - T: Images are displayed according to change in time.
4. Select the display method from the [Display Type] drop-down list.
5. Click the <New Page> button. A new [Display] panel appears showing the images displayed per channel.

NOTE Use the <Retile> button when it is required to re-arrange the images in the currently displayed [Display] panel.

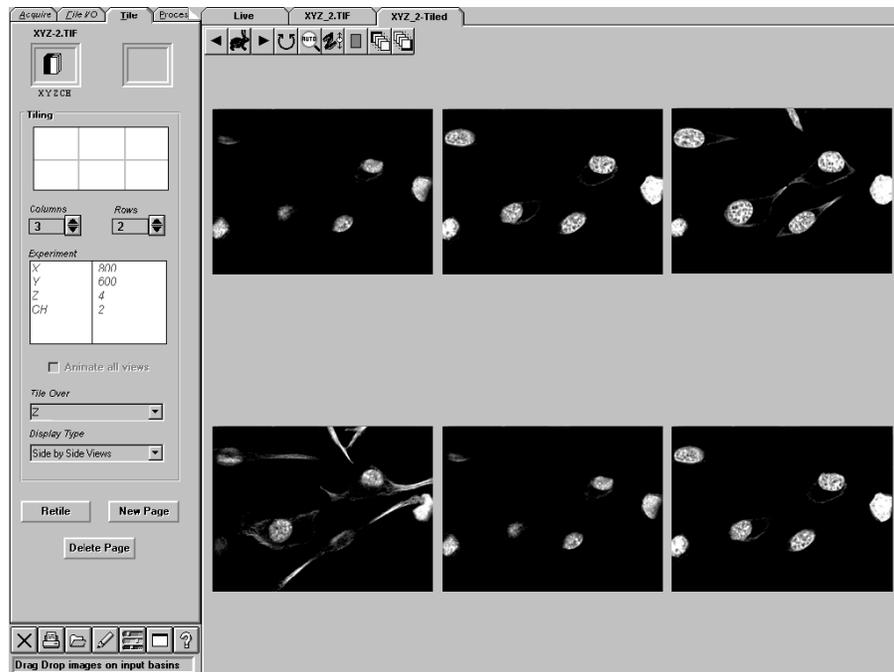


Fig. 2-75 Panel Displaying Images Per Channel

2-5-7-2 Displaying Images of Two Channels Together

Images acquired in a 2-channel mode can be displayed together for simultaneous view. The operation method is identical to the method for displaying images per channel except for the following point. See section 2-5-7-1, “Displaying Multiple Images Per Channel”.

- With images acquired in a multi-channel mode, select the display method from the [Display Type] drop-down list.

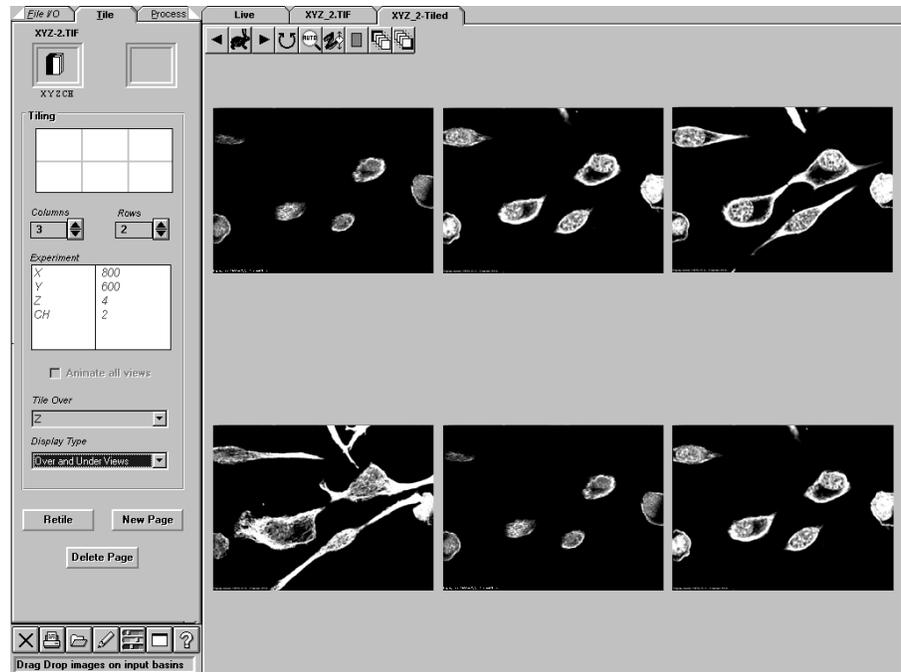


Fig. 2-76 Panel Displaying Images by Overlaying Different Channels

2-5-7-3 Displaying Time-Lapse Images

Multiple images acquired over time can be displayed side by side for simultaneous view.

1. Display the [Display] panel of one of the time-lapse images to be displayed together.
2. Set the number of images to be displayed together by using the <▲> and <▼> buttons in the [Columns] and [Rows] text boxes. How the images will be arranged can be confirmed in the gray box at the upper part of the [Tiling] group box.
3. Select [T] from the [Tile Over] drop-down list.
4. When the time-lapse images were acquired in a multi-channel mode, select the display method from the [Display Type] drop-down list.
5. Click the <New Page> button. A new [Display] panel appears showing the images displayed per channel.



Use the <Retile> button when it is required to re-arrange the images in the currently displayed [Display] panel.

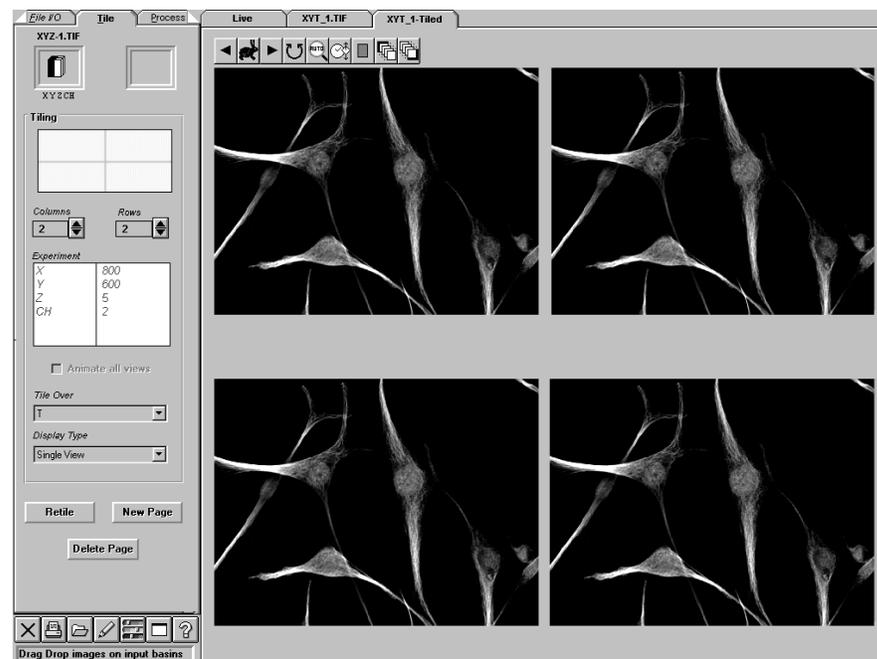


Fig. 2-77 Panel Showing Time-Lapse Images Together

2-5-7-4 Displaying Multiple Cross-Section Images

Images acquired from different cross-sections can be displayed together for simultaneous view.

The operation method is identical to the method for displaying time-lapse images except for the following point. See section 2-5-7-3, “Displaying Time-Lapse Images”.

- Select [Z] from the [Tile Over] drop-down list.

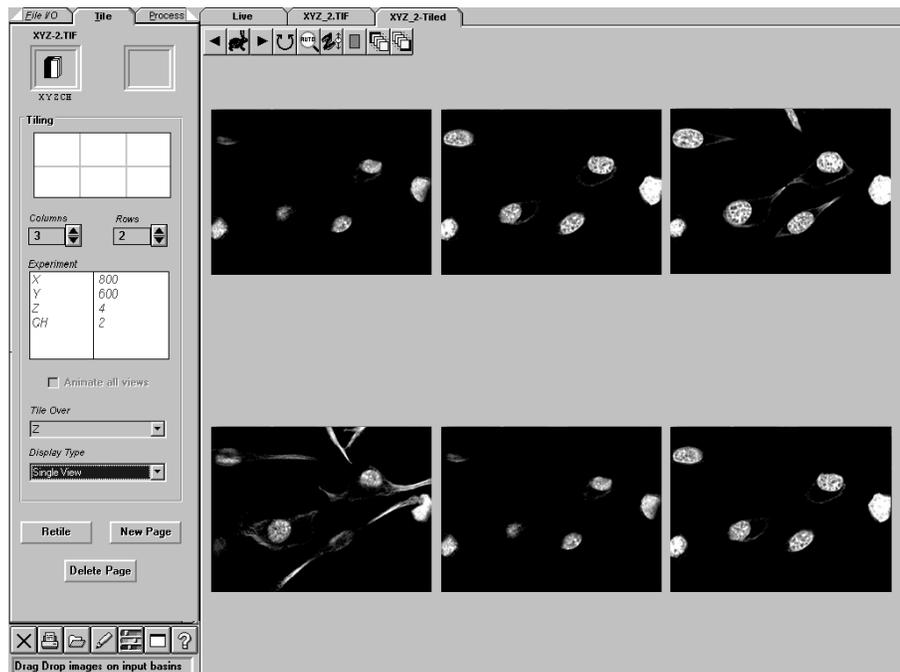


Fig. 2-78 Panel Displaying Different Cross-Section Images Together

2-5-7-5 Displaying Same Images in Different Display Methods

Images composed of multiple image slices can be displayed in more than one display methods together.

1. Display the [Display] panel of one of the images (image composed of multiple slices) to be displayed together.
The icon of the image is displayed in the frame at the top left of the [Tile] panel and the acquisition parameters used in image acquisition are displayed in the [Experiment] panel.
2. Set the number of images to be displayed together by using the <▲> and <▼> buttons in the [Columns] and [Rows] text boxes. How the images will be arranged can be confirmed in the gray box at the upper part of the [Tiling] group box.
3. Select [Z] or [T] from the [Tile Over] drop-down list.



- Click the <New Page> button. A new [Display] panel appears showing the same images as the displayed image. The number of the displayed images is as set in step 2 above.



NOTE Use the <Retile> button when it is required to re-arrange the images in the currently displayed [Display] panel.

- Click the top left image of the displayed images.
Buttons appear above the clicked image.
- Switch the image display using the displayed buttons.



TIP For details on the display switching method, see sections 2-5-3, “Switching the Displayed Channels”, 2-5-4, “Displaying Merged Image of Multiple Channels” and 2-5-5 “Switching the Display Method of Multiple Images”.

- Perform steps 5 and 6 above for each of the displayed images.

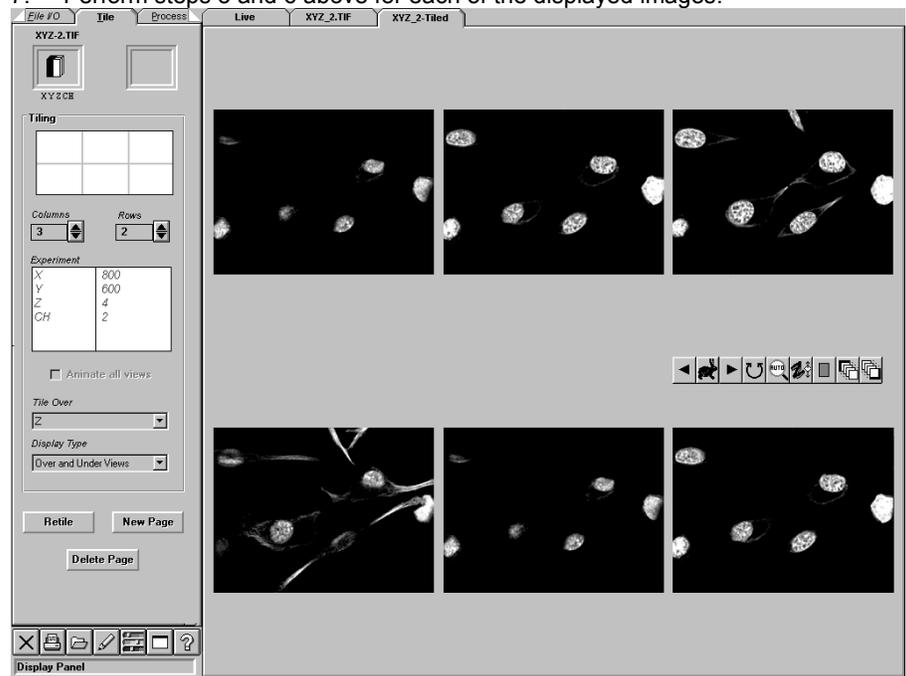


Fig. 2-79 Panel Showing 2-Channel Mode Images in Different Display Methods

Fig. 2-79 shows a panel where the display methods are varied as shown below.



2-5-7-6 Re-arranging Images Using the Same Display Method

All of the images displayed together in a [Display] panel can be rearranged simultaneously based on the same display method (channels, magnification, scroller position).

1. Click one of the images displayed together.
Two sets of buttons appear above and below the clicked image.
2. Change the image display method using the displayed buttons.
3. Click the <Retile> or <New Page> button. All of the images in the panel are re-displayed using the same display method as that set in step 2 above.

2-5-7-7 Displaying Different Images Together

Two completely different images can be displayed together.

The two images to be displayed together should be acquired by observation or loaded by opening a file. If two images are not available, prepare them by image acquisition or file opening.

1. Display the [Display] panel of either image to be displayed with another image.
The icon of the image is displayed in the frame at the top left of the [Tile] panel and the acquisition parameters used in image acquisition are displayed in the [Experiment] panel.



<Experiment List> button

2. Click the <Experiment List> button in the toolbar at the bottom of the [Tile] panel. The [Experiments in Memory] dialog box appears as shown below.

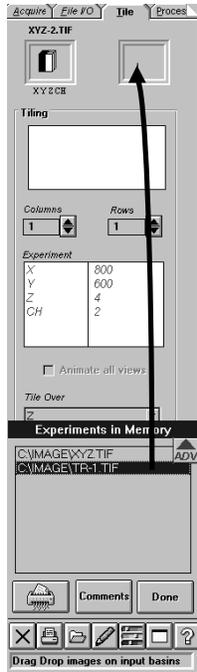


Fig. 2-80 [Experiments in Memory] Dialog Box

3. From the [Experiments in Memory] dialog box, select the file name of the second image to be displayed and drag it into the frame at the top right of the [Tile] panel. The icon of the second image is displayed in the frame at the top left of the [Tile] panel and the acquisition parameters used in the image acquisition are displayed in the [Experiment] panel.

TIP The mouse pointer turns into the image icon during dragging.

4. Click the <Done> button in the [Experiments in Memory] dialog box to close it.
5. Set the number of images to be displayed together by using the <▲> or <▼> buttons in the [Columns] and [Rows] text boxes. How the images will be arranged can be confirmed in the gray box at the upper part of the [Tiling] group box.
6. When there are multiple images to be displayed, select the following items in the [Tile Over] drop-down list.
 - Self: The same images as the image being displayed will be displayed.
 - Z: Images are displayed according to change in cross-section.
 - T: Images are displayed according to change in time.



7. When displaying images acquired in a multi-channel mode, select the display method from the [Display Type] drop-down list.

8. Click the <New Page> button. A new [Display] panel appears showing the two images one above the other.

The image of the file displayed in the frame at the top left of the [Tile] panel is displayed on the upper part of the [Display] panel, and that of the file displayed in the frame at the top right is displayed on the lower part.



Use the <Retile> button when it is required to re-arrange the images in the currently displayed [Display] panel.

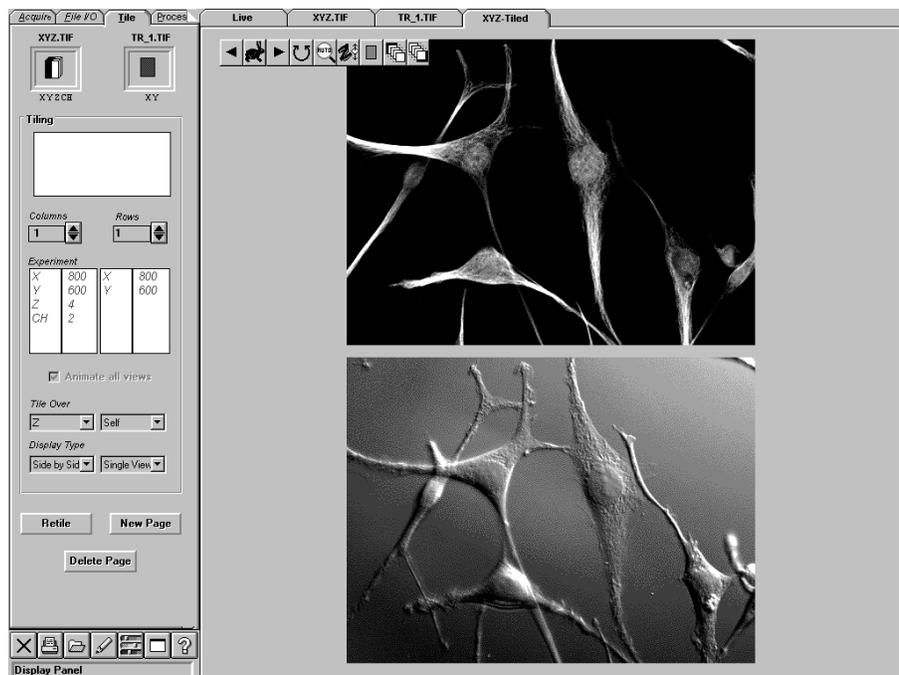


Fig. 2-81 Panel Displaying Two Different Images Together

2-5-8 Displaying an Image in Full Screen

Only the image itself can be displayed to fill the screen by erasing all other display components such as the toolbar, panel and status bar. This feature is useful for taking pictures using an analog printer for creation of a slide.



<Full Screen> button

1. Display the [Display] panel of the image to be displayed.
2. Click the <Full Screen> button in the toolbar at the bottom left of the screen.

TIP

When the mouse left button is clicked while the image is displayed full screen, the buttons which are usually displayed on the top of the [Display] panel (<Display> buttons, etc.) and those usually displayed on the bottom right (<Display channel switch> button) can be displayed in the image.

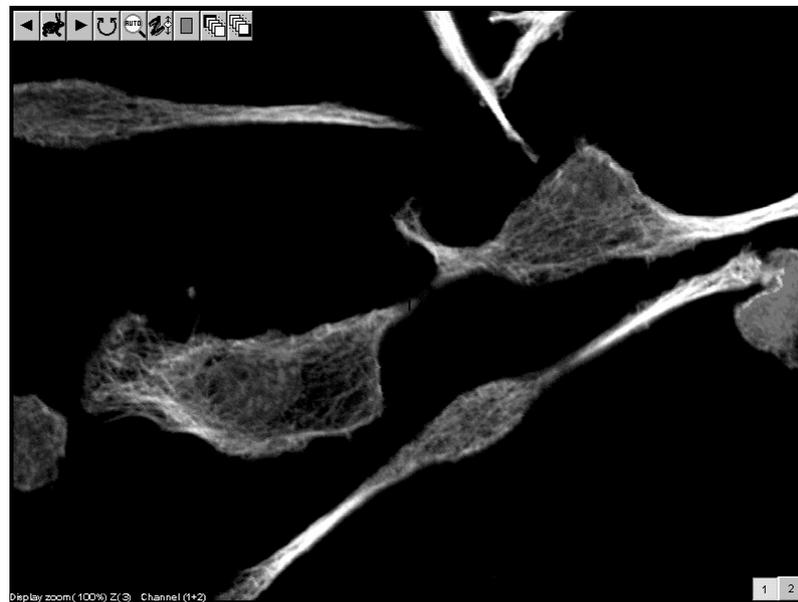


Fig. 2-82 Panel Showing the <Display> and Other Buttons

3. Click the mouse right button on the image to cancel the full-screen display.

One Point!

The image can also be displayed full screen by the mouse operation on the image.

1. Display the [Display] panel of the image to be displayed full screen, and click the mouse right button on the image.
2. A pop-up menu as shown below appears.
3. Select [FullScreen Display] from the menu.



2-5-9 Magnifying/Reducing an Image

The image can be magnified or reduced using the buttons displayed at the top of the [Display] panel. Magnification or reduction up to 3:1 or 1:3 the original image is possible.

1. Display the [Display] panel of the image to be magnified or reduced.
2. The buttons as shown below are displayed on the top of the [Display] panel.
(Usually, the <Auto> button is displayed, and clicking it displays the list of buttons shown below.) Use these buttons to magnify or reduce the image.

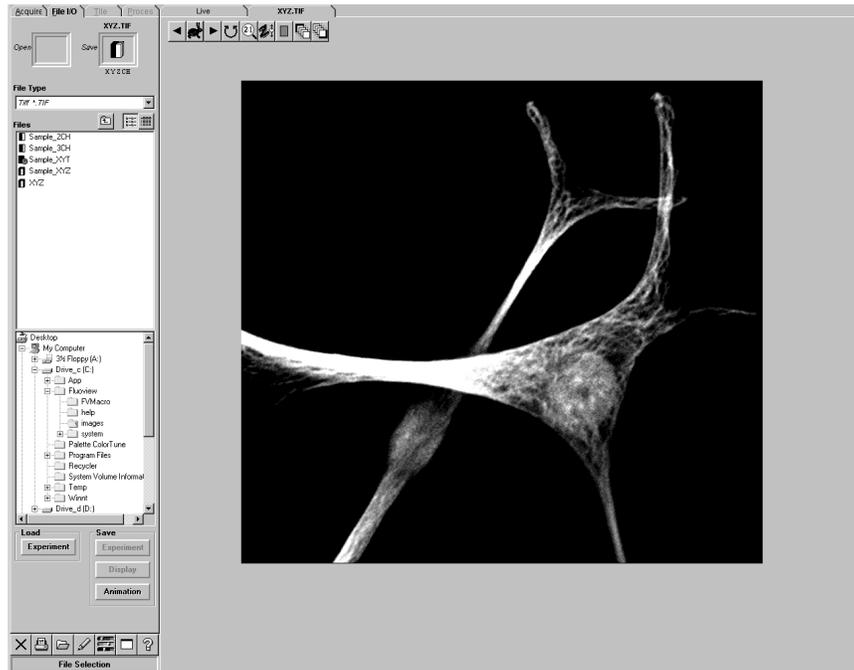
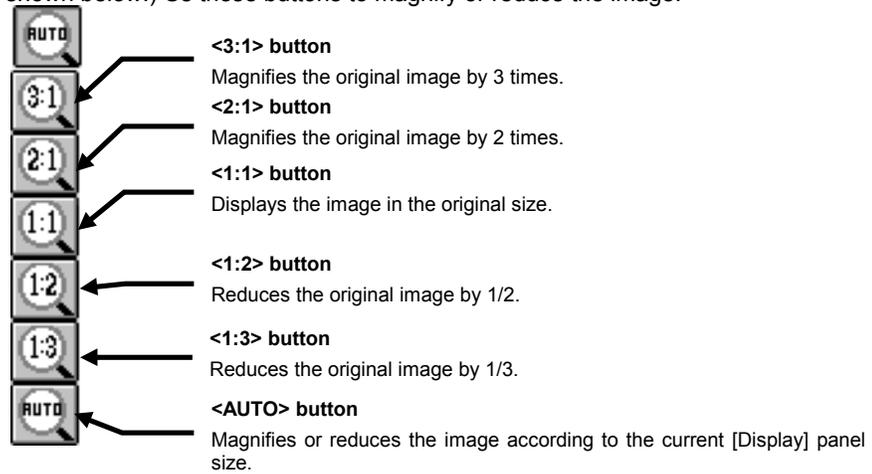


Fig. 2-83 Panel Showing a 2:1 Magnified Image

2-6 Image Processing

Images can be processed using the [Process] panel. Display the [Process] panel at the front.

2-6-1 Filtering

Use the [Filters] sub-panel in the [Process] panel to apply filtering to images.

1. Display the [Filters] sub-panel in the [Process] panel at the front.

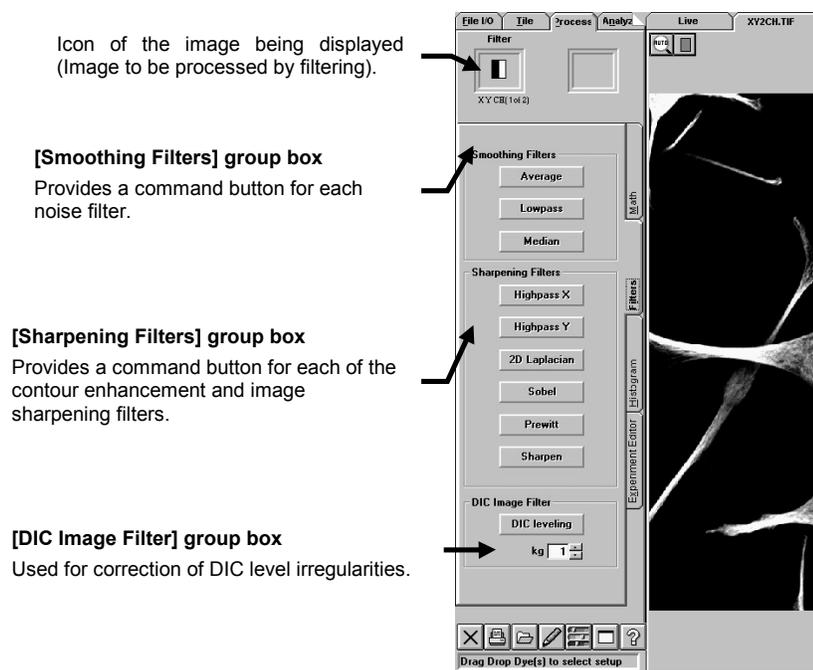


Fig. 2-84 [Filters]Sub-panel

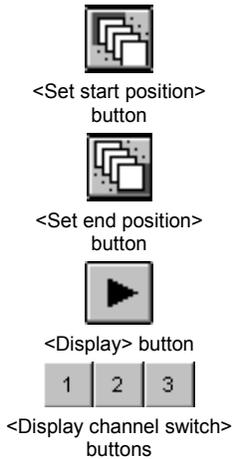
2-6-1-1 Contour Enhancement

When an image is blurred by the boundaries between image grains becoming unclear, it can be sharpened by applying contour enhancement. Five types of filters are provided for use in the contour enhancement.

1 Laplacian filter

This filter enhances the contours of the image grains. If the image contains noise, the noise is also enhanced. By adding the original image to the image processed with Laplacian filtering, it is possible to obtain an image with stronger contour enhancement. The filter format is as shown below.

$$\begin{bmatrix} -1 & -1 & -1 \\ -1 & 8 & -1 \\ -1 & -1 & -1 \end{bmatrix}$$



1. Display the [Display] panel of the image to be processed with Laplacian filtering.
2. When the image is composed of multiple image slices, the range of image slices to be filtered can be specified using the <Set start position> and <Set end position> buttons above the image. First display the image slice to start filtering using the <Display> button and click the <Set start position> button. Then, set the image slice to end filtering in the same way as above.
3. When the image to be filtered was acquired in the multi-channel mode, filtering is applied only to the channels being displayed.
Example) When only the Ch1 image is displayed, filtering is applied to the Ch1 image only.

TIP For the switching of channels, see section 2-4-3, "Switching the Display Channels".



4. Click the <2D Laplacian> button. A new [Display] panel having the page tab named [Filter] appears, showing the filtered image.

TIP During filtering, the status bar shows the progress of processing.

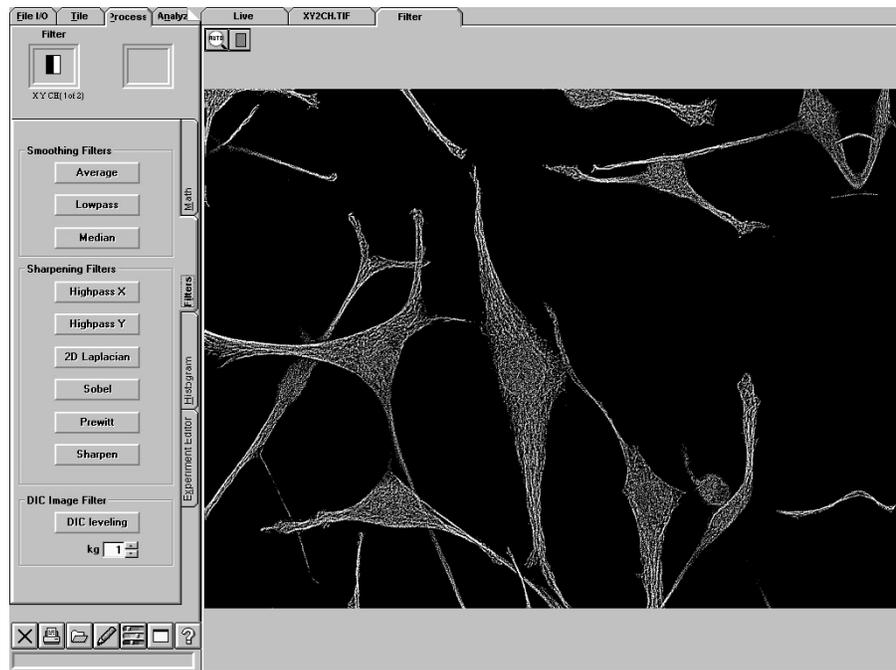


Fig. 2-85 Panel Displaying the Laplacian Filtered Image

2 Sobel filter

This filter enhances the contours of the image grains. If the image contains noise, the noise is also enhanced. It has two filter formats, X and Y, as shown below. The format providing the larger value after filtering is used.

$$\begin{pmatrix} -1 & -2 & -1 \\ 0 & 0 & 0 \\ 1 & 2 & 1 \end{pmatrix} \quad \begin{pmatrix} -1 & 0 & 1 \\ -2 & 0 & 2 \\ -1 & 0 & 1 \end{pmatrix}$$

The operation method is identical to Laplacian filtering except for the following point. See section 2-6-1-1-1, "Laplacian filter".



<Sobel> button

- Click the <Sobel> button.

3 High-pass X filter

The HIGH-PASS X filter passes the high-frequency structures in the X-direction of image. In this way, it can extract details by detecting positions with large variation. This processing is useful for making structures clear or extract the edges. The filter format is as shown below.

$$\begin{pmatrix} -1 & -1 & -1 \\ 0 & 0 & 0 \\ 1 & 1 & 1 \end{pmatrix}$$

The operation method is identical to Laplacian filtering except for the following point. See section 2-6-1-1-1, "Laplacian filter".



<Highpass X> button

- Click the <Highpass X> button.

4 High-pass Y filter

The HIGH-PASS Y filter passes the high-frequency structures in the Y-direction of image. In this way, it can extract details by detecting positions with large variation. This processing is useful for making structures clear or extract the edges. The filter format is as shown below.

$$\begin{pmatrix} 1 & 0 & -1 \\ 1 & 0 & -1 \\ 1 & 0 & -1 \end{pmatrix}$$

The operation method is identical to Laplacian filtering except for the following point. See section 2-6-1-1-1, "Laplacian filter".



<Highpass Y> button

- Click the <Highpass Y> button.

5 Prewitt filter

This filter enhances the contours of image grains in a similar way to the Sobel filter, but more strongly than it. It has two filter formats, X and Y, as shown below. The format providing the larger value after filtering is used.

$$\begin{matrix} & X & & & Y \\ \begin{pmatrix} -2 & -2 & -2 \\ 0 & 0 & 0 \\ 2 & 2 & 2 \end{pmatrix} & & & & \begin{pmatrix} -2 & 0 & 2 \\ -2 & 0 & 2 \\ -2 & 0 & 2 \end{pmatrix} \end{matrix}$$

The operation method is identical to Laplacian filtering except for the following point. See section 2-6-1-1-1, "Laplacian filter".



<Prewitt> button

- Click the <Prewitt> button.

2-6-1-2 Noise Reduction

When random noise interferes with an image, its irregularity increases and it become harder to see. Such noise can be reduced by means of filtering. Three kinds of filters are available for noise reduction.

1 Averaging filter

The averaging filter is used to eliminate details in image or reduce noise. However, as it makes everything in an image smooth, it also makes the edge sections dull, which sometimes result in the image resolution deterioration. The filter format is as shown below.

$$\begin{pmatrix} 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \end{pmatrix} \times 1/9$$

The operation method is identical to Laplacian filtering except for the following point. See section 2-6-1-1-1, "Laplacian filter".



<Average> button

- Click the <Average> button.

2 Median filter

The Median filter reduces noise in image while leaving the edges intact. However, it may be ineffective in case noise is concentrated in some positions or the image is very noisy.

The operation method is identical to Laplacian filtering except for the following point. See section 2-6-1-1-1, "Laplacian filter".



<Median> button

- Click the <Median> button.

3 Low-pass filter

The low-pass filter passes the low-frequency structures. In this way, it can eliminate small grains and provide smooth, noise-reduced image. filter format is as shown below.

$$\begin{bmatrix} 1 & 2 & 1 \\ 2 & 4 & 2 \\ 1 & 2 & 1 \end{bmatrix} \times 1/16$$

The operation method is identical to Laplacian filtering except for the following point. See section 2-6-1-1-1, "Laplacian filter".



<Lowpass> button

- Click the <Lowpass> button.

2-6-1-3 Image Sharpening

1 Sharpen filter

The sharpen filter turns blurred image into a clear image. The filter format is as shown below.

$$\begin{bmatrix} 0 & -1 & 0 \\ -1 & 5 & -1 \\ 0 & -1 & 0 \end{bmatrix}$$

The operation method is identical to Laplacian filtering except for the following point. See section 2-6-1-1-1, "Laplacian filter".



<Sharpen> button

- Click the <Sharpen> button.

2-6-1-4 Correcting DIC Level Irregularities

DIC level irregularities refers to uneven brightness of the image background which may be observed when a transmitted image is acquired in IDC observation.

The DIC level irregularities can be corrected to make the image easier to view.

If fluorescence observation is used, note that the correction is possible only with the transmitted images.

1. Display the [Filters] sub-panel in the [Process] panel.

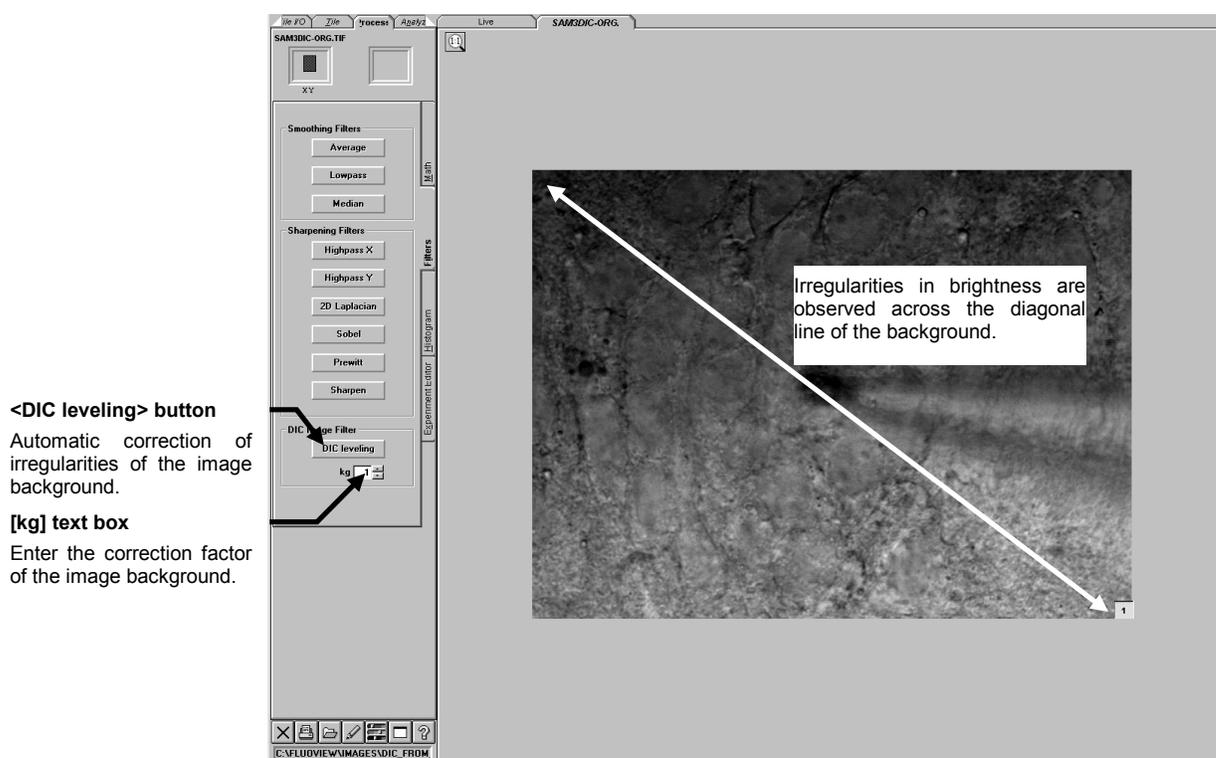


Fig. 2-86 [Filters] Sub-panel

2. Enter the appropriate correction factor for the image background in the [kg] text box.

TIP The correction factor can be set between 0 and 1 in steps of 0.1.
The standard factor is 1. If the effect is extreme, try using 0.5.



3. Click the <DIC leveling> button.

The [Level] panel is newly created in the [Display panel] and shows an image after the correction of the DIC level irregularities of the background.

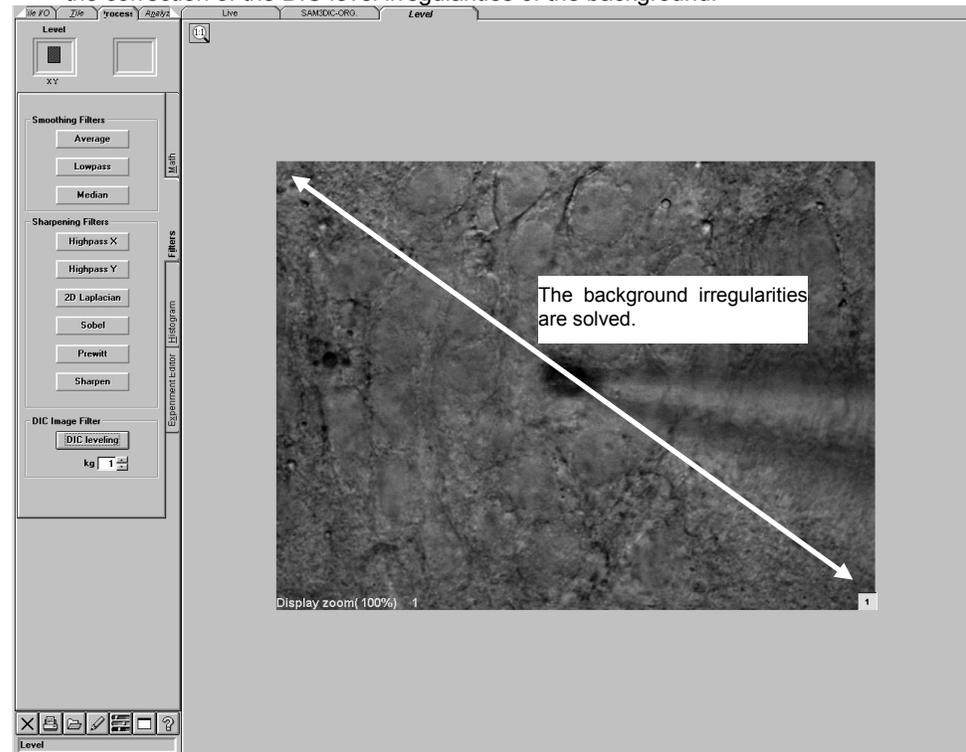


Fig. 2-87 [Level] Panel After DIC Level Correction

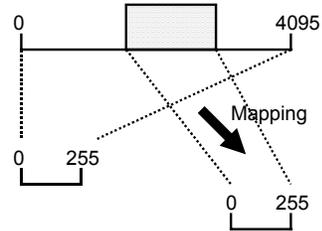
4. If the DIC level irregularities cannot be reduced, repeat steps 1 to 3 above by varying the correction factor.

2-6-2 Contrast Conversion

The LUT intensity can be mapped (re-assigned) while observing a histogram.

Mapping (re-assignment) results in changing the image contrast.

An image acquired by observation contains intensity information in values from 0 to 4095, but the intensity information used in actual display takes values from 0 to 255 by assigning the original values from 0 to 4095 to values from 0 to 255 usually. This facility changes the contrast by noticing a certain section between 0 and 4095 and mapping this section to values between 0 and 255.



1. Display the [Process] panel at the front.
2. Display the [Histogram] sub-panel of the [Process] panel at the front.

Sets the image to be subjected to contrast change. The icon of the image is displayed here.

[Channel] option button
Select the channel to be subjected to LUT intensity mapping (re-assignment).

<Compute Histogram> button
Displays the histogram.

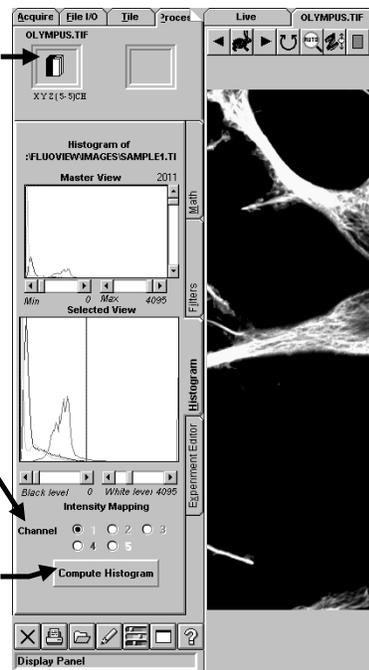


Fig. 2-88 [Histogram] Sub-panel

3. Display the [Display] panel of the image you want to change the contrast.
The icon of the image is displayed in the frame at the top left of the [Process] panel



<Display channel switch>
buttons

- When the image was acquired in the multi-channel mode, select whether the LUT intensity mapping (re-assignment) is applied to multiple channels simultaneously or to a single channel.

To select the target channel(s), use the <Display channel switch> buttons. The histogram of the selected channel(s) is displayed.

Example) When only the Ch1 image is displayed, the histogram of Ch1 is displayed and mapping of only the Ch1 image is possible.



For the switching of channels, see section 2-4-3, "Switching the Display Channels".

- Click the <Compute Histogram> button. A histogram as shown in Fig. 2-89 appears.

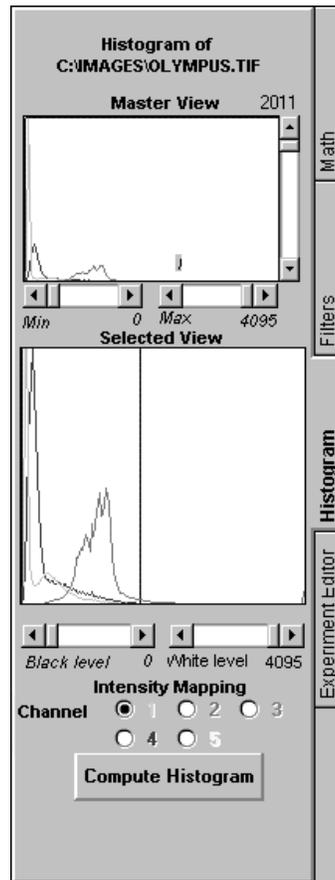


Fig. 2-89 Histogram

6. Enclose the histogram section of interest using the scale in the [Master View] field.
7. The magnified view of the region selected by the scale is shown in the [Selected View] field.

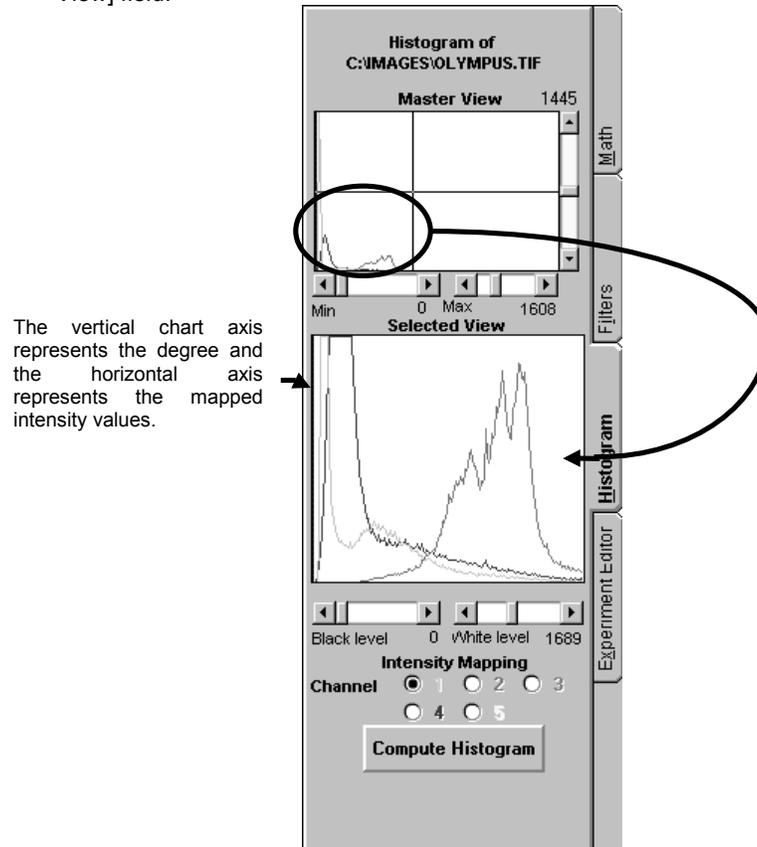


Fig. 2-90 Histogram of the Selected Region

8. When the image was acquired in the multi-channel mode, select the channel(s) to be subjected to LUT intensity mapping (re-assignment) using the [Channel] option buttons.
9. Select the region to be subjected to mapping (re-assignment) using the scale in the [Selected View] field. While moving the scale, confirm the change in contrast in the [Display] panel. The intensity values in the selected region are mapped (re-assigned) to intensity values from 0 to 255 and displayed.

2-6-3 Mathematical Operations Between Images

Arithmetic or logical operations can be applied between two different images or between an image and a constant.

2-6-3-1 Image Addition

Addition of an image to an image (constant to an image) is possible as described below.

1. Display the [Math] sub-panel of the [Process] panel.

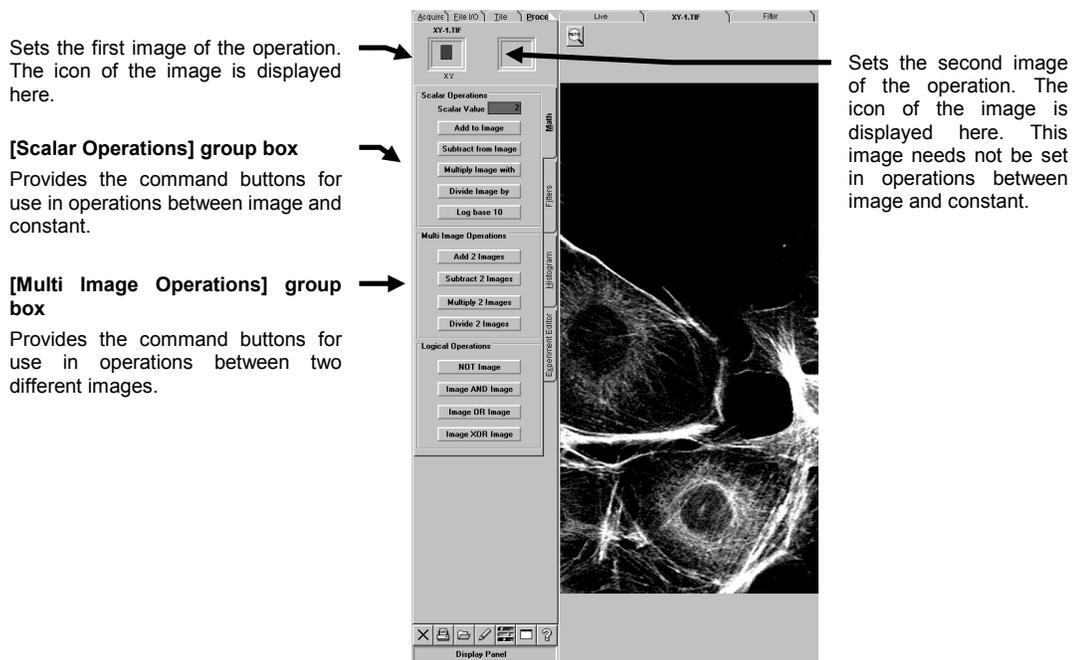


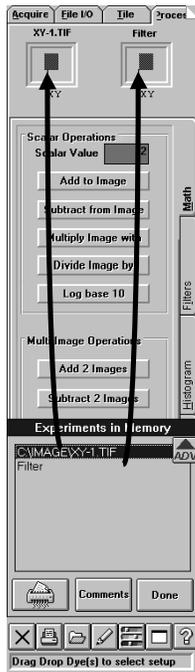
Fig. 2-91 [Math] Sub-panel



- Click the <Experiment List> button in the toolbar at the bottom of the [Process] panel. The [Experiments in Memory] dialog box appears as shown below.



Fig. 2-92 [Experiments in Memory] Dialog Box



- From the [Experiments in Memory] dialog box, select the file name of the first image and drag it to the frame at the top left of the [Process] panel. The icon of the image is displayed in the frame at the top left of the [Process] panel.

TIP Before the dragging and dropping, the frame at the top left shows the icon of the image file displayed in the Display panel.

TIP The mouse pointer turns into the image icon during dragging.

- From the [Experiments in Memory] dialog box, select the file name of the second file and drag it to the frame at the top right of the [Process] panel. The icon of the image is displayed in the frame at the top right of the [Process] panel.

(No second file dragging is needed when the image processing is by value.)

TIP The mouse pointer turns into the image icon during dragging.

- Click the <Done> button in the [Experiments in Memory] dialog box to close it.



6. Enter the constant for use in operation in the [Scalar Value] text box in the [Scalar Operations] group box.

(This step is required only for operation between an image and a constant.)

7. **To add an image to an image:**



Click the <Add 2 Images> button in the [Multi Image Operations] group box. A new [Display] panel showing [Image+Image] in the page tab appears, displaying the image obtained by the addition operation.



To add a constant to an image:

Click the <Add to Image> button in the [Scalar Operations] dialog box. A new [Display] panel showing [Image+Const] in the page tab appears, displaying the image obtained by the addition operation.

