

Time Lapse Imaging System BioStation IMQ CELL-S2 / CELL-S2-P Instructions

<Application Software>

Introduction

Thank you for purchasing a Nikon product.

This instruction manual is written for the users of the application software of the Time Lapse Imaging System BioStation IM/IMQ.

To ensure correct usage, read this manual carefully before operating the product.

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- Although every effort has been made to ensure the accuracy of this manual, errors or inconsistencies may remain. If you note any points that are unclear or incorrect, please contact your nearest Nikon representative.
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- If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.
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Notes on Handling the Software (Read the following before use.)

To ensure correct and safe operation, read this manual before using the product.

1. Disclaimer

Nikon shall not be liable for any damages and problems on user side or on a third-party side, which may result from the use of this software.

2. Notes on the control PC

Factory Setting

The software comes with a control PC. Do not use any other PC to operate the product. A change to the OS environment factory setting may lead to incorrect operation. The control PC must not be modified by the user.

Power-saving mode

Use of the control PC with power-saving mode turned on causes the time lapse to stop halfway. Be sure to set power-saving mode to OFF (factory setting) in using the PC.

Anti-virus software

Set real-time scanning of anti-virus software to OFF. Real-time scanning enabled may cause this software to run unstably.

3. File compatibility

Files created by the software of Ver. 1.* to Ver. 2.0 can be read into the software of Ver. 2.1 or later, whereas those created by the software of Ver. 2.1 or later cannot be read into the software of Ver. 1.*.

Additionally, if a file created by the software of Ver. 1.* to Ver. 2.0 is read into the software of Ver. 2.1 or later, it is automatically upgraded to the file format of the version currently used. Once upgraded, it cannot be read into the software of the version by which it was originally created.

4. About the example screens used in the manual

- This manual describes various operations in BioStation IM/IMQ (Referred to as the BioStation IM series together, hereafter.) using BioStation IMQ screens as examples. Procedures are virtually identical for BioStation IM series.
- This manual describes various operations in Windows XP/Vista/7 using Windows XP screens as examples. Procedures are virtually identical for Windows XP, Vista, and 7.
 Depending on the specific OS type or version, the actual appearance of the screen or operations may not correspond precisely to the example screens shown at various points throughout the manual. For information on operations or screens specific to your OS version, please consult the OS user's manual.

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This chapter describes the basic operations commonly used for starting and closing and all windows of the "BioStation IM"

1.1 Starting and Closing the Software

This section describes how to start and close this software installed in the control PC.

1.1.1 Starting the Software

Starting the software from the shortcut icon on the desktop

1. Double-click the Bio Station IM shortcut icon on the desktop of the control PC.

The BioStation IM starts up.



Figure 1.1-1 Shortcut icon of Bio Station IM

Starting the software from the start menu

1. Open the Start menu, and then click Bio Station IM.

The Bio Station IM starts up.



Figure 1.1-2 Start menu

For the BS-IM-C chamber, the Live observation screen appears first when this software starts.

For the BS-IM-MC MOT chamber with the AF (Auto focusing) mode enabled, the Reference mark register screen appears first. For registering the AF position of the reference mark on this screen, see Section 1.1.2, "Registering AF Position of the Reference Mark (BS-IM-MC MOT Chamber)".



Figure 1.1-3 Live observation screen

1.1.2 Registering AF Position of the Reference Mark (BS-IM-MC MOT Chamber)

When using the BS-IM-MC MOT chamber with the AF mode enabled, register the AF position of the reference mark as described below.

If the AF position of the reference mark is not registered, the New time-lapse setting screen cannot be displayed. To carry out time-lapse observation on the BS-IM-MC MOT chamber, be sure to register the AF position of the reference mark.

1. Check that the reference mark is displayed on the screen after the software is started, and click the Mark registration button.

To make observations with greater AF accuracy, check that the "Stable" is indicated on the screen display on the software.

When the Mark registration button is clicked, the AF positions of the reference marks for wells 1 to 4 are registered in order. This takes about four minutes.

When registration is complete, an image of well 1 is displayed on the Live observation screen.

When you carry out only live observation, click the Exit button; the screen is switched to the Live observation screen.

If the reference mark is not placed within the blue frame at the center of the screen, click the Mark registration button to register the AF positions of the reference marks.

For details see Section 7.4, "Setting up the BS-IM-MC MOT chamber", in the separate manual, "BioStation IMQ Instructions <System>".



Figure 1.1-4 Reference mark register screen

1.1.3 Closing the Software

1. Click the Close button (🔀) in upper right corner of the window.

This software closes, and the Windows desktop appears.

Perform this procedure when you detach the chamber.

For the BS-IM-MC MOT chamber, this procedure triggers the dish mounting part take the position in readiness to sustain dismounting of the chamber.

Close confirmation dialog box

If the Close button is clicked while captured images remain unsaved, the Close confirmation dialog box appears.

To save the captured image, click the No button to return to the Bio Station IM screen.

To close the software without saving the captured image, click the Yes button.



1.2 Common Function

The items shown on the top screen of the software are common functions for all screens.



Figure 1.2-1 Live observation screen

1.2.1 Screen Switch Buttons

Click these buttons to select each function of the BioStation IM series.

BioStation IM (Version:2.20 Build:140)						
Live observation	New	Time-lapse images	Time-lapse images			
	time-lapse setting	in process	Acquired			

Figure 1.2-2 Screen switch buttons

Table 1.2-1 Functions of screen switch button	s
---	---

Item	Function		
Live observation	Select to use the BioStation IM series as a microscope.		
New time-lapse setting	Select to perform time-lapse experiment. Select to start time-lapse experiment, after setting observation point, observation condition, and so on.		
Time-lapse image in process	The time-lapse experiment in progress is displayed. This screen automatically appears after time-lapse experiment starts on the New time-lapse setting screen. This screen cannot be displayed by operating any buttons.		
Time-lapse images Acquired	Select to playback performed time-lapse images. This screen automatically appears after time-lapse experiment ends. On this screen, loading and reproducing saved file of time-lapse experiment results are available.		

1.2.2 Status Display and Settings of the Microscope

This area shows the current temperatures and the temperature settings of the culture chamber and the humidifier water tank. The outside air temperature is also shown here.

For the temperature settings of the culture chamber and the humidifier tank, see Step 3 in Section 2.1, "Start-up", in the separate manual, "BioStation IMQ Instructions <System>".

Temp Chamber	37.0 37.0 🕂	Water 37.0 37.0	34.0 37.0 🕂
Outside	24.0 (unit:deg C) Stable	Unstable

Figure 1.2-3 Temperature status display of the microscope

Table 1 2-2	Functions on the status display and settings of the microscope
	i unctions on the status display and settings of the microscope

Item	Function			
Temp	since the softwa	Click the Temp button to display the Temperature dialog box. The temperature change since the software