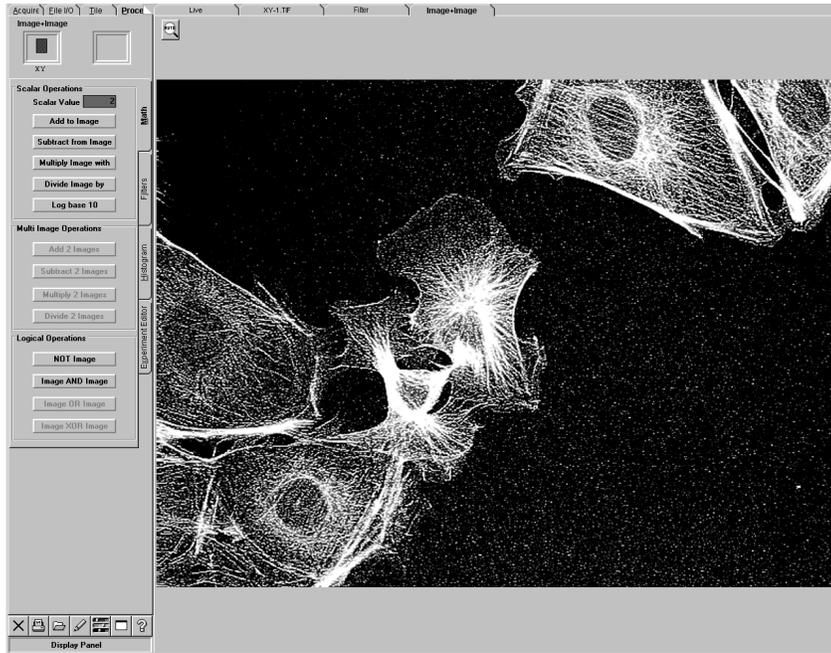


Fig. 2-93 [Image+Image] Panel

2-6-3-2 Image Subtract

Subtraction of an image from an image (constant from an image) is possible as described below.

The operation method is identical to Image + Image (Image + Constant) except for the following point. See section 2-6-3-1, "Image + Image (Image + Constant)".

Subtract 2 Images

<Subtract 2 Images>
button

- **To subtract an image from an image:**

Click the <Subtract 2 Images> button in the [Multi Image Operations] group box. A new [Display] panel showing [Image-Image] in the page tab appears, displaying the image obtained by the subtraction operation.

Subtract from Image

<Subtract from Image>
button

- **To subtract a constant from an image:**

Click the <Subtract from Image> button in the [Scalar Operations] group box. A new [Display] panel showing [Image-Const] in the page tab appears, displaying the image obtained by the subtraction operation.

2-6-3-3 Image Multiplication

Multiplication of an image by an image (image by a constant) is possible as described below.

The operation method is identical to Image + Image (Image + Constant) except for the following point. See section 2-6-3-1, "Image + Image (Image + Constant)".

Multiply 2 Images

<Multiply 2 Images>
button

- **To multiply an image by an image:**

Click the <Multiply 2 Images> button in the [Multi Image Operations] group box. A new [Display] panel showing [Image*Image] in the page tab appears, displaying the image obtained by the multiplication operation.

Multiply Image with

<Multiply Image with>
button

- **To multiply an image by a constant:**

Click the <Multiply Image with> button in the [Scalar Operations] group box. A new [Display] panel showing [Image*Const] in the page tab appears, displaying the image obtained by the multiplication operation.

2-6-3-4 Image Division

Division of an image by an image (image by a constant) is possible as described below.

The operation method is identical to Image + Image (Image + Constant) except for the following point. See section 2-6-3-1, "Image + Image (Image + Constant)".



Divide 2 Images

<Divide 2 Images>
button

● **To divide an image by an image:**

Click the <Divide 2 Images> button in the [Multi Image Operations] group box. A new [Display] panel showing [Image/Image] in the page tab appears, displaying the image obtained by the division operation.

Divide Image by

<Divide Image by>
button

To divide an image by a constant:

Click the <Divide Image by> button in the [Scalar Operations] group box. A new [Display] panel showing [Image/Const] in the page tab appears, displaying the image obtained by the division operation.

2-6-3-5 NOT Image

The NOT operation of an image allows the bright and dark areas of the image to be reversed.

1. Display the [Math] sub-panel of the [Process] panel at the front.

Sets the image to be subjected to operation. The icon of the image is displayed here.

<NOT Image> button
Executes a NOT operation.

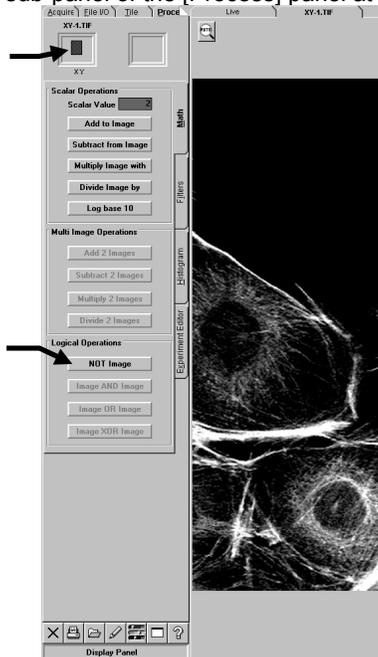


Fig. 2-94 [Math] Sub-panel

2. Display the [Display] panel of the image to be subjected to NOT operation at the front. The icon of the image is displayed in the frame at the top left of the [Process] panel.



- Click the <NOT Image> button. A new [Display] panel showing [NOT] in the page tab appears, showing the image obtained by the operation.

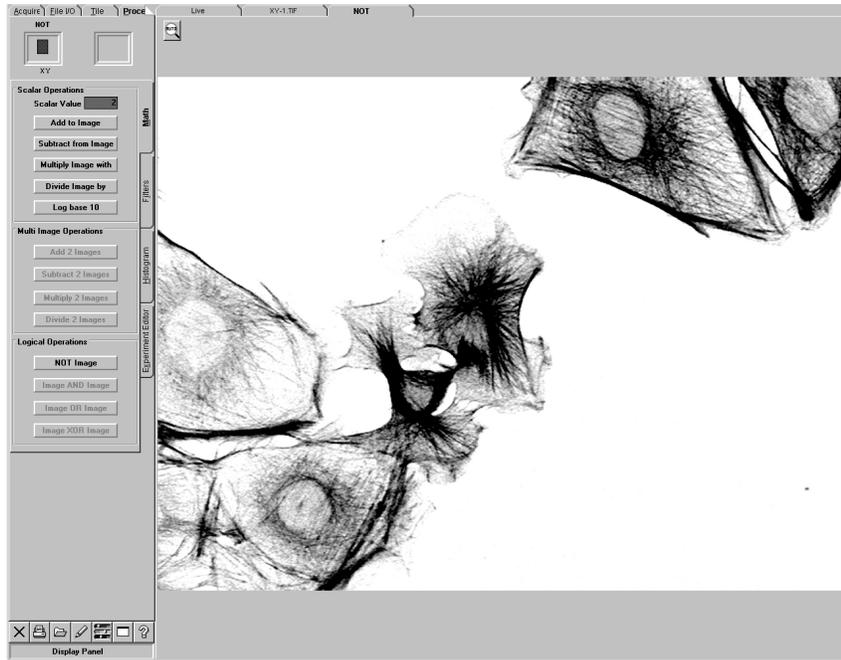


Fig. 2-95 [NOT] Panel

2-6-3-6 Image AND Image

Two different images can be ANDed.

1. Display the [Math] sub-panel of the [Process] panel at the front.

Sets the image to be subjected to operation. The icon of the image is displayed here.

Sets the second image of the operation. The icon of the image is displayed here.

<Image AND Image> button
Executes an AND operation.

<Image OR Image> button
Executes an OR operation.

<Image XOR Image> button
Executes an exclusive OR operation.

Fig. 2-96 [Math] Sub-panel

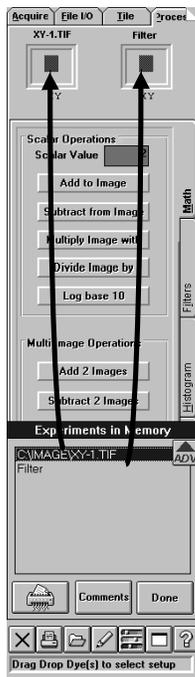


<Experiment List> button

2. Click the <Experiment List> button in the toolbar at the bottom of the [Process] panel. The [Experiments in Memory] dialog box appears as shown below.



Fig. 2-97 [Experiments in Memory] Dialog Box



- From the [Experiments in Memory] dialog box, select the file name of the first image and drag it to the frame at the top left of the [Process] panel. The icon of the image is displayed in the frame at the top left of the [Process] panel.

TIP

Before the dragging and dropping, the frame at the top left shows the icon of the image file displayed in the [Display] panel.

TIP

The mouse pointer turns into the image icon during dragging.

- From the [Experiments in Memory] dialog box, select the file name of the second file and drag it to the frame at the top right of the [Process] panel. The icon of the image is displayed in the frame at the top right of the [Process] panel.

TIP

The mouse pointer turns into the image icon during dragging.

- Click the <Done> button in the [Experiments in Memory] dialog box to close it.



Image AND Image
 <Image AND Image>
 button

- Click the <Image AND Image> button. A new [Display] panel showing [Image AND Image] in the page tab appears, showing the image obtained by the operation.

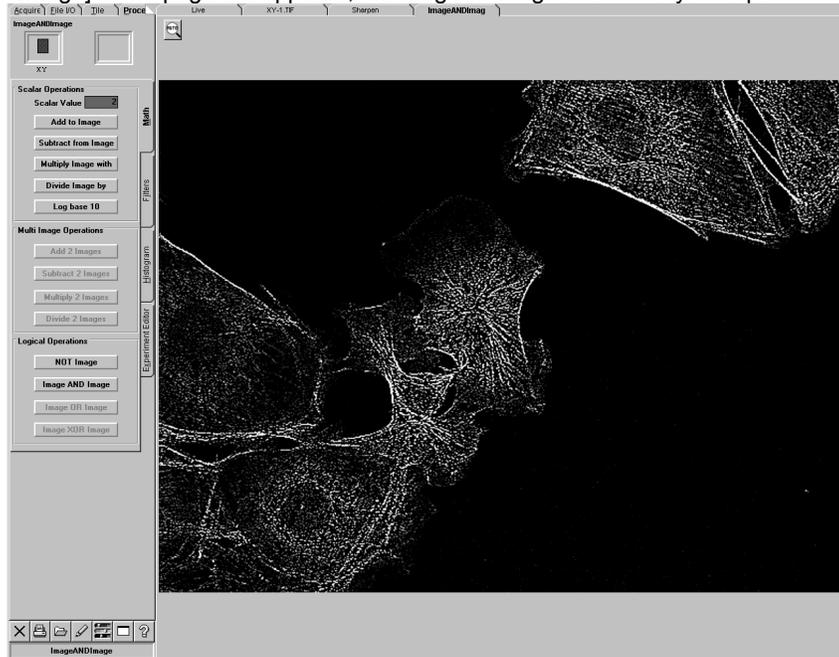


Fig. 2-98 [Image AND Image] Panel

2-6-3-7 Image OR Image

Two different images can be ORed.

The operation method is identical to the AND operation between two different images except for the following point. See section 2-6-3-6, "Image AND Image".

Image OR Image
 <Image OR Image>
 button

- Click the <Image OR Image> button. A new [Display] panel showing [Image OR Image] in the page tab appears, showing the image obtained by the operation.

2-6-3-8 Image XOR Image

Two different images can be XORed.

The operation method is identical to the AND operation between two different images except for the following point. See section 2-6-3-6, "Image AND Image".

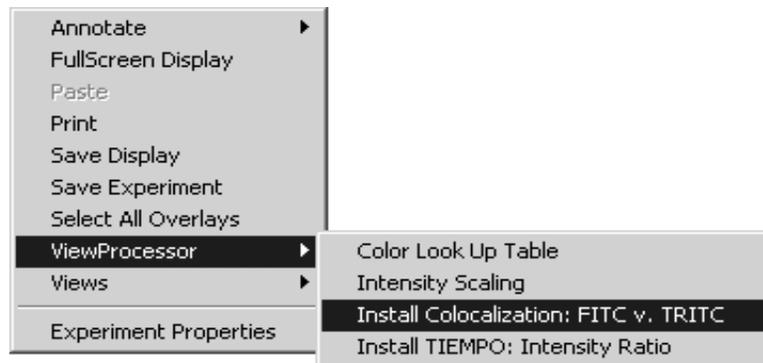
Image XOR Image
 <Image XOR Image>button

- Click the <Image XOR Image> button. A new [Display] panel showing [Image XOR Image] in the page tab appears, showing the image obtained by the operation.

2-6-4 Brightness overlap level between 2 channels (Colocalization)

This function enables to observe overlap intensity of two channel of image.

1. Acquires or opens images which more than two channels, and displays the images on [Display] panel.
2. On the [Display] panel, displays and overlaps 2 channel image that to be observed. See section 2-4-4-2 for overlapping the image display.
3. On the overlapped images, click right-mouse button to show the following pop up menu.



4. Select [Install Colocalization: ****.v ****] of [ViewProcessor]. Then [Colocalization Processor] appears.
[****] shows the dyeing method or its number of channel.

There are the following 4 Annotation Mode in [Colocalization Processor] dialog box.

- None Mode
- Threshold Mode
- Min-Max Bounds Mode
- Regions Mode

2-6-4-1 Annotation Mode

1 None Mode

None Mode is the default of [Colocalization Processor] dialog box. The mode can be changed by option button in [Annotation Mode] Group Box. The items described for this mode can be used in other modes.

[Data Selection] Group Box
<Disabled> Option Button
 Graph and Text box will disappear from [Colocalization Processor] dialog box and <Hide> button at lower right will change to <Close> button. When <Close> is clicked, the [Colocalization Processor] dialog will dismiss.

<Current slice> Option Button
 This button executes colocalization for images displayed on [Display] panel.

[Histogram] Graph
 It acquires brightness value of 1st channel selected on horizontal axis and brightness of value of other channel on vertical axis and displays brightness distribution. The point with the value which less than [LUT min] text box is colored with blue, and the point more than [LUT max] value is colored with in red; and other point will be colored by gray scale.

[Summary] Text Box
 Summary : Dye method of vertical and horizontal axis or channel number
 Mode : Option button selected at [Data Selection] group box
 Samples processed : Total numbers of pixels on whole image to be processed
 Binning : [Bin Width] Setting condition of text box
 Max. samples in any bin : Max. value in plot data on graph

[Annotation Mode] Group Box
 Select either [None], [Threshold], [Min-Max Bounds] or [Regions] option button. In case of [Threshold], [Min-Max Bounds] or [Regions] are selected, text box appears in [Annotation] Group Box. [Colocalization Processor] is displayed, [None] is selected.

<Accept Histogram> Button
 [Histogram] graph appears in [Display] panel. The graph can be handled same with the acquired image.

[Zoom] Text Box
 It sets display magnification of [Histogram] graph.

[Bin Width] Text Box
 It sets resolution of [Histogram] graph. For example, if the value is 16, it displays brightness in 16 gray-scale as one plot.

[LUT Max] Text Box
 Double-click inside text box so that the display can alternately be changed in two methods.
 1. All data uniform display
 2. Binary display of

[LUT Min] Text Box

<Hide> Button
 It hides [Colocalization Processor] dialog box.

 The [Colocalization Processor] dialog box appears again by pressing  [Colocalization Processor] button in [Display] panel.

Fig. 2-99 [Colocalization Processor] Dialog Box
 In case of Annotation Mode is [None].

2 Thresholds Mode

Specifies Threshold level on [Histogram] graph, then measures by use of the threshold level.

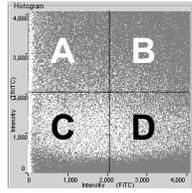
[Histogram] Graph
Threshold line vertical and horizontal red line) appears. The value can be adjust by dragging those lines.

[Annotation] Text Box
For details, refer to the explanation that appears in next page.

[Annotation mode] Group Box
When <Thresholds> option button is selected, this dialog box appears.

[Threshold] Text Box
Threshold values of X and Y axis are displayed on [Histogram] graph. It can also be input from the keyboard
The value entered will be automatically changed to the value that can be processed in software.

**Fig. 2-100 [Colocalization Processor] Dialog Box
In case of Annotation Mode is [Threshold].**



[Histogram] graph

Explanation of [Annotation] Text Box

Display in Text Box	Explanation
Threshold Annotation	Threshold Mode
Total samples in histogram:	Total numbers of image pixels to be processed
(Dye-name1)[Thresh:]	Name of Dye method. Threshold value in X-axis.
<threshold:	Total numbers of image pixels below the threshold value in X-axis $((A+C)/(A+B+C+D)) \times 100$ [%]
>=threshold:	Total numbers of image pixels above the threshold value in X-axis $((B+D)/(A+B+C+D)) \times 100$ [%]
(Dye-name2)[Thresh:]	Name of Dye method. Threshold value in Y-axis
<threshold:	Total numbers of image pixels below the threshold value in Y-axis $((C+D)/(A+B+C+D)) \times 100$ [%]
>=threshold:	Total numbers of image pixels above the threshold value in Y-axis $((A+B)/(A+B+C+D)) \times 100$ [%]
Upper-left	Upper left portion of [histogram] graph
Samples	Total numbers of image pixels contained in upper left portion
[of (Dye-name1) < threshold]	$(A/(A+C)) \times 100$ [%]
[of (Dye-name2) >= threshold]	$(A/(A+B)) \times 100$ [%]
Of all samples	$(A/(A+B+C+D)) \times 100$ [%]
Upper-right	Upper right portion of [histogram] graph
Samples	Total numbers of image pixels contained in upper right portion
[of (Dye-name1) >= threshold]	$(B/(B+D)) \times 100$ [%]
[of (Dye-name2) >= threshold]	$(B/(A+B)) \times 100$ [%]
Of all samples	$(B/(A+B+C+D)) \times 100$ [%]
Lower-left	Lower left portion of [histogram] graph
Samples	Total numbers of image pixels contained in lower left portion
[of (Dye-name1) < threshold]	$(C/(A+C)) \times 100$ [%]
[of (Dye-name2) < threshold]	$(C/(C+D)) \times 100$ [%]
Of all samples	$(C/(A+B+C+D)) \times 100$ [%]
Lower-right	Lower right portion of [histogram] graph
Samples	Total numbers of image pixels contained in lower right portion
[of (Dye-name1) >= threshold]	$(D/(B+D)) \times 100$ [%]
[of (Dye-name2) < threshold]	$(D/(C+D)) \times 100$ [%]
Of all samples	$(D/(A+B+C+D)) \times 100$ [%]

3 Min-Max Bounds Mode

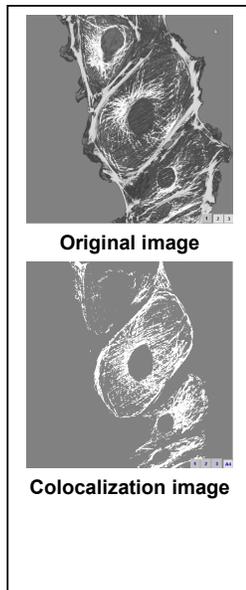
Specifies the rectangular on [Histogram] graph, and measures colocalization by use of the rectangular.

In addition, distribution level of brightness inside rectangular is displayed on [Display] panel. Color is the same as color of rectangular selected.

[Histogram] Graph
 Rectangle is displayed in graph. The rectangular can be positioned by dragging.

[Annotation] Text Box
 For details, refer to the explanation that appears in next page.

<Accept Annotation> button
 It extracts brightness only of image designated in rectangular on [Histogram] graph and creates new [Display] panel and displays the image.



[Annotation mode] Group Box
 When < Min-Max Bounds > option button is selected, this dialog will appear.

[Min.] Text Box
[Max.] Text Box
 Max. and Min. Positions of rectangle on [Histogram] graph will be displayed. You can input the values directly and change the values. The value entered will be automatically changed to the value that can be processed in software.

	Min.	Max.
FITC	608	2656
TRITC	200	2248

**Fig. 2-101 [Colocalization Processor] Dialog Box
 In case of Annotation Mode is [Min-Max Bounds].**



Explanation of [Annotation] Text Box

Display in Text Box	Explanation
Min-Max Annotation	Min-Max Bounds Mode
Total samples in histogram	Total numbers of image pixels to be processed
(Dye-name1) limits:	X-axis range of rectangular
Minimum:	Minimum value of X-axis rectangular
Maximum:	Maximum value of X-axis rectangular
(Dye-name2) limits:	Y-axis range of rectangular
Minimum:	Minimum value of Y-axis rectangular
Maximum:	Maximum value of Y-axis rectangular
RGB:	Color of rectangular: To be displayed in 256 gray scale (Red, Green, Blue)
Samples inside region:	Total numbers of image pixels inside rectangular and ratio to whole region
Samples outside region:	Total number of image pixels outside rectangular and ratio to whole region

4 Regions Mode

Specifies the arbitrary region in [Histogram], and measures colocalization within the region.

In addition, distribution level of brightness in draw pictorial figure is displayed on [Display] panel. Color is the same as color of draw pictorial figure selected.

[Histogram] Graph

You can draw a pictorial figure on [Histogram], using graphic button located at lower right side of dialog box. See 2-12 Entering Comment in Image for methods to delete graphic, to select plural numbers of graphics and to change color of graphics frame.

[Annotation mode] Group Box

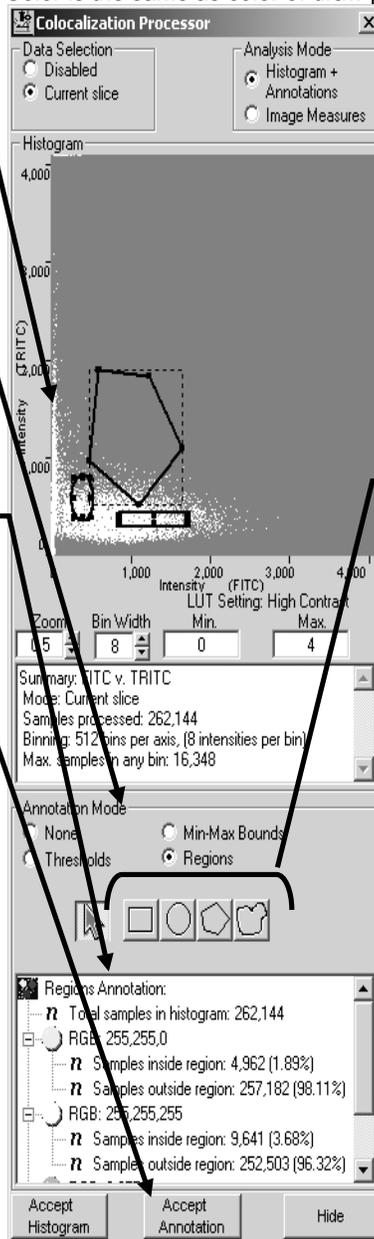
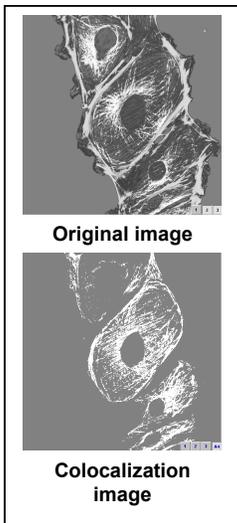
When < Regions > option button is selected, this dialog box will appear.

[Annotation] Text Box

For details, refer to the explanation that appears in next page.

<Accept Annotation> button

It extracts brightness only of image designated in rectangular on [Histogram] graph and creates new [Display] panel and displays the image.



<Rectangular> Button

Rectangular draw: On graph, drag diagonally from upper left to lower right to draw rectangular region.

<Circle> Button

Circle/Oval draw: Drag diagonally rectangle that circumscribes region of the circle to be drawn on the image.

<Poly region> Button

Polygon draw: Click each peak to draw a polygon. When the last peak is clicked, the peak clicked at first time will be connected with the last peak.

<Free Region> Button

Free draw: Specify region by dragging on image. Releasing the mouse button at the end of drag, end and start positions are connected.

**Fig. 2-102 [Colocalization Processor] Dialog Box
In case of Annotation Mode is [Regions]**



Explanation of [Annotation] Text Box (In case that 3 pictorial figures exist. Items (1) will be increased according to number of pictorial figures.)

Display in Text Box		Explanation
Regions Annotation		Regions Mode
(1) {	Total samples in histogram	Total numbers of image pixels to be processed
	RGB:	1st pictorial figure. Color of rectangular: To be displayed in 256 gray scale (Red, Green, Blue)
	Samples inside region:	Total numbers of image pixels inside rectangular and ratio to whole region
	Samples outside region:	Total numbers of image pixels outside rectangular and ratio to whole region
	RGB:	2nd pictorial figure. Color of rectangular: To be displayed in 256 gray scale (Red, Green, Blue)
	Samples inside region:	Total numbers of image pixels inside rectangular and ratio to whole region
	Samples outside region:	Total numbers of image pixels outside rectangular and ratio to whole region
	RGB:	3rd pictorial figure. Color of rectangular: To be displayed in 256 gray scale (Red, Green, Blue)
	Samples inside region:	Total numbers of image pixels inside rectangular and ratio to whole region
	Samples outside region:	Total numbers of image pixels outside rectangular and ratio to whole region

2-6-4-2 Colocalization for series image data set

Colocalization is also available for series image data set, i.e XYZ image data set.

[Data Selection] Group Box

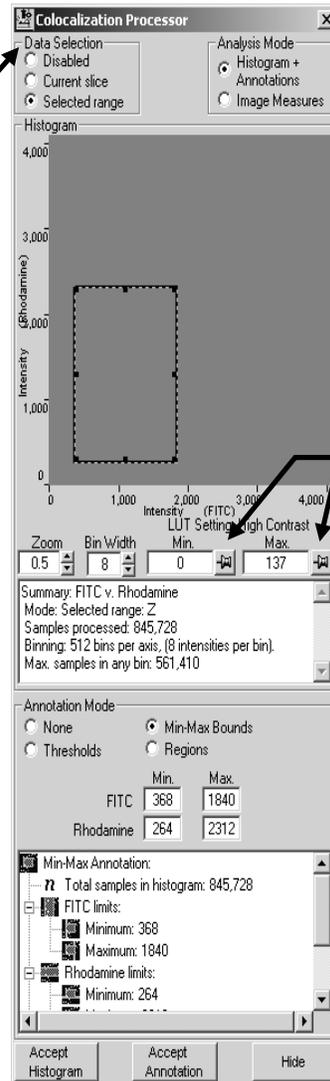
<Current slice> Option Button

The colocalization is processed for the image in a series data set which is displayed in [Display] panel.

<Selected range> Option Button

This button appears when series data set is in use.

The colocalization is processed on to the all image of the data set.



<Draw> Button

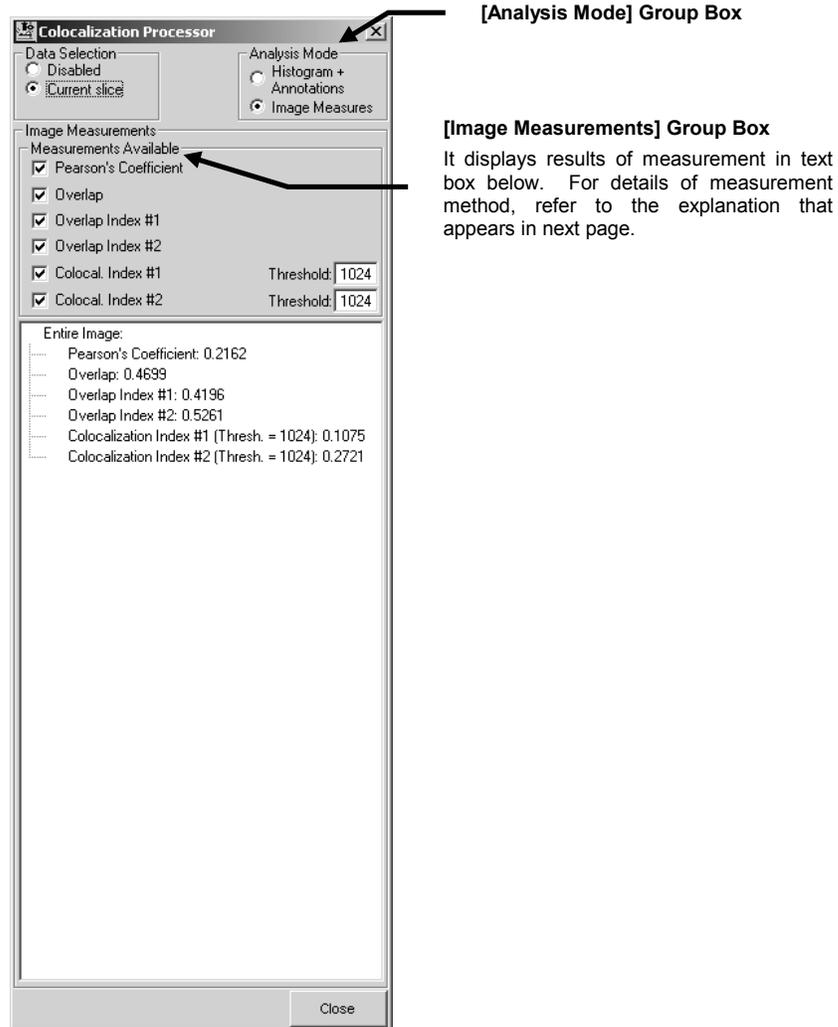
<Draw> button appears when image data set in use.

When <Current Slice> option button is selected at [Data Selection] group box, and <Draw> buttons at right side of text boxes – [LUT Max] and [LUT Min] are depressed, the values in text boxes will not change even if Current Slice image displayed on [Display] panel is changed. In case that <Draw> buttons are popped out, the values will change with the Current Slice change.

**Fig. 2-103 [Colocalization Processor] Dialog Box
Colocalization for series image data set**

2-6-4-3 Image measurement

When option button of [Image Measures] of [Analysis Mode] Group Box is selected, the dialog as shown below appears.



**Fig. 2-104 [Colocalization Processor] Dialog box
In case that Analysis Mode is Image Measures**

• **Pearson's Coefficient :**

In case that the value to be acquired is defined as r ,

$$r = \frac{\sum_i (F_{1,i} - \overline{F_{1,i}}) \cdot (F_{2,i} - \overline{F_{2,i}})}{\sqrt{\sum_i (F_{1,i} - \overline{F_{1,i}})^2 \cdot \sum_i (F_{2,i} - \overline{F_{2,i}})^2}}$$

However, legends used for image measurement are as follows:

$F_{1,i}$: Brightness of wavelength λ_1 at i -th pixel, $F_{2,i}$: Brightness of wavelength λ_2 at i -th pixel

$$\overline{F_{1,i}} = \frac{1}{N} \sum_i F_{1,i} : \text{Average brightness of wavelength } \lambda_1, \overline{F_{2,i}} = \frac{1}{N} \sum_i F_{2,i} : \text{Average}$$

brightness of wavelength λ_2

• **Overlap :**

In case that the value to be acquired is defined as r ,

$$r = \frac{\sum_i F_{1,i} \cdot F_{2,i}}{\sqrt{\sum_i F_{1,i}^2} \cdot \sqrt{\sum_i F_{2,i}^2}}$$

• **Overlap Index #1 :**

In case that the value to be acquired is defined as k_1 ,

$$k_1 = \frac{\sum_i F_{1,i} \cdot F_{2,i}}{\sum_i F_{1,i}^2}$$

• **Overlap Index #2 :**

In case that the value to be acquired is defined as k_2 ,

$$k_2 = \frac{\sum_i F_{1,i} \cdot F_{2,i}}{\sum_i F_{2,i}^2}$$

• **Colocalization Index #1 :**

In case that the value to be acquired is defined as m_1 ,

$$m_1 = \frac{\sum_{i \in A} F_{1,i}}{\sum_i F_{1,i}}$$

However, $i \in A$ should be i -th pixel that belongs to aggregation A that is greater than threshold value.

• **Colocalization Index #2 :**

In case that the value to be acquired is defined as m_2 ,

$$m_2 = \frac{\sum_{i \in A} F_{2,i}}{\sum_i F_{2,i}}$$

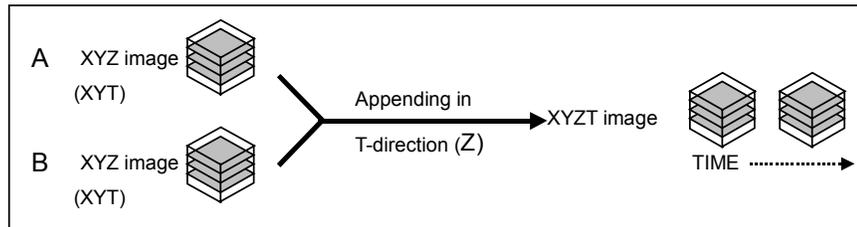
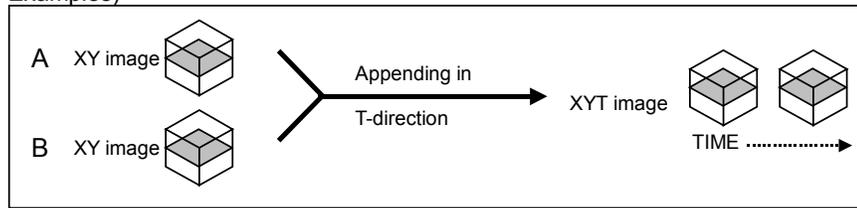
However, $i \in A$ should be i -th pixel that belongs to aggregation A that is greater than threshold value.

2-6-5 Appending image (Append)

2-6-5-1 Appending two images

Two images (A and B) acquired in XY or XYZ observation can be appended along the Z- or T-direction or as animation.

Examples)



The image files used in appending are subjected to the following restrictions.

- The sizes of the two image files should be identical.
- The numbers of channels of the two image files should be identical.
- The T- or Z-observation conditions (interval times, acquisition times, number of acquisitions, number of steps, etc.) of the two image files should be identical.
- The combination of the two image files should be a combination with which appending is permitted (see the table on page 2-272).



Two XY images can be appended in the T-direction with the following procedure.

1. Open the two image files (A and B) for appending.
2. Display the [display] panel of the first image (A) at the front.
3. Display the [Experiment Editor] sub-panel of the [Process] panel at the front.

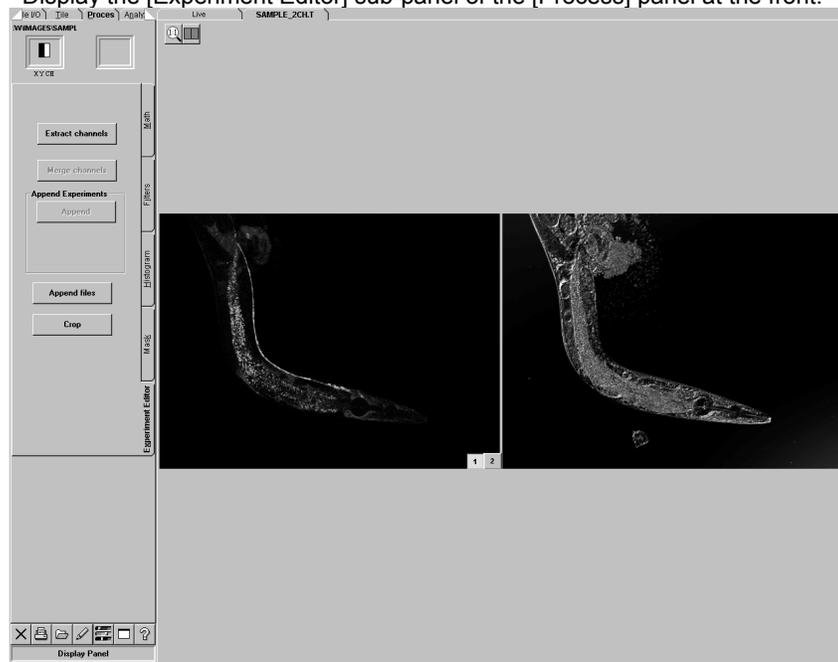


Fig. 2-105 [Experiment Editor] Sub-panel



<Experiment List> button

- In the toolbar at the bottom of the [Process] panel, click the <Experiment List> button.

The [Experiments in Memory] dialog box appears as shown below.

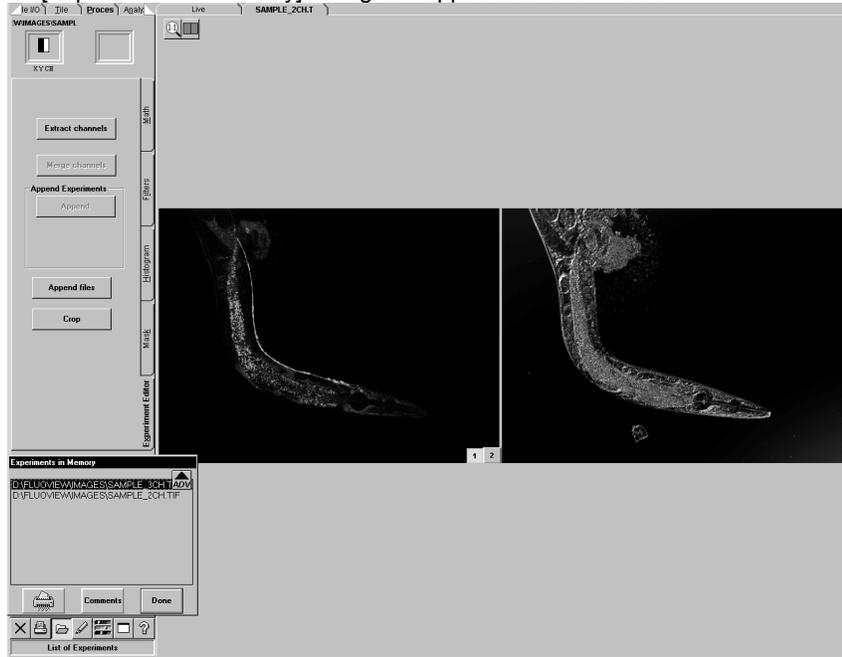
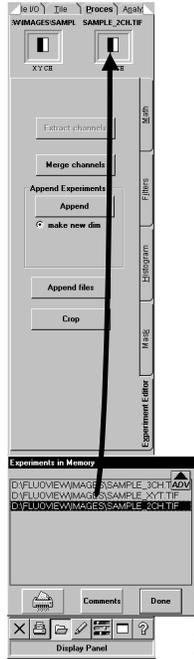


Fig. 2-106 [Experiments in Memory] Dialog Box



The icon of the image file being displayed at the front of the [display] panel (A) is shown in the frame at the top left of the [Process] panel.



- In the [Experiments in Memory] dialog box, select the file name of the second image (B) and drag it to the frame at the top right of the [Process] panel. The icon of the second image will be displayed in the frame at the top right of the [Process] panel.

TIP The mouse pointer turns into the image icon during dragging.

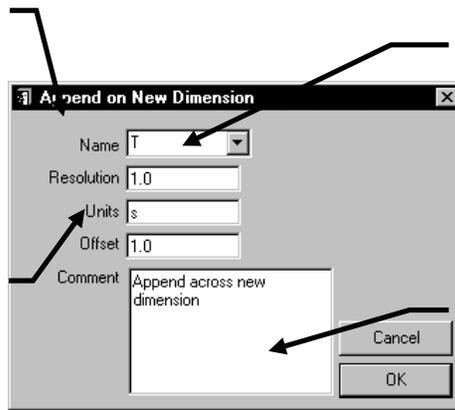
- Click the <Done> button in the [Experiments in Memory] dialog box to close it.
- Click the <Append> button in the [Experiment Editor] sub-panel.

The [Append on New Dimension] dialog box appears as shown below.

[Resolution] text box
Specify the interval time (in seconds) when "T" is selected in the [Name] drop-down list.

Specify the number of steps when "Z" is selected in the [Name] drop-down list.

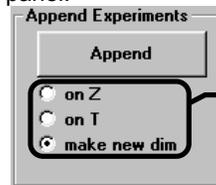
[Units] text box
Specify the unit of the figure specified in the [Resolution] text box.



[Name] drop-down list
Select the type of the image to be created by appending.
T: Time-lapse image (T-direction)
Z: Z-series image (Z-direction)
AN: Animation image
St: 3D image or stereo image to be viewed through color (red/green) eyeglasses

[Comment] text box
Enter the comment for the created image.

TIP Depending on the type of the image to be created by appending, various option buttons appears under the <Append> button in the [Experiment Editor] sub-panel.



Option buttons depending on the image mode.

- make new dim.. Create an image of new observation mode. (Select the type in the [Append on New Dimension] dialog box.)
- on Z..... Append along the Z-direction.
- on T..... Append along the T-direction.

8. A new [display] panel having [Append] as the window title is created, showing the image after appending.



TIP

For appending more than 3 images at a time, see section 2-6-5-2.

2-6-5-2 Appending image from several image data set

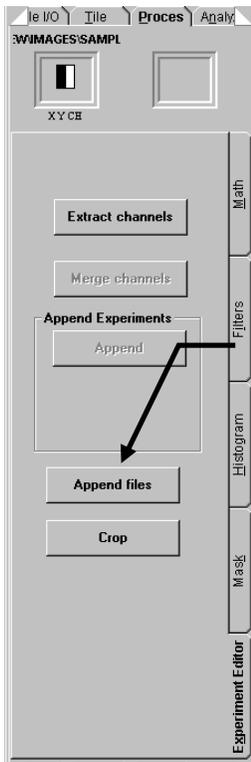
An image data set can be made from appending several image data set.



NOTE

There are the following restrictions when you append image files.

- Two image files must be the same image size.
- Two image files must be the same channel number.
- Two image files must be a combination that can be appended (see 2-253 page).



< Append files > button

1. Click <Append files> button on [Experiment Editor] sub panel of [Process] panel.

2. [Append on New dimension] dialog box appears as shown below.

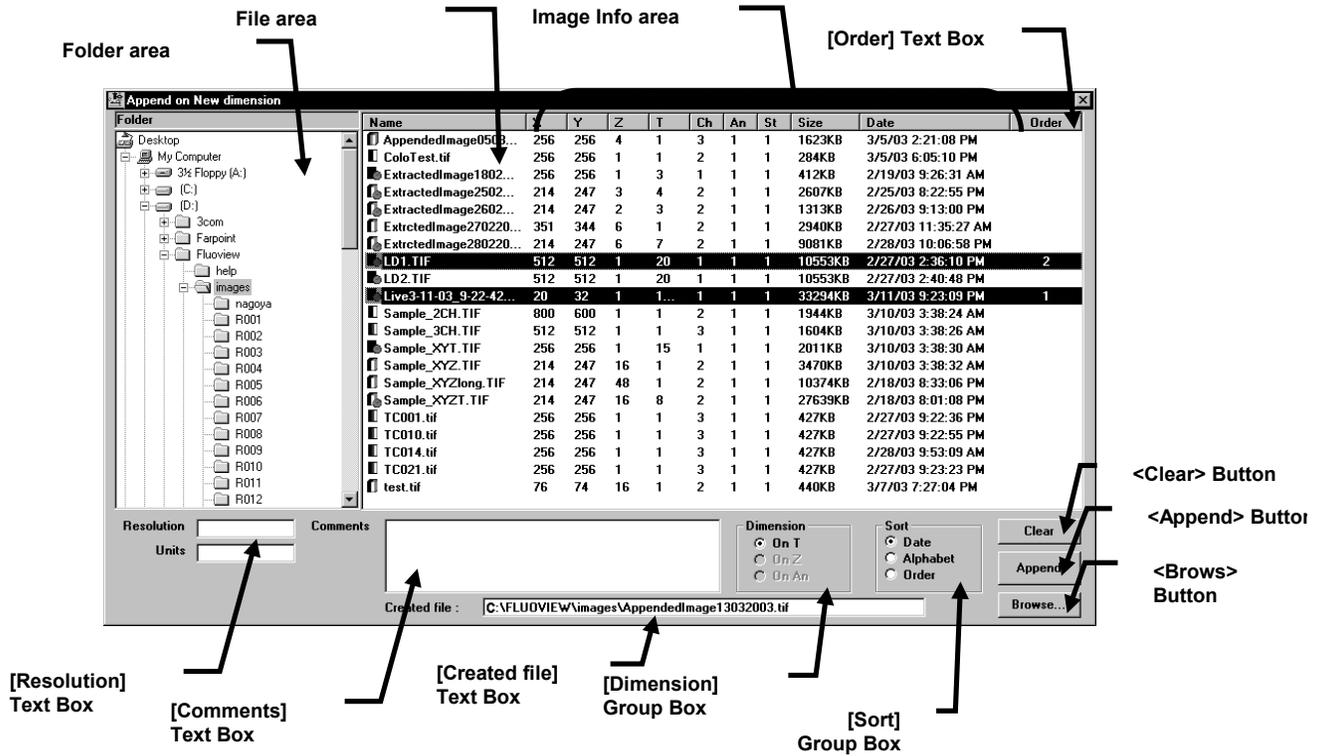


Fig. 2-107 [Append New dimension] Dialog Box

3. Select file folder on Folder area. Then, MultiTif image file name appears in File area.

4. Select image files to be appended.



Image files can be selected by dragging file name in File area.
 Several image files can assign by using Click and Shift-key + Click in File area.
 Several image files can also assign by Click with Ctrl-key.

Order of the file selection appears in [Order] text box area.



5. Select Series data set type that will be created in [Dimension] Group Box.

TIP The option buttons work as the followings.

- on T Appends file in T direction.
- on Z Appends file in Z direction.
- on An Appends file as animation.

TIP Time information of each file stays as its original, in case of T direction appending.

Resolution information will be taken over when file of different Z Resolution information is appended, in case of Z direction appending.

6. Select the order for appending image data with [Sort] Group Box.

TIP The option buttons work as the followings.

- Date Appends in order of date.
- Alphabet Appends in alphabetical order.
- Order Appends in order which is specified at[Order].

7. Enter comment into [Comments] dialog box.

TIP Comment entered can be reviewed as follows.

- 1.Display appended image on [Display] panel.
- 2.Do mouse-right-click over the image and select [Experiment Properties] from pop up menu. Dialog box – [Image Comments] will appear.



3. Select tab – [Comments] on [Image Comments] dialog box so that comment will be displayed in the text box.



8. Enter folder and file name into [Create file] text box.
By pressing <Browse...> button, folder appears for easy folder selection.
9. Click <Append> button, then new appended image created and stored.



Pressing <Clear> button clears the file assignment.



[Resolution] text box works as follows.

-In case of T in [Dimension] group box:

All images are time-series: Input not possible.

Image is not time-series: The setting is utilized for the time stamp interval that starts with 0 sec.

-In case of Z in [Dimension] group box

The setting is utilized for the Z step interval that starts with 0 um.

-In case of on An in [Dimension] group box:

The setting is utilized for pixel intensity step that starts with intensity 0.

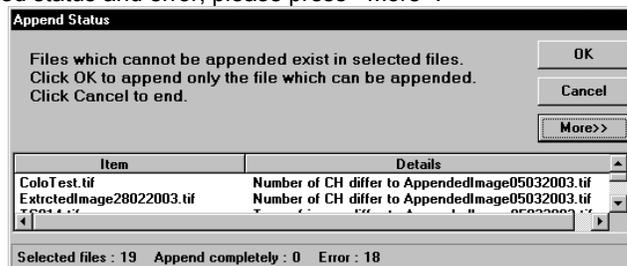


Depending upon image size and number of slices to be appended, it may take a few minutes or several tens of minutes to process for appending. Progress bar will appear to indicate the progress during appending.

One Point!

[Append Status] dialog box appears when images can not appended. Pressing <OK>, the image is appended only for those are possible to be appended.

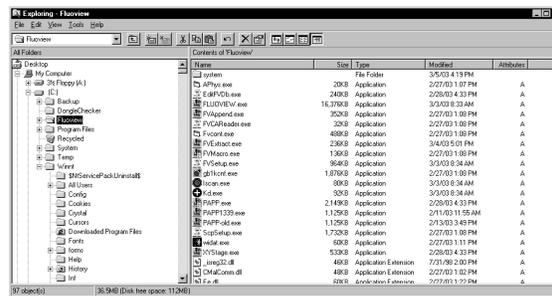
To see detailed status and error, please press <More>.



One Point!

Plural numbers of images can be appended without starting FLUOVIEW software.

1. Select <Start> button at bottom of Windows screen.
2. In case of Windows NT, select [Programs] – [Windows NT Explorer] command from the [Start] menu. In case of Windows 2000, select [Accessories] - [Windows Explorer] command from the [Start] menu. As shown below, Windows NT or Windows 2000 Explorer will appear.

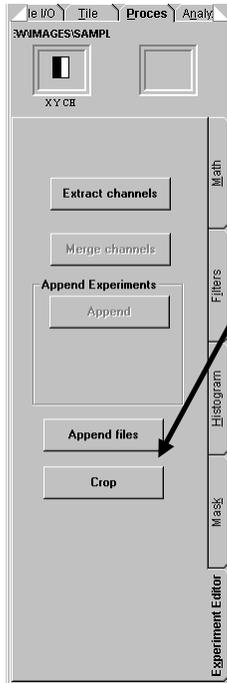


3. Double click [FVAppend.exe] in [FLUOVIEW] folder at [C:] drive. Dialog box - [Append on New dimension] will appear.
4. In case that the same operation as the procedures described in this manual is done, the image will be appended and a new file will be created.

2-6-6 Extract image (Crop)

2-6-6-1 Extract image data from an image data set

New image data with selected slice image from an image data set can be made by this function.



The following is an example for Image extraction from an XYZT image data set.

1. Display the image to be extract in [Display] panel.
2. Select [Experiment Editor] sub panel in [Process] panel.
3. By clicking <Crop> button. [Extract] dialog box appears.

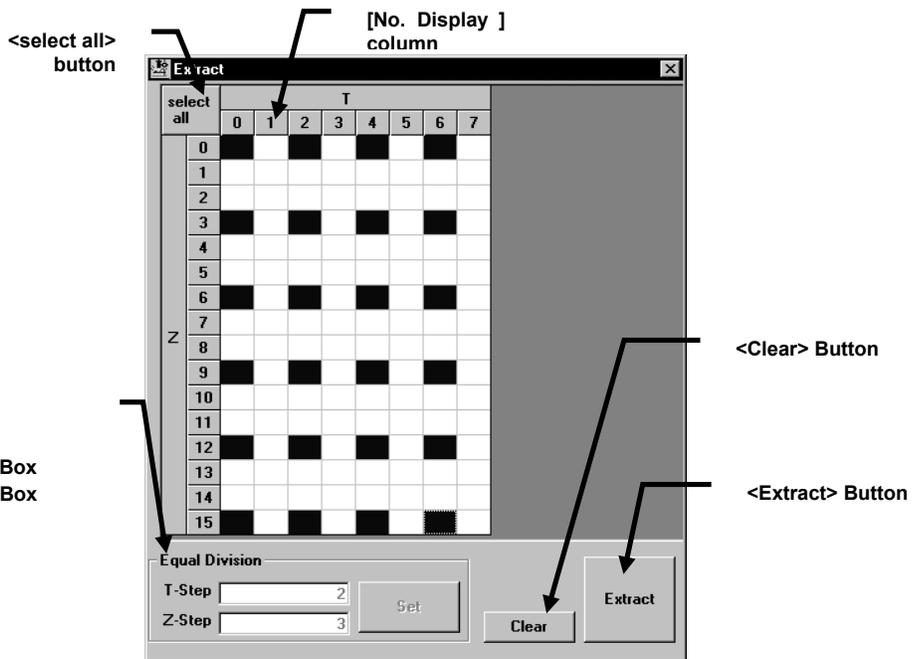


Fig. 2-108 [Extract] Dialog box

4. Select image slices by pressing on [Extract] dialog box.



TIP

The following method of slice selection is also available.

- All of image slices can be select by pressing <select all> button.
- All of row or column slices can be selected by pressing [No. Display].
- Image slices with a certain time interval and/or Z step can be selected by pressing <Set> after set [T-Step] and/or [Z-Step] in [Equal Division].
- Image slices with a certain rectangular area on [Extract] panel can be selected by dragging from top-left to bottom-right.

TIP

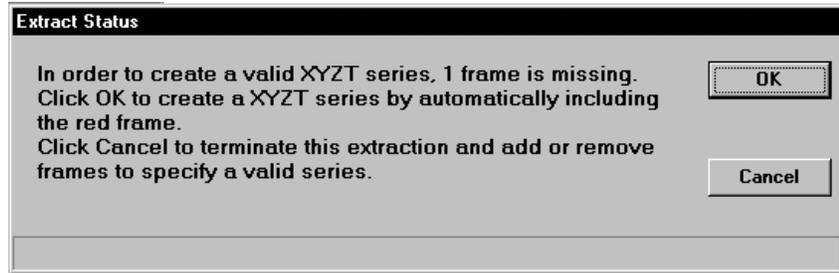
The slice assignment cleared by clicking <clear> button in [Extract] dialog box.

5. Click <Extract> button the extracted image appears in [Display] panel with named [Crop].

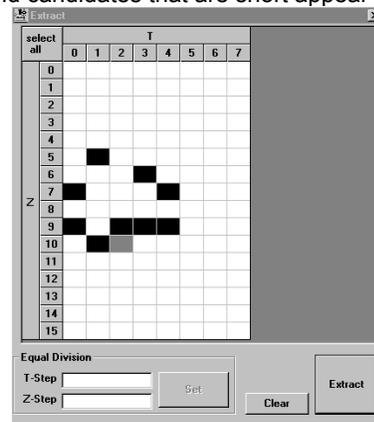


One Point!

When arbitrary portion of XYT image is extracted and plural numbers of [No. Display] columns at T columns are selected, the dialog box as shown below will appear in case that number of units at Z portion of [No. Display] on all T columns are not the same number of units.



In [Extract] dialog box, grid candidates that are short appear in red.



Selection of grid candidates that are short is done as follows.

1. Verification will be done whether or not the same numbers of Z elements are selected against all grids in T direction.
2. When there are grids in which Z elements are short, the selection of Z element position equal to Z element that corresponds to previous T element will automatically be done.

When <OK> button of [Extract Status] dialog box is clicked, the extraction for grids including the grids displayed in red will be done . When <Cancel> button is clicked, extraction will be aborted.



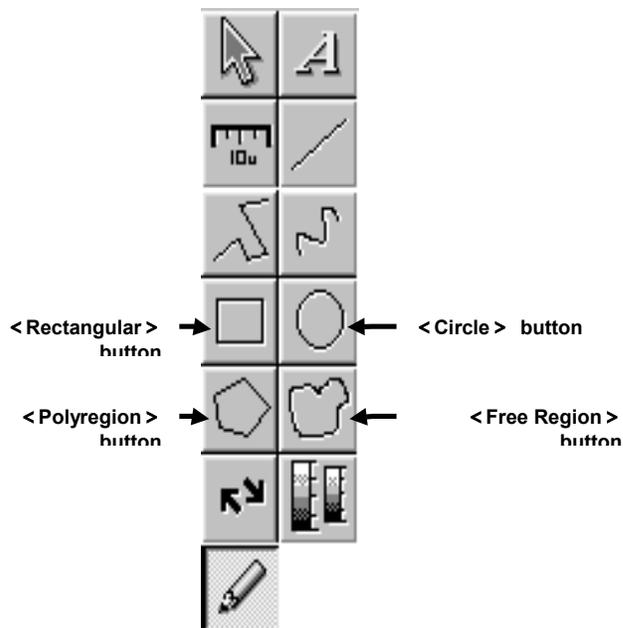
One Point!

It is possible to cut out the image and extract it by specifying arbitrary region. Image cut out is done as follows.



<Annotate> button

1. Select [Annotate] button in tool bar.



2. Select either <Rectangular>, <Circle>, <Polyregion> or <Free Region> to assign clipping area.

When option other than <Rectangular> is selected, the cut out (clipping) will be done against rectangle that circumscribes the specified profile.

3. Follow procedures subsequent to 3 of section 2-7-6-1 and extract the image. The image will be cut out and extracted and new image will be displayed.

One Point!

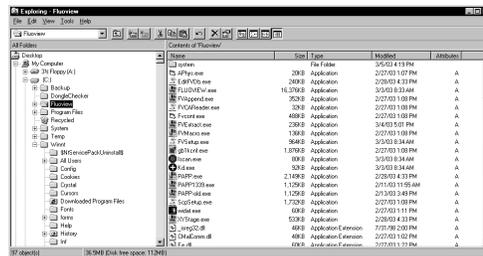
Slice extract function works even if FLUOVIEW software is not running. However, clipping function works only with FLUOVIEW software.

1. Press Windows <Start> button.

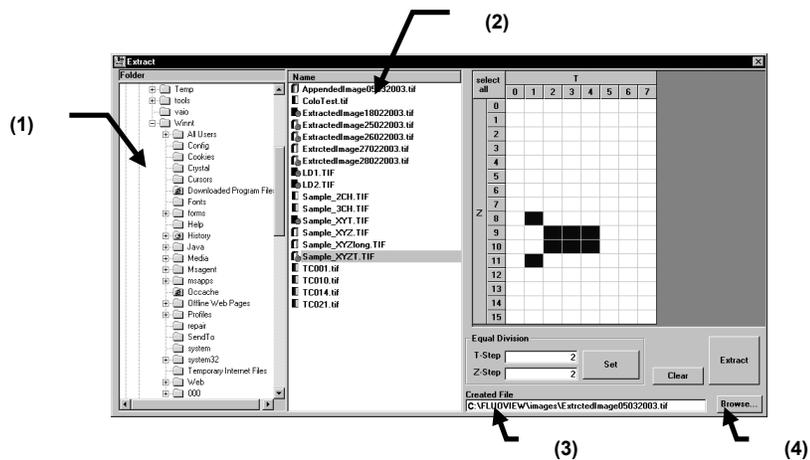
2. In case of Windows NT, select [Windows NT Explorer] command in [Programs].

In case of Windows 2000, select [Windows Explorer] command in [Accessories].

Then, Windows Explore appears



3. Double-click FVExtract.exe in Fluoview of C: Drive..

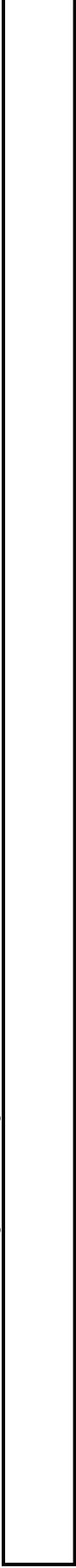


4. Assign folder (1) Folder area to be image data stored.

5. Select image data in (2) File area to be extracted.

6. File and its folder name appears in <Created file> text box, after pressing <Browse...> and set file and folder name to be stored.

7. Image is extracted and new image data file is created by following procedures this section.



Combinations of images with which appending are possible

B \ A	XY	XYZ	XYZ	XYT	XYZT	XZ	XZT	XT	Pt-Pt	XY-AN
XY	XYZ XYT XY-AN	XYZ	XYZ	XYT						XY-AN
XYZ	XYZ	XYZ XYZT			XYZT					
XYT	XYT		XYT							
XYZT		XYZT			XYZT					
XZ						XZT	XZT			
XZT						XZT	XZT			
XT								XT-AN		
Pt-Pt									Pt-Pt-AN	
XY-AN	XY-AN									XY-AN

Pt..... Point-scanned image

AN Animation image

3D image or stereo image to be viewed through color (red/green) eyeglasses

2-7 Image Analysis

Images can be analyzed using the [Analyze] panel. Display the [Analyze] panel at the front.

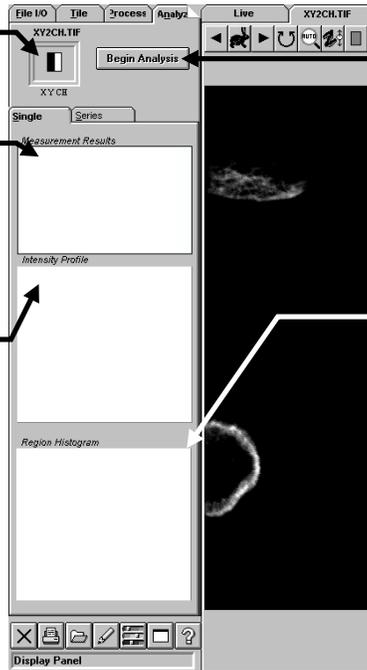
Displays the icon of the image being displayed (image to be subjected to analysis).

[Measurement Results] box

Shows the measurement data of the specified line or region.

[Intensity Profile] box

Shows the intensity profile chart of the specified line or region. When a line is specified, the line profile is displayed, and double-clicking this field displays the [Enhanced Profile Plot] window. When a region is specified, the bird's eye view is displayed, and double-clicking this field displays the [Intensity Map] window.



<Begin Analysis> button
Starts analysis.

[Region Histogram] box
Shows the histogram of the specified line or region. Double-clicking this field displays the [Enhanced Histogram Plot] window.

Fig. 2-109 [Analyze] Panel

2-7-1 Checking the Intensity of a Specific Part

2-7-1-1 Intensity Values on a Line (Line Profile)

The intensity values on a line in an image can be displayed graphically.

1. Display the [Single] sub-panel at the front.
2. Display the [Display] panel of the image to be subjected to the intensity checking at the front.
3. Click the <Annotate> button in the toolbar at the bottom of the [Analyze] panel. A list of buttons appears as shown below.



<Annotate> button



4. From the displayed buttons, click the <Line> button, <Poly Line> button or <Free Line> button.
5. Specify the straight line, polygonal line or free line on the image in the [Display] panel.

They can be specified as described below.



<Line> button

- To specify a straight line:
On the image, place the mouse pointer on the point you want to start the straight line and drag until the point you want to end it.



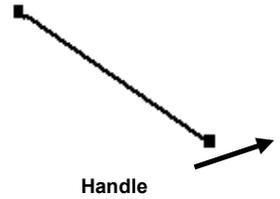
<Poly Line> button

- To specify a polygonal line:
On the image, click the points corresponding to the start point, peak points and end point of the desired polygonal line, then click the right button of the mouse to set the specification.



<Free Line> button

- To specify a free line:
On the image, drag the mouse pointer along the line to be checked.
The line is displayed on the image together with the handles on it. The intensity profile can be displayed while the handles are displayed.



NOTE When the mouse is clicked in other place than on the specified line, the handles on the line disappear. The intensity profile cannot be displayed when the handles are not displayed.



TIP The checked line can be moved, deleted or changed of size or color. This is possible with the same method as entering comment in the image.
For details, see sections 2-12-6, 2-12-7, 2-12-8 and 2-12-9 in section 2-12, "Entering Comment in Image".



<Annotate> button

6. Click the <Annotate> button so that the list of buttons disappears.
7. Click the <Begin Analysis> button. The intensity profile of the specified line will be displayed in the [Intensity Profile] box of the [Analyze] panel.

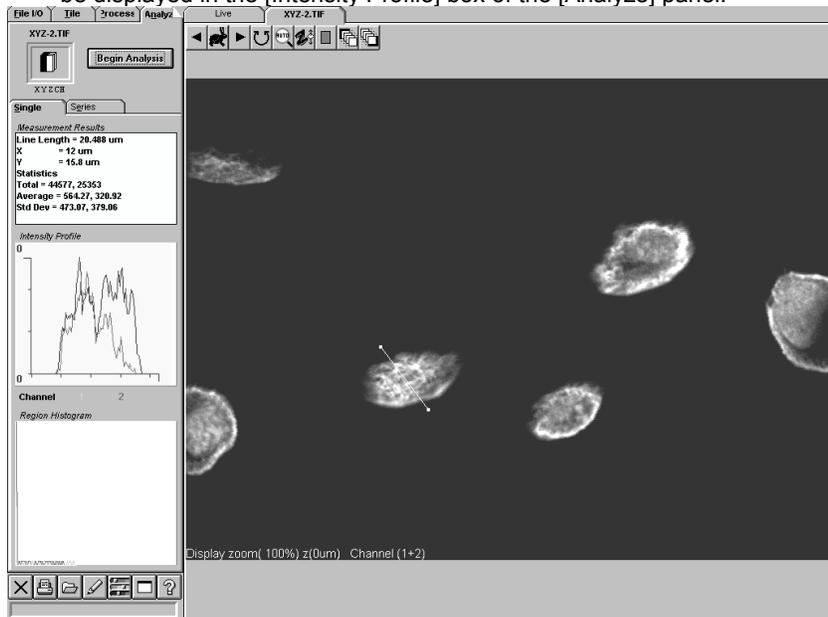


Fig. 2-110 Panel After Analysis (Line Specification)

8. Double-click the [Intensity Profile] button. The [Enhanced Profile] window appears as shown below.

<Properties> button

Displays the [Editing] dialog box for use in detailed setting of the chart or change of the chart display.
See section 2-15, "Changing the Chart Display Method" for details.

<Copy> button

Copies the plotted image in the clipboard.

<Save> button

Saves the profile data in a file using an Excel-compatible format.

<Close> button

Quits the [Enhanced Profile Plot] window and returns to the [Analyze] panel.

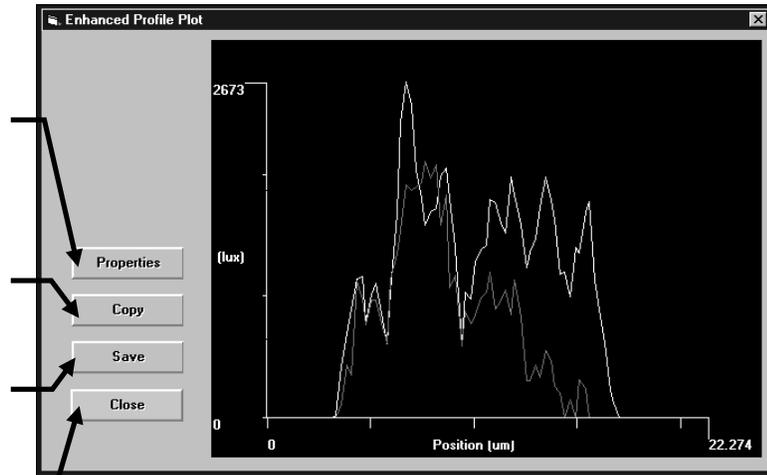


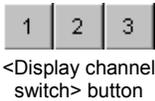
Fig. 2-111 [Enhanced Profile Plot] Window

- TIP** When a desired area is specified by dragging the left button of the mouse on the graph, the specified area can be magnified.
- TIP** When the right button of the mouse is dragged on the graph, the graph can be scrolled.
- TIP** The magnification or scrolling of the graph can be canceled by dragging the left button of the mouse from the bottom left to the right of the magnified graph.
- TIP** When the mouse pointer is placed on a graph line while the **Ctrl** or **Alt** key is held depressed, the coordinates can be displayed.
- TIP** The displayed data can be applied to other applications.
See section 2-11, "Transferring Data to Another Application" for details.

2-7-1-2 Intensity Values on a Planar Region (Bird's Eye View)

The intensity values on a region in an image can be displayed graphically.

1. Display the [Single] sub-panel at the front.
2. Display the [Display] panel of the image to be subjected to the intensity checking at the front.
3. When the image was acquired in the multi-channel mode, select whether the multiple channels are analyzed simultaneously or only one channel is analyzed.



To select the target channel(s), use the <Display channel switch> buttons. Only the channel(s) being displayed will be analyzed.

Example) When only the Ch1 image is displayed, only the Ch1 image is analyzed.



For the switching of channels, see section 2-5-3, "Switching the Display Channels".



<Annotate> button

4. Click the <Annotate> button in the toolbar at the bottom of the [Analyze] panel. A list of buttons appear as shown below.



5. From the displayed buttons, click the <Rectangular> button, <Circle> button or <Polyregion> button.



6. Specify the region to be checked in the image in the [Display] panel.

They can be specified as described below.



<Rectangular> button



<Circle> button



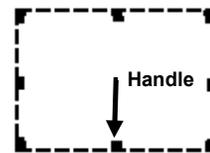
<Polyregion> button



<Free Region> button

- To specify a rectangle:
On the image, drag the mouse pointer along the diagonal line of the desired rectangle, from the top left corner to the bottom right corner.
- To specify a circle or ellipse:
On the image, assume a rectangle circumscribing the circle to be checked and drag the mouse pointer along the diagonal line between opposite corners of the rectangle.
- To specify a polygonal region:
On the image, click the points corresponding to the corners of the polygon to be checked. After clicking the last corner point, click the right button of the mouse to connect the last clicked point to the first clicked point.
- To specify a free region:
On the image, specify a region by dragging. Then release the mouse button to complete dragging. The point where the dragging was ended will be connected to the point where it was started.

A region is displayed on the image together with handles on the perimeter. The region is selected as the target of the bird's eye view while the handles are displayed.



NOTE

If the mouse is clicked in other place than inside the region specified on the image, the handles will disappear. The bird's eye view cannot be displayed while the handles are not displayed.

TIP

The checked region can be moved, deleted or changed of size or color. This is possible with the same method as entering comment in the image. For details, see sections 2-12-6, 2-12-7, 2-12-8 and 2-12-9 in section 2-12, "Entering Comment in Image".



<Annotate> button

7. Click the <Annotate> button so that the list of buttons disappears.
8. Click the <Begin Analysis> button. The bird's eye view of the specified region will be displayed in the [Intensity Profile] box of the [Analyze] panel.



The [Measurement Results] box in the [Single] sub-panel shows the measurement results such as the area (Area), horizontal and vertical lengths (X/Y, Z or T) and perimeter length (Perimeter) of the region, and the statistic result of each channel such as the total (Total), average (Average) and standard deviation (Std Dev).

9. When the image was acquired in the multi-channel mode, the channel(s) to be subjected to the bird's eye view display can be selected using the [Channel] option buttons.

Shows:
Measurement results including;
Perimeter
· Area
· X/Y, Z or T
Statistical channel data including;
· Total
· Average
· Std Dev

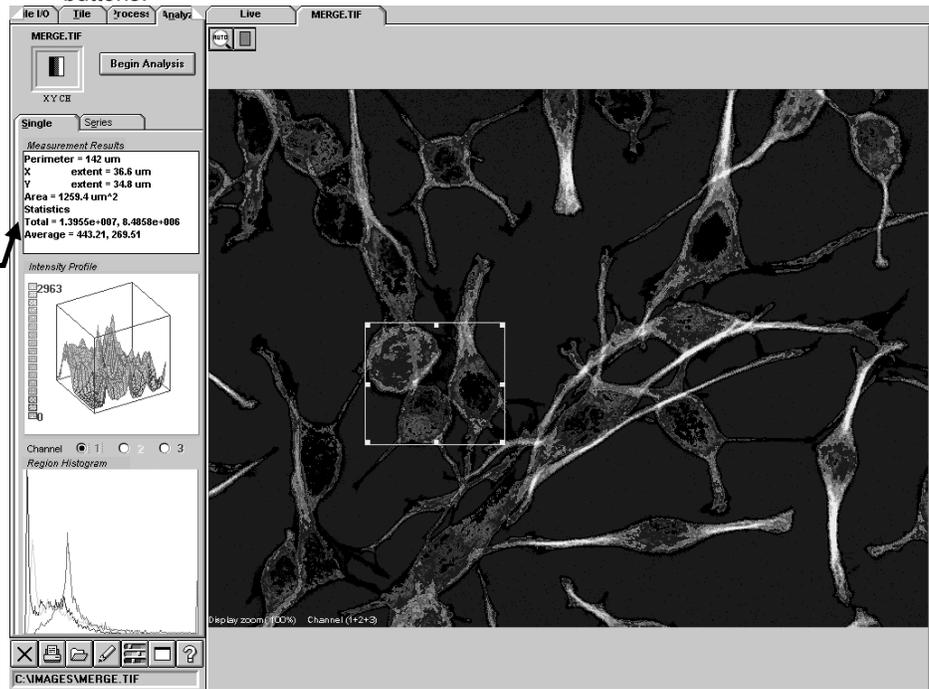


Fig. 2-112 Panel After Analysis (Region Specification)

10. Double-click the [Intensity Profile] button. The [Intensity Map] window appears as shown below.

[Angle] scale

Sets the angle in the horizontal direction. The result can be confirmed with the small bird's eye view in the frame on the

[Tilt] scale

Sets the angle in the vertical direction. The result can be confirmed with the small bird's eye view in the frame on the top left.

<Plot> button

Displays the bird's eye view with the angles set above.

<Spin> button

Spins the bird's eye view by one turn. The spinning starts from the front.

<Copy> button

Copies the plotted image in the clipboard.

<Save> button

Saves the profile data in a file using an Excel-compatible format.

<Close> button

Quits the [Enhanced Profile Plot] window and returns to the [Analyze] panel.

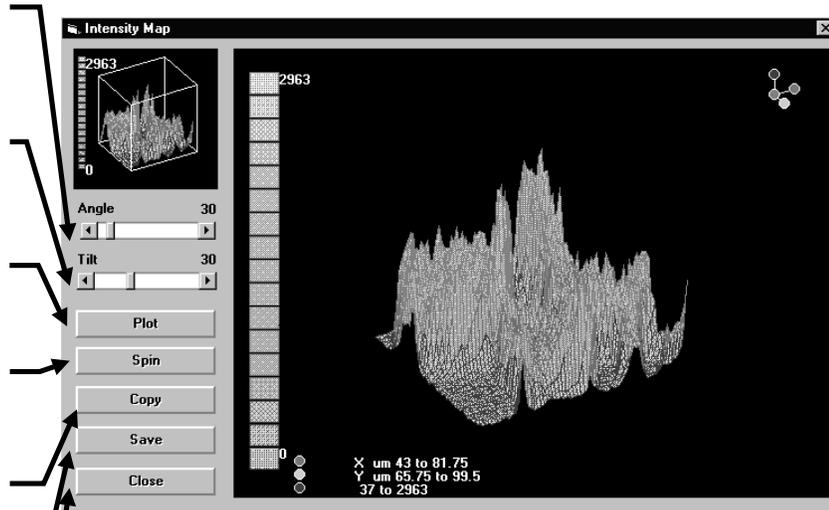


Fig. 2-113 [Intensity Map] Window



The displayed data can be utilized in other applications. See section 2-11, "Transferring Data to Another Application" for details.

2-7-2 Checking the Intensity Distribution of a Specific Part

2-7-2-1 Intensity Distribution on a Line (Histogram)

The histogram on a line in an image can be displayed.

The histogram is displayed in the [Region Histogram] box in the [Single] sub-panel.

The operation method is identical to displaying the intensity profile on a line. See section 2-7-1-1, "Intensity Values on a Line (Line Profile)".

Double-click the [Region Histogram] window. The [Enhanced Histogram Plot] windows appears as shown below.

<Properties> button

Displays the [Editing] dialog box for use in detailed setting of the chart or change of the chart display.

See section 2-15, "Changing the Chart Display Method" for details.

<Copy> button

Copies the plotted image in the clipboard.

<Save> button

Saves the profile data in a file using an Excel-compatible format.

<Close> button

Quits the [Enhanced Profile Plot] window and returns to the [Analyze] panel.

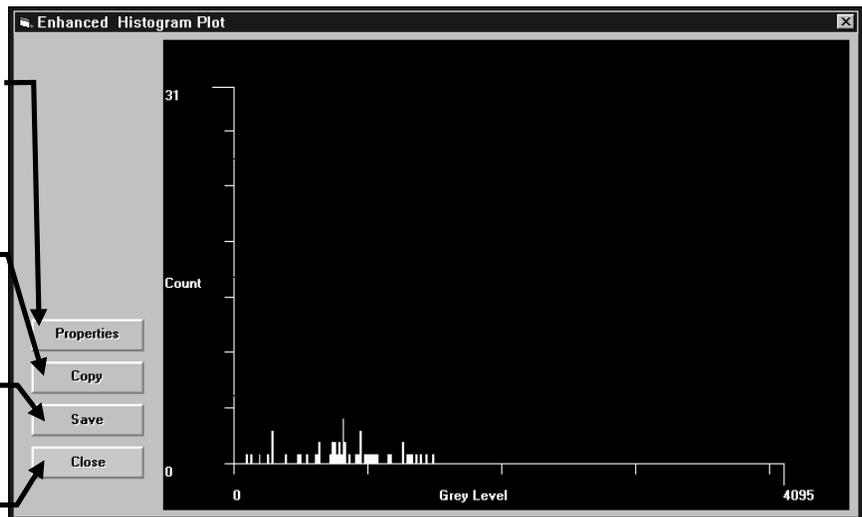


Fig. 2-114 [Enhanced Histogram Plot] Window (Line Specification)



When a desired area is specified by dragging the left button of the mouse on the graph, the specified area can be magnified.



When the right button of the mouse is dragged on the graph, the graph can be scrolled.



TIP The magnification or scrolling of the graph can be canceled by dragging the left button of the mouse from the bottom left to the right of the magnified graph.

TIP When the mouse pointer is placed on a graph line while the **Ctrl** or **Alt** key is held depressed, the coordinates can be displayed.

TIP The displayed data can be applied to other applications. See section 2-11, "Transferring Data to Another Application" for details.

2-7-2-2 Intensity Distribution on a Planar Region (Histogram)

The histogram on a region in an image can be displayed.

The histogram is displayed in the [Region Histogram] box in the [Single] sub-panel.

The operation method is identical to displaying the intensity profile on a line. See section 2-7-1-2, "Intensity Values on a Planar Region (Bird's Eye View)".

Double-click the [Region Histogram] window. The [Enhanced Histogram Plot] windows appears as shown below.

- <Properties> button**
Displays the [Editing] dialog box for use in detailed setting of the chart or change of the chart display.
See section 2-15, "Changing the Chart Display Method" for details.
- <Copy> button**
Copies the plotted image in the clipboard.
- <Save> button**
Saves the profile data in a file using an Excel-compatible format.
- <Close> button**
Quits the [Enhanced Profile Plot] window and returns to the [Analyze] panel.

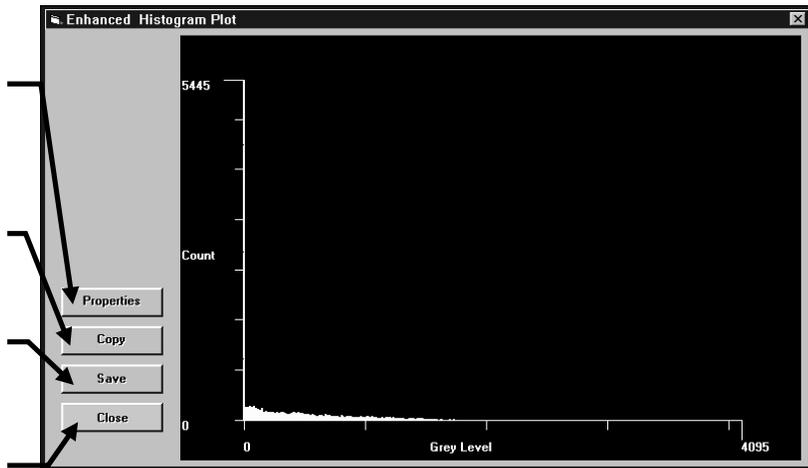


Fig. 2-115 [Enhanced Histogram Plot]Window (Line Specification)

TIP

When a desired area is specified by dragging the left button of the mouse on the graph, the specified area can be magnified.

TIP

When the right button of the mouse is dragged on the graph, the graph can be scrolled.

TIP

The magnification or scrolling of the graph can be canceled by dragging the left button of the mouse from the bottom left to the right of the magnified graph.

TIP

When the mouse pointer is placed on a graph line while the **Ctrl** or **Alt** key is held depressed, the coordinates can be displayed.

TIP

The displayed data can be applied to other applications.
See section 2-11, "Transferring Data to Another Application" for details.

2-7-3 Measuring a Part

2-7-3-1 Length Measurement

The length between two points in an image or the perimeter of a region in an image can be measured.

The [Measurement Results] box in the [Single] sub-panel shows the measurement results such as the length between 2 points (Length) or perimeter of the region (Perimeter) and the horizontal and vertical lengths (X/Y, Z or T) of the region, and the statistic result of each channel such as the total (Total), average (Average) and standard deviation (Std Dev).

The operation method for measuring the length between 2 points is identical to that for displaying the intensity profile of a line. See section 2-7-1-1, "Intensity Values on a Line (Line Profile)". The operation method for measuring the perimeter of a region is identical to that for displaying the bird's eye view of a region. See section 2-7-1-2, "Intensity Values on a Planar Region (Bird's Eye View)".



The measurement results can be written in comment by copying and pasting them in the [Image Comments] dialog box in the [Experiments in Memory] dialog box.

See section 2-3-4, "Saving Comment Together with Image" for details.

2-7-3-2 Area Measurement

The area of a region in the image can be measured.

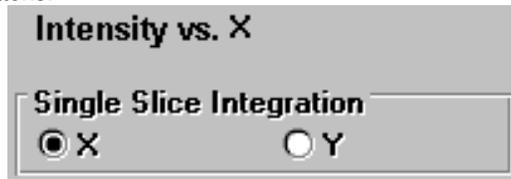
The [Measurement Results] box in the [Single] sub-panel shows the measurement results such as the area of the region (Area), perimeter of the region (Perimeter) and the horizontal and vertical lengths (X/Y, Z or T) of the region, and the statistic result of each channel such as the total (Total), average (Average) and standard deviation (Std Dev).

The operation method is identical to that for displaying the bird's eye view of a region. See section 2-7-1-2, "Intensity Values on a Planar Region (Bird's Eye View)".

2-7-3-3 Measuring the Change in Mean Value of Intensity

The mean value of the intensity in a region specified in an image can be measured and displayed graphically.

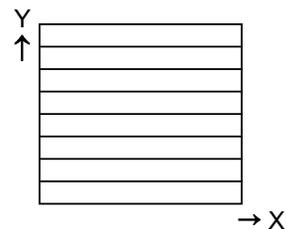
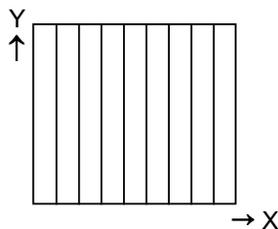
1. Display the [Series] sub-panel at the front.
2. Display the [Display] panel of the image that you want to check the intensity at the front.
3. When the image is composed of multiple image slices, operations will be applied to each slice of the image. When the image is composed of a single image slice or when one of the multiple image slices is selected, the [Single Slice Integration] group box appears as shown below. Select the direction of interest (X or Y) using the option buttons.



NOTE When the selected image was acquired in the XY observation mode, the [Single Slice Integration] group box shows the option buttons for selection of the X- or Y-direction. When the selected image was acquired in the XT observation mode, the option buttons for selection of the X- or T-direction are displayed.

Example) When the direction of interest is selected in the [Single Slice Integration] group box, line-by-line computation operation starts on the perpendicular lines to the selected direction. For example, an image acquired in XY observation is checked as shown below.

(When the X-direction is selected) (When the X-direction is selected)





<Set start position>
button



<Set end position>
button



<Successive display>
button



<XYZ series>
button



<XYT series>
button



<Display channel switch>
buttons

TIP

When the image is composed of multiple image slices, the range of image slices to be subjected to the operation can be set using the <Set start position> and <Set end position> buttons above the images. First display the image slice to start the operation using the <Display> button and click the <Set start position> button. Then, set the image slice to end the operation in the same way as above.

TIP

With an image acquired in XYZT observation, the slice images (cross-section (Z)/time lapse (T)) to be subjected to the operation can be selected using the <XYZ series> and <XYT series> buttons above the image.

4. When the image was acquired in the multi-channel mode, select whether the multiple channels are operated simultaneously or only one channel is operated. To select the target channel(s), use the <Display channel switch> buttons. Only the channel(s) being displayed will be analyzed.
Example) When only the Ch1 image is displayed, only the Ch1 image is analyzed.

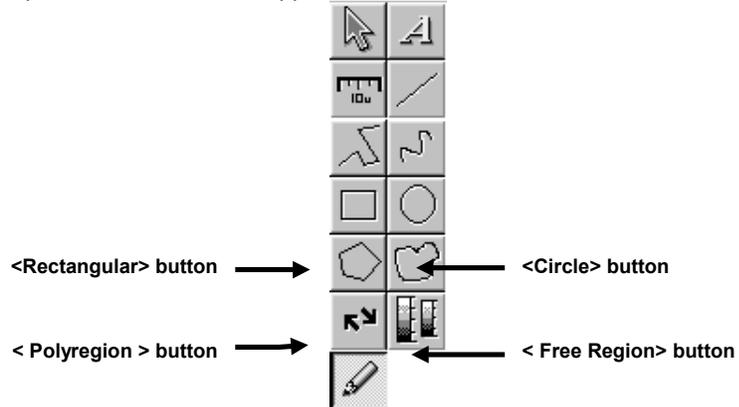
TIP

For the switching of channels, see section 2-5-3, "Switching the Display Channels".



<Annotate> button

5. Click the <Annotate> button in the toolbar at the bottom of the [Analyze] panel. A list of buttons appear as shown below.

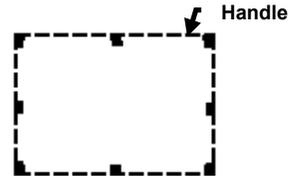


6. From the displayed buttons, click the <Rectangular> button, <Circle> button, <Polyregion> button or <Free Region> button.

7. Specify the region to be checked in the image in the [Display] panel.

For the specification method, see section 2-7-1-2, "Intensity Values on a Planar Region (Bird's Eye View)".

A region is displayed on the image together with handles on the perimeter. The region is selected as the target of mean value computation operation while the handles are displayed.

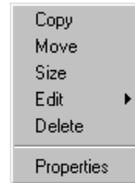


NOTE

If the mouse is clicked in other place than inside the region specified on the image, the handles will disappear. On the contrary, clicking the mouse in a region displays handles around it. The operation cannot be executed while the handles are not displayed.



8. Click the mouse. A pop-up menu as shown below appears. Select [Properties] from the menu.



9. The [Properties] dialog box as shown below appears. Display the [Color] panel at the front.

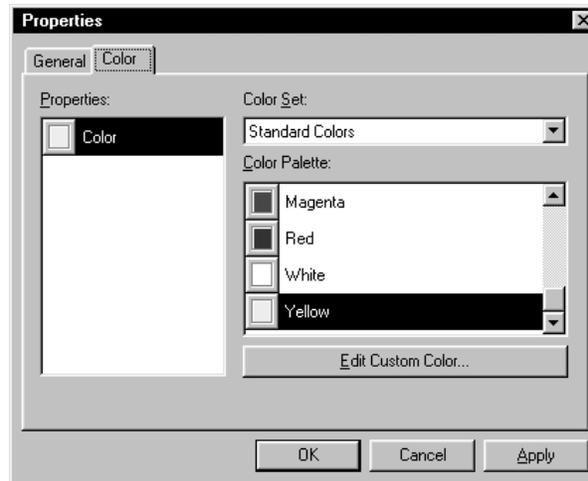


Fig 2-116 [Properties] Dialog Box

10. Select the desired color from the [Color Palette] list box.
11. It is also possible to specify more than one region simultaneously and display their operation results together. First specify the regions by repeating steps 7 and 8 above for each. Use different colors for the regions. After having set the regions, click the mouse in the first region. With the second regions and after, click the mouse while pressing the **Shift** key depressed.



<Annotate> button

12. Click the <Annotate> button so that the list of buttons disappears.
13. Using the scale in the [Threshold] group, set the threshold value for the intensity values used in operation. The intensity data above the threshold values set here will be used in the operation.



14. Click the <Begin Analysis> button. The mean value of the specified regions will be displayed graphically in the [Mean Intensity] box.



NOTE

The colors of the chart lines corresponding to the colors assigned to the regions.



NOTE

When the image was acquired in the multi-channel mode, the channel number is displayed to the right of each chart line.

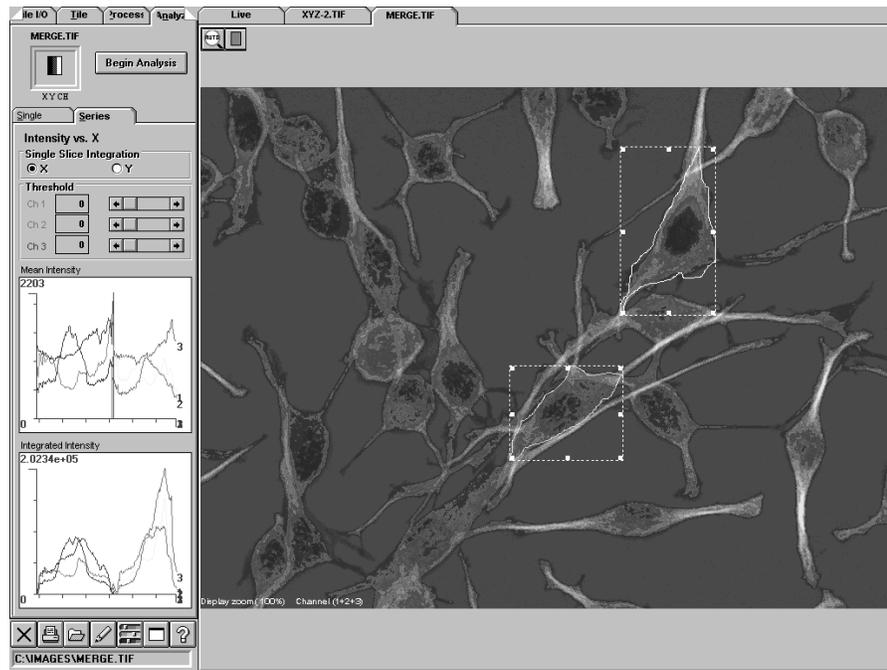


Fig. 2-117 Panel After Analysis

- Double-click the [Mean Intensity] box. The [Average Intensity Trace] window appears as shown below.

<Properties> button

Displays the [Editing] dialog box for use in detailed setting of the chart or change of the chart display.

See section 2-15, "Changing the Chart Display Method" for details.

<Copy> button

Copies the plotted image in the clipboard.

<Save> button

Saves the profile data in a file using an Excel-compatible format.

<Close> button

Quits the [Average Intensity Trace] window and returns to the [Analyze] panel.

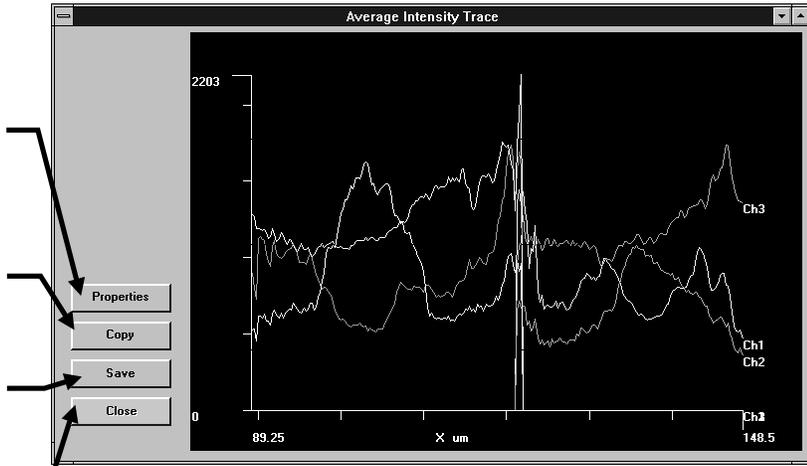


Fig. 2-118 [Average Intensity Trace] Window

TIP When a desired area is specified by dragging the left button of the mouse on the graph, the specified area can be magnified.

TIP When the right button of the mouse is dragged on the graph, the graph can be scrolled.

TIP The magnification or scrolling of the graph can be canceled by dragging the left button of the mouse from the bottom left to the right of the magnified graph.

TIP When the mouse pointer is placed on a graph line while the **Ctrl** or **Alt** key is held depressed, the coordinates can be displayed.

TIP The displayed data can be applied to other applications. See section 2-11, "Transferring Data to Another Application" for details.

2-7-3-4 Measuring the Change in Integrated Intensity

The total value of the intensity in a region specified in an image can be measured and displayed graphically.

The operation results are displayed graphically in the [Integrated Intensity] box in the [Series] sub-panel.

The operation method is completely identical to that for obtaining the mean value of intensity. See section 2-7-3-3, "Measuring the Change in Mean Value of Intensity".

Double-click the [Integrated Intensity] box. The [Integrity Intensity Trace] window appears as shown below.

<Properties> button

Displays the [Editing] dialog box for use in detailed setting of the chart or change of the chart display.

See section 2-15, "Changing the Chart Display Method" for details.

<Copy> button

Copies the plotted image in the clipboard.

<Save> button

Saves the profile data in a file using an Excel-compatible format.

<Close> button

Quits the [Integrated Intensity Trace] window and returns to the [Analyze] panel.

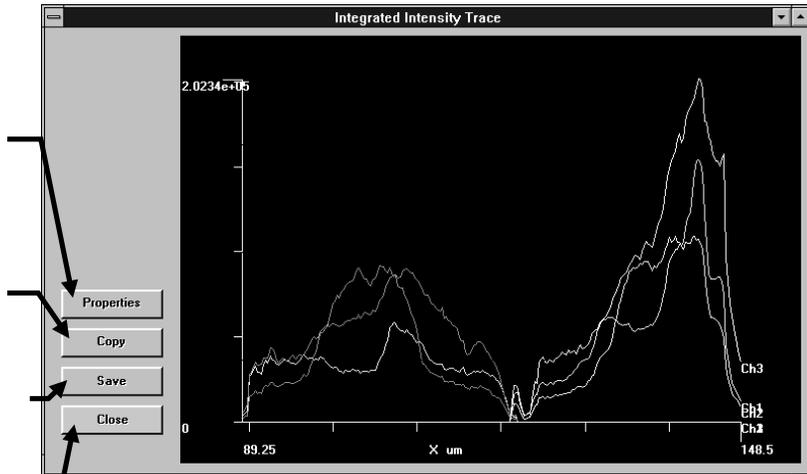


Fig. 2-119 [Integrated Intensity Trace] Window

- TIP** When a desired area is specified by dragging the left button of the mouse on the graph, the specified area can be magnified.
- TIP** When the right button of the mouse is dragged on the graph, the graph can be scrolled.
- TIP** The magnification or scrolling of the graph can be canceled by dragging the left button of the mouse from the bottom left to the right of the magnified graph.
- TIP** When the mouse pointer is placed on a graph line while the **Ctrl** or **Alt** key is held depressed, the coordinates can be displayed.
- TIP** The displayed data can be applied to other applications. See section 2-11, "Transferring Data to Another Application" for details.

2-8 Building an Image from a Different Viewpoint

2-8-1 Building Extended Focus Image from XYZ Image

2-8-1-1 Display Switching to Built Image

An extended focus image can be built from XYZ (multiple sections) images and the display can be switched to show the built image.

1. Display the [Display] panel of the XYZ (multiple sections) image.
2. The following button is displayed at the top of the [Display] panel.
(Usually, only the <XYZ series> button is displayed. When it is clicked, a list of buttons appears as shown below.)



<XYZ series> button

Displays only one of multiple sections image slices.

<Extend> button

Displays the extend image.



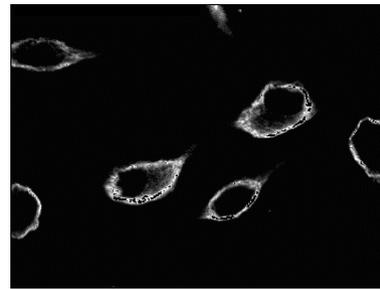
<Display>button

3. Click the <Extend> button.
4. Click the <Display> button at the top of the [Display panel] repeatedly to build the extended-focus image.

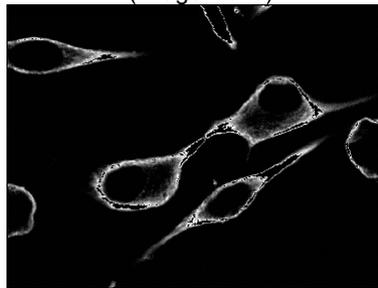
(The extended-focus image can also be displayed by using the <Display> button to start successive display.)



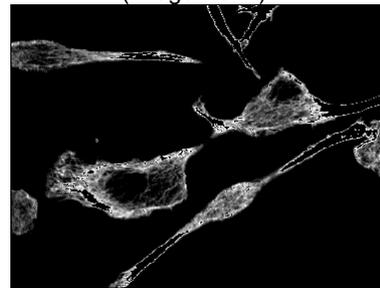
(Image No. 0)



(Image No. 1)



(Image No.2)



(Image No. 3)

Fig. 2-120 Four Images Used in Building Extended-Focus Image

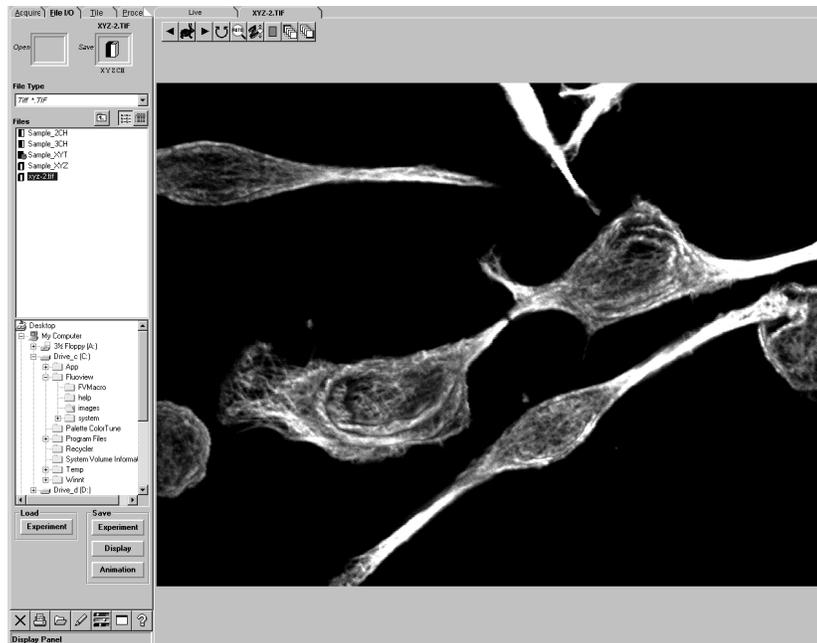


Fig. 2-121 Panel Showing an Extended-Focus Image



Among the multiple image slices composing the XYZ image, the range of image slices to be used in extend image building can be specified.



<Set start position>
button



<Set end position>
button

1. Display the image slice to be set as the start image by using the <Display> buttons at the top of the [Display] panel.
2. Click the <Set start position> button at the top of the [Display] panel.
3. Display the image slice to be set as the end image by using the <Display> buttons at the top of the [Display] panel.
4. Click the <Set end position> button at the top of the [Display] panel.

NOTE

The <Set start position> and <Set end position> buttons are valid in the pushed-in condition. To cancel a previously set start or end position, click the <Set start position> or <Set end position> button again.

NOTE

When it is required to analyze the built extended-focus image or save it in the Fluoview Multi Tiff format, create the extended-focus image as a separate image from the original image. See section 2-8-1-2, "Turning Built Image into Single Image".

2-8-1-2 Turning Built Image into Single Image

From XYZ (multiple sections) image, an extended-focus image can be built as a separate image from the original image.

Use the [Visualize] panel to build the image.

First display the [Visualize] panel.

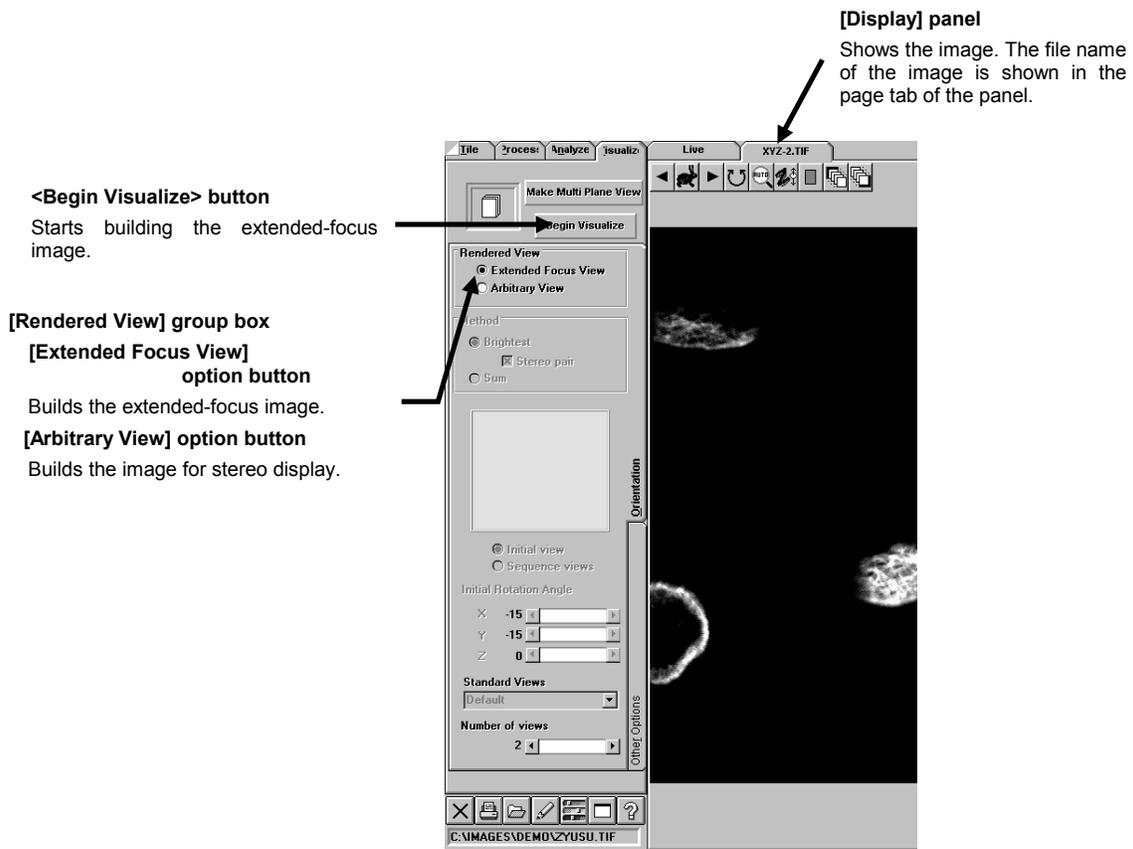


Fig. 2-122 [Visualize] Panel

1. Display the [Display] panel of the XYZ (multiple sections) image.
2. Click the [Extended Focus View] option button in the [Rendered View] group box.
3. Click the <Begin Visualize> button to start the image building. When it completes, the built image is displayed in the [Extended] panel.

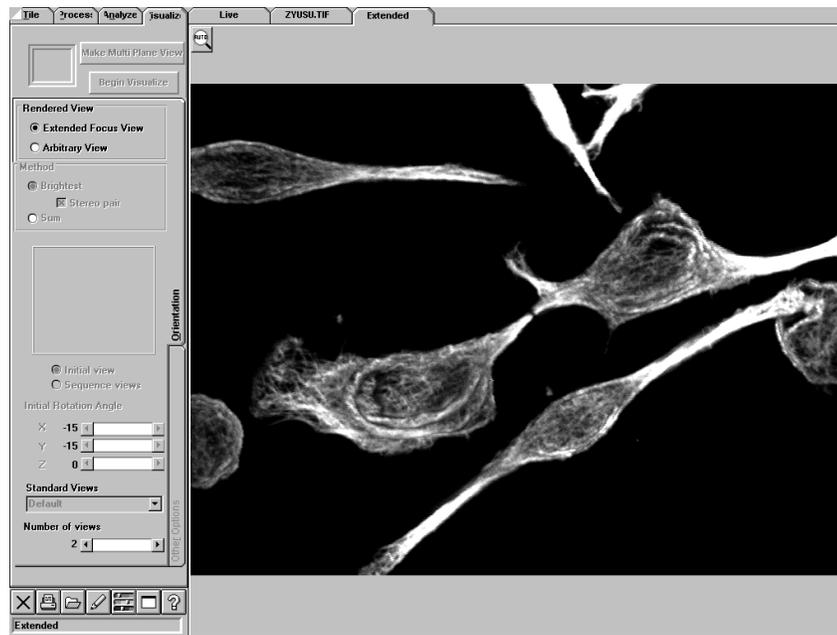


Fig. 2-123 Panel Showing Extended-Focus Image

2-8-1-3 Turning Built Image into time series image

From XYZT image, an extended-focus image can be built as a separate time series image from the original image.

Use the [Visualize] panel to build the image.

First display the [Visualize] panel.

1. Display the [Display] panel of the XYZT image.
2. Click the [Extended Focus View] option button in the [Rendered View] group box.
3. Click the <Begin Visualize> button to start the image building. When it completes, the built image is displayed in the [Extended] panel.

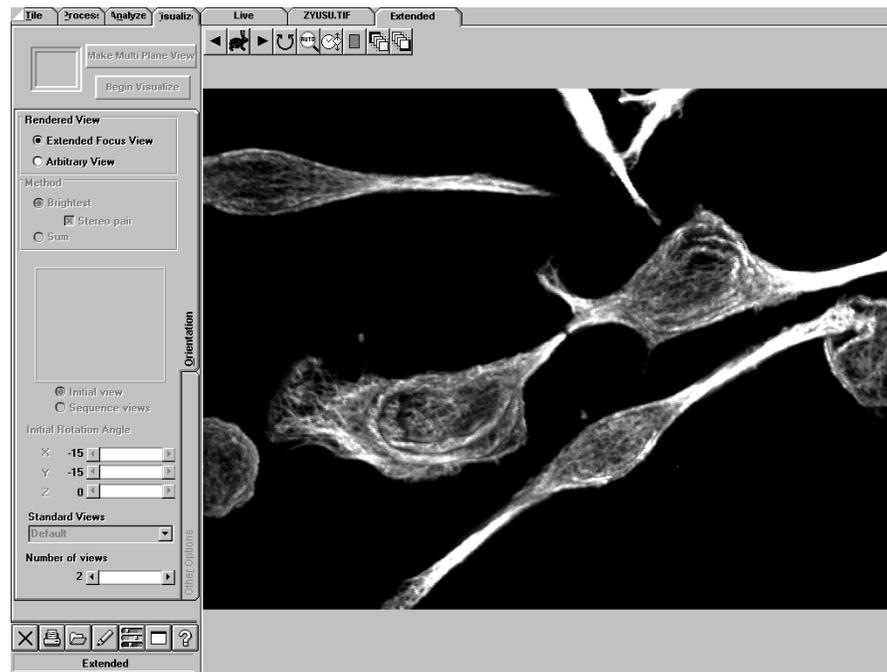


Fig. 2-124 Panel Showing Extended-Focus Image

2-8-2 Building line images to be viewed in Z direction

The images acquired by cutting the XYZ image off vertically and horizontally can be displayed with the information on each line.

Each line images is created as a separate image from the original one.

To display the line images, use the [Visualize] panel.

Display the [Visualize] panel.



Make Multi Plane View

<Make Multi Plane View>
button

1. Display the [Display] panel of the XYZ (multiple sections) image.
2. Click the <Make Multi Plane View> option button in the [Rendered View] panel.
The [3D-] panel is created to start the line image building.

Image display in XY-direction

The line image to be displayed in XZ or YZ-direction can be moved by dragging the red line (XZ) or brown line (YZ) on the XY-direction image.

Line image display in XZ-direction

Display the image of the line specified on the XY-direction image.

Line image display in YZ-direction

Display the image of the line specified on the XY-direction image.

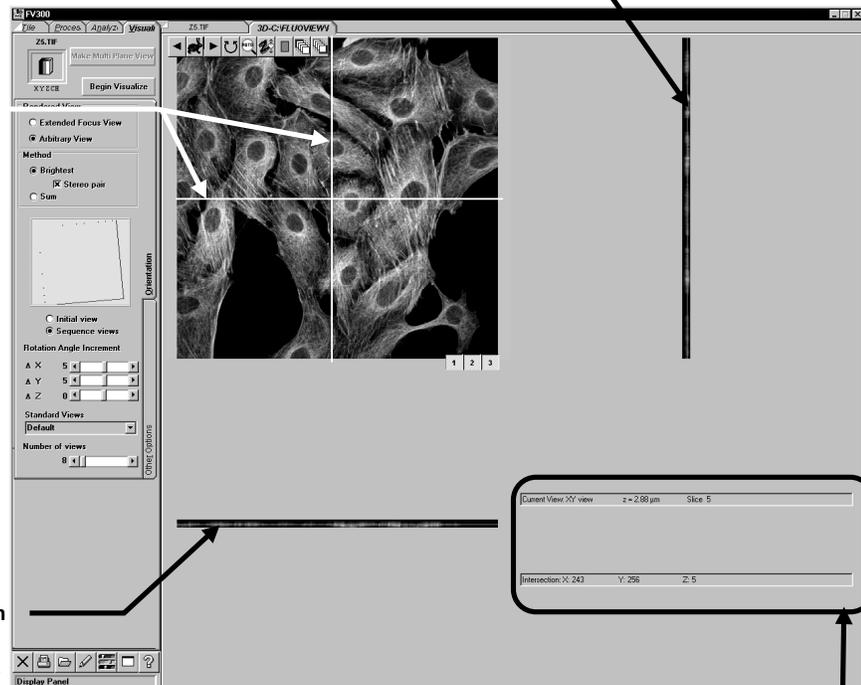


Fig. 2-125 Panel showing the line images to be viewed in Z direction

Upper row: shows the current slice and the steps in Z-direction acquiring the image.

[z=]: the number of current steps

[Slice]: the number of current slice

Lower row: shows the current X and Y coordinates and Z position on the XY-direction image.

[X.]: current X position

[Y.]: current Y position

[Z.]: current Z position



If you change the slice to be displayed with the <Display> button, [Slice] and [Z:] aren't effected.

[Slice] and [Z:] show current slice and Z position according to moving the red or brown line indicating XZ or YZ-direction on the XY-direction image displayed left above.

2-9 Viewing 3D Image

Use the [Visualize] panel to view an image three-dimensionally.

First display the [Visualize] panel.

[Begin Visualize] button

Starts building the images for 3D display.

[Method] group box

[Brightest] option button

Builds the image by accumulating the intensity value.

[Stereo Pair] check box

To be checked when building a pair of stereo 3D images or a 3D image to be viewed through color (red/green) eyeglasses.

[Sum] option button

Builds the image by adding the intensity values.

[Initial view] option button

Sets the angle at which the rotation should start. The angle itself can be set using the [Initial Rotation Angle] scale immediately below the option button.

[Sequence views] option button

It is selected to determine per what degree the view should be rotated. The [Rotation Angle Increment] scale and the [Total angle] scale appear as shown in lower right figure and each angle factor can be set.

[Display] panel

Shows the image. The file name of the image is shown in the page tab of the panel.

[Rendered View] group box

[Extended Focus View] option button

Checking this option button changes the check box in the [Method] group as shown in the lower left figure.

[Arbitrary View] option button

Builds the image for 3D display.

[Initial Rotation Angle] Scale

This appears when [Initial View] option button is selected. Angle can be set.

[Rotation Angle Increment] scale

Set the angles

[Standard Views] drop-down list

Selects the rotation direction. It is possible to set an arbitrary direction.

[Number of views] scale

Sets the number of views displayed during rotation.

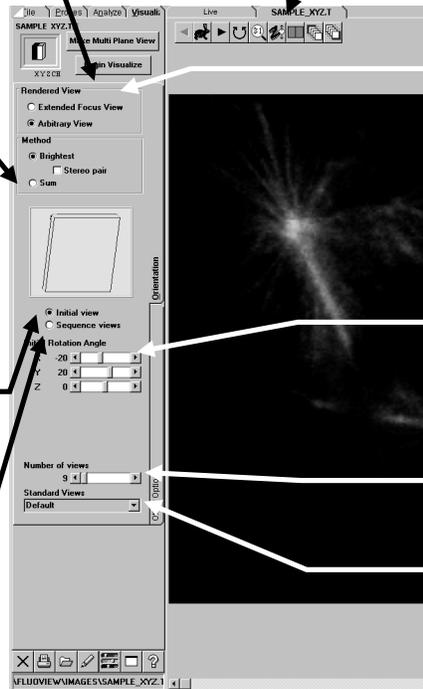


Fig. 2-128 [Visualize] Panel and [orientation] Sub-panel

[Method] Group Box

When [Extended Focus View] option button is selected, [Method] group box will change as shown in figure.

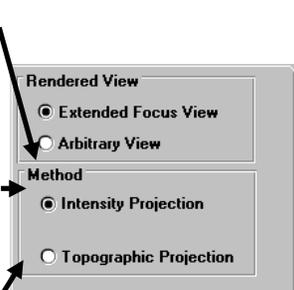
[Intensity Projection] Option Button

Builds an image that focused on each cross section as shown in Fig. 2-108.

[Topographic Projection] Option Button

Builds an image that indicates which cross section the focal point is located as shown in Fig. 2-109.

Fig 2-126 when [Extended Focus View] option button is selected



[Rotation Angle Increment] Scale

Set an angle increment for the view rotation.

[Total angle] Scale

Set the total angle from 1st view to the last one.

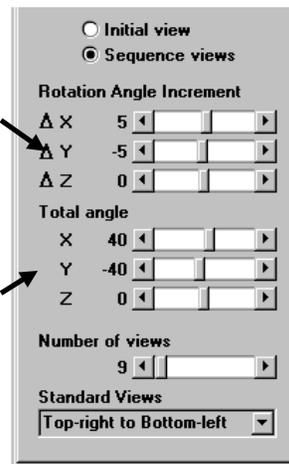


Fig 2-127 when [Sequence Views] option button is selected



Fig 2-129 Example of Intensity Projection

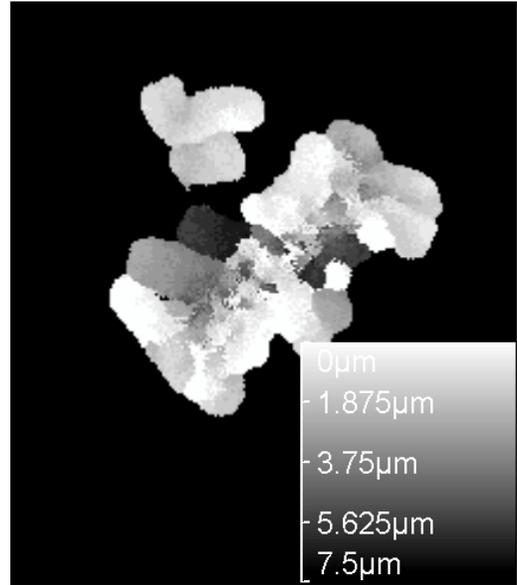


Fig 2-130 Example of Topographic Projection

[Threshold range Low / High] scale

Sets the range of intensity values to be used in image building.

[Depth Weight] scale

Sets the weighting in the depth direction. By increasing this setting, it is possible to provide the image with a perspective by darkening the far objects and brightening near objects.

[Stereo Factor] text box

Sets the deviation between the left and right eyes when building a pair of stereo 3D images or a 3D image to be viewed through color (red/green) eyeglasses.

[Z stretch Factor] text box

Provides each multiple sections image with a feeling of thickness. The value displayed here in advance has been calculated by the system so that the scale in the planar, or XY-direction of the image is identical to the scale in the depth, or Z-direction. Usually, this value does not need to be changed.

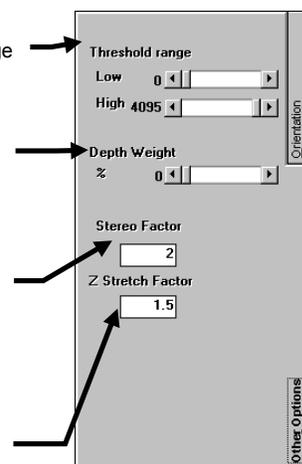
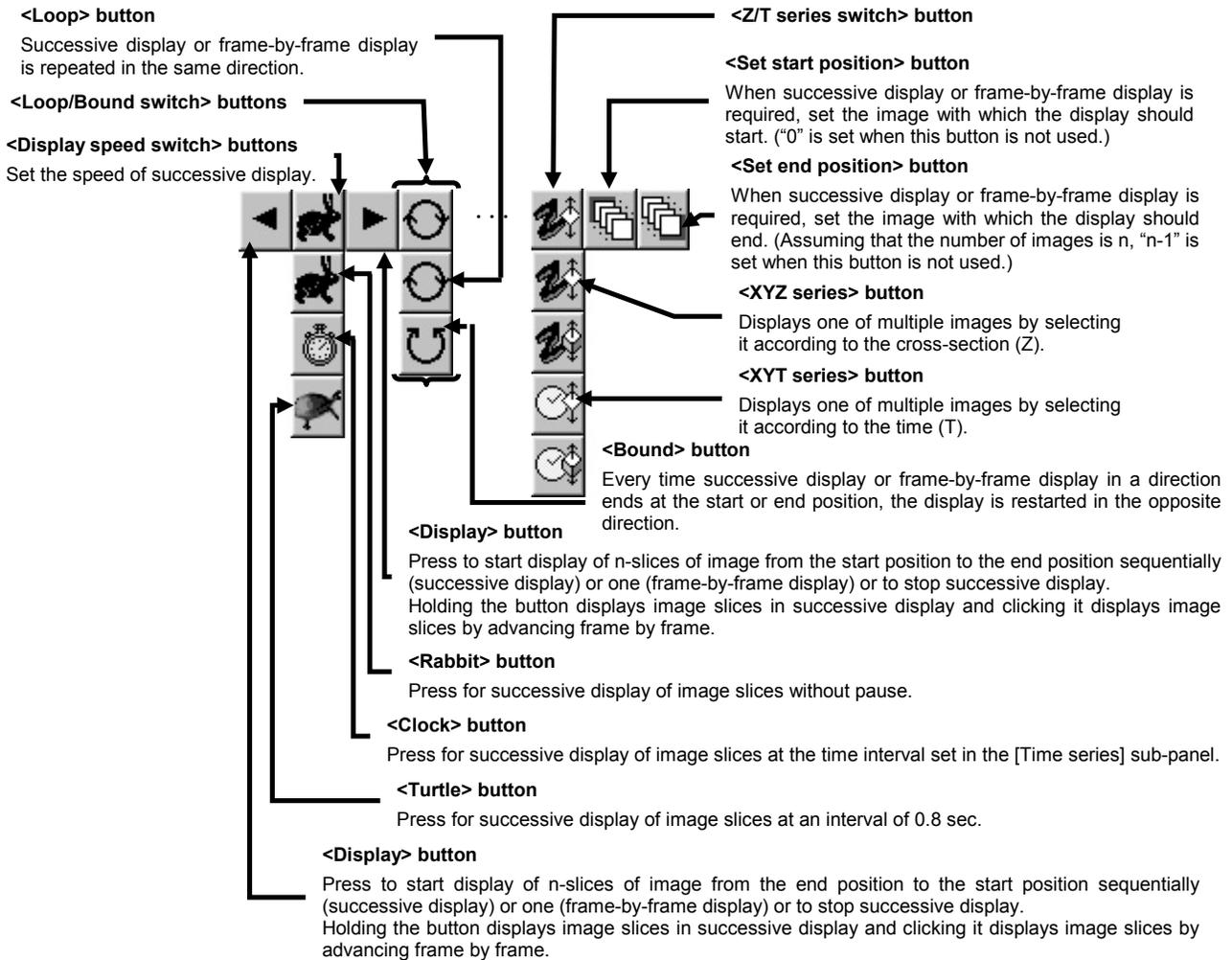


Fig. 2-131 [Other options] Sub-panel of [Visualize] Panel

2-9-1 Successive Display of Images

Images composed of multiple image slices acquired by varying the multiple sections (XYZ observation, XYZT observation) can be displayed successively using the buttons at the top of the [Display] panel as shown below.

1. Display the [Display] panel of the image composed of multiple image slices.
2. The buttons as shown below are displayed at the top of the [Display] panel. If the <XYZ series> button is not displayed under the <Z/T series switch> button while the images were acquired in XYZT observation mode, click the <Z/T series switch> button and click the <XYZ series> button in the displayed list of buttons.
3. Display the image slice to start the successive display by using the <Display> button at the top of the [Display] panel.



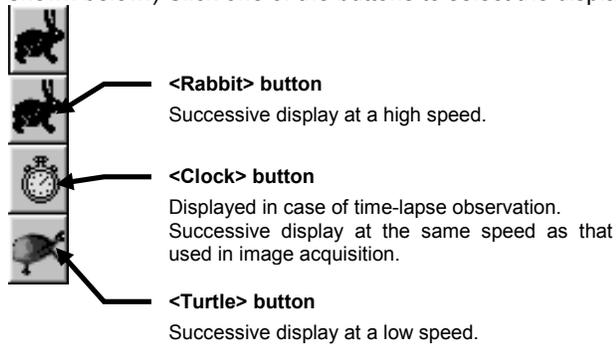


4. Click the <Set start position> button. (If the start position is not set, image No. 0 becomes the start image automatically.)
5. Display the image slice to end the successive display by using the <Display> button at the top of the [Display] panel.
6. Click the <Set end position> button. (If the end position is not set, image No. n-1, assuming that the number of images is n, becomes the start image automatically.)
7. Click and hold the <Display> button. The image slices will be displayed successively from the start position to the end position.
To stop the successive display, click the <Display> button again.

2-9-1-1 Changing the Successive Display Speed

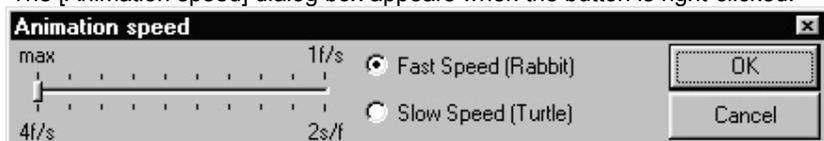
The buttons on the top of the [Display] panel can be used to vary the speed of successive display of multiple image slices.

1. Display the [Display] panel of the image to be subjected to successive display speed change.
2. The buttons as shown below are displayed at the top of the [Display] panel. (Usually, the <Rabbit> button is displayed. Clicking it displays a list of buttons as shown below.) Click one of the buttons to select the display speed.



3. The display speed provided by the <Rabbit> or <Turtle> button can be varied by clicking the mouse right button on the button.

The [Animation speed] dialog box appears when the button is right-clicked.



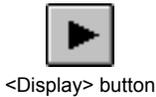


4. Select the option button of the speed to be varied.
5. Set the desired display speed in the scale on the right.
6. Click the <OK> button to close the [Animation speed] dialog box.

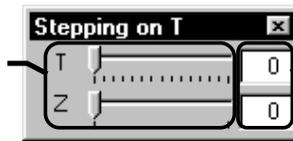
2-9-1-2 Changing the successive image display position

The successive display position of multiple image slices in an image can be changed using a bottom displayed at the top of the [display] panel.

1. Display the [display] panel of the image subjected to successive display position change at the front.
2. Click the mouse right button on the <Display> button at the top of the [Display] panel to display a scale. (The following figure shows the scale with an XYZT image.)



Drag the scale or click a point on it to change the display position to a position before or after the current position.



The display position can also be changed by direct entry of the value.

3. Drag the scale to another position to move the successive display position to the position.
Entering the value directly in the text box can also move the display position.

2-9-2 Animation

Images composed of multiple image slices acquired by varying the multiple sections (XYZ observation, XYZT observation) can be built into animation image, which can be displayed in 3D by rotating images.

1. Display the [Display] panel of the image composed of multiple image slices.
2. When the images were acquired in the multi-channel mode, select whether animation is built from images of more than one channel or from an image of only one channel.



To select the target channel(s), use the <Display channel switch> buttons. Only the channel(s) being displayed will be used in the animation building.

Example) When only the Ch1 image is displayed, only the Ch1 image is used.

TIP

For the switching of channels, see section 2-5-3, "Switching the Display Channels".

3. Click the [Arbitrary View] option button in the [Rendered View] group box.
4. Click the [Brightest] or [Sum] option button in the [Method] group box.
5. Select the image rotation direction from the [Standard Views] drop-down list.
6. Click the [Initial view] option button.
7. Set the angle at which the rotation should start using the [Initial Rotation Angle] scale.
8. Click the [Sequence views] option button.
9. Set the angle per rotation step using the [Rotation Angle Increment] scale.
10. Set the number of images to be rotating using the [Number of views] scale.
11. Click the <Begin Visualize> button to start building the animation. When the building completes, the built image is displayed in the [3D Animation] panel.

TIP

The status bar shows the progress of building processing.



During the building, the <Begin Visualize> button turns into the <Cancel Visualize> button. Click the <Cancel Visualize> button if you want to cancel the building.

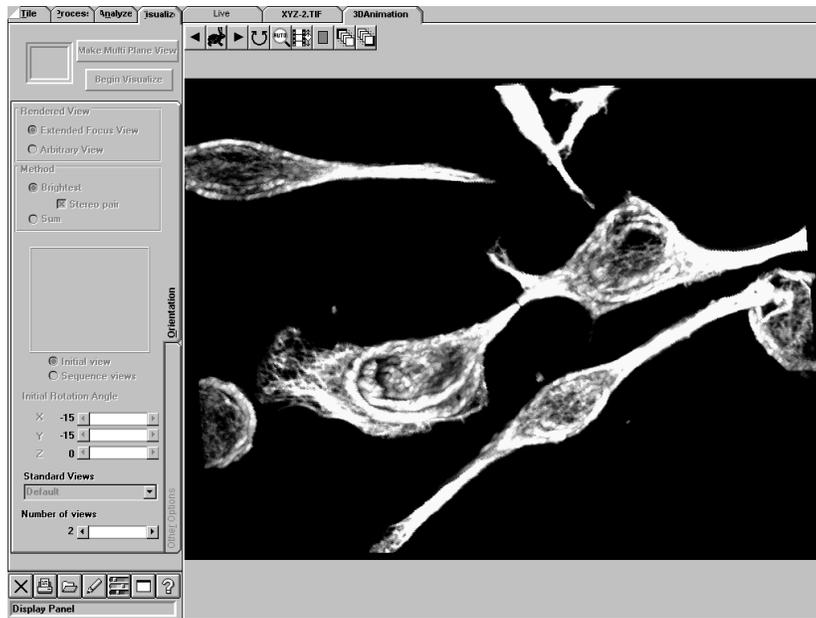


Fig. 2-132 Panel Showing the Built Image



- Click and hold the <Display> button at the top of the [3D Animation] panel. The images will rotate so that they can be viewed three-dimensionally.



Click the <Display> button to stop the image rotation.

2-9-3 Building Stereo 3D Images

Images composed of multiple image slices acquired by varying the multiple sections (XYZ observation) can be built into a pair of stereo 3D images. These images can be viewed three-dimensionally by watching them with two eyes for a while.

The operation is similar with animation. Perform steps 1 to 10 in section 2-9-2, "Animation", then proceed to the following steps.

1. Check the [Stereo Pair] check box.
2. Click the <Begin Visualize> button to start building the images. When the building completes, the built images are displayed in the [3D Animation] panel.



The status bar shows the progress of building processing.



During the building, the <Begin Visualize> button turns into the <Cancel Visualize> button. Click the <Cancel Visualize> button if you want to cancel the building.



<Cancel Visualize>
button

3. The following buttons are displayed at the top of the [Display] panel.



Stereo 3D images.

These buttons can be used to switch the stereo 3D images between the Ch1-only images, Ch2-only images, Ch3-only images and images overlaying multiple channels. Select the channel(s) of interest.

4. Watch the two images with two eyes for a while. They will look as a single 3D image.

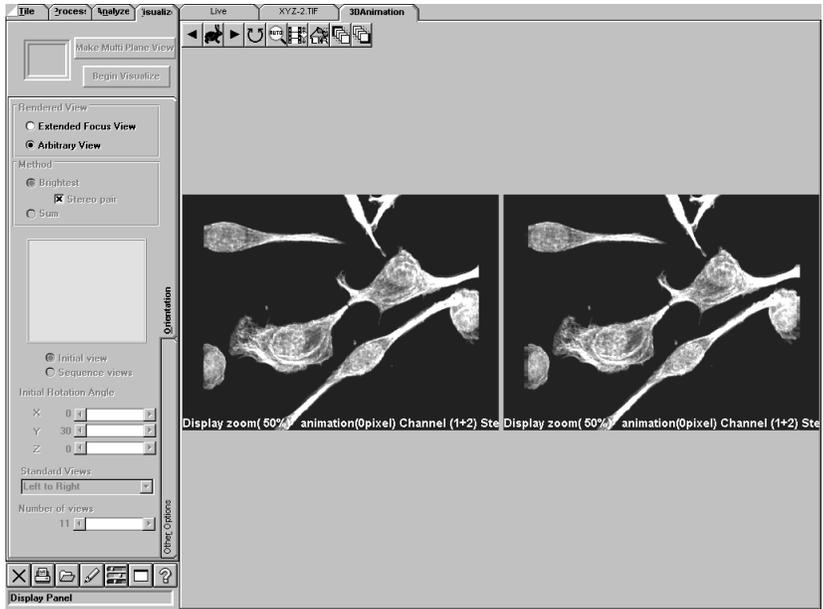


Fig. 2-133 Panel Showing Stereo 3D Images

2-9-4 Building a 3D Image to be Viewed Through Color (Red/Green) Eyeglasses

Images composed of multiple image slices acquired by varying the multiple sections (XYZ observation) can be built into an image which looks 3D when viewed through a pair of color (red/green) eyeglasses. This image can be viewed three-dimensionally by watching them with two eyes for a while.

The operation is similar with animation. Perform steps 1 to 10 in section 2-9-2, "Animation", then proceed to the following steps.

1. Check the [Stereo Pair] check box.
2. Click the <Begin Visualize> button to start building the images. When the building completes, the built image is displayed in the [3D Animation] panel.



The status bar shows the progress of building processing.



<Cancel Visualize>
button



During the building, the <Begin Visualize> button turns into the <Cancel Visualize> button. Click the <Cancel Visualize> button if you want to cancel the building.

3. The following buttons are displayed at the top of the [Display] panel.



3D image for color (red/green) eyeglasses.

These buttons can be used to switch the 3D images between the Ch1-only image, Ch2-only image and Ch3-only image. Select the channel(s) of interest.

4. Watch the image through a pair of color (red/green) eyeglasses.



The color (red/green) eyeglasses can also be used to view the animation. To start animation, click the <Successive display> button.

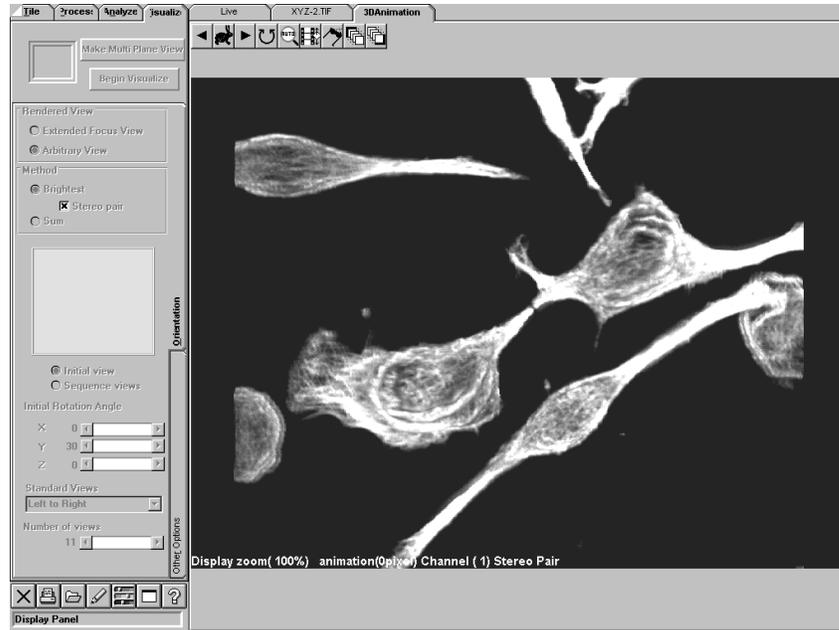


Fig. 2-134 Panel Showing 3D Image to be Viewed Through Color Eyeglasses

2-10 Viewing Images Following the Progress of Time

Images composed of multiple image slices (XYT, XYZT or XZT observation) can be displayed following the time lapse to show the change over time.

2-10-1 Displaying Images Together

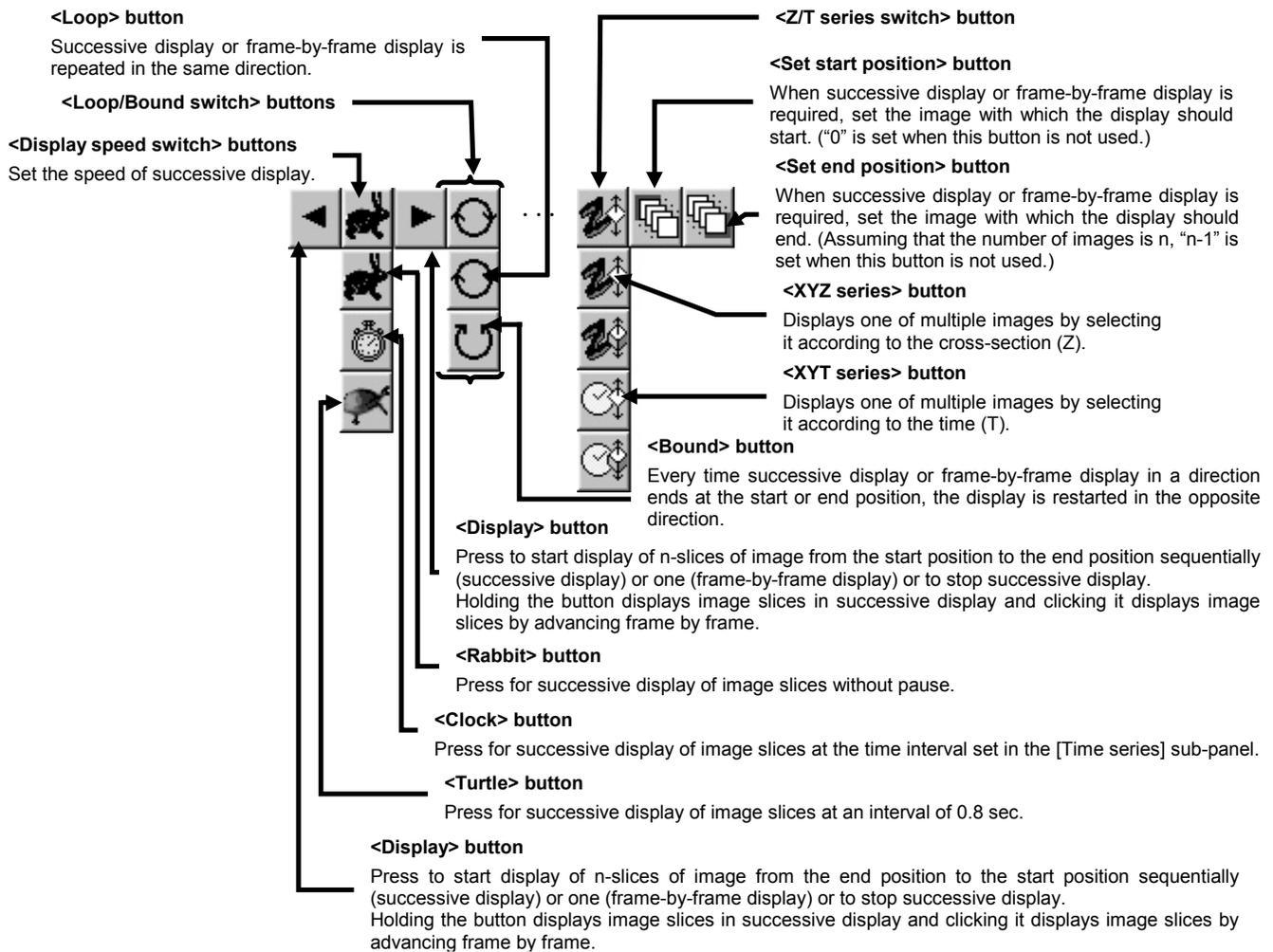
The change over time can be viewed at a glance by displaying multiple image slices together. For the detailed operation method, see section 2-5-7, "Displaying Multiple Image Slices Together".

2-10-2 Displaying Images Successively

The change over time can also be displayed as animation.

With images composed of multiple slices, such as time-lapse images the buttons as shown below are displayed at the top of the [Display] panel.

1. Display the [Display] panel of the image composed of multiple image slices.
2. To display XYZT observation images by noticing the progress of time, it is required to select the <XYT series> button under the <Z/T series switch> button at the top of the [Display] panel. If the <XYT series> button is not displayed, click the <Z/T series switch> button and click the <XYT series> button in the displayed list of buttons.
3. Display the image slice to start the successive display by using the <Display> button at the top of the [Display] panel.



4. Click the <Set start position> button. (If the start position is not set, image No. 0 becomes the start image automatically.)
5. Display the image slice to end the successive display by using the <Display> button at the top of the [Display] panel.
6. Click the <Set end position> button. (If the end position is not set, image No. n-1, assuming that the number of images is n, becomes the start image automatically.)
7. Click and hold the <Display> button. The images will be displayed successively from the start position to the end position.
Click the <Display> button to stop the successive display.

2-11 Transferring Data to Another Application

2-11-1 Transferring Analysis Data to Another Application

Analysis data can be transferred to Excel.

1. Display the [Analyze] panel and executes analysis. After it, display the [Enhanced Profile Plot], [Intensity Map], [Enhanced Histogram Plot], {Average Intensity Trace} or [Integrated Intensity Trace].



For the operation method, see section 2-7, "Image Analysis".

<Save> button

Saves the profile data in a file using an Excel-compatible format.

<Close> button

Quits the [Enhanced Profile Plot] window and returns to the [Analyze] panel.

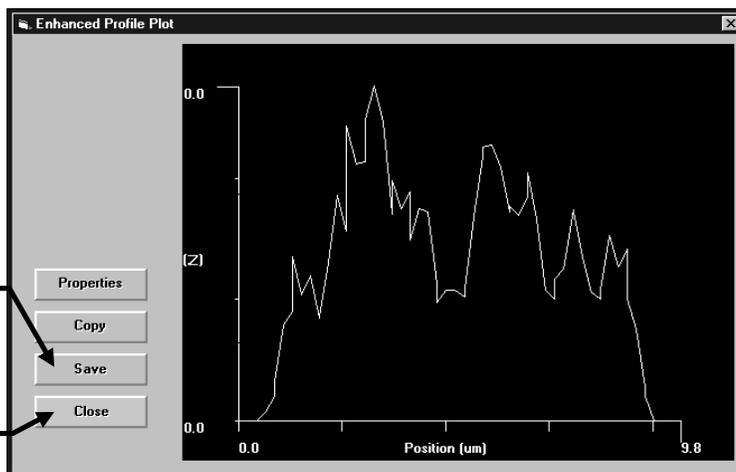


Fig. 2-135 [Enhanced Profile Plot] Window



Microsoft Excel is not included in the FLUOVIEW FV300 system.
Please purchase it separately.



- Click the <Save> button. When the [Save As] dialog box appears as shown below, set the file name and click the <OK> button to save the analysis data.

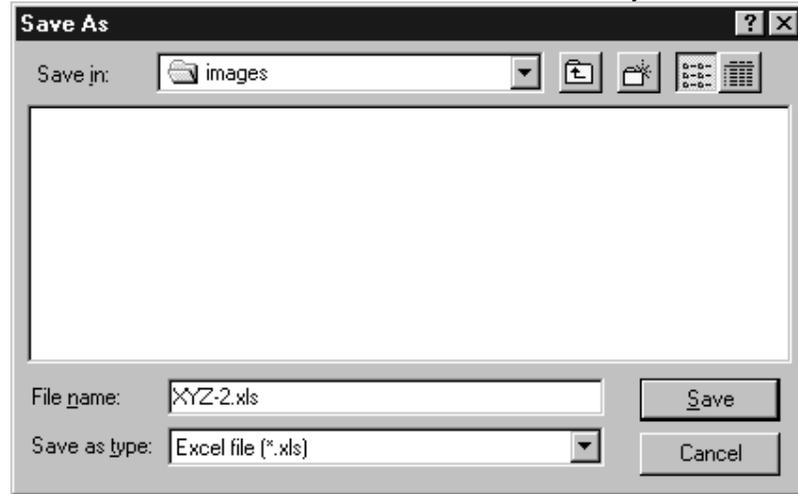


Fig. 2-136 [Save As] Dialog Box

- Exit from FLUOVIEW or display the [Start] menu by pressing the **Ctrl** + **Esc** keys.
- Select [Programs] and issue the [Microsoft Excel] command.
- From the [File] menu of Excel, select the [Open] command and open the file saved in step 2.
- When the dialog box as shown below appears, click the [Delimited] option button in the [Original Data Type] group box, then select [Windows (ANSI)] from the [File Origin:] drop-down list.

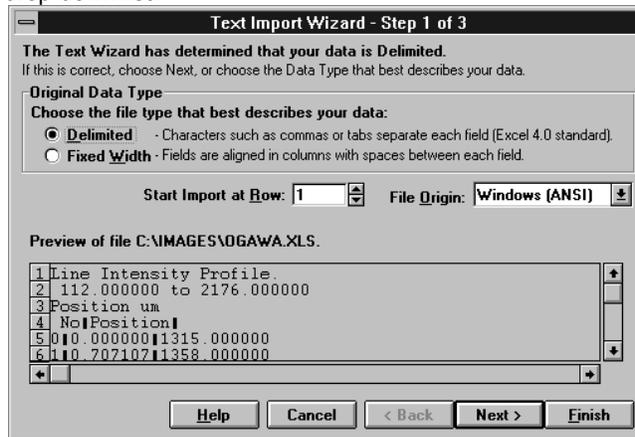


Fig. 2-137 Dialog Box When the File is Opened by Excel 1/3

7. Click the <Next> button. When the dialog as shown below appears, check the [Tab] check box in the [Delimiters] group box, then select [[none]] from the [Text Qualifier:] drop-down list.

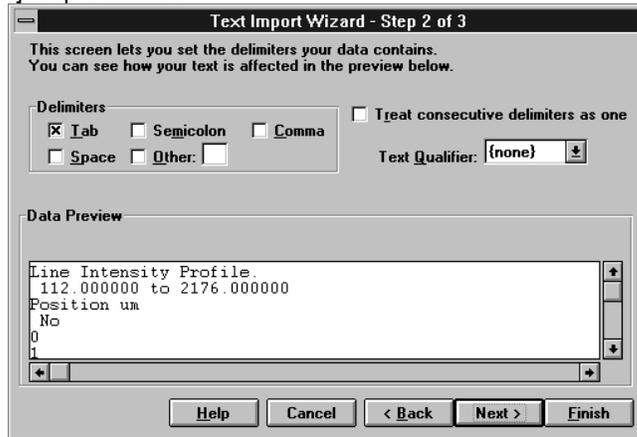


Fig. 2-138 Dialog Box When the File is Opened by Excel 2 / 3

8. Click the <Next> button. When the dialog as shown below appears, select the [General] option button in the [Column Data Format] group box, then click the <Finish> button.



Fig. 2-139 Dialog Box When the File is Opened by Excel 3 / 3



TIP

For detailed operation procedures of Excel, refer to the [Excel manuals].

	A1			Line Intensity Profile					
	A	B	C	D	E	F	G	H	
1	Line Intensity Profile.								
2	112.000000 to 2176.000000								
3	Position um			0.000000 to 181.048776					
4	No	Position							
5	0	0	1315						
6	1	0.707107	1358						
7	2	1.414214	1353						
8	3	2.12132	1334						
9	4	2.82132	1365						
10	5	3.328427	1404						
11	6	4.035534	1276						

2-11-2 Transferring the Plot Image of Analysis Data to Another Application

The plot image of analysis data can be transferred to an application handling images, such as Paint. The following description takes Paint as example.

1. Display the [Analyze] panel and executes analysis. After it, display the [Enhanced Profile Plot], [Intensity Map], [Enhanced Histogram Plot], [Average Intensity Trace] or [Integrated Intensity Trace].

TIP

For the operation method, see section 2-7, "Image Analysis".

<Copy> button

Copies the plot image to the clipboard.

<Close> button

Quits the [Enhanced Profile Plot] window and returns to the [Analyze] panel.

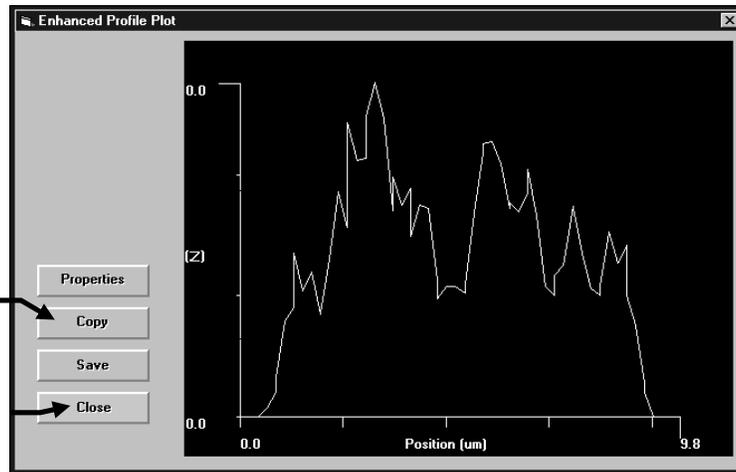


Fig. 2-140 [Enhanced Profile Plot] Window

2. Click the <Copy> button to copy the plot image to the clipboard.
3. Exit from FLUOVIEW or display the [Start] menu by pressing the Windows key or the **Ctrl** + **Esc** keys.
4. Select [Programs]-[Accessories] and issue the [Paint] command.
5. From the [Edit] menu of Paint, select the [Paste] command and paste the plot image which has been copied to the clipboard in step 3.

TIP

For detailed operation procedures of Paint, refer to the [help provided by Paint].

2-11-3 Transferring Image Data to Another Application (microVoxel, Paint, etc.)

To transfer image data to another application, the image data should be saved in a file and the file should be transferred.

1. Save an image using one of the formats that can be handled by the destination application. See sections 2-3, "Saving, Opening and Shredding Images" and 2-3-1, "Saving Images" for the image saving procedure.
2. Exit from FLUOVIEW or display the [Start] menu by pressing the Windows key or the **Ctrl** + **Esc** keys.

NOTE

To transfer data to microVoxel, it is required to exit from FLUOVIEW, exit from Windows and restart OS/2.

3. Start the image transfer destination application (Paint, etc.).
4. In the application, open the file saved in step 1.

2-12 Entering Comment in Image

Comment can be entered in an image for use in presentation or slide creation.



<Annotate>button

Use the <Annotate> button in the toolbar at the bottom left of the screen. Click the <Annotate> button. A list of buttons appears as shown below.



2-12-1 Writing Characters in Image

This facility is used to enter the title, acquisition parameters and/or notes in an image. Some labels are provided in advance. Characters can be written either by using these labels or entering desired characters at will.



<Text>botton

1. Display the [Display] panel of the image in which you want to write characters.
2. Click the <Text> button in the displayed list of buttons. The dialog as shown below appears.

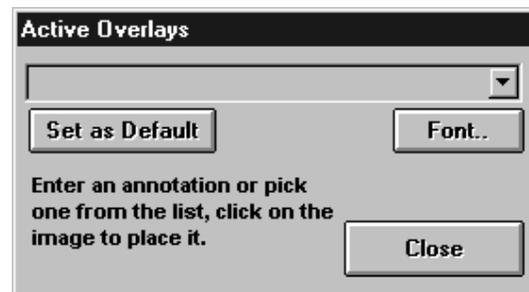


Fig. 2-141 [Active Overlays] Dialog Box



3. From the drop-down list in the dialog box, select one of the following labels.
 - Z Position <z>
 - Time Instant <t>
 - Channel # <channel>
 - Display Zoom <display zoom>
 - Is bounded <bounds>
 - Experiment Name <name>
 - <display zoom> <z> <t> <animation> <channel> <stereo> <bounds>

TIP

Desired characters can also be entered.

1. Click the character in the drop-down list of the dialog box.
2. Delete the character by pressing the **Delete** or **Back Space** key.
3. Enter characters from the keyboard.

TIP

When the <Set as Default> button is pressed after having selected a label, it is set as the label displayed permanently at the bottom left of the [Display] panel.

4. Click the button. The dialog box as shown below appears.

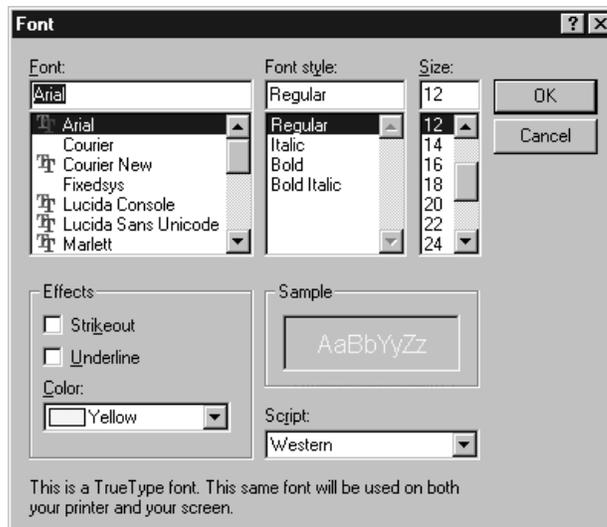


Fig. 2-142 [Font] Dialog Box



5. Select the character font and size using the [Font], [Font Style] and [Size] list boxes.
6. Click the <OK> button to close the [Font] dialog box.
7. Place and click the mouse pointer on the image position you want to enter characters.
8. Click the <Close> button to close the [Active Overlays] dialog box.



Refer to "Appendix I List of Functions in the [Active Overlays] Dialog Box" for other functions of the [Active Overlays] dialog box.

2-12-2 Displaying the Image Intensity

The intensity of any pixel of an image can be displayed without using the [Analyze] panel.

1. Display the [Display] panel of the image that you want to display the intensity at the front.
2. In the list of buttons displayed, click the <Text> button. The dialog box as shown below appears.



<Text> button

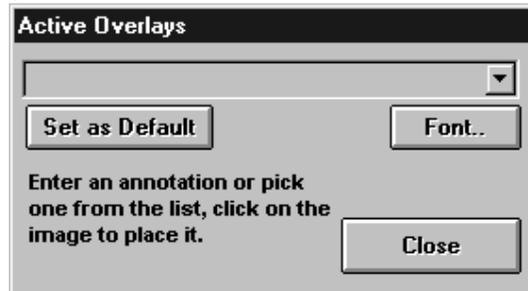


Fig 2-143 [Active Overlays] Dialog Box

3. In the drop-down list inside the dialog box, select "I = <intensity hotspot value>".
4. On the image, place the mouse pointer on the position that you want to display the intensity and click the mouse.
5. Click the <Close> button to close the [Active Overlays] dialog box.

2-12-3 Displaying the X-coordinate/Y-coordinate of the Image

The X-coordinate position or the Y-coordinate position of any pixel of an image can be displayed.



<Text> button

1. Display the [Display] panel of the image that you want to display the X-coordinate position or the Y-coordinate position at the front.
2. In the list of buttons displayed, click the <Text> button. The dialog box as shown below appears.

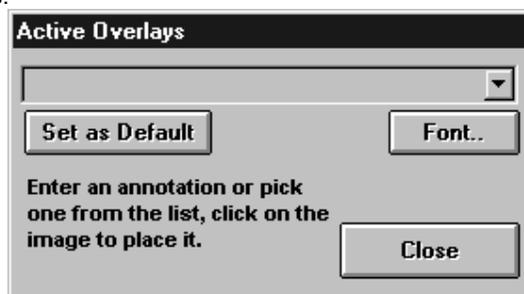


Fig 2-144 [Active Overlays] Dialog Box

3. In the drop-down list inside the dialog box, select "x = <x hotspot value "" units> " to display the X-coordinate position or "y = hotspot value "" units>" to display the Y-coordinate position.
4. On the image, place the mouse pointer on the position that you want to display the X-coordinate position or the Y-coordinate position and click the mouse.
5. Click the <Close> button to close the [Active Overlays] dialog box.



The displayed value indicates the distance from the origin when assuming the upper left of the image as the origin.

2-12-4 Drawing a Figure in Image

This facility is used to draw figures in the image. FLUOVIEW provides seven figure drawing modes.

1. Display the [Display] panel of the image in which you want to draw figures.
2. Select one of the following command buttons and draw a figure in the image using the mouse. The operation methods of the command buttons are described below.



<Line> button

To draw a straight line:

On the image, click the point that you want to start the straight line, and drag the mouse from there to the point you want to end it.



<Poly Line> button

To draw a polygonal line:

On the image, click the points corresponding to the start point, peak points and end point of the desired polygonal line, then click the right button of the mouse to set the specification.



<Rectangular> button

To draw a rectangle:

On the image, drag the mouse pointer along the diagonal line of the desired rectangle, from the top left corner to the bottom right corner.



<Circle> button

To specify a circle or ellipse:

On the image, assume a rectangle circumscribing the circle to be checked and drag the mouse pointer along the diagonal line between opposite corners of the rectangle.



<Polyregion> button

To specify a polygonal region:

On the image, click the points corresponding to the corners of the desired polygon. After clicking the last corner point, click the right button of the mouse to connect the last clicked point to the first clicked point.



<Free Region> button

To specify a free region:

On the image, specify a region by dragging. Then release the mouse button to complete dragging. The point where the dragging was ended will be connected to the point where it was started.



<Free Line> button

To specify a free line:

On the image, drag the mouse pointer along the desired line.

2-12-5 Drawing a Scale in Image

A scale can be drawn between two points in an image.



<Scale> button

1. Display the [Display] panel of the image in which you want to draw a scale.
2. Click the <Scale> button in the displayed list of buttons.
3. Click the image position you want to draw a scale.
4. Change the scale size. See 2-11-8, "Changing the Comment Size" for the operation procedure.

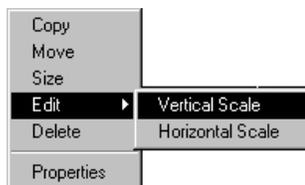
NOTE

To display a correct scale, it is required that the image has been acquired while the objective magnification setting in the software matches the magnification of the actually used objective.

TIP

A vertical scale can be drawn as well as a horizontal scale.

1. Click the mouse on the scale to turn the scale active (i.e. handles displayed around the scale).
2. Click the right mouse button.
3. Select [Edit] from the displayed menu. A sub-menu as shown below appears.



4. Select [Vertical Scale] to display a vertical scale.



<Text> button



The size of characters in the scale can also be changed.

The size of characters in the scale is determined by the size of characters written using the <Text> button. Perform the following operation before starting to draw the scale.

1. Click the <Text> button in the list of buttons.
2. When the <Annotation> dialog box is displayed, click its button. The [Font] dialog box will appear.
3. Change the character font and size using the [Font], [Font Style] and [Size] list boxes.
4. Click the <OK> button to close the [Font] dialog box.
5. Click the <OK> button to close the [Annotation] dialog box.

2-12-6 Drawing an Arrow in Image

This facility is used to draw an arrow for indicating a point in interest in image or adding explanation in it.

1. Display the [Display] panel of the image you want to draw an arrow.
2. Click the <Arrow> button in the displayed list of buttons.
3. Drag the mouse pointer from the start point to the end point of the desired arrow.
4. To change the arrow size, see section 2-11-8, "Changing the Comment Size".

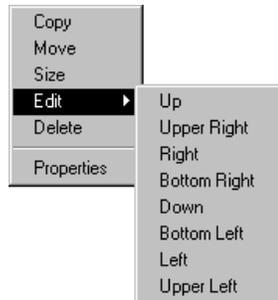


<Arrow> button

TIP

The direction indicated by a previously drawn arrow can be changed.

1. Click the mouse on the arrow to make the arrow active (i.e. handles displayed on the arrow).
2. Click the right button of the mouse.
3. Select [Edit] from the displayed menu. A sub-menu as shown below appears.



4. Select the desired arrow direction from the sub-menu.

2-12-7 Drawing Color Bars in Image

This facility is used to draw color bars in an image.

1. Display the [Display] panel of the image you want to draw color bars.
2. Click the <Color Bar> button in the displayed list of buttons.
3. Draw color bars in the image by dragging the mouse pointer along the desired position in the image.
4. To change the color bar size, see section 2-11-8, "Changing the Comment Size".



<Color Bar> button

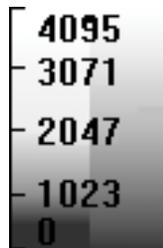


The labels of the color bars can be switched to display or hide.

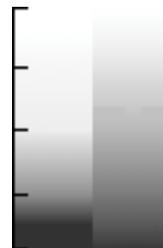
1. Click the mouse on/inside the color bars to select. (The handles appear around the color bars.)
2. Right-click the mouse.
3. Select [Edit] in the menu to display the sub-menu as shown below.



4. In the sub-menu, select "Show Labels" to display the labels or "Hide Labels" to hide the labels.



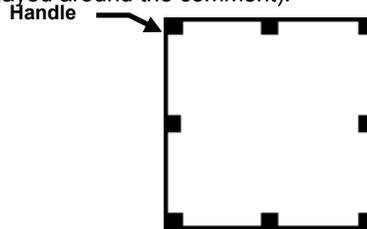
The labels are shown



The labels are hidden

2-12-8 Deleting Comment

1. Click the mouse on the comment to be deleted to make the comment active (i.e. handles displayed around the comment).



2. Click the right button of the mouse. A pop-up menu as shown below appears.

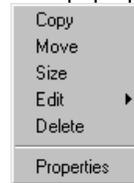


Fig. 2-145 Pop-up Menu

3. Select [Delete] from the menu.



To delete more than one comment simultaneously:

Select multiple comments and select [Delete] from the pop-up menu.

To select, click the mouse on or in the middle of one of the comments and click the mouse while holding the **Shift** key to select the second comment and after.

After making all of the comments to be deleted active (i.e. handled displayed around them), click the right button of the mouse on one of the comments and select [Delete] from the displayed pop-up menu.

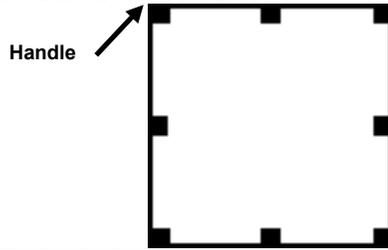
2-12-9 Moving Comment

1. Click the mouse on the comment to be moved to make it active (i.e. handles displayed around it).
2. Click the right button of the mouse. A pop-up menu as shown in Fig. 2-145 appears.
3. Select [Move] from the menu.
4. The mouse pointer turns into a cross (+). Move the mouse to move the comment together with the mouse pointer.
5. Click the left button of the mouse to determine the new position.



TIP

A comment can also be moved by selecting it, placing the mouse pointer on it so that the mouse pointer turns into a cross (+), then dragging the mouse. In this case, the final positioning of the comment can be determined by placing the mouse pointer outside the areas enclosed by the handles and clicking the left button of the mouse.

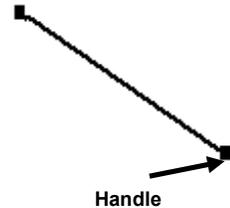


NOTE

The mouse pointer may be hardly visible depending on the image being displayed. In this case, use the method of displaying the pop-up menu.

2-12-10 Changing the Comment Size

1. Click the mouse on the comment to be resized to make it active (i.e. handles displayed around it).
2. Click the right button of the mouse. A pop-up menu as shown in Fig. 2-145 appears.
3. Select [Size] from the menu.
4. The menu pointer turns into . When the mouse is moved, the comment is magnified or reduced according to the mouse pointer.
5. Click the left button of the mouse to determine the size.



TIP

A comment can also be magnified or reduced by selecting it, placing the mouse pointer on one of the handles so that the mouse pointer turns into , then dragging the mouse.

2-12-11 Changing the Comment Color

1. Click the mouse on the comment to be changed of color to make the comment active (i.e. handles displayed around it).
2. Click the right button of the mouse. A pop-up menu as shown in Fig. 2-145 appears.
3. Select [Properties] from the menu. The [Properties] dialog box as shown below appears. Display the [Color] panel at the front.

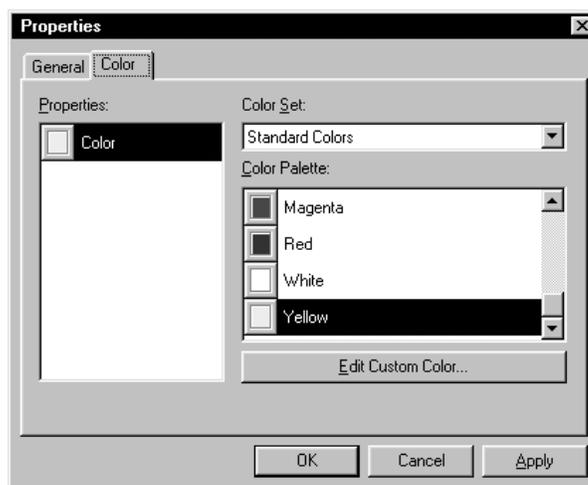


Fig. 2-146 [Properties] Dialog Box

4. Select the desired color from the [Color Palette] list box.

TIP

To change the comment color automatically, set in the [FLUOVIEW Setup] dialog box.

For details, see the description on the [Software] panel in section 1-3-1 in MAINTENANCE, "Overall Setting of FLUOVIEW" for detailed operations.

2-12-12 Changing the Comment Font

1. Click the mouse on the comment to be changed of font to make the comment active (i.e. handles displayed around it).
2. Click the right button of the mouse. A pop-up menu as shown in Fig. 2-145 appears.
3. Select [Properties] from the menu. The [Properties] dialog box as shown below appears. Display the [Font] panel at the front.

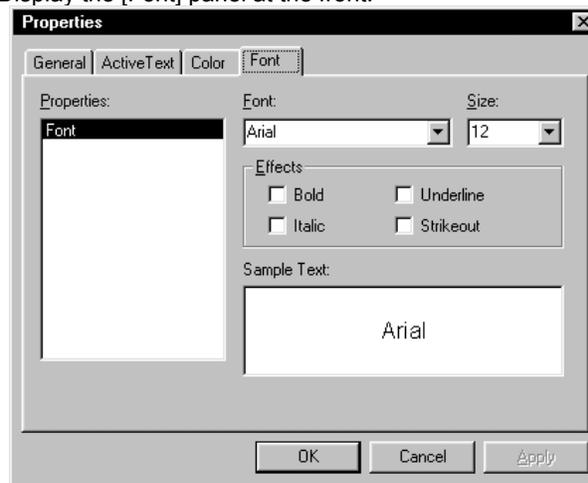


Fig 2-147 [Properties] Dialog Box

4. Set the font type and size using the drop-down lists and select the effect.

2-13 Image Output at Printer



<Print> button

1. Display the [Display] panel of the image to be output at the printer.
2. Click the <Print> button in the toolbar at the bottom left of the panel. A dialog box as shown below appears.

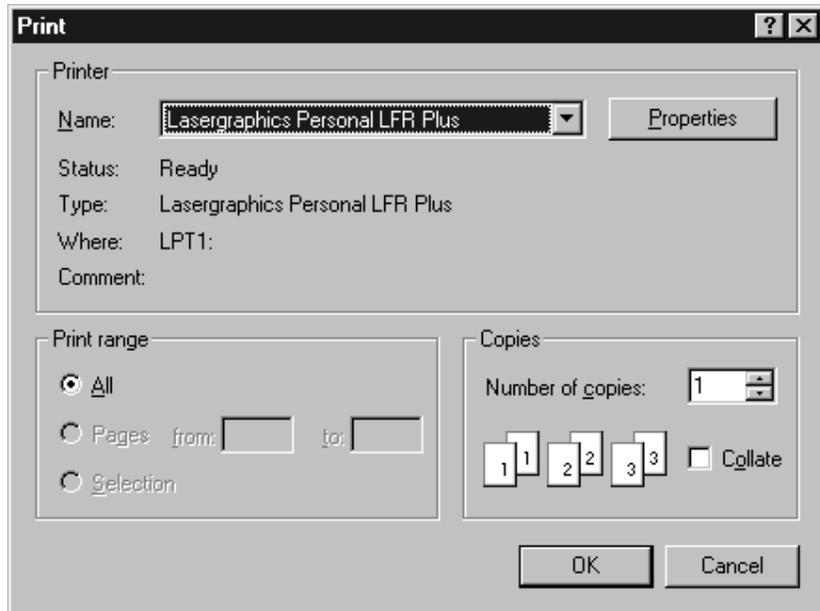


Fig. 2-148 [Print] Dialog Box

3. Select the connected printer name from the [Name] drop-down list.
4. If it is required to set the detailed data of the printer, click the <Properties> button in the dialog box.
5. Click the <OK> button of the dialog box.

NOTE

It is required to install and select the printer driver before the above operation. Refer to the Windows manuals for details.

For the printer operation procedures, refer to the printer manuals.

One Point!

The [Print] dialog box can also be displayed by mouse operation on the image.

1. Display the image to be output at the printer at the front of the [Display] panel, and click the right button of the mouse on the image.
2. A pop-up menu as shown below appears.
3. Select [Print] from the menu.

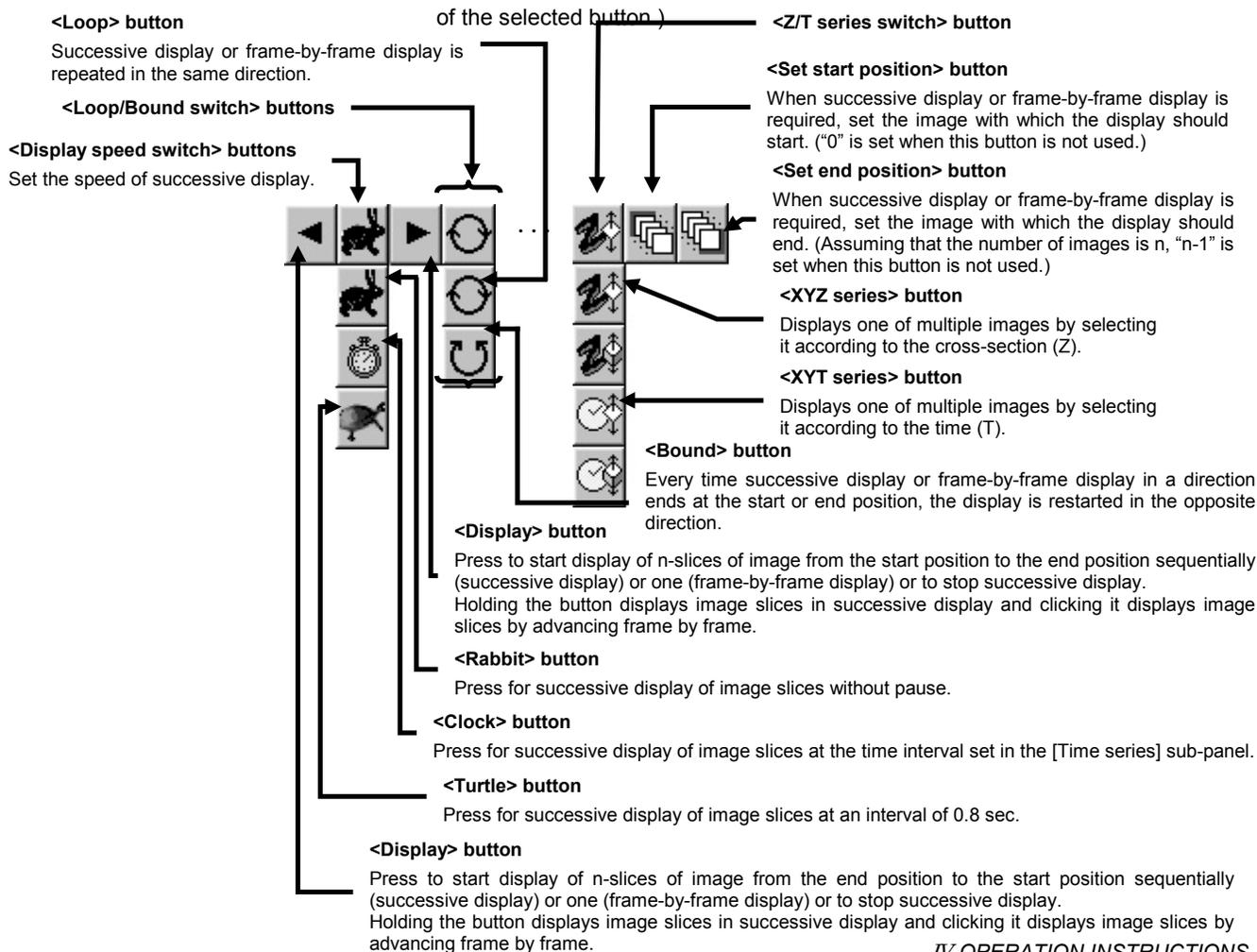


2-14 Merger/Extraction of Image Channels

2-14-1 Setting the Range of Multiple Image Slices

When merging or extracting channels in images composed of multiple image slices, such as time-lapse images and images acquired by varying the cross-sections, it is possible to select only some of image slices as the target of channel merger or extraction. This section describes how to set the range of target image slices.

1. Display the [Display] panel of the images composed of multiple image slices.
2. The buttons as shown below are displayed at the top of the [Display] panel. To switch to the image slice of another cross-section, click the <Z/T series switch> button then click the <XYZ series> button in the displayed list of buttons. To switch to the image slice of another instant in the elapsed time, click the <Z/T series switch> button then click the <XYT series> button in the displayed list of buttons. (In these operations, the icon in the <Z/T series switch> button changes to the icon



3. Display the image slice to start the range at the front using the <Display> button at the top of the [Display] panel.
4. Click the <Set start position> button. (If the start position is not set, image No. 0 becomes the start image automatically.)
5. Display the image slice to end the range at the front using the <Display> button.
6. Click the <Set end position> button. (If the end position is not set, image No. n-1, assuming the number of image slices is n, becomes the start image automatically.)

2-14-2 Merging Image Channels

For instance, when a specimen is imaged with two scans for 2-channel fluorescence observation and 1-channel transmitted light observation, the transmitted light image can be merged to the fluorescence images to create a 3-channel image. Note that the display gradation of the images obtained by overlaying 3 channels may be poor than original.

1. Open the two files to be used in creating a new image.



The image files used in overlay are subjected to the following restrictions.

- **The sizes of the images in the two image files should be identical.**
- **The number of data bits in the two image files should be identical.**

(For example, it is not possible to overlay a Fluoview Multi Tiff file with a Single TIF(S) 8-bit file.)

TIP

When the image in one of the image files is composed of multiple image slices, it is possible to use only some of the slices by setting a slice range. See section 2-14-1, "Setting the Range of Multiple Image Slices" for the operation procedure.

When the images in both image files are composed of multiple image slices, the overlaid image will have the same number of image slices as the file with the fewer image slices.

Example 1: XYZ image + XY image = XY image

(The first image slice of the XYZ image is overlaid with the XY image.)

Example 2: XYZ image with 10 slices + XYZ image with 5 slices = XYZ image with 5 slices

(The first five image slices are overlaid.)

2. Display the [Experiment Editor] sub-panel in the [Process] panel.

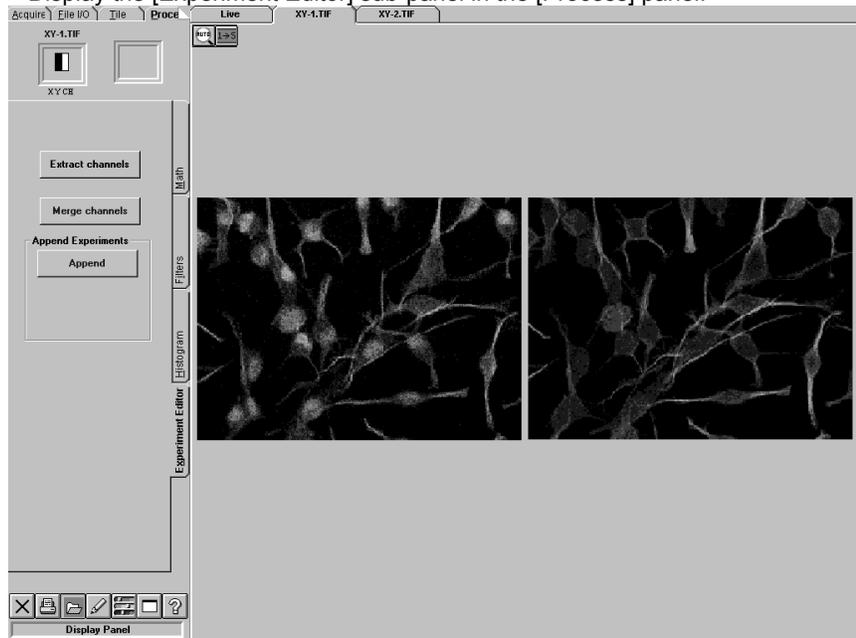


Fig. 2-149 [Experiment Editor] Sub-panel



<Merge channels> button

3. Click the <Merge channels> button.

The [Experiments in Memory] dialog box appears as shown below.

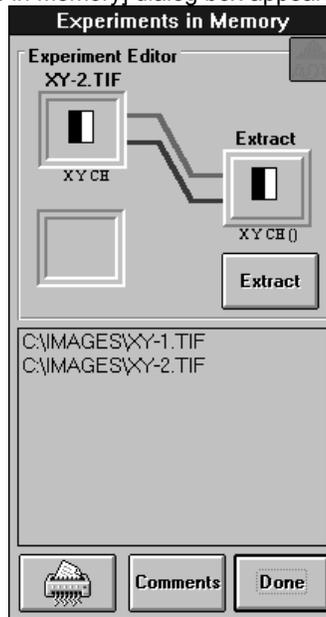
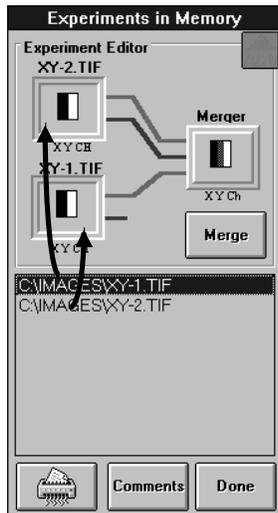


Fig. 2-150 [Experiment Editor] Group Box

TIP The frame at the top left of the [Experiments in Memory] dialog box shows the icon of the image file displayed at the front of the [Display] panel.



4. From the file list in the [Experiments in Memory] dialog box, select the file name of the first image and drag it into the frame at the top. The icon of the first image is displayed in the frame at the top left.

TIP The mouse pointer turns into the image icon during dragging.

5. From the file list in the [Experiments in Memory] dialog box, select the file name of the second image and drag it into the frame at the top left. The icon of the second image is displayed in the frame at the top left of the [Experiments in Memory] dialog box.

TIP The mouse pointer turns into the image icon during dragging.



When the image of the second selected image file is composed of multiple image slices, the setting of the image slice range is ineffective even when the range is set.

6. Up to 3 channels are selected automatically beginning with the first image set in the [Experiments in Memory] dialog box, and connected to [Merger] by lines.

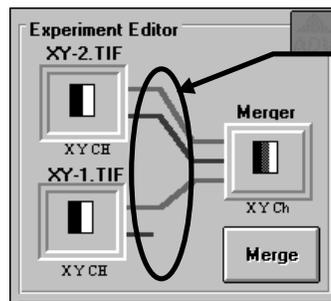


The lines of channels are colored as shown below.

- Ch1: Navy blue
- Ch2: Light blue
- Ch3: Green
- Ch4: Yellow green
- Ch5: Orange
- Ch6: Red



When the mouse pointer is approached to the icon-side end of a line connected to [Merger], the color of the line end turns into yellow. Clicking the line in this condition switches the channel between the selected and deselected status alternately. The lines connected to [Merger] indicate the selected channels.



Clicking a channel line in this area switches the channel between the selected and deselected status.



Up to 6 channels can be selected together.
To select another channel after having selected 6 channels, deselect the unnecessary channels before selecting required channels.

- Click the <Merge> button in the [Experiments in Memory] dialog box. A new [Display] panel showing [Merge] in the page tab appears and the images including newly merged channel(s) are displayed in the panel.

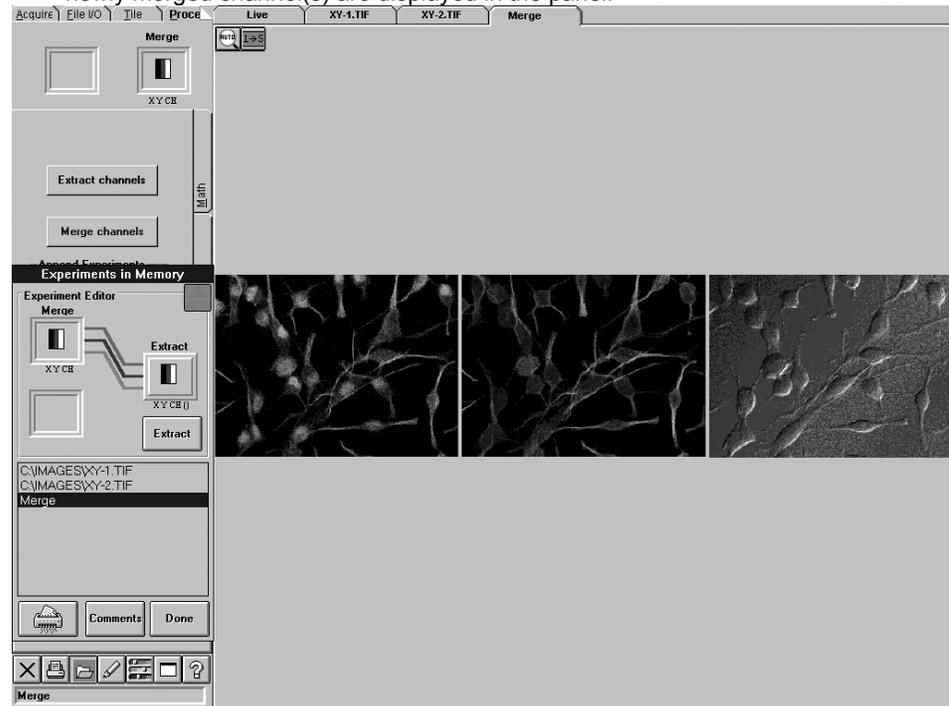


Fig. 2-151 [Merge] Panel

2-14-3 Extracting Channels from Image

Desired channels can be extracted from an image. Use this facility to extract only the images of required channels from an image acquired from more than one channel.

1. Open the image file of the image to extract channels in advance.

TIP

When the image has been saved in more than one image file, it is possible to use only some of the slices by setting a slice range. See section 2-14-1, "Setting the Range of Multiple Image Slices" for the operation procedure.

NOTE

The channel extraction cannot change the image type (XYZ, XYT, etc.). Therefore, it is not possible, for example, to extract an XY image from an XYZ or XYT image.

2. Display the [Experiment Editor] sub-panel in the [Process] panel.

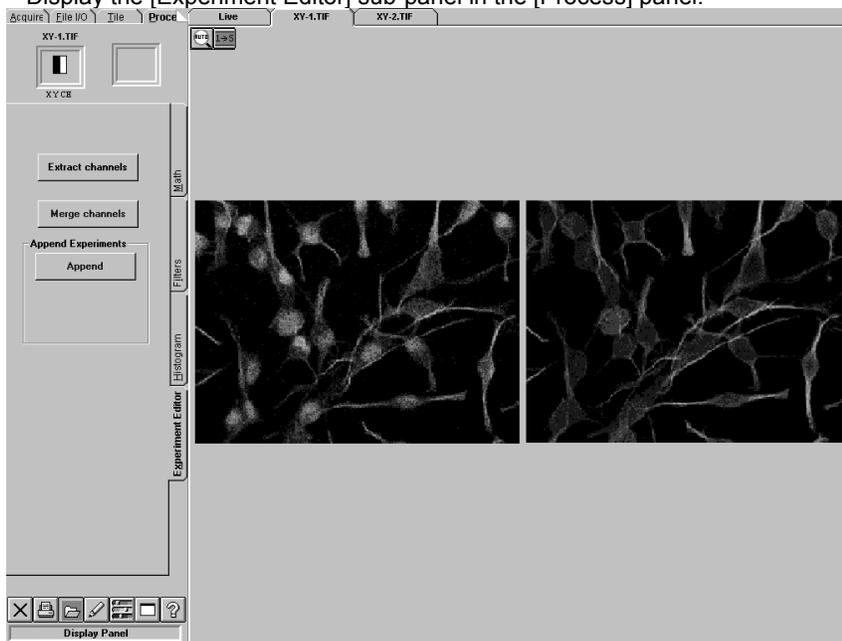


Fig. 2-152 [Experiment Editor] Sub-panel



Extract channels

<Extract channels> button

3. Click the <Extract channels> button. The [Experiments in Memory] dialog box appears as shown below.

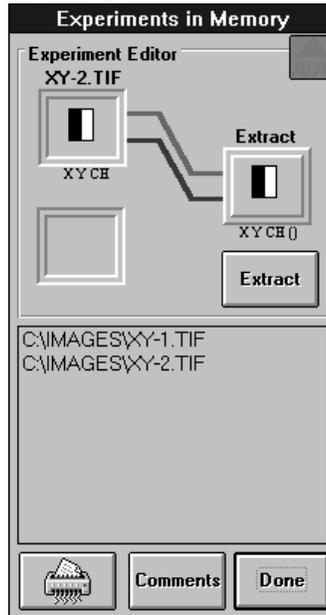
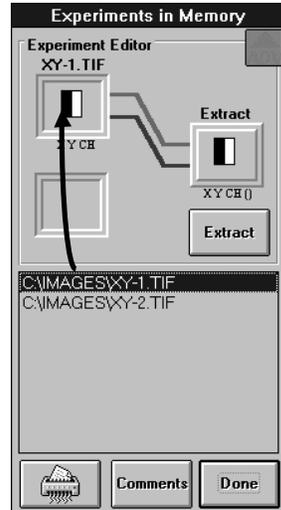


Fig. 2-153 [Experiment Editor] Group Box

TIP

The frame at the top left of the [Experiments in Memory] dialog box shows the icon of the image file displayed at the front of the [Display] panel.



- From the file list in the [Experiments in Memory] dialog box, select the file name of the image from which to extract channels and drag it into the frame at the top left. The icon of the image is displayed in the frame at the top left.

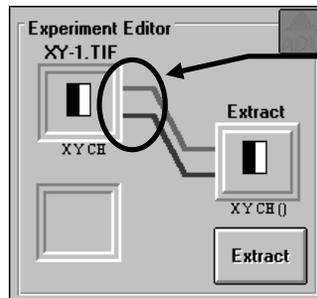
TIP The mouse pointer turns into the image icon during dragging.

- The channels of the image set in the [Experiments in Memory] dialog box are connected to [Extract] by lines.

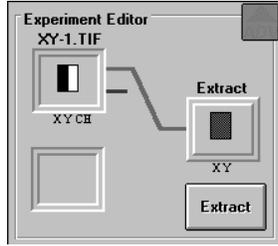
NOTE The lines of channels are colored as shown below.

- Ch1: Navy blue
- Ch2: Light blue
- Ch3: Green
- Ch4: Yellow green
- Ch5: Orange
- Ch6: Red

TIP When the mouse pointer is approached to the icon-side end of a line connected to [Extract], the color of the line end turns into yellow. Clicking the line in this condition switches the channel between the selected and deselected status alternately. The lines connected to [Extract] indicate the selected channels.



Clicking a channel line in this area switches the channel between the selected and deselected status.



6. Among the channel lines connected to [Extract], deselect the unnecessary channels as described in the TIP on the previous page so that only the necessary channels are selected.
7. Click the <Extract> button in the [Experiments in Memory] dialog box. A new [Display] panel showing [Extract] in the page tab appears and the image of the extracted channel(s) is displayed in the panel.

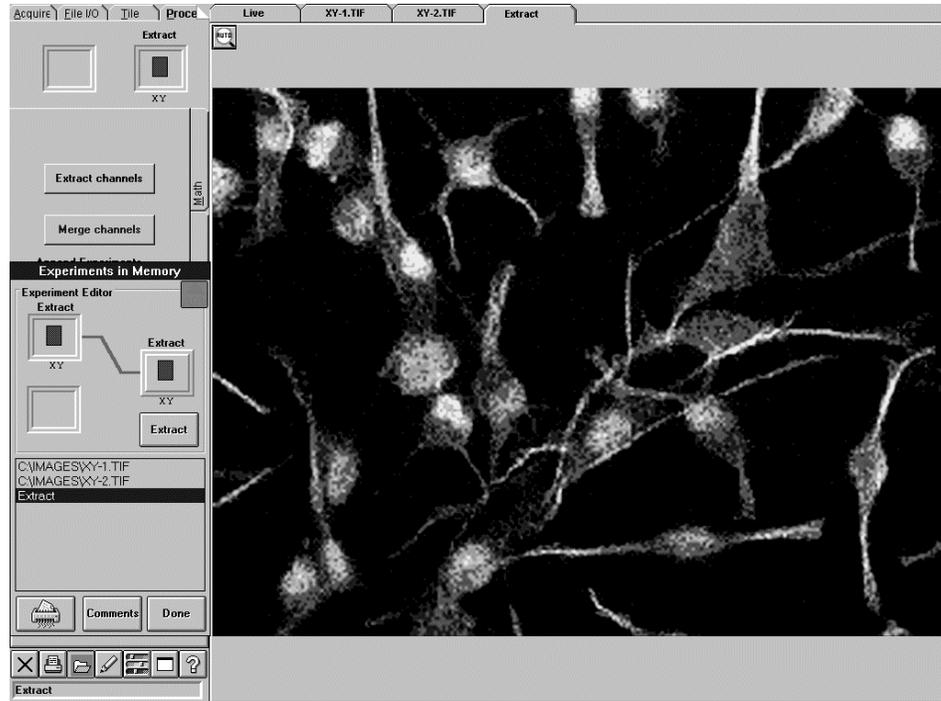


Fig. 2-154 [Extract] Panel

2-15 Changing the Chart Display Method

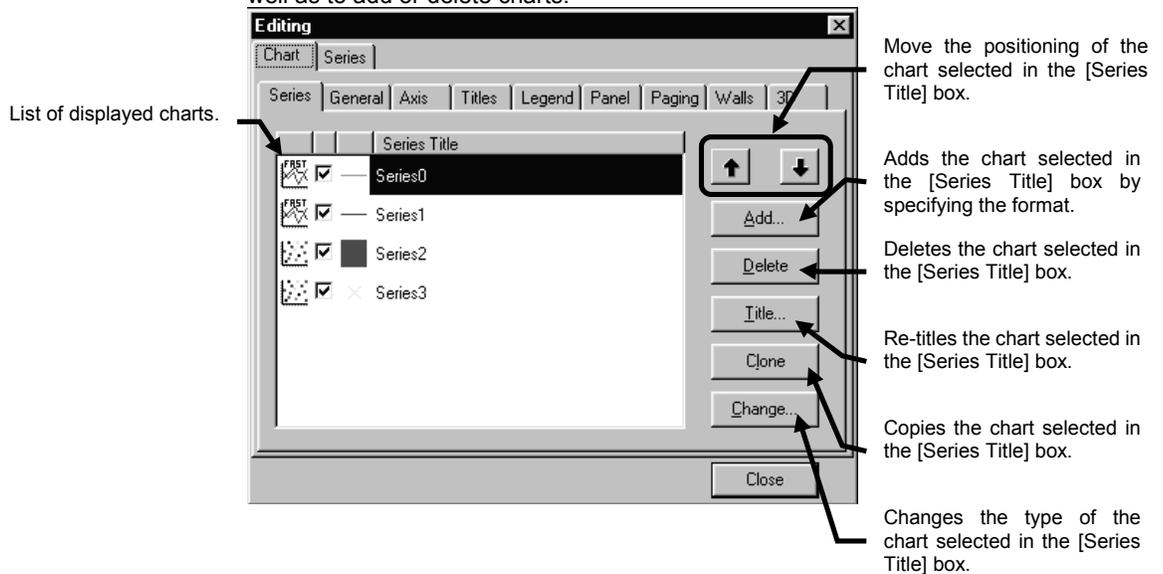
When the processed analysis data chart in the [Analyze] panel is double-clicked, the [Enhanced Profile Plot] dialog box appears.

By clicking the <Properties> button, the [Editing] dialog box can be displayed, allowing the detailed chart settings and chart display method to be changed.

2-15-1 [Chart] Panel

- [Series] panel

This panel is used to change the type, title or other settings of the displayed chart as well as to add or delete charts.



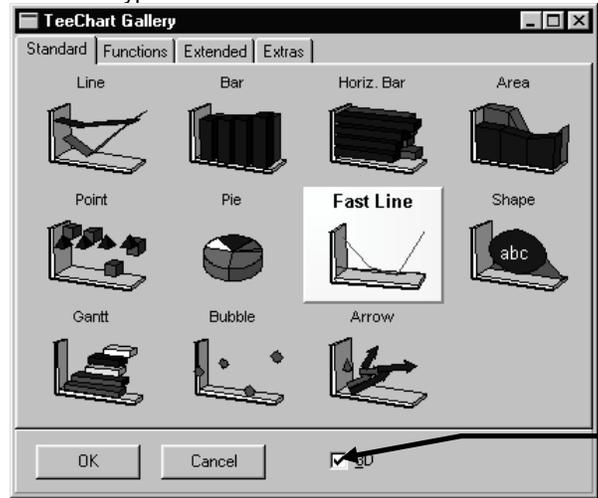


- [TeeChart Gallery] sub-panel

Displayed when the <Add> or <Change> button is clicked.

Used to set the chart type.

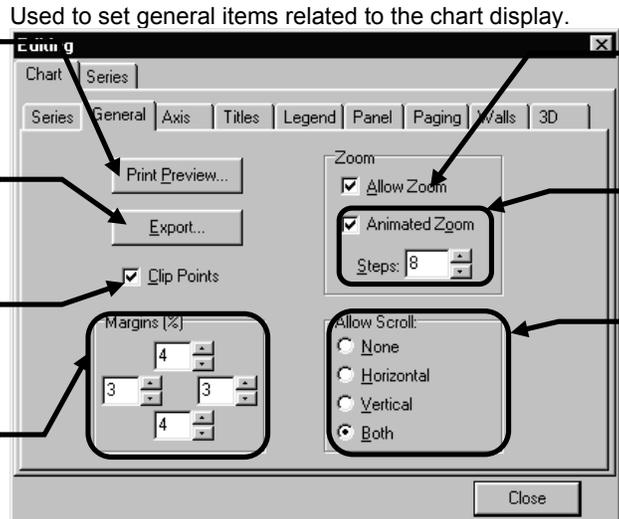
The chart types that cannot be set cannot be selected.



Enable or disable the 3D display.

- [General] sub-panel

Preview the printed chart or sets the orientation and margins of the print paper.



Save the chart in the format of an image file or in the clipboard.

When a chart is displayed by zooming or scrolling, select whether the data of only the area contained in the chart axes is to be displayed or not.

Set the margins from the edges of the area in which the chart is displayed.

Used to set general items related to the chart display.

Enables or disables zooming.

When zooming is enabled, enable or disable gradual zooming according to the step count set in the [Steps:] dialog box.

Set the chart scrolling direction.

● [Axis] sub-panel

Used to set the coordinate axes of the chart.

Enable or disables the display of chart axes.

Select the chart axes to be set in the [Scales], [Title], [Labels], [Ticks] and [Position] panels.

Enable or disable the chart axis display set in the [Axis:] group box.

Display the chart axes by selecting the optimum scale automatically.

Set the maximum and minimum values of the chart axes either automatically or manually.

Set the number of steps of the values displayed in the chart axis labels.

Enable or disable the inverted chart display.

Enable or disables the logarithmic chart display.

● [Title] sub-panel

Used to set the title of the coordinate axis.

Set the angle of displaying the coordinate axis title.

Set the angle of displaying the coordinate axis title.

Set the title of the coordinate axis.

Set the title character font.



• [Labels] sub-panel

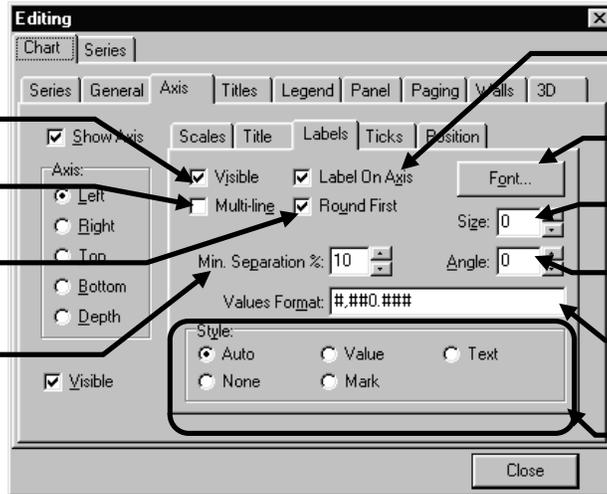
Used to set the label of the coordinate axis.

Enable or disable the label display.

Not used with the present application.

Enable or disable the scale display according to the maximum and minimum values in the chart.

Set the distance between labels.



Enable or disable the label display on a point of crossing with another axis.

Set the label character font.

Set the distance from the title to the label display position.

Set the angle of label display.

Set the label display format.

Set the label type. Select [Auto] or [None].

• [Ticks] sub-panel

Used to set the coordinate axis line.

Set the type, color and width of the coordinate axis.

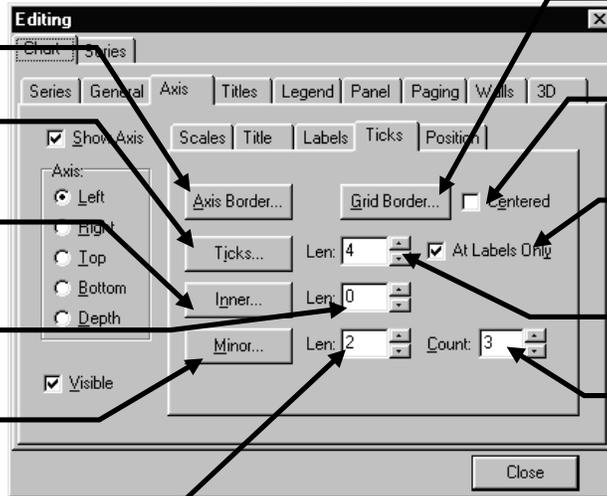
Set the type, color and width of the scale line on the label side of the coordinate axis.

Set the type, color and width of the scale line on the chart side of the coordinate axis.

Set the length of the scale line from the coordinate axis to the chart side.

Set the type, color and width of the scale line between labels.

Set the length from the coordinate axis of the scale line between labels to that on the label side.



Set the type, color and width of the line along the label.

Enable or disable the display of coordinate axis scales in the intermediate position between labels.

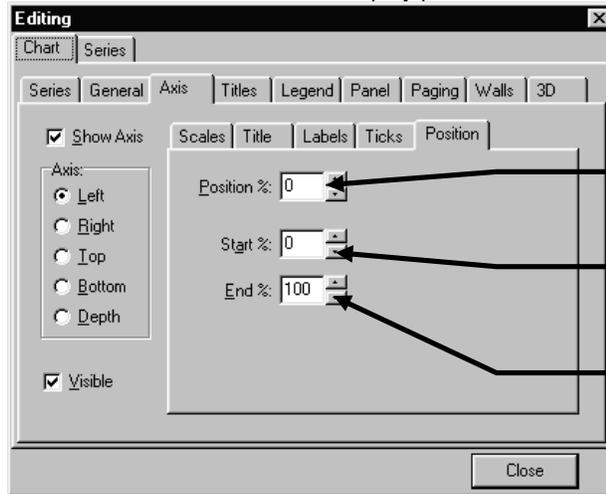
Enable or disable the display of scale line in the positions where labels are displayed.

Set the length of the scale line from the coordinate axis to the label.

Set the number of division between labels.

- [Position] sub-panel

Used to set the coordinate axis display position.



Set the coordinate axis display position with respect to the chart.

Set the coordinate axis display range with reference to the start point.

Set the coordinate axis display range with reference to the end point.

- [Titles] sub-panel

Used to set a chart title.

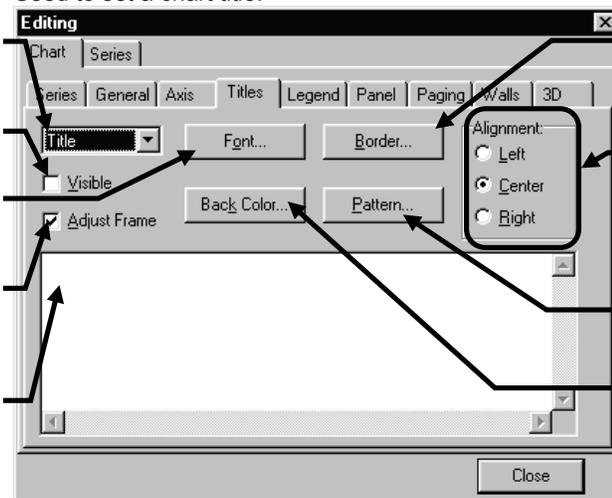
Switch the title display position between the header and footer.

Enable or disable the title display.

Set the title character font.

Switch the title background display range between the entire width of the chart display area and the character area.

Enter the title to be displayed here.



Set the type, color and width of the title frame.

Set the title display position.

Set the pattern and color of the title background.

Set the title background color.

● [Legend] sub-panel

Used to set the chart legend display.

Enable or disable the legend display.

Set the legend background color.

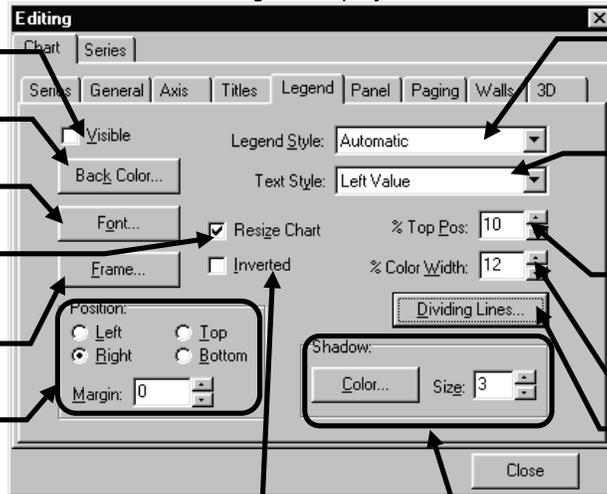
Set the legend character font.

Set whether or not the chart is resized according to the legend display.

Set the type, color and width of the legend frame line.

Set the legend display position and the margin between the chart display area and legend display area.

Enables or disables the inversion of the display order of legend items.



Set the items to be displayed in the legend. Usually set [Automatic].

Set the legend display style.

This setting is effective when the numerical value display is selected in the [Legend Style] pull-down menu.

Fine-adjust the positioning of the legend display from the top edge.

Set the width of the lines displayed in the legend column.

Set the type, color and width of the lines dividing the legend items.

Set the color and width of the shadow area of the shadowed display of legend area.

● [Panel] sub-panel

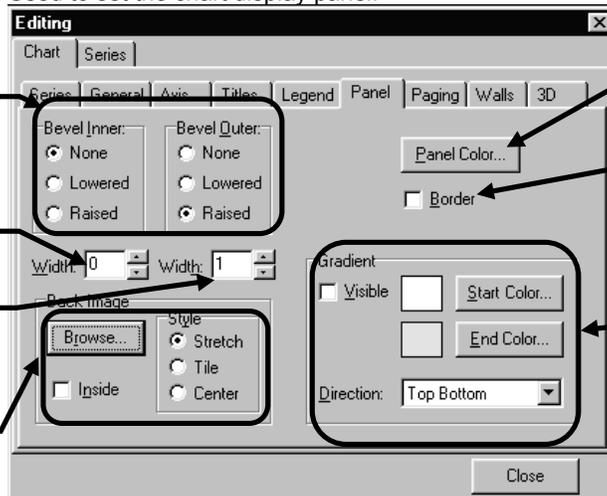
Used to set the chart display panel.

Set the format of the borders of the chart display area. [Bevel Inner] sets the inner borders of the chart display area and [Bevel Outer] sets the outer borders of the chart display area.

Sets the width between the inner and outer borders.

Sets the width of the inner and outer borders.

Specifies the background image of the chart display area, sets whether it is displayed inside the chart or in the entire chart display area, and sets its display position.



Set the background color of the chart display area.

Enable or disable the display outside the outer frame of the chart display area.

Set whether or not the chart display area background is displayed with gradations, and sets the colors and type of the background.

● [Paging] sub-panel

Used to paginate a chart for detailed viewing.

Sets the number of horizontal axis points displayed per page.

Sets the current page number and total number of pages.

Moves to the first page.

Moves to the previous page.

Enables or disables the change in scaling in the last page.

Moves to the next page.

Moves to the last page.

● [Walls] sub-panel

Used to set the background of the axis of XYZ or XYt observation chart.

Enables or disables the chart axis background display.

Changes color of Left, Bottom and Back wall.

<Background> button
Sets the background color.

<Border> button:
Sets whether or not the background frame is displayed, and sets its color, width and type.

<Pattern> button:
Sets whether or not the background pattern is displayed, and sets its color, width and type.

[Transparent] check box:
Enables or disables the transparent background display.

[Size] text box:
Sets the background thickness.

[Dark 3D] check box:
Enables or disables the shadowed, 3D display. This check box can be selected when the background thickness is specified.



● [3D] sub-panel

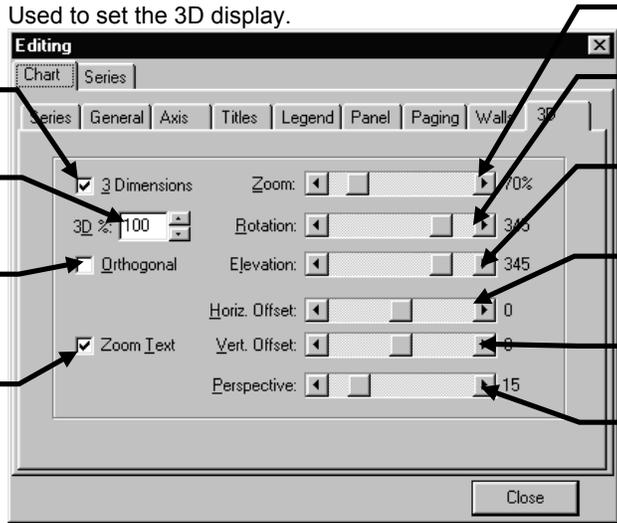
Used to set the 3D display.

Switches between the 3D and 2D chart display.

Sets the 3D chart display effect by varying the angle.

Enables or disables the 3D display in the depth-wise direction.

Enables or disables the zooming display of character strings in the chart, such as label characters, together with the chart display.



Change the zoom ratio.

Rotates the chart in the horizontal direction with respect to the bottom side.

Rotates the chart in the vertical direction with respect to the bottom side.

Moves the chart display position in the horizontal direction.

Moves the chart display position in the vertical direction.

Distorts the chart to change the display effect.

2-15-2 [Series] Panel

- [Format] sub-panel

Used to set the display method of a series of images.

The set items are variable depending on the type of the chart.

The following descriptions take a Line 3D chart and Point 3D chart as examples.

This is the [Format] sub-panel when the Line 3D chart is selected.

Select the chart series to be set in the [Series] panel.

Sets whether or not the outer frame of the chart is displayed, and sets the color, width and type of the frame.

Sets the chart color.

Enables or disables the stairstep type chart display.

Enables or disables the shadowed 3D display.

Enables or disables the coloring per coordinate axis.

Sets the chart pattern.

This is the [Format] sub-panel when the Point 3D chart is selected.

Sets the chart color.

Enables or disables the display varying the color at every point in the chart.

Sets whether or not chart lines are displayed, and sets the color, width and type.

Sets the depth of points in the chart.



- [Points] sub-panel

Used to set the inflection points in the chart.

Enables or disables the inflection point display.

Enables or disables the 3D inflection point display.

Selects whether the ends of the inflection points are aligned with the coordinate axis (when checked) or the centers of the inflection points are aligned with the coordinate axis (when unchecked).

Enables or disables the dark shadowed 3D display of inflection points.

Sets the inflection point background color.

Resets the background color to the default color.

Sets the width of inflection points.

Sets the height of inflection points.

Sets the type of inflection points.

Sets whether or not the inflection point borders are displayed, and sets the color, width and type.

- [General] panel

Used to set the general items.

Enables or disables the chart display inside the legend.

Sets the shape that the cursor changes when it is placed on the chart.

Sets the display formats when the label display is set to the numerical value display or percent display.

Selects whether the labels are displayed on or below the chart.

Usually leave this box unchecked.

Selects whether the labels are displayed to the left or right of the chart.

Usually leave this box unchecked.

- [Marks] sub-panel

Used to set the items to be displayed along the coordinate points.

Enables or disables the item display in the chart.

Enables or disables the transparent background display.

Enables or disables the item display of lines outside the coordinate axis.

Sets the background color of the item display area.

Sets the item character font.

Sets the contents of the displayed item.

Sets the color, width and type of the outer frame of the item display area.

Sets the color, width, type and length of the arrows indicating the item display area.

- [Data Source] sub-panel

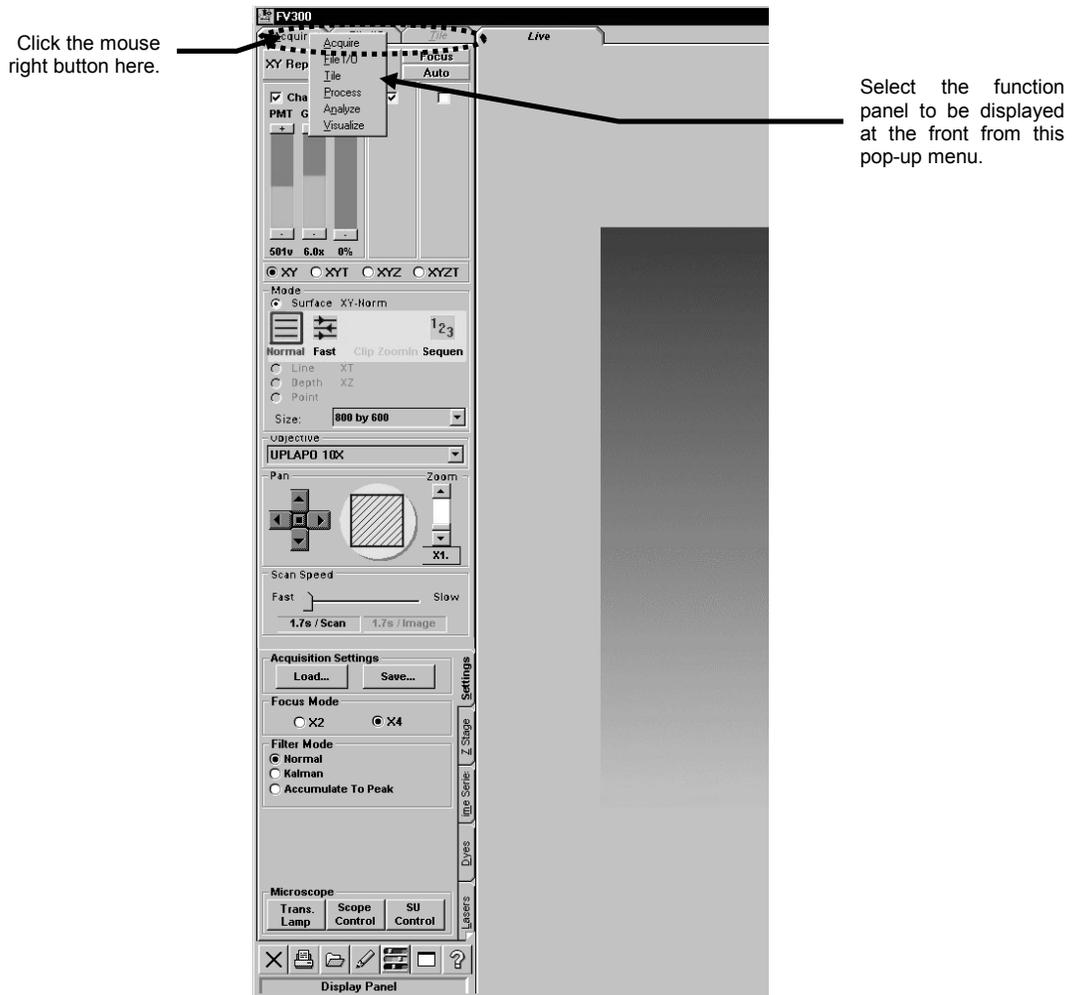
Do not change the settings here but leave them in the initial condition.

2-16 Pop-up Menu

Selection of function panels and display panels and other frequently-used FLUOVIEW functions (full-screen display, printer output, image save, LUT setting, comment setting) can be controlled by clicking the right button of the mouse, without selecting specific page tabs or buttons.

- Pop-up menu of function panel

When the right button of mouse is clicked on the page tab of a function panel, a pop-up menu appears to allow selection of the function panel to be displayed at the front.





● Pop-up menu of display panel

When the right button of mouse is clicked on the page tab of a [Display] panel, a pop-up menu appears to allow selection of the [Display] panel to be displayed at the front.

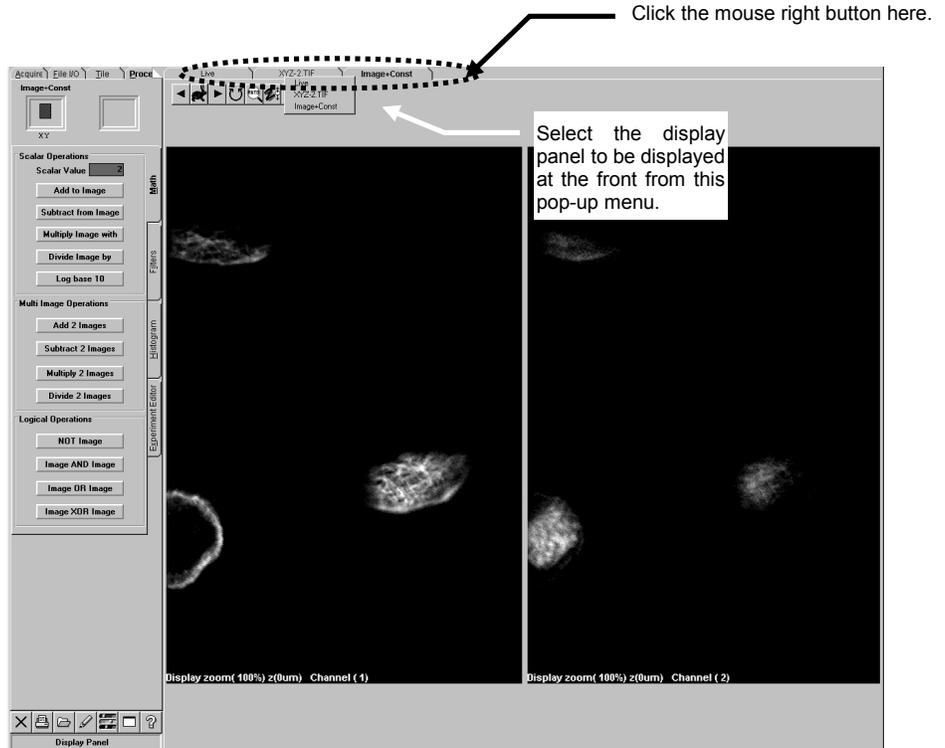


Fig. 2-155 [Display] Panel Showing Pop-up Menu



●Pop-up menu of comment

When the right button of mouse is clicked on an comment in image, a pop-up menu appears to allow editing or deleting the specified comment.

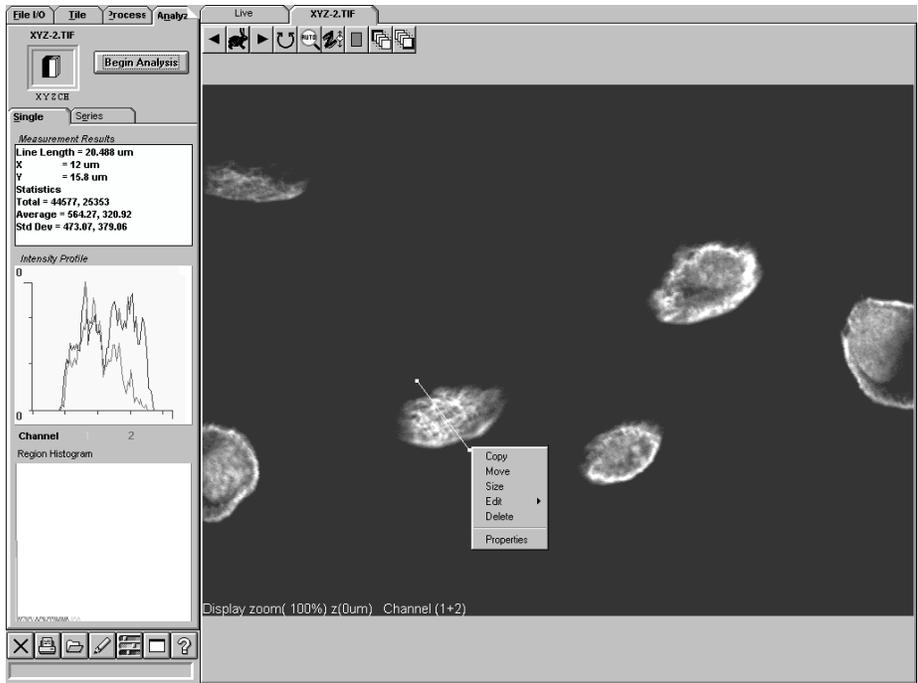


Fig. 2-156 Image Comment with Pop-up Menu

Copy	Copies the comment.
Move	Moves the comment.
Size	Resizes the comment.
Edit	Edits the selected arrow or scale.
Delete	Deletes the comment.
Properties	Edits the comment color and font.



●Pop-up menu of image

When the right button of mouse is clicked on the image, a pop-up menu appears to allow selection of image operations (full-screen display, printer output, image save, LUT setting, number of image divisions, comment editing).

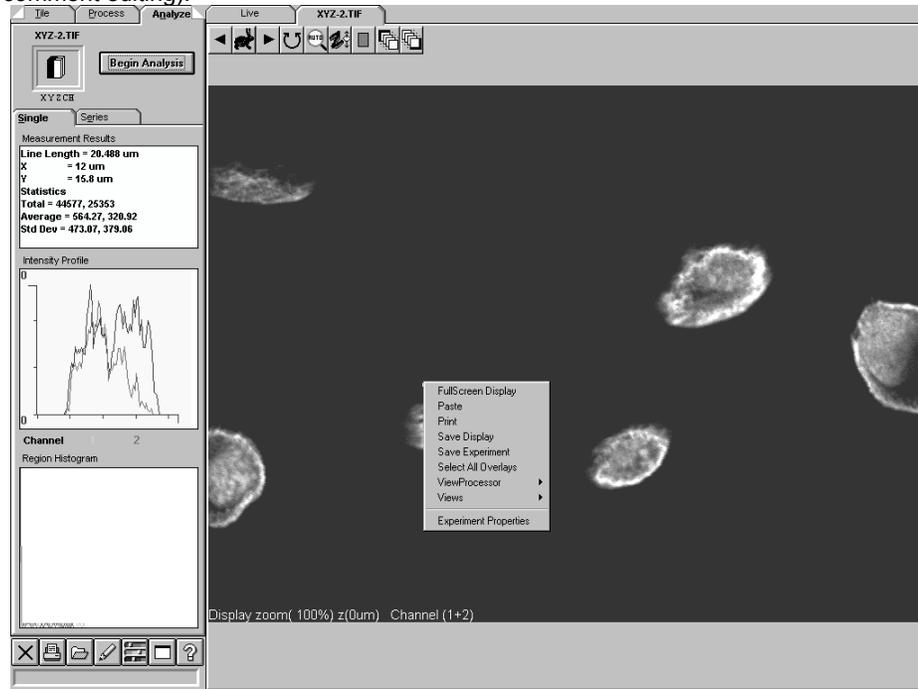


Fig. 2-157 [Display] Panel Showing Pop-up Menu

FullScreen Display	Displays the image full-screen.
Paste	Pasts the comment copied on the image.
Print	Outputs the image at the printer.
Save Display	Saves the image as a single image.
Save Experiment	Saves the image as a series of images.
Select All Overlays	Selects all the comments overlaid on the image.
ViewProcessor	Edits the image LUT.
Views	Sets the number of image divisions when displaying more than one image.
Experiment Properties	Edits the image comment.



●Pop-up menu of the [Experiments in Memory] dialog box

When the right button of mouse is clicked in the frame for opening an image file in the [File I/O], [Tile] or [Process] panel, a pop-up menu appears. This makes it possible to select the file to be opened when performing tile display, inter-image operations, image channel merger/extraction, etc.

Click the mouse right button here.

The pop-up menu appears showing the file names that can be selected.

Select the file name to be processed.

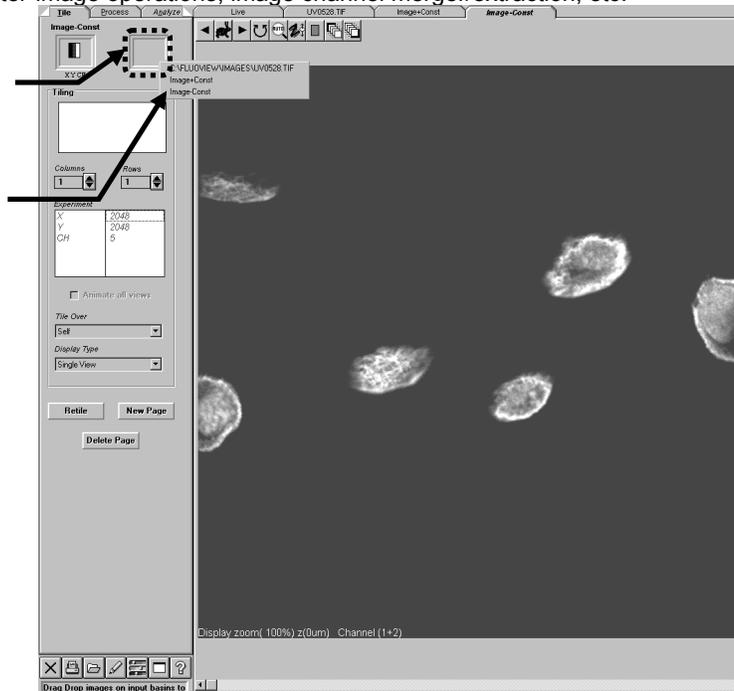


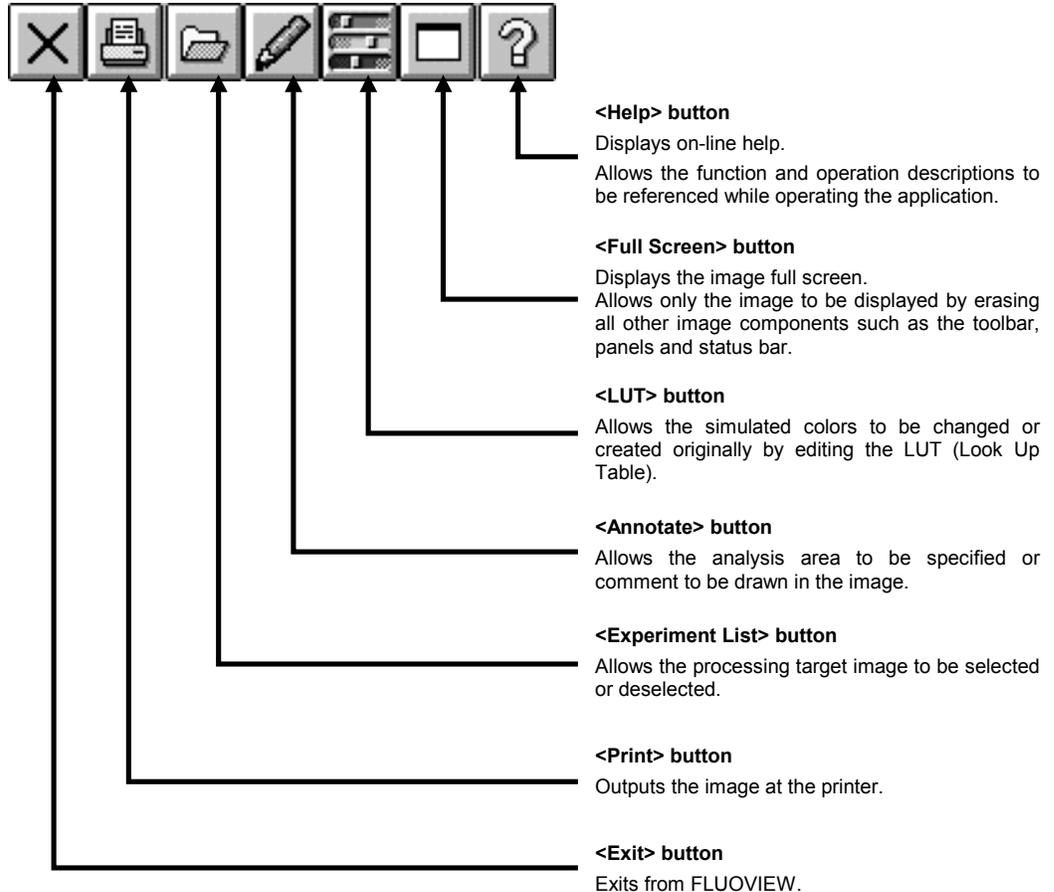
Fig. 2-158 Function Panel Showing Pop-up Menu

Appendix A List of Tools

Appendix A-1 List of Tools

Appendix A-1-1 Toolbar

The horizontal array of buttons at the bottom left of the panel form the toolbar. The most frequently-used FLUOVIEW functions are gathered here.



Appendix A-1-2 Tools at the Top of Display Panel

These refer to the horizontal array of buttons at the top of the display panel. The functions frequently used with images are gathered here.

<Set end position> button

When successive display or frame-by-frame display is required, set the image with which the display should end. ("n-1", assuming that the number of images is n, is set when this button is not used.) This button is also used to set the image for ending a processing operation such as image save.

<Set start position> button

When successive display or frame-by-frame display is required, set the image with which the display should start. ("0" is set when this button is not used.) This button is also used to set the image for starting a processing operation such as image save.

<Display switching> button

Used to display more than one channel simultaneously.

<Z/T series switch> button

When an image is composed of multiple image slices, this button switches between the display according to multiple sections and that according to the progress of time. This button is also used to display an extended image.

<Magnify/reduce> button

Magnifies or reduces the image. Magnification or reduction by 3:1 or 1:3 the original image size is possible.



<Bound>/<Loop> buttons

Set the repetition direction of the successive display or frame-by-frame display.

<Display> button

Press to start display of n-slices of image from the start position to the end position sequentially (successive display) or one by one (frame-by-frame display) or to stop successive display.

Holding the button displays image slices in successive display and clicking it displays image slices by advancing frame by frame.

<Display speed switch> button

Set the speed of successive display.

<Display> button

Press to start display of n-slices of image from the end position to the start position sequentially (successive display) or one by one (frame-by-frame display) or to stop successive display.

Holding the button displays image slices in successive display and clicking it displays image slices by advancing frame by frame.

Appendix B List of Hot Keys

Frequently-used FLUOVIEW functions (scanning, panel switching) can be controlled from the keyboard without using the mouse.

- Image acquisition-related keys

Image acquisition channel selection

Key	Target Operation
Ctrl + 1	Switches the status of the Ch1 check box (Ch1 scanned/not scanned).
Ctrl + 2	Switches the status of the Ch2 check box (Ch2 scanned/not scanned).
Ctrl + 3	Switches the status of the Ch3 check box (Ch3 scanned/not scanned).

PMT voltage/gain/offset adjustments

Key	Target Operation
←	Enables and makes variable the LED slider on the left.
→	Enables and makes variable the LED slider on the right.
↓	Decreases the value of an enabled LED slider. (Fine adjustment)
Shift + ↓	Decreases the value of an enabled LED slider. (Coarse adjustment)
↑	Increases the value of an enabled LED slider. (Fine adjustment)
Shift + ↑	Increases the value of an enabled LED slider. (Coarse adjustment)

NOTE

An LED slider is enabled and variable when its setting values are displayed in red.

Image acquisition (scanning)

Key	Target Operation
F1	Displays the on-line help screen.
F2	Performs repeated scanning. [XY Repeat]
F3	Starts scanning according to the current scan mode. [Scan Once]
F4	Performs focus scanning. [Focus]
F6	Acquires another image after completing series image acquisition. [Append Next]

Appendix B List of Hot Keys

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Key	Target Operation
F7	Completes image acquisition after completing series image acquisition. [Series Done]
Spave	Stops scanning. [Stop Scan]

Scanning speed and area setting

Key	Target Operation
Ctrl + ←	Decreases the scan speed.
Ctrl + →	Increases the scan speed.
Ctrl + ↓	Decreases the zoom ratio.
Ctrl + ↑	Increases the zoom ratio.

Z stage setting

Key	Target Operation
Page Up	Moves the Z stage up. (Fine adjustment)
Shift + Page Up	Moves the Z stage up. (Coarse adjustment)
Page Down	Moves the Z stage down. (Fine adjustment)
Shift + Page Down	Moves the Z stage down. (Coarse adjustment)
Ctrl + Page Up	Sets the Z stage to the Stop Z position.
Ctrl + Page Down	Sets the Z stage to the Start Z position.
Insert	Switches the [Locked] check box (motor excitation) status.
Ctrl + Delete	Cancels the Start and Stop settings. <Set Zero> Z motor: The current position becomes 0.0. Piezo: Returned to the 0.0 position.
Home	Moves the stage till the Stop Z position.
End	Moves the stage till the Start Z position.



● Transmitted illumination lamp ON/OFF keys

Key	Target Operation
Ctrl + L	Switches the transmitted illumination ON/OFF. <Trans. Lamp>

● Image Saving

Key	Target Operation
Ctrl + S	Save the image in the [Display] panel being displayed as series images. <SAVE>

● [Hi-Lo]LUT switchover key

Key	Target Operation
Ctrl + H	It alternatively switches over between the previously-assigned LUT and [Hi-Lo] LUT on the channel, whose PMT adjustment window ([PMT], [Gain] and [Offset]) is opening on the [Acquire] panel during the acquisition period.
Ctrl + Shift + H	It alternatively switches over between the previously-assigned LUT and [Hi-Lo] LUT on all the channels acquiring images.

● Panel select keys

Main panel

Key	Target Operation
Alt + A	Selects the [Acquire] panel.
Alt + F	Selects the [File I/O] panel.
Alt + T	Selects the [Tile] panel.
Alt + P	Selects the [Process] panel.
Alt + N	Selects the [Analysis] panel.
Alt + V	Selects the [Visualize] panel.

Appendix B List of Hot Keys



[Acquire] sub-panels

Key	Target Operation
Alt + S	Selects the [Settings] sub-panel.
Alt + Z	Selects the [Z Stage] sub-panel.
Alt + M	Selects the [Time Series] sub-panel.
Alt + D	Selects the [Dyes] sub-panel.
Alt + L	Selects the [Lasers] sub-panel.
Alt + E	Selects the [TIEMPO] sub panel . (It is an option panel with TIEMPO available).

[Process] sub-panels

Key	Target Operation
Alt + M	Selects the [Math] sub-panel.
Alt + I	Selects the [Filter] sub-panel.
Alt + H	Selects the [Histogram] sub-panel.
Alt + K	Selects the [Mask] sub panel. (It is an option panel with TIEMPO available).
Alt + X	Selects the [Experiment Editor] sub panel.

[Analysis] sub-panels

Key	Target Operation
Alt + S	Selects the [Single] sub-panel.
Alt + E	Selects the [Series] sub-panel.
Alt + I	Select the [Isoplot] sub panel. (It is an option panel with TIEMPO available).

[Visualize] sub-panels

Key	Target Operation
Alt + O	Selects the [Orientations] sub-panel.
Alt + R	Selects the [Other options] sub-panel.

Appendix C Glossary

A

AOTF

AOTF represents Acoustic Optical Tunable Filter. AOTF is the sound optical element having optical anisotropy. When a sound wave is spread in the element, the element behaves as a phase grid and only the light of the wavelength corresponding to the wavelength of a sound wave is diffracted in the specific direction. Therefore, it is possible to control selection of laser wavelength and adjustment of laser intensity at high speed.

Active

Status of being selected or executable. An active window can be distinguished from other windows by the color of the title bar.

Application

Same as “software”. Among software, refers to the software used directly by the users.

B

Backlash

The quantity of play (or loosening between gear teeth and part) which is produced when the stage is moved up or down by the Z-motor.

Button

→Command button.

C

Check box

A small square which can be either checked with **X** or cleared. The check box indicate an item which can be enabled or disabled. The item is enabled when it is checked with X.

Clear

Action of removing check mark **X** from a check box to disable the item. To clear a check box checked with **X**, click the check box.

Click

Action of pressing then releasing the button of the mouse.

Clipboard

The place which relays data when an operation such as “Copy”, “Cut” and “Paste” is executed.

Command button

A figure in the shape of button in the window.

Clicking a command button with the mouse allows the function indicated on the button to be executed.

Confocal

Signifies the possibility of obtaining data on the plane where the irradiated laser beam is focused.

Contrast

Variation (change) between the brightest and darkest areas in an image.

Control menu

The menu displayed when the control menu box at the left end of the title bar of a window is clicked. When the window is minimized, the control menu can be displayed by clicking the icon. The control menu contains commands for controlling the window.

Control menu box

The square button at the left end of the title bar of a window. Clicking this box opens the control menu.

Copy

Action of placing selected data in the clipboard so that the data can be placed in other place later.

Cursor

Blinking | which indicates the area where a character can be input. When a keyboard key is pressed, the character is entered in the position of the cursor.

D

Dialog box

Some functions require fine settings so that they can be executed, and some functions require the confirmation of settings before being executed. The dialog box is a sub-window displayed in such cases.

Directory

Hierarchical classification of the space inside a disk so that the files can be arranged in a significant manner according to their categories.

Disk drive

The storage device storing the files. Some disk drives such as the hard disk drive and floppy disk drive are capable of both input and output, and some such as the CD-ROM drive is designed for read only.

Dot

→Pixel.

Double-click

Action of pressing and releasing the mouse button quickly twice, without moving the mouse.

Drag

Action of placing the mouse pointer on the target function, pressing the mouse button, moving the mouse while keeping the mouse button pressed, and releasing the mouse button at the destination position.

Drive

→Disk drive.

Drive name

Character such as "A" and "C", assigned to each drive. → Disk drive.

E

Extended focus

View of an XYZ image obtained by projection in the Z-direction.

Extension

Up to 3 characters after a period, which are attached at the end of a file name. The extension usually represents the type of the file or directory.

F

File

Group of information which is named and saved in a disk

G

Gain

This function brightens the image by the ratio set at the time of image acquisition. Use Gain when a bright image cannot be obtained even by setting PMT Voltage to 800 V.

Group

Refers to the applications registered in the program manager. When a group is opened on the program manager, more than one icon is displayed in the window. When the group is closed, the group becomes an icon of the window.

I

Icon

An icon is a small figure with characters below it. The icon indicates the status in which the window is closed (or minimized).

Iconize

This refers to turn a window into an icon display by using the iconize button of the [Iconize] command in the control menu. The application continues to run even after it has been turned into an icon. Selecting an icon returns it to the active application.

Intensity

Brightness of each pixel in an image.

Items displayed in pale color

The menu commands and buttons which cannot be used are displayed in less visible way, i.e. in a pale color or gray.

K

Keyboard input

Action of inputting an alphanumeric character from the keyboard of the computer.

L

LUT(LookUpTable)

The image acquired by observation (input) has 12 bits of brightness data per pixel. Meanwhile, the brightness data which can actually be displayed (output) consists of 8 bits per pixel for each of R, G and B. The LUT is the tabulation of this relationship between the input and output.

M

Macro

Record of a series of operations. When a macro is executed, the series of operations defined for it are executed.

Maximize button

The button showing an upward triangle at the right of the title bar. Clicking this button displays the window full screen. The same operation is also available using the [Maximize] command in the control menu.

Mouse

A device which was named because it looks like a mouse. It is used to give instructions in the window.

Mouse pointer

When the mouse is moved on the desk, arrow  moves in the display along the movement of the mouse. This "" is called the mouse pointer.

O**Offset**

This function darkens the image by the ratio set at the time of image acquisition.
(Offset should be used before using Gain.)

On-line help

Manual displayed on the screen, that is built into software.

Option button

Small circular button inside a rectangular frame in a window. Only one option button (item) can be selected from the items enclosed in the frame.

P**Panel**

The large rectangle with a page tab in a window. The panel is provided on a per-function basis, and clicking the page tab displays the panel of the selected function.

Paste

Action of placing the data in the clipboard in an application.

Piezo-

Abbreviation of piezoelectric, which is the phenomenon of generating electricity when a force is applied. This phenomenon is utilized in electric spark generators for use with gas appliances, etc.

Pixel

The minimum graphic unit of screen display. Also referred to as the dot.

PMT Voltage

Increasing this setting increases the sensitivity. When a bright image cannot be obtained even by setting PMT Voltage to 800 V, do not vary PMT Voltage any more but increase Gain. This usually provides a better effect than increasing PMT Voltage above 800 V.

R**Resolution**

Number of dots composing the image on a screen or printer. When the number of dots is increased, i.e. when the resolution is increased, the gradation can be displayed in more details.

Reversed display

Display method which indicates that an item or character string is selected and has become the target of the user's next operation. The reversed displayed characters are shown in a different color from other characters.

S**Scroll**

Action of moving text or a picture up or down in order to view the other part of information than the information which can be displayed at once in the window.

Scroll bar

The bar displayed at the bottom or right of a window containing more information than can be displayed at once. The scroll bar has a knob and two arrow buttons. Dragging the knob scrolls the information directly and clicking one of the arrow buttons scrolls the information line by line. Holding an arrow button scrolls the information continuously.

Simulated colors

Colors used to display the image data acquired by observation on a display. Original simulated colors can be created by editing the LUT.

Status bar

The line showing information at the bottom of a window. It shows the information on the operation or the description of the function selected with the mouse pointer.

T**Text**

A file expressed with the ASCII codes such as characters and numerals, and with some control codes such as the line feed code. This format is referred to as the text format. The text is usually input from the keyboard.

Appendix C Glossary

Text box

A box in the window that accepts the input of character strings. Clicking a text box displays blinking |. This “|” indicates the position where the input character is inserted.

Title bar

The horizontal bar at the top of the window, that shows the title of the window or dialog box.

Toolbar

The toolbar provides frequently used functions in the form of buttons. It can be used any time during execution of any function.



Window

A large rectangle with a title. A window can be opened or closed.

Appendix D Formatting of Magnetic Optical Disk

Use the Disk Administrator tool of Windows for formatting a magnetic optical (MO) disk. Note that this tool can be used only by a person who have the authority of Administrator.

1. Click the <start> button at the bottom of the Windows screen.
2. When the [start] menu is displayed, select commands [Programs] - [Administrative Tools (Common)] - [Disk Administrator]. The window as shown below appears.

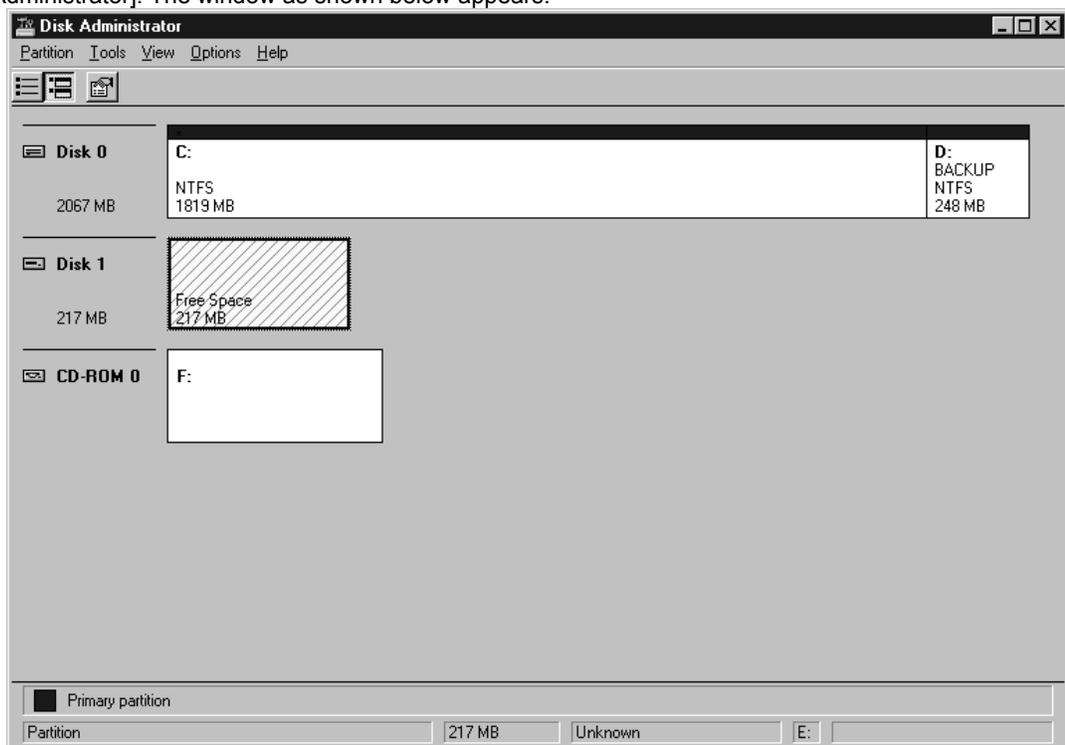


Fig. Appendix-D-1 [Disk Administrator] Window

3. Select the MO disk drive in the [Disk Administrator] window.

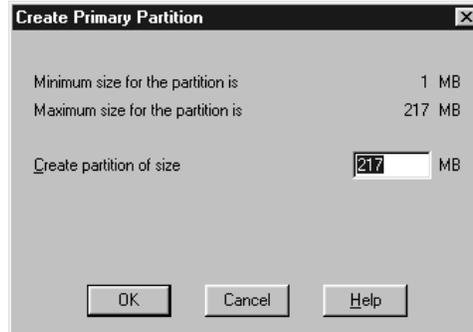
NOTE

The drive to which the MD disk is assigned is variable depending on computers.

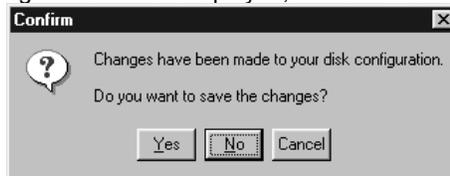


Appendix D Formatting of Magnetic Optical Disk

- From the [Partition] menu of the [Disk Administrator] window, select the [Create] command. The dialog box as shown below appears.



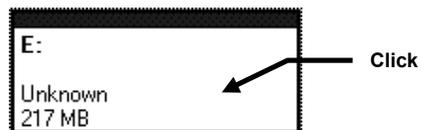
- Set the value of the [Create partition of size] edit box in the [Create Primary Partition] dialog box to "Maximum size", and click the <OK> button.
- From the [Partition] menu of the [Disk Administrator] window, select the [Commit Change know] command.
- When the confirmation message as shown is displayed, click the <Yes> button.

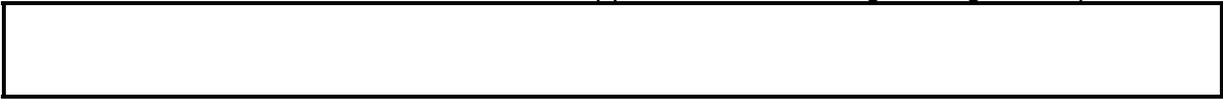


- When the following message is displayed, click the <OK> button.



- In the [Disk Administrator] window, select the MO disk drive.





- From the [Tool] menu of the [Disk Administrator] window, select the [Format] command. The dialog box as shown below appears.

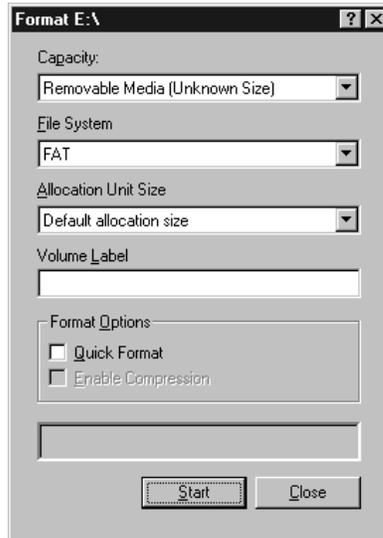
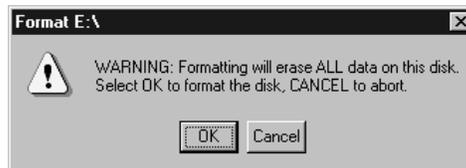
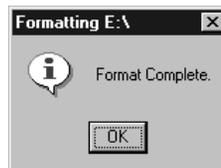


Fig. Appendix D-2 [Format] Dialog Box

- Select [Removable Media (Unknown Size)] from the [Capacity] drop-down list in the [Format] dialog box. Then select [FAT] from the [File System] drop-down list and select [FAT] and select [Default Allocation Unit Size] from the [Allocation Unit Size] drop-down list.
- Click the <Start> button.
- When the confirmation message as shown below is displayed, click the <OK> button.



- When the following message is displayed after completion of formatting, click the <OK> button.



- Click the <Close> button in the [Format] dialog box, then select [Partition] - [Exit] in the [Disk administrator] window to exit from the operation.

Appendix E Converting Analysis Data into a Chart Using EXCEL

1. Start up Excel.
2. From the [File] menu of Excel, select the [Open] command to open the analysis data file saved after analysis using FLUOVIEW.
3. When the dialog box as shown below appears, click the [Delimited] option button in the [Original Data Type] group box, then select [Windows (ANSI)] from the [File Origin:] drop-down list.

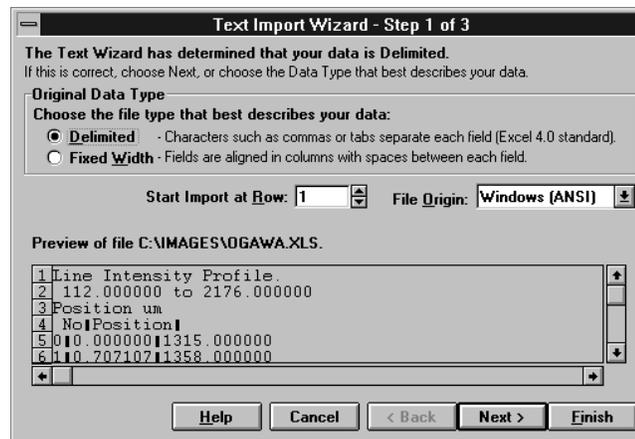


Fig. Appendix E-1 Dialog Box Displayed When File is Opened with Excel (1/3)

4. Click the <Next> button. When the dialog box as shown below appears, check the [Tab] check box in the [Delimiters] check box, then select [(none)] from the [Text Qualifier:] drop-down list.

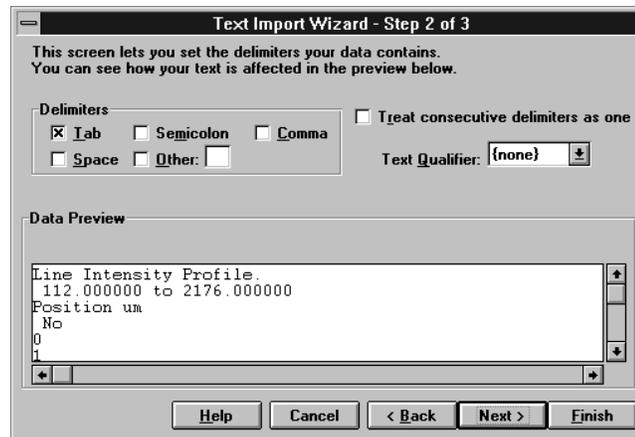


Fig. Appendix E-2 Dialog Box Displayed When File is Opened with Excel (2/3)

Appendix E Converting Analysis Data into a Chart Using EXCEL

- Click the <Next> button. When the dialog box as shown below appears, click the [General] option button in the [Columns Data Format] group box, then click the <Finish> button.

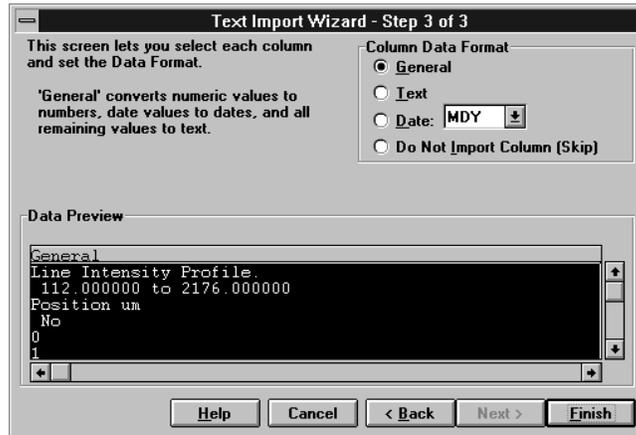


Fig. Appendix E-3 Dialog Box Displayed When File is Opened with Excel (3/3)

- Drag the data to be converted into a chart.

	A	B	C	D	E
1	Line Intensity Profile.				
2	73.000000 to 1658.000000				
3	Position um				
4	No	Position			
5	0	0	73		
6	1	0.25	104		
7	2	0.5	130		
8	3	0.75	108		
9	4	1	83		
10	5	1.25	121		
11	6	1.5	100		
12	7	1.75	110		
13	8	2	90		
14	9	2.25	87		
15	10	2.5	119		
16	11	2.75	124		
17	12	3	88		
18	13	3.25	234		
19	14	3.5	492		
20	15	3.75	1356		
21	16	4	1658		
22	17	4.25	1635		

Sheet



<ChartWizard> button

7. Click the <ChartWizard> button.
8. On the sheet, drag the area where the chart is to be inserted.
The dialog box as shown below appears.

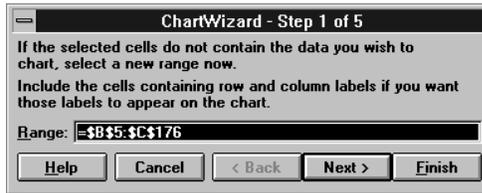


Fig. Appendix E-4 Dialog Box of Chart Wizard (1/5)

9. Click the <Next> button. The dialog box as shown below appears.

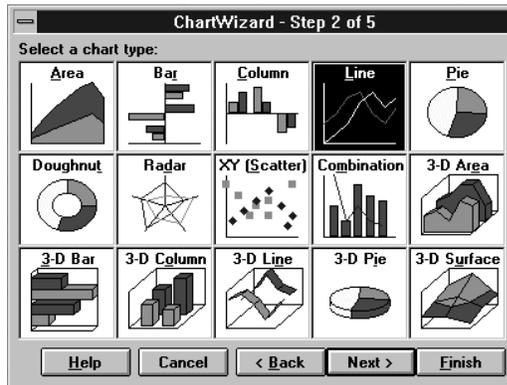


Fig. Appendix E-5 Dialog Box of Chart Wizard (2/5)

10. Select the desired chart type and click the <Next> button. The dialog box as shown below appears.

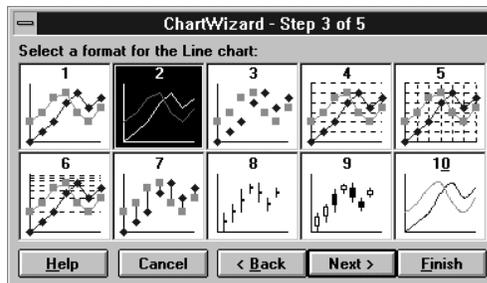


Fig. Appendix E-6 Dialog Box of Chart Wizard (3/5)



11. Select the desired chart format and click the <Next> button. The dialog box as shown below appears.

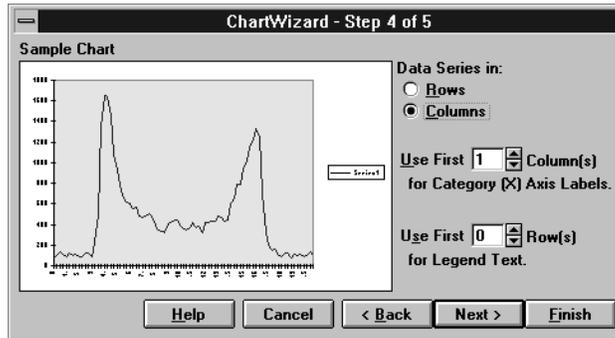


Fig. Appendix E-7 Dialog Box of Chart Wizard (4/5)

12. Click the [Columns] option button under [Data Series in:].
13. Set the data column number that you want to use as the X-axis label position in the [Use First Column(s)] text box.
14. Click the <Next> button. The dialog box as shown below appears.

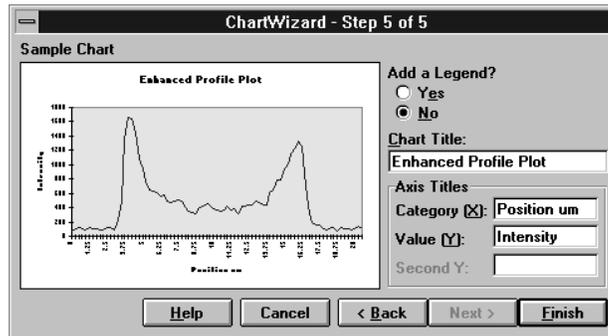


Fig. Appendix E-8 Dialog Box of Chart Wizard (5/5)

15. Enter the chart title in the [Chart Title] text box.
16. Enter the X-axis label in the [Category (X):] text box and the Y-axis label in the [Value (Y):] text box.

17. Click the <Finish> button. A chart will be drawn on the sheet.

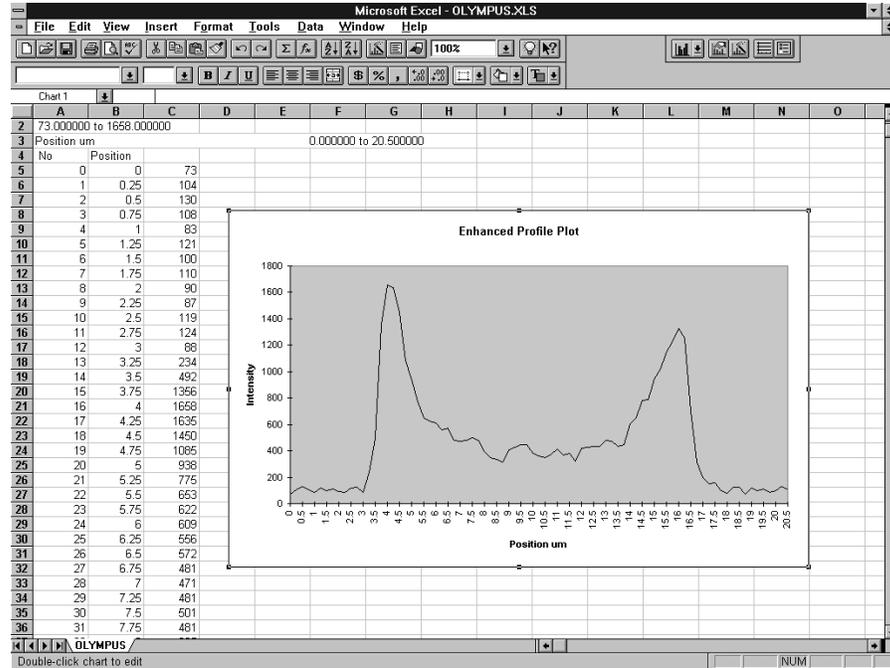


Fig. Appendix E-9 Sheet with Inserted Chart



When the mouse is clicked on the chart, black handles will appear around the chart.

The following operations are available in this condition.

- Moving the chart
Place the mouse pointer on the chart and drag it.
- Resizing the chart
Place the mouse pointer on one of the handles around the chart and drag it.

To return to the original condition, click a place outside the chart.

To enter reference text, use the "reference text" style.

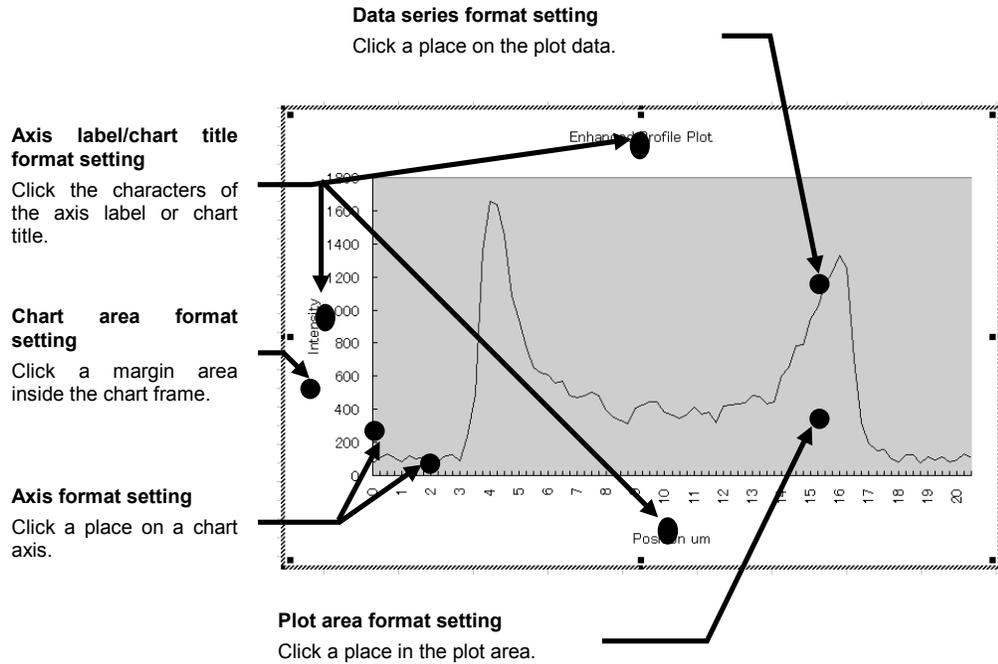


TIP

When the mouse is double-clicked on the chart, a frame composed of oblique lines appear around the chart. (The frame may become a window when the chart is large.)

The format settings are available in this condition.

1. Click one of the following positions to display black handles.



2. Double-click. The dialog box for setting the selected format appears. Set the desired format and click the <OK> button.

(Refer to the Excel manuals for details.)

To return to the original condition, click a place outside the chart.

Appendix F User Registration

Windows NT or Windows 2000 manages the system setting data on a per-user basis. Use the following procedure to register yourself as a user.

TIP

Log in the system with Administrator.

Register yourself as a new user.

1. From the [Start] menu, select [Programs] - [Administrator] - [User Manager].
2. The [User Manager] window appears.

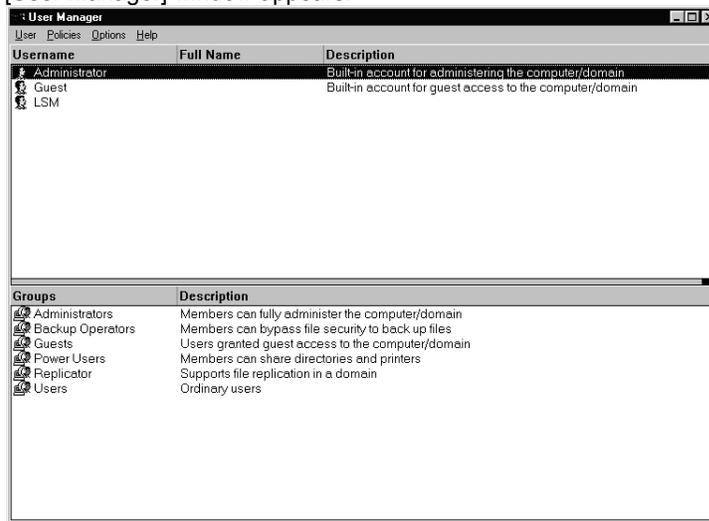


Fig. Appendix F-1 [User Manager] Window

3. Select [New User] from the [User] menu.
4. The [New User] dialog box appears.

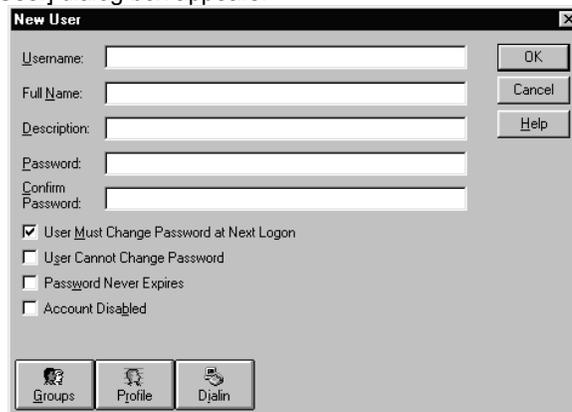


Fig. Appendix F-2 [New User] Dialog Box



5. Enter the user name in the [Username] text box.



It is not permitted to enter an existing user name or the same user name as the group name. The user name can be a character string of up to 20 characters and can contain any uppercase or lowercase characters except for the following characters.

“ / \ ¥ [] : ; | = , + × ? < >

A user name composed only of periods (.) or space () is not acceptable.

6. Enter the full name of the user in the [Full Name] text box.
7. Enter the description on the user in the [Description] text box.
8. Enter a password with up to 14 characters in the [Password] and [Confirm Password] text boxes.
9. Click the [User Must Change Password at Next Login] check box to check it.



The next time you log in with the registered user name, you can change the password.



<Group> button

10. Click the <Group> button.
The [Group Memberships] dialog box appears.

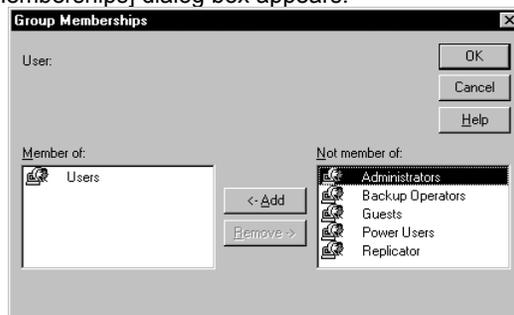


Fig. Appendix F-3 [Group Memberships] Dialog Box

11. Select [Administrators] in the [Net member of] list box.
12. Click the <Add> button.
13. Click the <OK> button.
14. Click the <OK> button.

To register other users, repeat steps 5 to 13 for each.

Appendix G USER REGISTRATION OF FV300

FLUOVIEW FV300 can store the system setup information (PMT Voltage, Gain, Offset, etc.) on a per-user basis.

To make this possible, you have to register yourself as a user and log in personally when starting up the FV300 software.

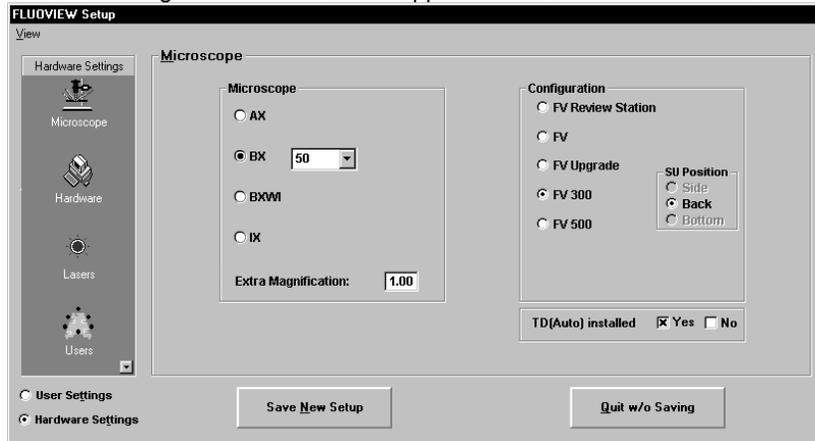
Appendix G-1 User Registration

Register yourself as a new user.

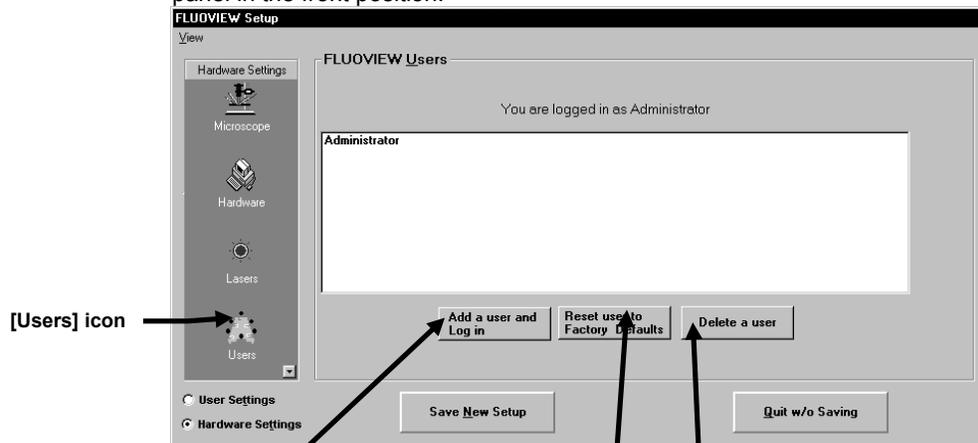


[FLUOVIEW Setup] icon

1. Double-click the [FLUOVIEW Setup] icon on the desktop.
Then the dialog box as shown below appears.



2. Click the [Users] icon in [Hardware Settings] to display the [FLUOVIEW Users] panel in the front position.



<Add a user and Log in> button
Used to add a new user.

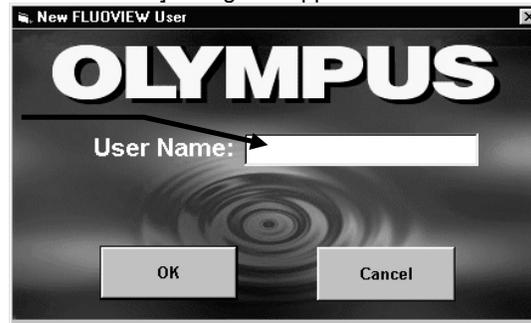
<Reset user to Factory Defaults> button
Resets the system setups of the users selected in the list box to the factory defaults.

<Delete a user> button
Deletes the user selected in the list box. (The Administrator and the user who log in now cannot be deleted.)

Appendix G USER REGISTRATION OF FV300/User Registration

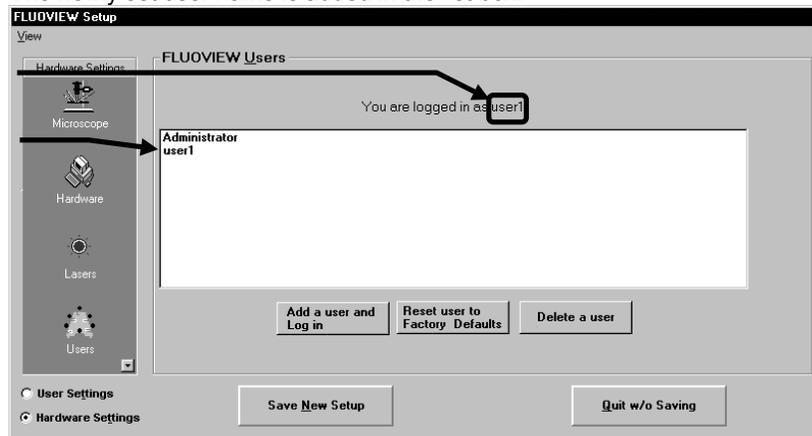
3. Click the <Add a user and Log in> button.
4. The [New FLUOVIEW User] dialog box appears.

[User Name:] text box
Enter the user name to be registered.



5. Enter the user name in the [User Name] text box and click the <OK> button.
The newly set user name is added in the list box.

The registered user name is shown here.
The user name is added.



6. To register other users, repeat steps 3 to 5 for each one.
7. Click the <Save New Setup> button or <Quit w/o Saving> button to close the dialog box.



The Administrator is the user name which saves the factory defaults of the system setup.

Appendix G-2 Logging into the FV300

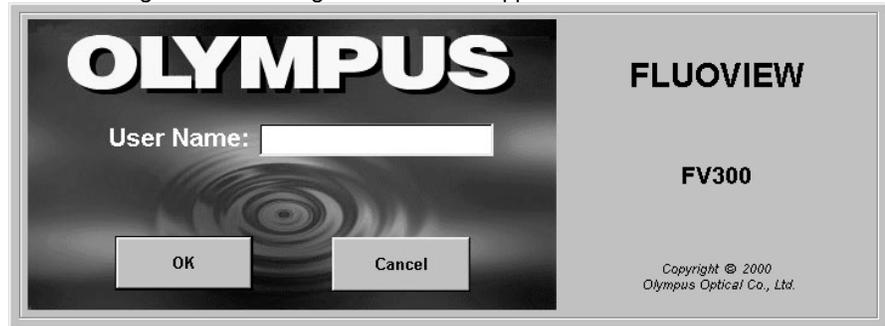
After the user name has been registered and the FV300 started, a dialog box for entering the user name appears.

Enter the user name to log in the FV300.



1. Double-click the [FLUOVIEW] icon on the desktop.

The dialog box for entering the user name appears as shown below.



2. Enter the user name in the [User Name:] text box and click the <OK> button.
3. The FLUOVIEW software starts.



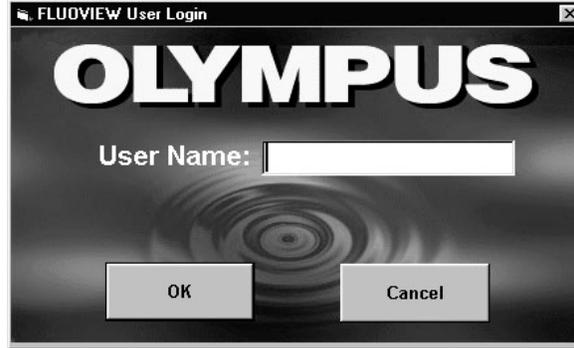
If no user name has been registered, the [FLUOVIEW User Login] dialog box is not displayed, but the system is automatically started for the Administrator.

Appendix G-3 Deleting a User

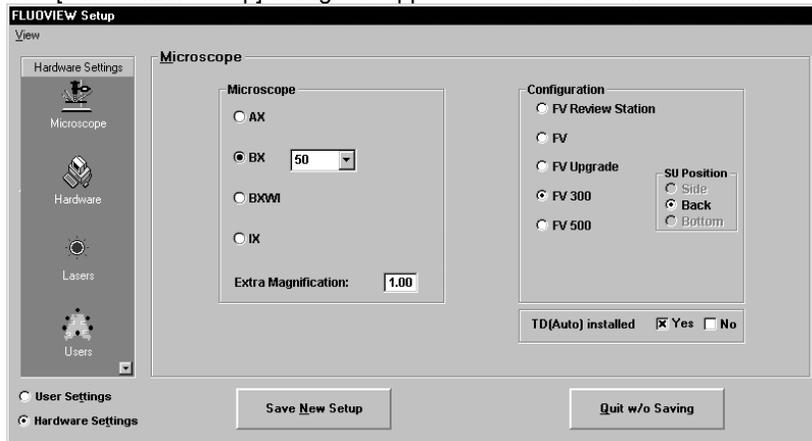


[FLUOVIEW Setup] icon

1. Double-click the [FLUOVIEW] icon on the desktop.
The dialog box for entering the user name appears as shown below.



2. Enter "Administrator" in the [User Name:] text box and click the <OK> button.
The [FLUOVIEW Setup] dialog box appears as shown below.





- Click the [Users] icon in [Hardware Settings] to display the [Users] panel at the front.

[Users] icon

<Add a user and Log in> button
Used to add a new user.

<Reset user to Factory Defaults> button
Resets the system setups of the users selected in the list box to the factory defaults.

<Delete a user> button
Deletes the user selected in the list box.
(The Administrator and the user who log in now cannot be deleted.)

- Select the user name to be deleted from the list box.
- Click the <Delete a user> button.



The Administrator cannot be deleted from the list.

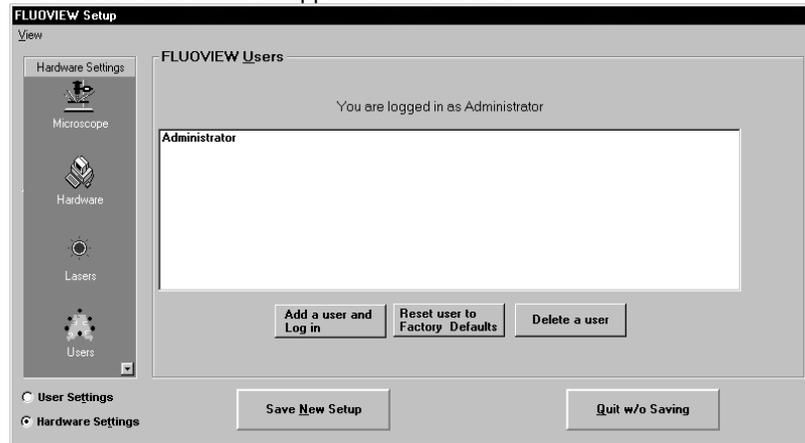
- The [Delete a user] dialog box appears to ask if you really want to delete the user.





7. Click the <Yes> button if you want to delete the user or the <No> button if you do not.

The deleted user name disappears from the list.



The user is deleted at the moment the <OK> button is clicked.

8. Click the <Save New Setup> button or <Quit w/o Saving> button to close the dialog box.

Appendix H Change of Default Folder for [File I/O] Panel

FLUOVIEW FV300 usually opens the default folder for the [File I/O] panel (C:\FLUOVIEW \IMAGES) to save acquired images or load saved images.

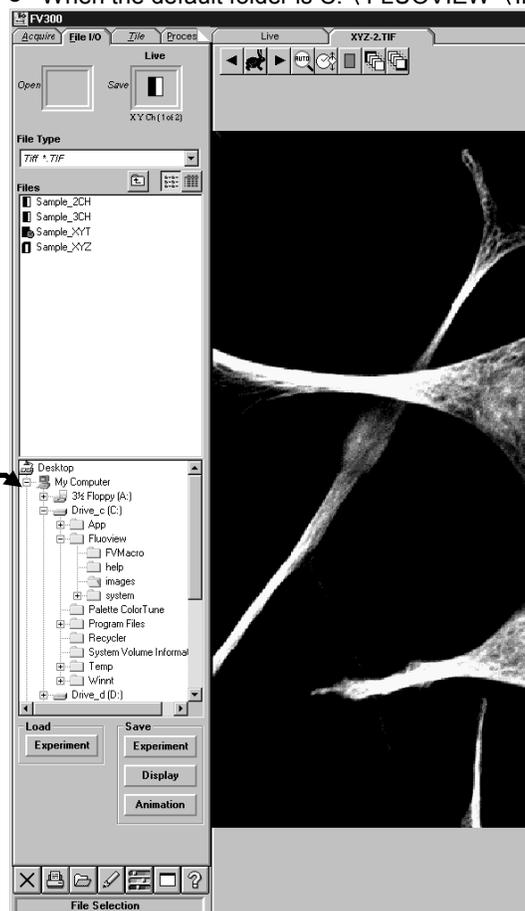
The default folder for saving an image can be changed or a desired folder can be specified directly when loading a saved image.

TIP Change the default folder only when required.
There is no need of change if folder C:\FLUOVIEW \IMAGES is all right.

TIP Folder names are delimited with “ \”.
For example, “C:\FLUOVIEW \IMAGES” means folder “IMAGE” in folder “FLUOVIEW” in “drive C”.

- When the default folder is C:\FLUOVIEW \IMAGES

Default folder “C:\FLUOVIEW \IMAGES” is being open.

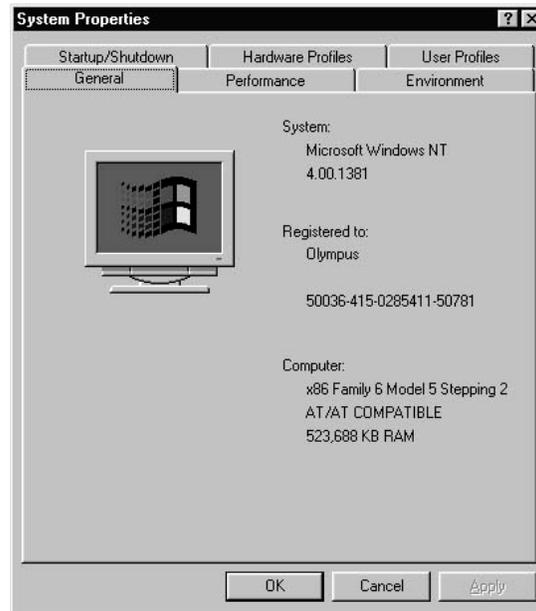


A different default folder can be set for each of the users logging in Windows NT or Windows 2000.

Appendix H Change of Default Folder for [File I/O] Panel

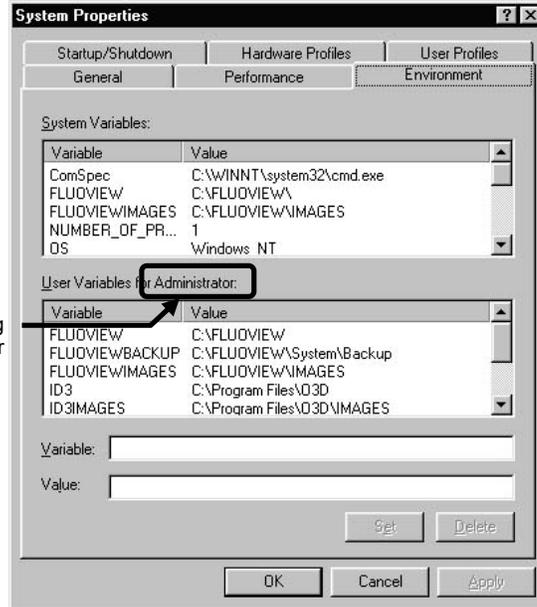


1. Click the [Start] button to open the [Start] menu. Then select commands [Settings] - [Control Panel] - [System]. The [System Properties] dialog box appears as shown below.

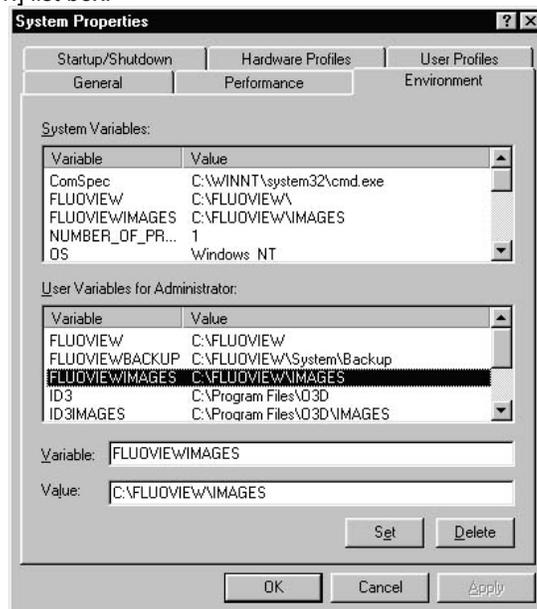


2. Select the [Environment] sub-panel.

User name logging in Windows NT or Windows 2000.



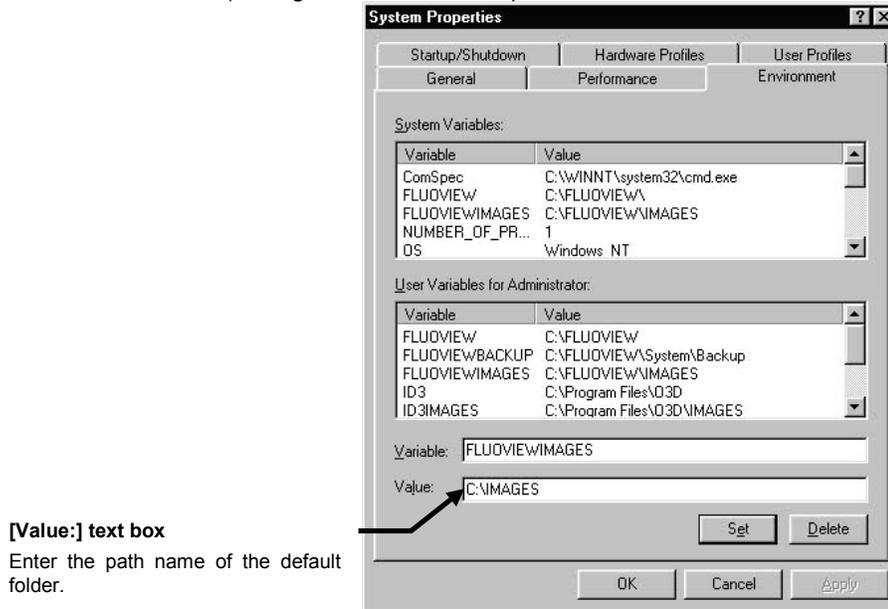
3. Select [FLUOVIEWIMAGES] under [Variable] in the [User Variables for Administrator:] list box.



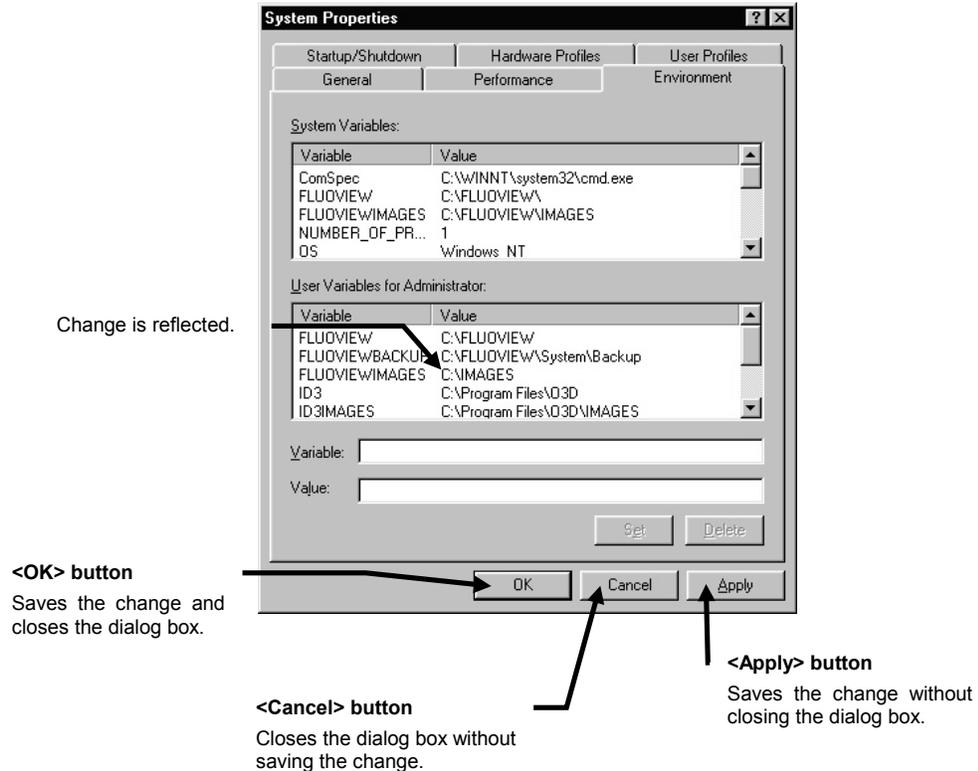
“Administrator” of [User Variables for Administrator:] is variable depending on the user name logging in Windows NT or Windows 2000.

Appendix H Change of Default Folder for [File I/O] Panel

- In the [Value:] text box, enter the path name of the default folder to be changed by delimiting the drive and folder names using backslashes (\).
(The figure shows an example in which folder IMAGE in drive C is entered.)



- Click the <Set> button

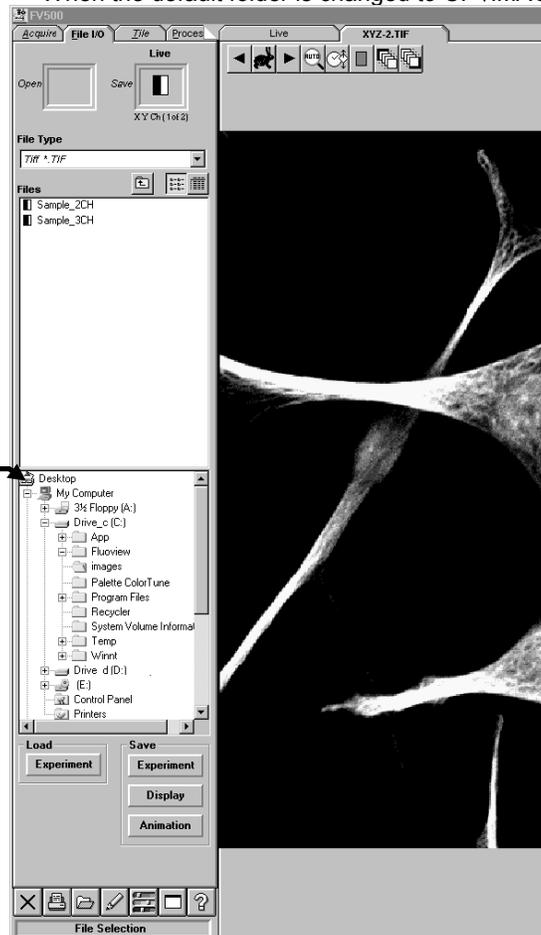




6. Click the <OK> button to close the [System Properties] dialog box.
Now, the default folder for the [File I/O] panel will be changed from the next time you start FLUOVIEW.

● When the default folder is changed to C: \ IMAGES

New default folder C \ IMAGES is being open.



To reset the default folder to the factory setup, enter “C: \ FLUOVIEW \ IMAGES” in the [Value] text box.



Do not change the setup after “FLUOVIEWIMAGES” in the [User Variables for <username>] list box. If erroneous setting is made here, the system will be unable to be started up.

Appendix I List of Functions in the [Active Overlays] Dialog Box

Active Overlays is a kind of overlay function displayed on an image.

Active Overlays does not simply show the entered characters, but searches the image data related to the keyword specified in < > and displays the data values on the image.

The following setups are required to enable the use of Active Overlays.

Appendix I-1 Coordinate Position Data

Appendix I-1-1 X-Coordinate

The X-coordinate position with respect to the top left corner of the screen is displayed.

«Syntax»

<x[hotspot] [raw/calibrated] value [units]>

Arguments inside [] can be omitted.

If [raw/calibrated] is omitted, the same setting as when [calibrated] is specified will be applied.

«Setup Procedure»

1. Enter "x" as the first character inside < >.
2. Add the following characters to set the display method.
 - hotspot : Display measurement points with "+" markings.
 - raw : Display data in pixel values.
 - calibrated : Display data in numerical values (μm).
 - units : Display the unit (pixels/ μm).

«Examples»

<Input character strings>	<Displayed strings (** represent figures.)>
<x raw value>	**
<x hotspot raw value>.....	+**
<x hotspot calibrated value units>.....	+** μm



The detailed display procedure is described in section 2-11-3, "Viewing the X- or Y-Coordinate Position of Image" in Volume [OPERATION].

Appendix I-1-2 Y-Coordinate

The Y-coordinate position with respect to the top left corner of the screen is displayed.

«Syntax»

<y [hotspot] [raw/calibrated] value [units]>

Arguments inside [] can be omitted.

If [raw/calibrated] is omitted, the same setting as when [calibrated] is specified will be applied.

«Setup Procedure»

1. Enter “y” as the first character inside < >.
2. Add the following characters to set the display method.
 - hotspot : Display measurement points with “+” markings.
 - raw : Display data in pixel values.
 - calibrated : Display data in numerical values (µm).
 - units : Display the unit (pixels/µm).

«Examples»

<Input character strings>	<Displayed strings (** represent figures.)>
<y raw value>	**
<y hotspot raw value>.....	+**
<y hotspot calibrated value units>.....	+**µm
<x hotspot raw value>,<y raw value>	+(**, **)



The detailed display procedure is described in section 2-11-3, “Viewing the X- or Y-Coordinate Position of Image” in Volume [OPERATION].

Appendix I-1-3 Other

Positions can also be displayed by entering the following keywords in the syntax in place of the X- and Y-coordinate positions described in sections J-1-1 and J-1-2.

1 Z Position

With cross-section related images such as XYZ images, the Z position with respect to the first image can be displayed.

«Syntax»

<z [raw/calibrated] value [units]>

Arguments inside [] can be omitted.

If [raw/calibrated] is omitted, the same setting as when [calibrated] is specified will be applied.

2 T Position

With time-related images such as XYZ images, the T position with respect to the first image can be displayed.

«Syntax»

<t [raw/calibrated] value [units]>

Arguments inside [] can be omitted.

If [raw/calibrated] is omitted, the same setting as when [calibrated] is specified will be applied.

3 Animation

With animation images created in the [Visualize] panel, the position with respect to the first image can be displayed.

«Syntax»

<Animation [raw/calibrated] value [units]>

Arguments inside [] can be omitted.

If [raw/calibrated] is omitted, the same setting as when [calibrated] is specified will be applied.

Appendix I-2 Intensity Data

The intensity value can be displayed.

When images are overlapped, the intensity value of each image is accompanied with the channel number placed after it.

«Syntax»

<intensity[[hotspot] [raw/calibrated] value [units]]>

Arguments inside [] can be omitted.

If [raw/calibrated] is omitted, the same setting as when [calibrated] is specified will be applied.

«Setup Procedure»

1. Enter "intensity" as the first character inside < >.
2. Add the following characters to set the display method.
 - hotspot : Display measurement points with "+" markings.
 - raw : Display data in values between 0 and 4095.
 - calibrated : Display data in numerical values (µm).
 - units : Display the unit if this has been set.

(Same as the concentration computation in FV-TIEMPO)

«Examples»

<Input character strings>	<Displayed strings (** represent figures.)>
<intensity raw value>	**
<intensity hotspot raw value>	**(1),**(2)

(When images are overlapped)



The detailed display procedure is described in section 2-11-2, "Viewing the Intensity Value of Image" in Volume [OPERATION].

Appendix I-3 Other

The following image data can be displayed in addition to the X- and Y-coordinate positions and intensity value.

Appendix I-3-1 Channel Number

The channel number can be displayed.

When images are overlapped, the displayed channel numbers are connected by the “+” markings.

«Syntax»

<Channel>

Appendix I-3-2 Objective Power

The objective power used in image capturing can be displayed.

«Syntax»

<Objective>

Appendix I-3-3 Date of Image Capturing

The date at which the image was captured can be displayed.

«Syntax»

<Date>

Appendix I-3-4 Time of Image Capturing

The time of the day at which image was captured can be displayed.

«Syntax»

<Time>

Appendix I-3-5 Image File Name

The image file name can be displayed.

«Syntax»

<Name>

Appendix J Hand Switch and Microscope Frame Function Allocation

When the U-HSTR2 hand switch, the BX51/BX61/IX81 microscope frames, and U-FH focus adjustment knob are shipped from the factory, the following functions have been allocated to their control buttons.



NOTE These function allocations are valid only when the FLUOVIEW software is started.

Appendix J-1 Hand Switch Functions

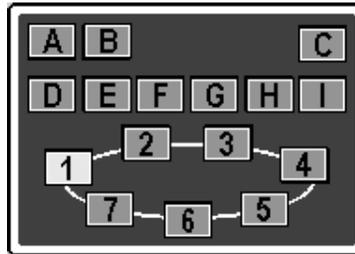


Fig. Appendix J-1 Hand Switch

Button	Function
A	Reflected light shutter OPEN/CLOSE switching
B	Transmitted light (LG-PS2) ON/OFF switching
C	Stage escape/return
D – I	Dichroic mirror setting (Cube positions 1 to 6)
1 – 6	Objective setting
7	Not used.

Appendix J-2 Microscope Frame Functions

Appendix J-2-1 BX

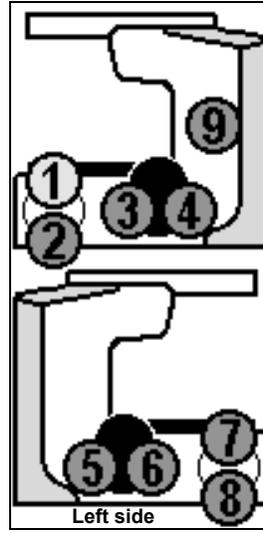


Fig. Appendix J-2 BX Microscope Frame

Button	Function
1	Stage escape/return switching
2	Focus fine/coarse adjustment switching
3	Z position upper limit setting in Z-observation
4	Z position lower limit setting in Z-observation
5	Excitation DM position up
6	Excitation DM position down
7	Stage escape/return switching
8	Focus fine/coarse adjustment switching
9	Focus fine/coarse adjustment switching

Buttons 3 and 4 are located on the right side of the microscope frame.

Appendix J-2-2 IX

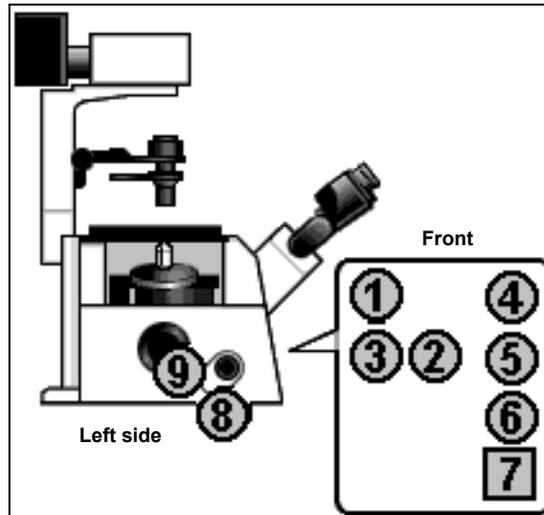


Fig. Appendix J-3 IX81 Microscope Frame

Button	Function
1	Focus fine/coarse adjustment switching
2	Excitation DM position up
3	Excitation DM position down
4	BI/LSM light path switching
5	Objective revolving nosepiece position up
6	Objective revolving nosepiece position down
7	Transmitted light (LG-PS2) ON/OFF switching
8	Z position upper limit setting in Z-observation
9	Z position lower limit setting in Z-observation

Appendix J-2-3 Focus Adjustment Knob

1 BX

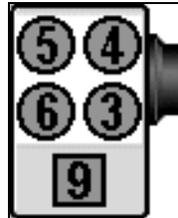


Fig. Appendix J-4 Focus Adjustment knob (BX)

Button	Function
3	Z position upper limit setting in Z-observation
4	Z position lower limit setting in Z-observation
5	Excitation DM position up
6	Excitation DM position down
9	Focus fine/coarse adjustment switching

2 IX



Fig. Appendix J-5 Focus Adjustment knob(IX)

Button	Function
1	Focus fine/coarse adjustment switching
2	Excitation DM position up
3	Excitation DM position down
8	Z position upper limit setting in Z-observation
9	Z position lower limit setting in Z-observation

V. MAINTENANCE

On This Volume

This volume describes the user maintenance procedures of the FLUOVIEW FV300 system.
Please read this volume so that you can use the system for an extended period of time.

1 Software Setup **1-1**

1-1 Re-setup or Updating of the Software	1-1
1-2 New Setup of the Software	1-3
1-3 Setting the System Configuration.....	1-6
1-3-1 Overall Setting of FLUOVIEW	1-6
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2 Maintenance of Major System Units **2-1**

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2-2 Laser Scanning Microscope	2-2

1 Software Setup

The FLUOVIEW software has been set up before it is delivered to the user.

A CD-ROM containing the software program is provided with FLUOVIEW. This CD-ROM is intended for use when re-setup is required due to a fault or when the user wants to set up the software newly.

When the FLUOVIEW software is updated, the user is also requested to update the software in use accordingly. For re-setup or updating of the software, see section 1-1, "Re-Setup or Updating of the Software" below and follow the procedures given in it.

When you want to set up the software newly, see section 1-2, "New Setup of the Software" below and follow the procedures given in it.

TIP

To set up the FLUOVIEW software, it is necessary that the computer in use already has Microsoft Windows NT Version 4.0 (English version) or Microsoft Windows 2000 (English version) installed in its hard disk.

A vacant space of 100 MB is required for the setup.

1-1 Re-setup or Updating of the Software

1. Insert the FLUOVIEW setup CD-ROM in the CD-ROM drive.

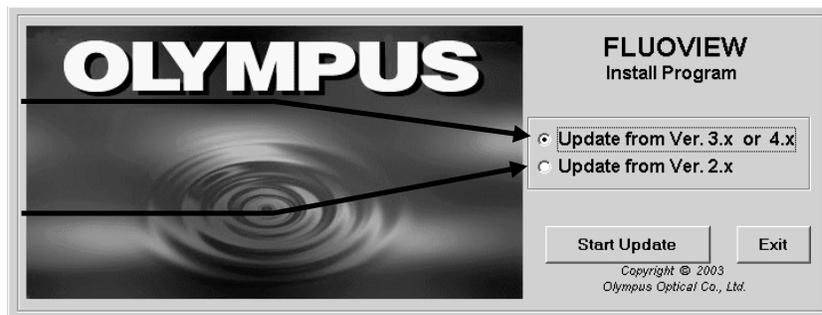
Then the dialog box as shown below appears.

[Update from Ver.3.x] option button

When updating from FV software after ver3.0, select this option button.

[Update from Ver.2.x] option button

When updating from FV software of ver2. x, press this option button.



TIP

If the dialog box doesn't appear, run the 'Install.exe' file that is present in the root directory of the CD-ROM.

2. Click the option button of the version of FV software installed, and click the <Start Update> button.
3. [Update FV Software] dialog box is displayed and starts the current settings. For save, carry out according to a wizard.

Software Setup/Re-setup or Updating of the Software



4. Continuously, deletion of software is started.
For deletion, carry out according to a wizard.

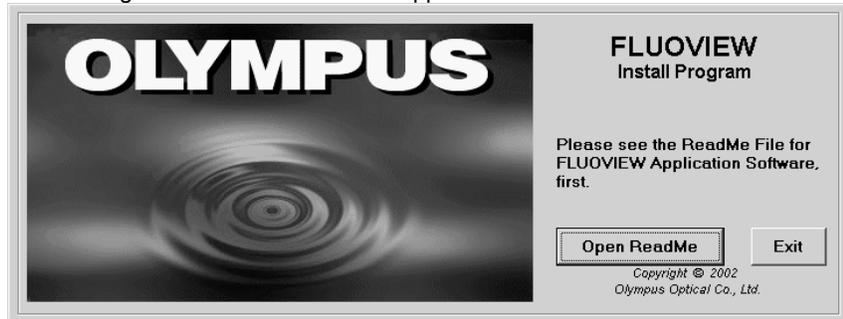
5. Continuously, installation of the software of a new version is started.
Refer to 1-2 “New Setup of the Software” for details.

1-2 New Setup of the Software

The following procedure allows you to set up the FLUOVIEW software in a computer you use.

1. Load the FLUOVIEW setup CD-ROM in the CD-ROM drive.

The dialog box as shown below will appear.



TIP

If the dialog box doesn't appear, run the 'Install.exe' file that is present in the root directory of the CD-ROM.

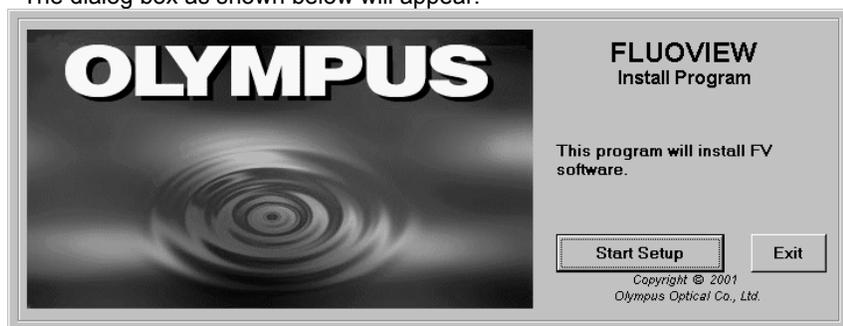
2. Select the <Open Readme> button.



<Close> button

3. When Readme.txt is displayed, read it carefully. Click the <Close> button on the top left of the window to close Readme.txt.

The dialog box as shown below will appear.



4. Select the <Start Setup> button.



5. When the [Choose Destination Location] dialog box appears, confirm the setup destination drive name and directory and select the <Next> button.





6. When the setup has completed, the [Setup Complete] dialog box appears. Select the [Yes, I want to restart my computer now.] option button and press the <Finish> button. This will restart the computer.



1-3 Setting the System Configuration

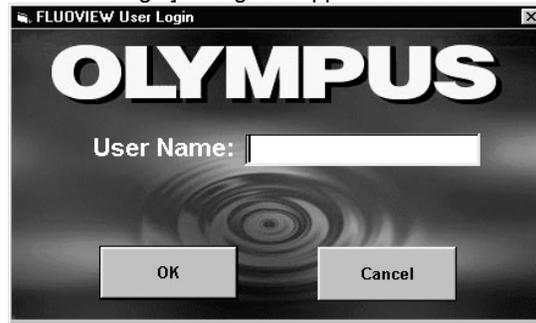
If it is required to change the settings after having set up the software, perform the following procedure.

1-3-1 Overall Setting of FLUOVIEW



[FLUOVIEW Setup] icon

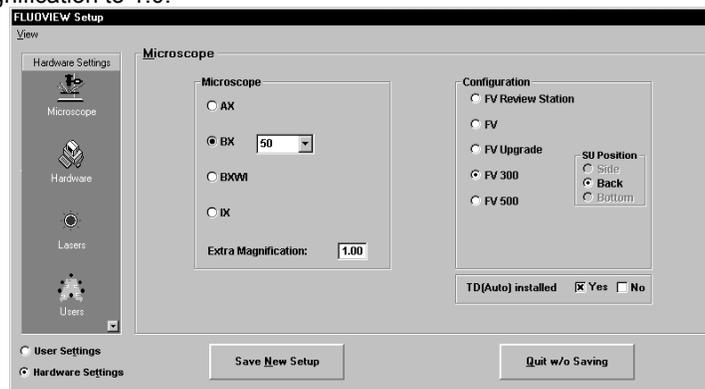
1. Double-click the [FLUOVIEW Setup] on the desktop.
The [FLUOVIEW User Login] dialog box appears as shown below.



2. Enter the user name in the [User Name:] text box and click the <OK> button to log into FLUOVIEW FV300.

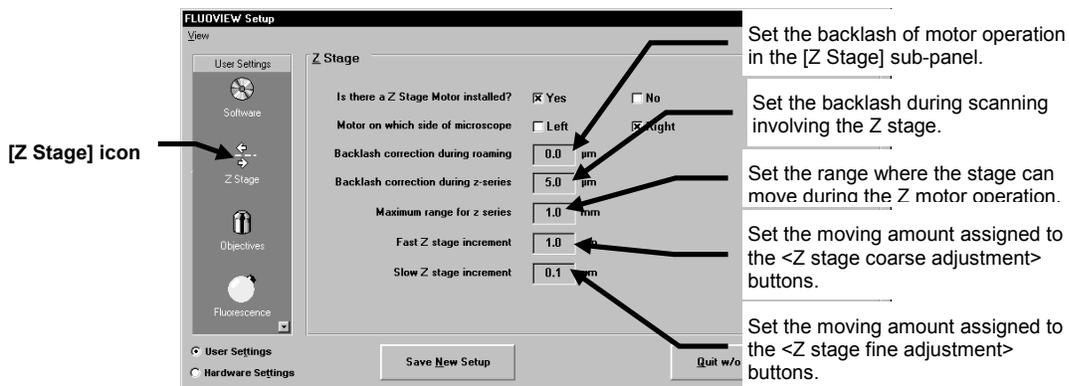
For details, see Appendix G-2, “Logging into the FV300”.

3. The dialog as shown below appears for use in saving the system configuration. First, select the type of microscope and set the extra (intermediate attachment) magnification to 1.0.



Selecting “BX51, 52”, “BX51,52WI” ,or “IX71” and “Automatic” in the [Microscope] group box or “BX61, 62”, “BX61,62WI”, or “IX81” in the [Microscope] group box shows the [Microscope Setup] dialog box to edit the BX or IX operation panel (Microscope Control Panel). For details, see section 1-3-2 “Setting the [Microscope Control Panel]”.

- Click the [Z Stage] icon in [User Settings] to display the [Z Stage] panel in the front position, and check and set the presence of motor controller, positioning of the motor controller, backlash and moving amount of the Z motor.

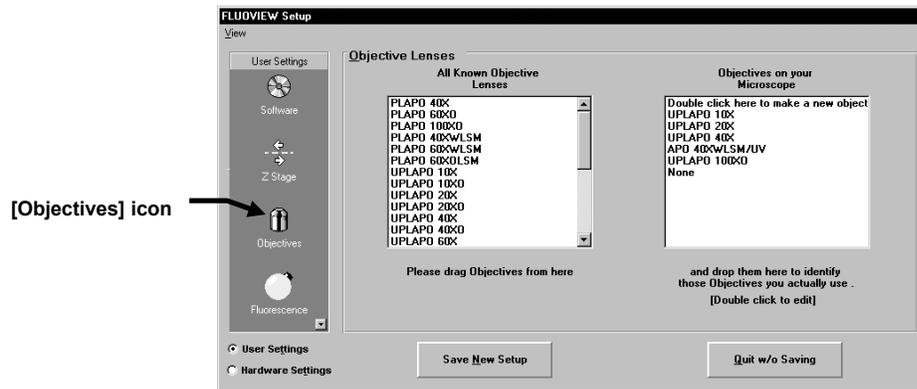


The [Maximum range for Z series (mm)] text box accepts a setting value between 1.0 and 3.0 (mm).
 The values in the [Start Z] and [Stop Z] text boxes in the [Z Stage] sub-panel are set according to the value set there.
 For example:
 When “1.0” (mm) is set as the maximum range for Z series, the minimum value for Z starting becomes -500 (μm) and the maximum value for Z stopping becomes 500 (μm).



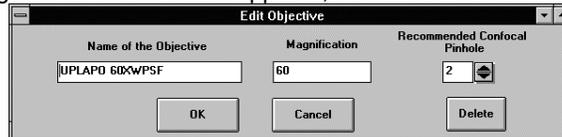
- Click the [Objectives] icon in [User Settings] to display the [Objectives] panel in the front position.

In this panel, select the items to be included in the list of objectives used by the FLUOVIEW application. (Each user should set the objectives that the user wants to use with FLUOVIEW.)



<To delete an unnecessary objective from the list>

In the list on the right, double-click the objective to be deleted from the list. When the dialog box as shown below appears, select the <Delete> button.



<To change the details (objective name, pinhole No., magnification) of an objective>

Double-click the objective for which you want to change the detailed information. When the dialog box appears, change the desired information items and select the <OK> button.

<To add an objective to the list>

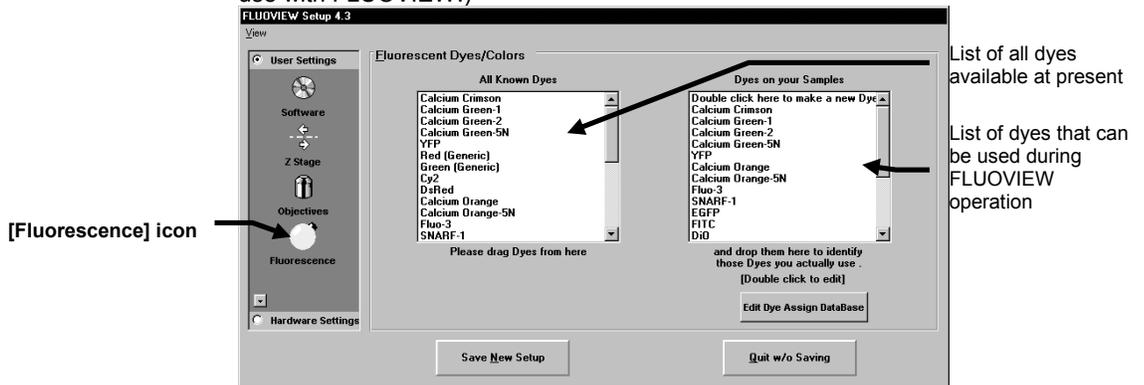
Double-click “Double Click here...” at the top of the list on the right. When the dialog box appears, set the detailed information on the new objective and select the <OK> button.

<To add one of the objectives in the list on the left to the list on the right>

Place the mouse pointer on the objective to be added in the list on the left, and drag it to the list on the right.

- Click the [Fluorescence] icon in [User Settings] to display the [Fluorescent Dyes/Colors] panel in the front position.

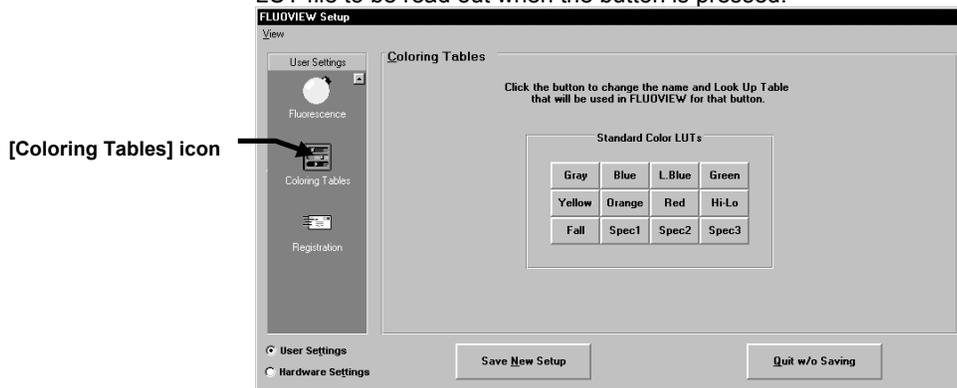
In this panel, select the items to be included in the list of dyes used by the FLUOVIEW application. (Each user should set the dyes that the user wants to use with FLUOVIEW.)



The setting procedure is similar to the objective setting procedure ([Objectives] panel).

- Click the [Coloring Tables] icon in [User Settings] to display the [Coloring Tables] panel in the front position.

In this panel, select the characters to be displayed in each of the 8 buttons displayed in the [Color Tool] dialog box of the FLUOVIEW application and the LUT file to be read out when the button is pressed.

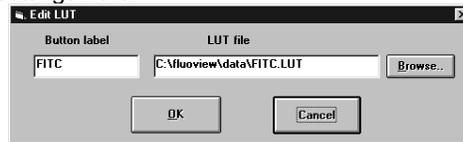




<To change the button settings>

Press the button to indicate that you want to change the setting. When the dialog box as shown below appears, specify the button name and LUT file name and select the <OK> button.

The LUT files which have been used up to Ver 1.0* (FITC, etc.) are supplied with this version in the same names as before. If you want to use such files, change their settings here.



- Click the [Software] icon in [User Settings] to display the [Software] panel in the front position.

In this panel, select the initial magnification status when images are to be displayed in [Display] panels. This panel is also used to set the mode of comment overlay in image and to set the numbers of [Function] and [Display] panels that can be displayed together and enable/disable the automatic black-level adjustment.

[Default Image Zoom in Single View] group box
Select the initial status of display magnification when a single image is to be displayed in a single [Display] panel.

[Default Image Zoom in Multiple View] group box
Select the initial status of display magnification when multiple images are to be displayed by displaying multiple [Display] panels together.

[Software] icon

[Coloring for new overlays] group box
Set the color of the comment to be overlaid in image in the [Display] panel.

[Overlay creation] group box
When comment is to be overlaid in image, select either the mode allowing repeated entries of comment per Annotation tool selection or the mode allowing single entry of comment.

[Automatic Black-level adjustment] group box
Select Enabled/Disabled to enable/disable the automatic black-level adjustment.

[Detect PMT Overrange] group box
Select [Enabled]/[Disabled] to enable/disable display the message which notifies that the Output excessive light to PMT.

Tab Display
Number of tabs on Function Panel visible at once: 4
Max number of tabs on Display Panel visible at once: 8

Automatic Black-level adjustment
 Enabled
 Disabled

Detect PMT Overrange
 Enabled
 Disabled

Coloring for new overlays
 Uses default color (yellow)
 Uses most recent color
 Uses next color in sequence

Overlay creation
 Requires click on tool for each
 Repeats with each click on image

Default Image Zoom in Single View
 Auto
 1:1

Default Image Zoom in Multiple View
 Auto
 1:1

User Settings
Software
Z Stage
Objectives
Fluorescence

Buttons: Save New Setup, Quit w/o Saving

9. Display the [Hardware] panel in the front position.

In this panel, select Enabled/Disabled to enable/disable the automatic black-level adjustment, enter the values for delay timing for TD unit, and enter the maximum duration for continuous bi-directional scanning when setting fast scan mode.

[Delay Timing for TD Unit] group box

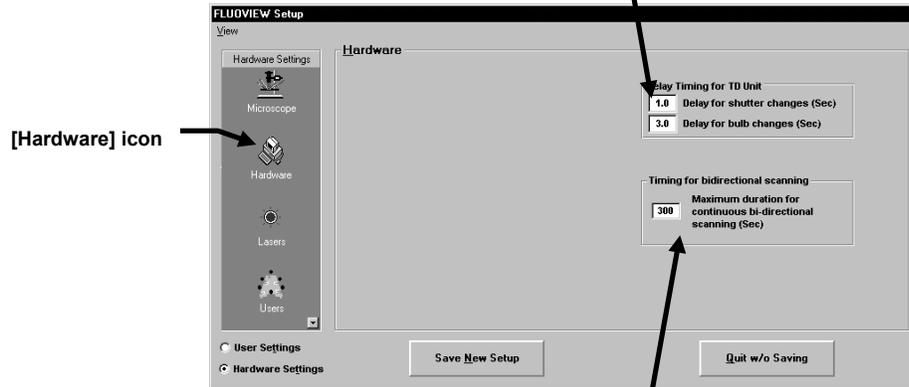
This data should usually be left in the default values and does not have to be changed.

[Delay shutter changes (sec)] text box

Enter the opening/closing wait time of the shutter in the TD unit in seconds.

[Delay for bulb changes (sec)] text box

Enter the ON/OFF wait time of the lamp in the TD unit in seconds.



[Timing for bidirectional scanning] group box

Enter the maximum duration for continuous bi-directional scanning (in seconds) when setting fast scan mode.

- Click the [Lasers] icon in [Hardware Settings] to display the [Lasers] panel in the front of position.

Set the lasers to be used and set its default intensity.

[Lasers Installed] check box
Select and check the lasers to be used with the FLUOVIEW application.

[Multi Lines] check box
Check when using Multiline Ar laser, then the scale appears to the right

[Lasers] icon

[Detail Setting] button

[Default Laser Intensity] scale
Set the initial ND filter value at the start of the FLUOVIEW application.

Appears when checking the [Multi Lines] check box.
The laser wave length is popped up when placing the mouse pointer onto the text box.

[Laser Intensities at Startup] group box
Set the laser intensities when starting up the FLUOVIEW application.
[User default intensities] option button
Select to use the default intensity set in the [Default Laser Intensity] scale above.
[Use most recent intensities] Option button
Set to use the intensity that was used in the last observation.

- Click the [Detail Setting] button to display [Laser Setting] dialog box.

[Name] drop down list
Select laser name. The name can be also input from the keyboard.

[Text] button
Set color of characters to display laser intensity.

[Background] button
Set background color of laser intensity display.

Input laser wavelength to be displayed in pop up dialog in laser intensity display.

This value is utilized to determine filter setting in accordance with dye, and it does not need to change usually.

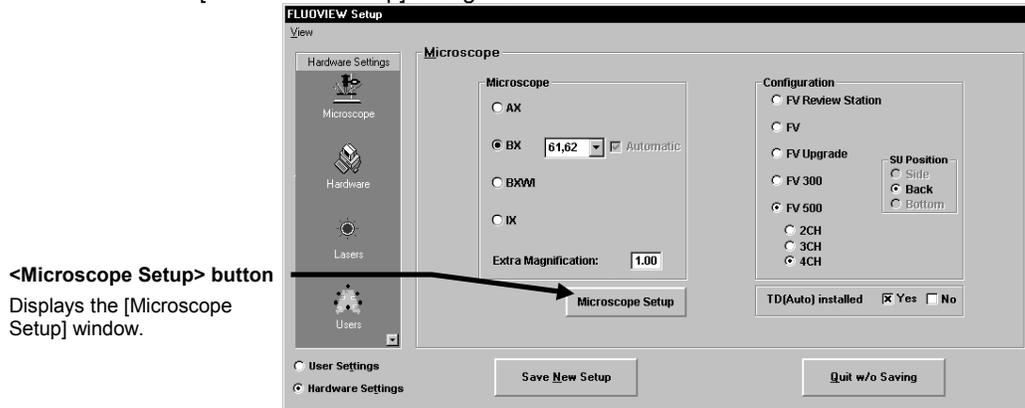
Check this box if dedicated AOTF is not equipped to the laser unit selected from drop down list of [Name].



12. The [Registration] panel have been set at the factory and do not need to be changed here.
13. After completing the setup, select the <Save New Setup> button on the bottom left of the panel.
(Selecting <Quit w/o Saving> exits the panel without saving the system setup.)

1-3-2 Setting the [Microscope Control Panel]

The <Microscope Setting> button as shown below appears when selecting “BX 51,52”, “BX51,52WI”, or “IX71” and checking the [Automatic] check box or selecting “BX61,62”, “BX61,62WI”, or “IX81” in the [Microscope] group box in the [Microscope] panel in the [FLUOVIEW Setup] dialog box.



<Microscope Setup> button
Displays the [Microscope Setup] window.

Clicking the <Microscope Setup> button displays the [Microscope Setup] window as shown below.

[Filter Turret] group box

Sets up the name and color of the filter turret for reflected light observation.

[Filter Turret] group box

Sets up the name and color of the filter turret for visual observation.

[Mirror Unit] group box

Sets up the name and color of the cube turret.

[Nosepiece] group box

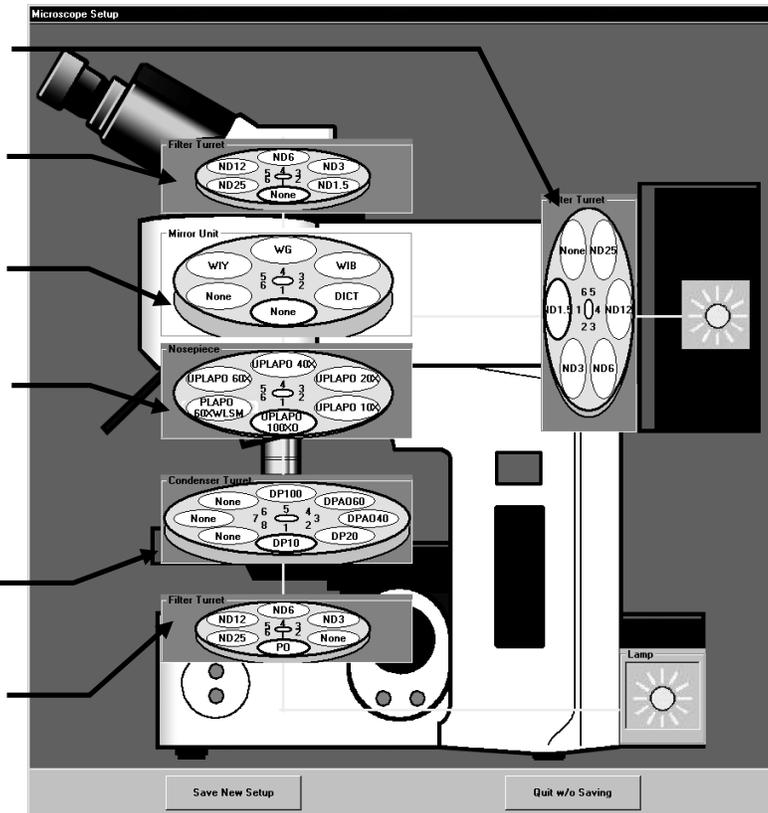
Sets up the name, color of the objective and sets the magnification, N.A, refractive index (n), number of the confocal pinhole, and the condenser worked with to each objective.

[Condenser Turret] group box

Sets up the name and color of the universal condenser.

[Filter Turret] group box

Sets up the name and color of the filter turret for transmitted observation.



(Combination with BX)

[Shutter] group box

Sets up to use the shutter for transmitted observation.

[Filter Turret] group box

Sets up the name and color of the filter turret for transmitted o
Select the initial status of display magnification when multiple images are to be displayed by displaying multiple [Display] panels together.

[Condenser Turret] group box

Sets up the name and color of the universal condenser.

[Nosepiece] group box

Sets up the name, color of the objective and sets the magnification, N.A., refractive index (n), number of the confocal pinhole, and the condenser worked with to each objective.

[Filter Turret] group box

Sets up the name and color of the filter turret for visual observation.

[Mirror Unit] group box

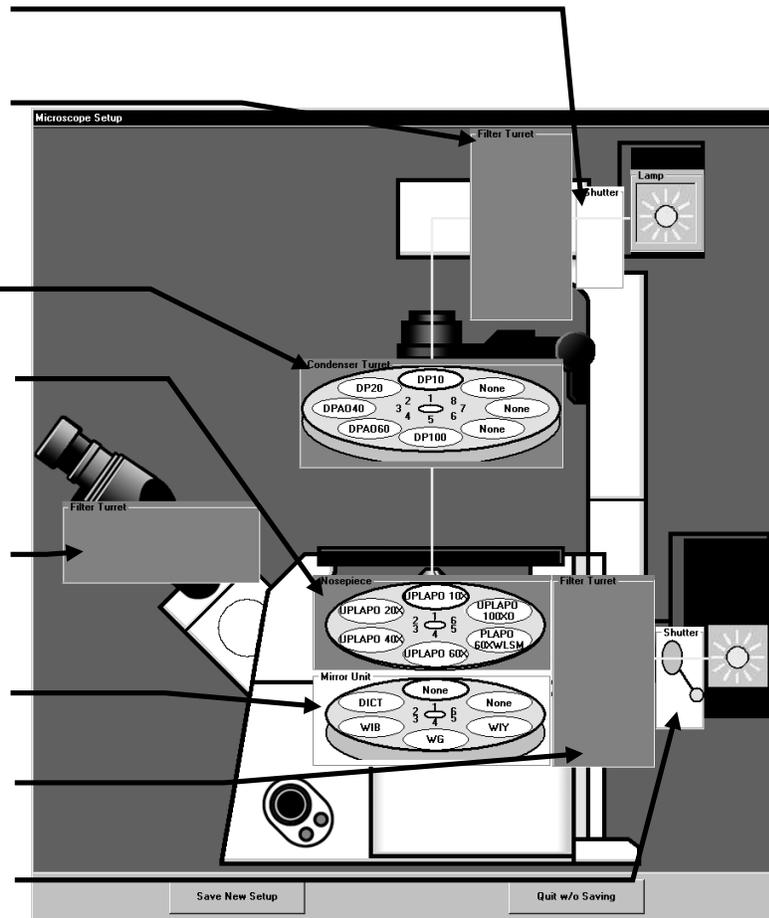
Sets up the name and color of the cube turret.

[Filter Turret] group box

Sets up the name and color of the filter turret for reflected light observation.

[Shutter] group box

Sets up to use the shutter for reflected light observation.



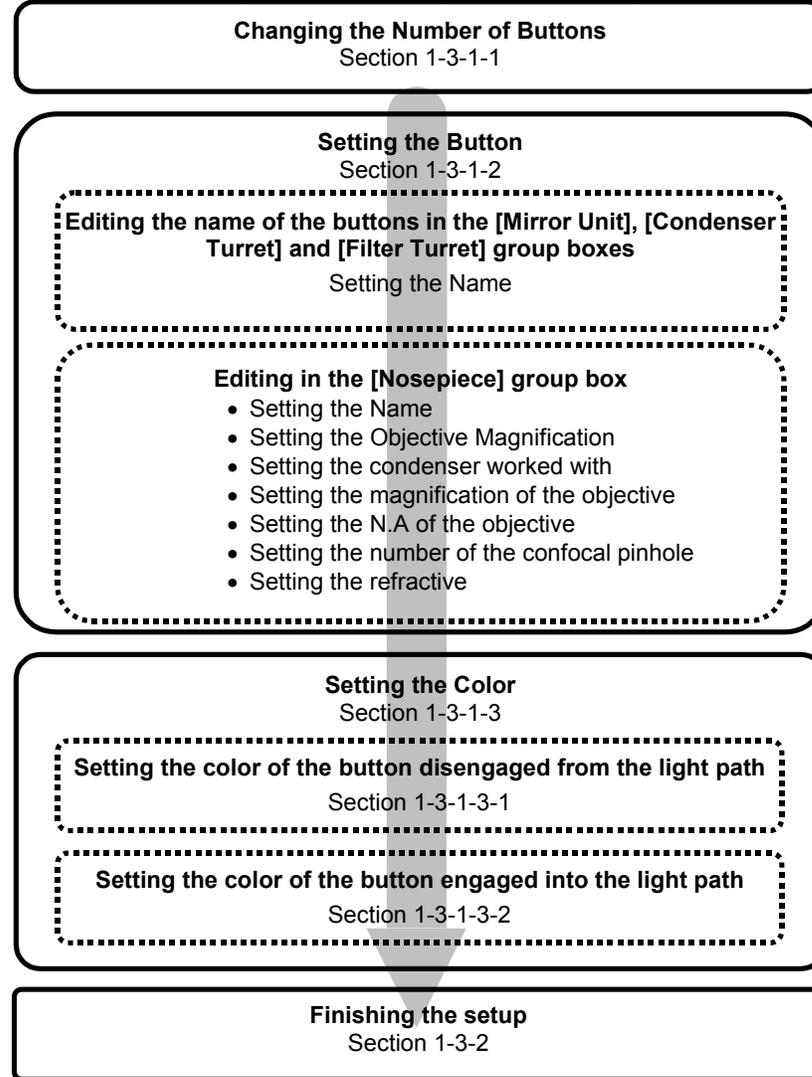
(Combination with IX)



NOTE When using IX, three parts can be motorized among the filter turrets, mirror unit, and condenser turret.

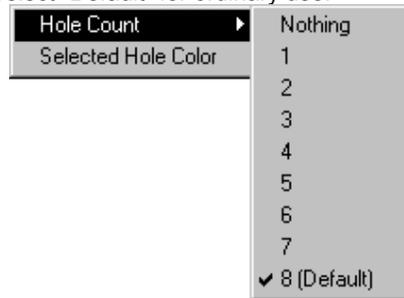


The example of setting procedure in the [Microscope Setup] window.



1-3-2-1 Setting the Number of Buttons

1. Right-click the mouse on the area outside of the buttons in the group box where the number of buttons to be displayed is changed. The pop-up menu as shown below appears. Select "Hole Count" and select the number of the buttons to be displayed in the sub-menu. Select "Default" for ordinary use.



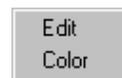
TIP

Selecting "Nothing" disables the turret itself.

1-3-2-2 Setting the Name

1 Editing the name of the buttons in the [Mirror Unit], [Condenser Turret], and [Filter Turret] group boxes

1. In the [Mirror Unit], [Condenser Turret], or [Filter Turret] group box, right-click the mouse on the button whose name is to be edited. The pop-up menu as shown below appears.



2. Select "Edit". The [Edit] dialog box as shown below appears.



3. Enter a name into the text box and click the <OK> button.

2 Editing in the [Nosepiece] group box

1. In the [Nosepiece] group box, right-click the mouse on the button whose name is to be edited. The pop-up menu as shown below appears.





2. Select "Edit". The [Edit] dialog box as shown below appears.

[Magnification] text box
Enter the magnification of the objective with keyboard.

[Recommended Confocal Pinhole] text box
Enter the number of the confocal pinhole with keyboard.

[Name of the Objective] drop-down list
Select the name of the objective in the list or enter the name with keyboard.

[Name of the Linked Condenser] drop-down list
Select the condenser worked with.

[Jog Fine Sensitivity] drop-down list
Set the amount of the fine movement of the revolving nosepiece per rotation.

[N.A.] text box
Enter N.A. of the objective with keyboard.

[n] text box
Enter refractive index with keyboard.

3. Click the <OK> button after setting is completed.

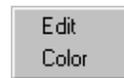


The initial values are automatically set in the [Magnification], [N.A.], [n], and [Recommended Confocal Pinhole] text boxes after the objective is selected in the [Name of the Objective] drop-down list.

1-3-2-3 Setting the Color

1 Setting the color of the button disengaged from the light path

1. Right-click the mouse on the button whose color is to be changed. The pop-up menu as shown below appears.



2. Select "Color". The [Color] dialog box as shown below appears.

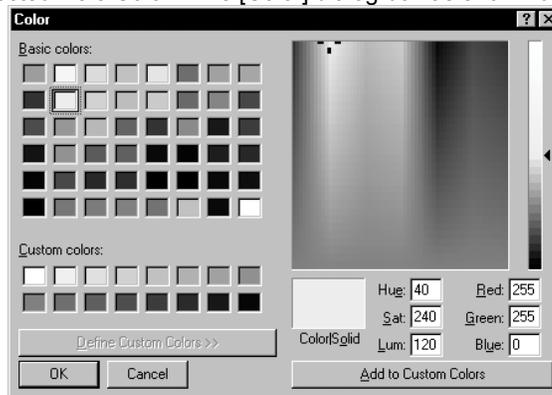
3. Select the color which you want to set in the color palette and click the <OK> button.

2 Setting the color of the button engaged into the light path

1. In [Microscope Control Panel] window on the software, specify the color of the button of the cube, the objective, and the condenser engaged into the light path. Right-click the mouse on the area outside of the buttons in the [Mirror Unit], [Nosepiece], [Condenser Turret], or [Filter Turret] group box.



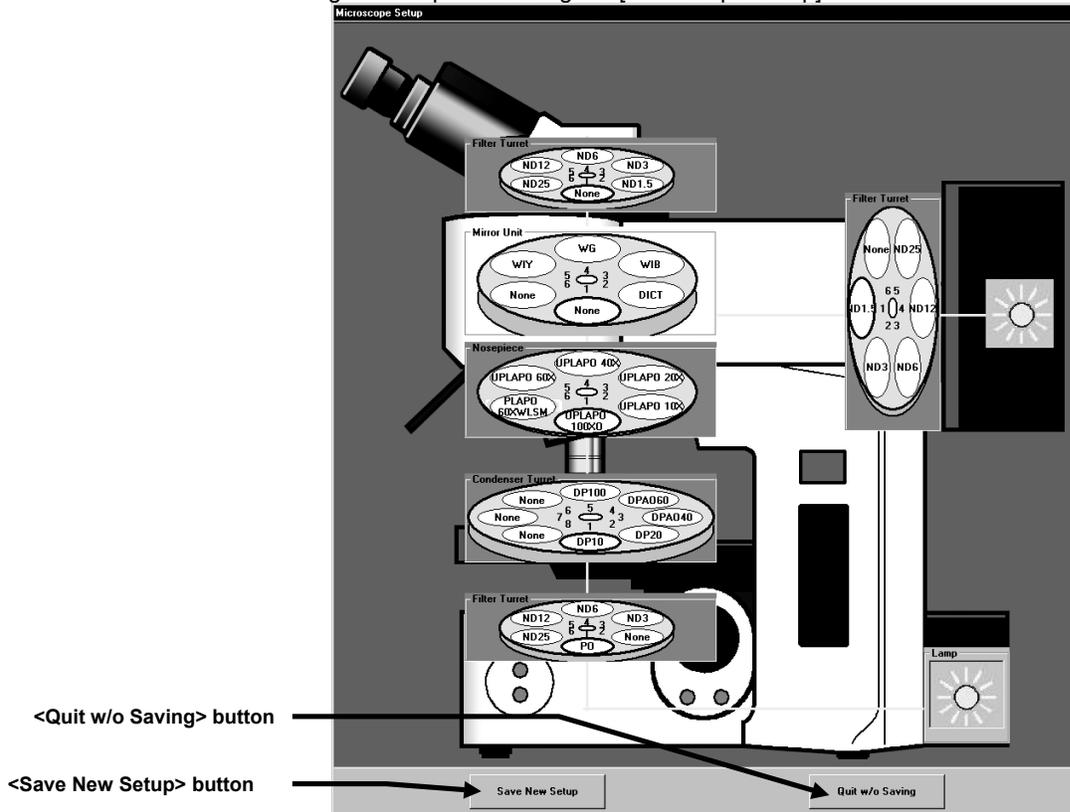
2. Select "Selected Hole Color". The [Color] dialog box as shown below appears.



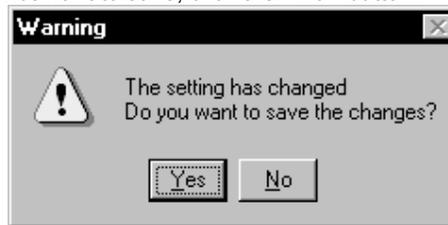
3. Select the color which you want to set in the color palette and click the <OK> button.

1-3-2-4 Finishing the setting

Finishing the setup and closing the [Microscope Setup] window.



1. Click the < Save New Setup> button to save the setup and close the window.
Or click the <Quit w/o Saving> button to cancel the setup and close the window.
Then the dialog box as shown below appears. If you want to save, click the <Yes> button, if you do not want to save, click the <No> button.



NOTE After saving is selected in the [Microscope Setting] window, the original setting is not returned even if the <Quit w/o Saving> is clicked in the [FLUOVIEW Setup] dialog box.

1-4 Adding the dyeing method

Newly add a dyeing method and set laser type, excitation wavelength, and emission wavelength.

This section describes a simple example of adding CFP as a dyeing method and setting laser type, excitation wavelength, and emission wavelength.

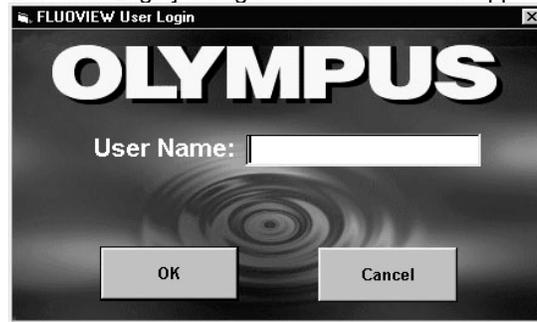
Add the dyeing method using the [Fluorescent Dyes/Colors] panel in the [Fluoview Setup] dialog box.



[FLUOVIEW Setup] icon

1. Double-click the [FLUOVIEW Setup] icon on the desktop.

The [FLUOVIEW User Login] dialog box as shown below appears.



2. Enter user name into the [User Name:] text box and click the <OK> button to log into FLUOVIEW FV500.

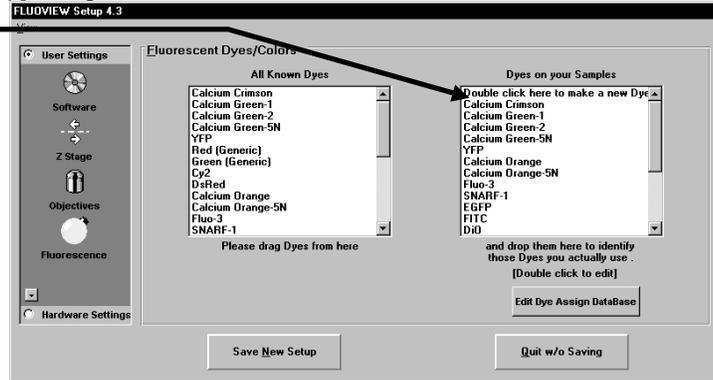


When using the system for one user, the [FLUOVIEW User Login] dialog box does not appear.



3. Display the [Fluorescent Dyes/Colors] panel at the front position in the [FLUOVIEW Setup] dialog box.

Double-click here to add the dyeing method.

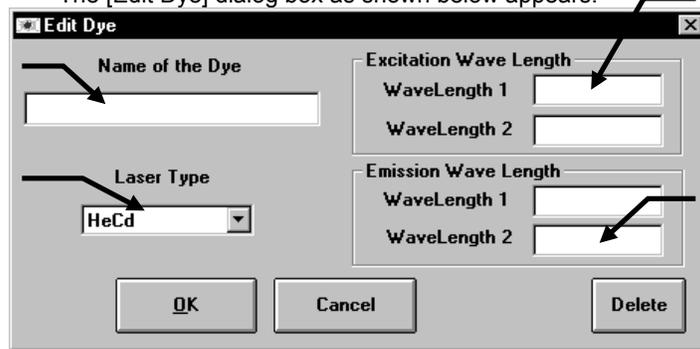


4. Double-click the “Double click here to make a new Dye” in the [Dyes on your Samples] list.

The [Edit Dye] dialog box as shown below appears.

[[Name of the Dye] text box
Enter the name of the dyeing method.

[Laser Type] drop-down list
Select the laser to use.



[Excitation Wave Length] group box

Enter the excitation wavelength. In case of the 2-wavelength and 1-photometry, enter the value into the [[WaveLength2] text box

[Emission Wave Length] group box

Enter the emission wavelength.

In case of the 1-wavelength and 2-photometry, enter the value into the [[WaveLength2] text box too.

5. Enter the name of the dyeing method into the [Name of the Dye] text box (e.g. CFP).
6. Select the laser to use using the [Laser Type] drop-down list (e.g. HeCd).



As the laser to use, helium-cadmium laser should be set in advance. When “HeCd” is not displayed, check the [HeCd] check box in the [Laser Equipment] panel.

7. Enter the value of the excitation wavelength into the [WaveLength 1] text box in the [Excitation Wave Length] group box (e.g. 442).

8. Enter the value of the emission wavelength into the [WaveLength 1] text box in the [Emission Wave Length] group box (e.g. 480).



photometry, enter the value of the wavelength into the [WaveLength 2] text box too.

9. Click the <OK> button to close the [Edit Dye] dialog box.
10. Select the <Save New Setup> button to close the [FLUOVIEW Setup] dialog box.



Some dyeing method does not appear in [Available Dyes] list box in [Dyes] subpanel.

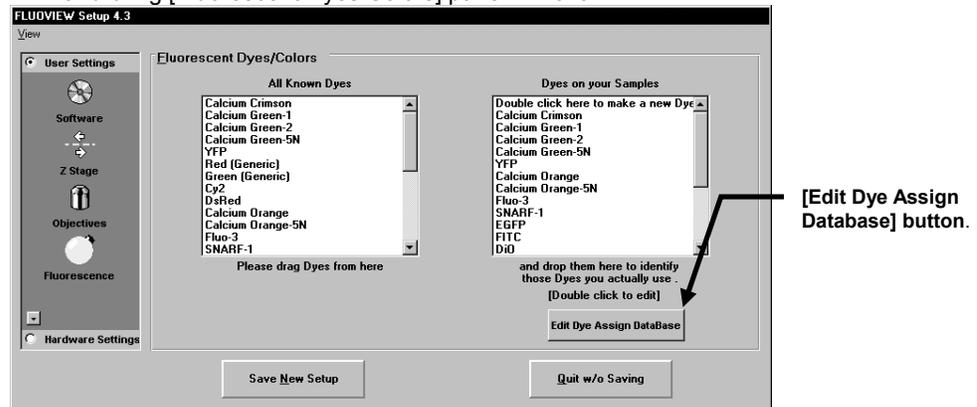
It happens when there is no relation between the dyeing method and image acquisition channel.

The relation of dye and the channel can be done by check [Assign dyes manually] check box, or see section 1-5, "Associating a Detection Channel and Filter to the Dyeing Method" for the setting.

1-4-1 Associating a Detection Channel and Filter to the Dyeing Method

The detection channel and filter can be associated to a dyeing method using the [Optical System Configuration Setup] dialog box.

1. Display [FLUOVIEW Setup] dialog. See section 1-3-1 of this manual to display [FLUOVIEW Setup] dialog.
2. Click [Fluorescence] icon located at [User Settings] in [FLUOVIEW Setup] dialog and bring [Fluorescent Dyes/Colors] panel in front.



- Click **[Edit Dye Assign Database]** button.

[Optical System Configuration Setup] dialog box appears as shown below.

[Beam Splitter] drop-down list
Select the beam splitter for each channel.
Select the blank when the selection is not required.

[Barrier Filter] drop-down list
Select the barrier filter for each channel.
Select the blank when the selection is not required.

[Excitation DM] drop-down list
Select the excitation dichroic mirror for each channel.

[Dye Select] group box
The valid dyeing methods (those for which the laser wavelengths are defined) defined in the database (Probedb.mdb) are displayed.
Clicking the <Clear Selection> button clears the dyeing method selection in the list box.

[TD Unit] group box
Displayed when the transmitted light unit is installed.
The channel No. is displayed together with "Transmitted".

[Dyes] drop-down list
Select the dyeing method detected in each channel.
Select the blank when the selection is not required.

[Laser]
Shows the lasers set for the channels.

Channel numbers.

The screenshot shows the 'Optical System Configuration Setup' dialog box. It features a 'Dye Select' list box on the left containing various dyes like Calcium Crimson, CFP, and Rhodamine-Phalloidin. A 'Clear Selection' button is below it. To the right, there are several drop-down menus: 'Excitation DM' (set to DM488/515), 'Beam Splitter' (set to Mirror), 'Barrier Filter' (set to BA480-495), 'Dye' (set to CFP), and 'Laser' (set to M_Ar1). Below these are two channel selection boxes labeled '1' and '2'. A 'TD Unit' section shows a 'Transmitted' indicator with the number '3'. At the bottom are 'Save' and 'Exit' buttons.

- In the list box in the [Dye Select] group box, select the dyeing method to be changed or created (CFP in this example). When a dyeing method is selected and if there is information associated with the selected dyeing method, the [Excitation DM] drop-down list shows the excitation dichroic mirrors, the [Beam Splitter] drop-down list shows the beam splitters, the [Barrier Filter] drop-down list shows the barrier filters, the [Dye] drop-down list shows the dyeing methods and the [Laser] group box shows the set laser types, all on the per-channel basis.

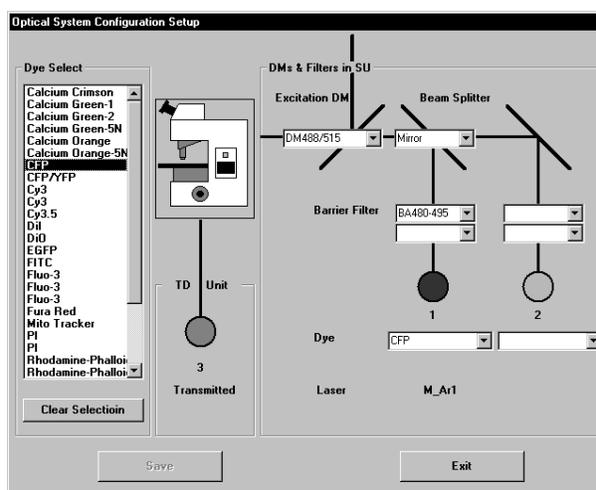


To select more than one dyeing method, select the required methods while holding the **[Ctrl]** key.



TIP Associations between dyeing methods and detection channels are saved per dyeing method combination, and applied when the combination is selected in the [Dyes] sub-panel of the FLUOVIEW software.

TIP Associations between dyeing methods and filters are saved per dyeing method combination, and displayed as setting guide on [Microscope configuration] window when the said combination is selected in the [Dyes] sub-panel of the FLUOVIEW software.



5. To change the excitation dichroic mirror, use the [Excitation DM] drop-down list. When it is not required to specify excitation dichroic mirror, select the blank. In this example, select "DM488/515".
6. To change the beam splitter, use the [Beam Splitter] drop-down list. When it is not required to specify a beam splitter mirror, select the blank. In this example, select "Mirror" for Channel 1.
7. To change the barrier filter, use the [Barrier Filter] drop-down list. When it is not required to specify a barrier filter, select the blank. In this example, select "BA480-495" for channel 1.
8. To change the dyeing method detection channel, set the [Dye] drop-down list of the previous channel to blank and select the dyeing method in the [Dye] drop-down list for the new channel. When it is not required to specify the detection channel, select



the blank.

In this example, select CFP for channel 1.

9. After completing the above, click the <Save> button to close the [Optical System Configuration Setup] dialog box.

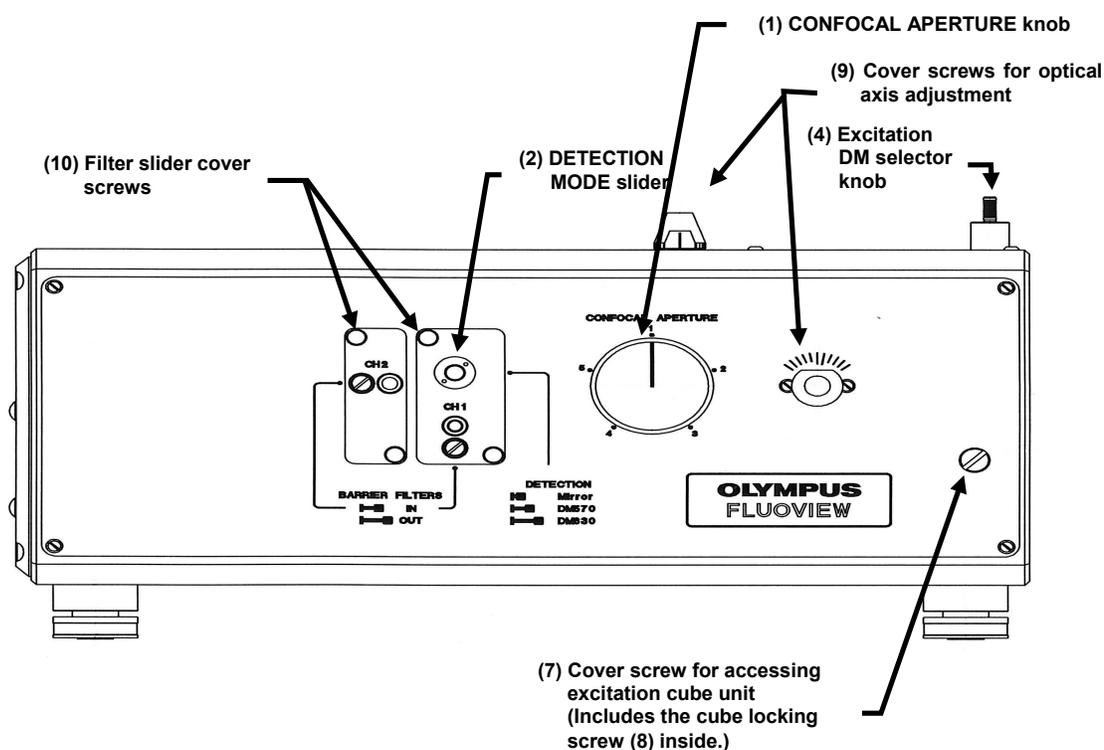
2 Maintenance of Major System Units

This section describes the maintenance of the following units.

- Scan unit
- Laser scanning microscope

2-1 Scan Unit

The scan unit used with this system has been designed to allow replacement of the BARRIER FILTER slider and DETECTION MODE slider in order to accommodate a wide range of research. This section describes procedures for replacing these parts and for adjusting the light axis after replacement.



2-1-1 Replacing the Barrier Filters Slider

To replace the BARRIER FILTERS slider, loosen the filter slider cover screws (10) and remove the slider together with the slider cover.

Then replace the BARRIER FILTER slider, fit the filter cover, and tighten the screws.

NOTE

Be sure to tighten the screws firmly, or noise may interfere with images.

2-2 Laser Scanning Microscope

According to IEC60825 "Safety of Laser Products" and EN60825, this product is classified as a CLASS 3B laser product. However, as it belongs to "CLASS 3B with a laser wavelength range from 400 to 700 nm, which is no more than 5 times the AEL of CLASS 2", all of the microscope modules listed below can be attached or removed as a part of their maintenance activities. Nevertheless, do not attach or remove any of the following units before turning all the laser units, or the dangerous laser come out.

For details, refer to the instruction manual of your microscope.

The other hand, in the United States of America, this product is classified as a CLASS IIIa laser product according to the CDRH laser safety regulation. Attachment or removal of the microscope modules listed below is not permitted as a part of user maintenance activities. Such attachment and removal should be performed as a part of service activities.

For detailed information, refer to our service representatives.

- 1) Transmitted lamp housing
- 2) Transmitted light fiber cable
- 3) Cube turret of reflected light fluorescence unit
- 4) Objective revolving nosepiece
- 5) Objectives
- 6) Condenser
- 7) Differential interference slider
- 8) Excitation Cube (Inside the scan unit)
- 9) Detection mode slider

The definitions of the term “maintenance” by EN60825 and CDRH 21CFR are quoted below for reference.

IEC60825(EN60825)

Maintenance:

The performance of those adjustments or procedures specified in user information provided by the manufacturer with the laser product, which are to be performed by the user for the purpose of assuring the intended performance of the product. It does not include operation or service.

The definitions of the term “maintenance” are quoted below for reference.

CDRH

“Maintenance” means performance of those adjustments or procedures specified in user information provided by the manufacturer with the laser product which are to be performed by the user for the purpose of assuring the intended performance of the product. It does not include operation or service as defined in paragraph (b) (27) and (38) of this section.

VI. TROUBLESHOOTING

On This Volume

This volume describes the treatment against possible troubles.

In case of trouble, please read volume before calling for service. If the normal operation cannot still be restored, please contact your local Olympus representative.

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1 TROUBLESHOOTING GUIDE

Under certain conditions, performance of this system may be adversely affected by factors other than defects. If a problem occurs, please review the following list and take remedial action as needed.

If you cannot solve the problem after checking the entire list, please contact your local Olympus representative for assistance.

Before contacting Olympus, please fill the "Inquiry Table" at the end of this volume and inform Olympus of its contents.

Phenomenon	Cause	Treatment	Manual Ref. Pages
1. Fluorescence image cannot be observed.	Laser is not oscillating.	Turn the laser unit ON. Ensure that the emission key is set to the ON position.	1-2-1, OPERATION INSTRUCTIONS
	The light path selector knob of the microscope (or light path selector button on an inverted microscope) is not set to the LSM light path.	Set the light path selector to the LSM light path.	1-1-2 & 1-2-4, OPERATION INSTRUCTIONS
	An excitation tube or analyzer for visual observation is engaged in the microscope.	Disengage the excitation tube and analyzer from the light path.	1-2-4, OPERATION INSTRUCTIONS
	The objective is not engaged in the light path.	Stop the objective at a click position.	Instruction manual of microscope
	The light path is blocked by the confocal aperture knob.	Stop the confocal aperture knob at a click position.	1-1-1, 1-2-6, OPERATION INSTRUCTIONS
	The confocal aperture number is small.	Increase the confocal aperture.	1-1-1, OPERATION INSTRUCTIONS
	The light path is blocked by barrier filters.	Push the barrier filters into the specified position.	1-1-1 & 1-2-8, OPERATION INSTRUCTIONS
	The light path is blocked by the light path detection switch.	Stop the light path detection switch at a click position.	1-1-1 & 1-2-7, OPERATION INSTRUCTIONS
	The fluorescent dye and excitation wavelength does not match.	Select a laser suitable for the fluorescent dye method. See 2-2-1-2, "Configuring the Scan Unit" in OPERATION INSTRUCTIONS and follow instructions of the [Microscope Configuration] window.	1-3-2 & 2-2-1-2, OPERATION INSTRUCTIONS

TROUBLESHOOTING GUIDE



Phenomenon	Cause	Treatment	Manual Ref. Pages
1. Fluorescence image cannot be observed	The fluorescent dye does not match the barrier filter.	Select the barrier filter matching the fluorescent dye. See 2-2-1-2, "Configuring the Scan Unit" in OPERATION INSTRUCTIONS and follow instructions of the [Microscope Configuration] window.	2-2-2-1, OPERATION INSTRUCTIONS 1-2-8 & 1-3-2, OPERATION INSTRUCTIONS
	Focusing is not correct.	Adjust focusing.	1-2-3, OPERATION INSTRUCTIONS
	The photomultiplier tube voltage of the detector is low.	Increase the photomultiplier tube voltage using the [PMT] scale in the [Acquire] panel.	2-2-1-3-9, OPERATION INSTRUCTIONS
	The OFFSET value is too high.	Decrease OFFSET to an optimum value.	2-2-1-3-9, OPERATION INSTRUCTIONS
	The photomultiplier tube of the channel set with the detection mode setting slider is not set.	Check the [Ch1], [Ch2] or [Ch3] check box in the [Acquire] panel.	1-1-1 & 2-2-1-3-3, OPERATION INSTRUCTIONS
2. Transmitted image cannot be observed.	The transmittance detection channel is not selected.	In the [Acquire] panel, check the check box of the [Ch] with which "Transmitted" is displayed.	2-2-3-3, OPERATION INSTRUCTIONS
	The transmitted light filter is engaged in the light path of the microscope.	Disengage the filter from the light path.	1-1-2 & 1-2-4, OPERATION INSTRUCTIONS
3. Image is disturbed.	The installation location is subject to large vibrations.	Consult Olympus.	-
	External light from a fluorescent lamp, etc. is detected.	Darken the room before acquiring image.	-
		Ensure that the top cover of the scan unit is closed tightly.	1-1-1, OPERATION INSTRUCTIONS
4. Image is striped.	The laser reflection light is penetrating.	The barrier filter selection is erroneous.	1-2-8, OPERATION INSTRUCTIONS
5. Image looks poor.	The analyzer is engaged in the light path.	Disengage the analyzer from the light path.	1-2-3 & 1-2-4, OPERATION INSTRUCTIONS
	The scan speed is high.	Decrease the scan speed to an optimum speed or integrate the image.	2-2-1-3-10 & 2-2-1-5, OPERATION INSTRUCTIONS

Phenomenon	Cause	Treatment	Manual Ref. Pages
5. Image looks poor.	The fluorescent dye does not match the barrier filter.	Select the barrier filter matching the fluorescent dye. See 2-2-1-2, "Configuring the Scan Unit" in OPERATION INSTRUCTIONS and follow instructions of the [Microscope Configuration] window.	2-2-1-2, OPERATION INSTRUCTIONS 1-2-8, 1-2-7 & 1-3-2, OPERATION INSTRUCTIONS
	The objective is stained.	Wipe lightly with a piece of gauze. When dirt cannot be taken, moisten the gauze with a slight amount of 3:7 alcohol:ether mixture or benzene.	-
	The installation location is subject to large vibrations.	Consult Olympus.	-
6. Image is irregularly blurred or the brightness is uneven.	The specimen is tilted.	Set the specimen horizontally.	Instruction manual of microscope
7. Image is flared.	Non-fluorescent glass is not used.	Use non-fluorescent glass.	-
	The cover glass thickness is not optimum.	Use a cover glass with a thickness of 0.17 mm.	-
	The specimen is over-stained.	Stain it properly again or increase the OFFSET value.	2-2-1-3-9, OPERATION INSTRUCTIONS
8. Circular flare is observed at the center of image.	The excitation wavelength does not match the barrier filter type.	Select suitable barrier filter for the excitation length.	2-2-1-2, OPERATION INSTRUCTIONS 1-2-8, OPERATION INSTRUCTIONS
	The barrier filter is not engaged.	See 2-2-1-2, "Configuring the Scan Unit" in OPERATION INSTRUCTIONS and follow instructions of the [Microscope Configuration] window.	1-3-2 & 2-2-1-2, OPERATION INSTRUCTIONS
9. Image is blurred or out of focus.	The focusing is not correct.	Adjust focusing with visual observation.	Instruction manual of microscope
	The correct confocal aperture knob is not selected.	Select the correct confocal aperture knob.	1-2-6, OPERATION INSTRUCTIONS
10. Image is dark and noisy.	The fluorescent dye is pale.	Apply optimum fluorescent dye.	-

TROUBLESHOOTING GUIDE



Phenomenon	Cause	Treatment	Manual Ref. Pages
10. Image is dark and noisy.	The confocal aperture is too small.	Adjust the confocal aperture to an optimum size.	1-2-6, OPERATION INSTRUCTIONS
	The HV of photomultiplier tube exceeds 800.	Set HV at no more than 800. If the image is still dark, adjust GAIN.	2-2-1-3-9, OPERATION INSTRUCTIONS
	The scan speed is too high.	Decrease the scan speed or integrate the image.	2-2-1-3-10 & 2-2-1-5, OPERATION INSTRUCTIONS
11. The position reproduction of the Z-motor is poor.	The Z-position fluctuates because the coarse movement knob of microscope is too light.	Make the microscope's coarse movement knob heavier by turning the microscope's rotation tension adjustment ring.	Instruction manual of microscope
12. The [Acquire] panel cannot be displayed.	The power unit is not recognized.	Check the connection between the computer and power unit. Turn the power unit ON.	1-2-1, OPERATION INSTRUCTIONS
13. The scale of the [Z Stage] sub-panel in the [Acquire] panel cannot be moved.	The Z-stage (Z revolving nosepiece) motor is not excited.	Check the [Locked] check box in the [Z Stage] sub-panel.	2-2-1-3-7, OPERATION INSTRUCTIONS
14. The fine movement knob of microscope cannot be turned or is not smooth.	The Z-stage (Z revolving nosepiece) motor is excited.	Clear the [Locked] check box in the [Z Stage] sub-panel.	2-2-1-3-7, OPERATION INSTRUCTIONS
15. The image cannot be saved in the disk.	The disk drive is not ON.	Ensure that the MO disk drive is ON	Instruction manual of MO disk
	The MO disk is not recognized.	Check the cable connection.	Instruction manual of MO disk
	The disk is not loaded in the drive.	Insert the disk properly.	Instruction manual of MO disk
	The disk is write-protected.	Release the write protection.	Instruction manual of MO disk
	The disk is not formatted. (either floppy disk or MO disk).	Format the disk before use.	Appendix D, OPERATION INSTRUCTIONS
	The available space in disk is insufficient.	Increase the available space in disk by deleting unnecessary files or use another disk (be sure to format a new disk).	- Appendix D, OPERATION INSTRUCTIONS
16. The image cannot be output at the printer.	The printer is not recognized.	Ensure that the printer is ON.	Instruction manual of printer
	The printer is not recognized.	Check the cable connection.	Instruction manual of printer
	The printer is out of paper.	Supply paper.	Instruction manual of printer

Phenomenon	Cause	Treatment	Manual Ref. Pages
17. FLUOVIEW software cannot be started.	The FLUOVIEW software has already been started.	Press Alt + Tab on the keyboard to switch to FLUOVIEW.	
	Another application is running.	Exit from the running software before starting the FLUOVIEW software.	1-2-2, OPERATION INSTRUCTIONS
18. The mouse pointer in the screen cannot be moved by moving the mouse.	The software is malfunctioning.	Press Ctrl + Alt + Delete on the keyboard and exit from FLUOVIEW by following the displayed messages. Then exit from windows and reboot the computer.	1-2-1 & 1-2-2, OPERATION INSTRUCTIONS
19. The power supply of Power Unit(FV5-PSU) is not turned on.	The fuse has run out.	Contact your local Olympus representative for assistance then replace the fuse.	-
20. Bios some check error message appears at the moment of starting up the computer.	The battery maintains Bios set value has run out.	Contact your local Olympus representative for assistance to replace the battery.	-
	Bios has broken.	Consult Olympus.	-

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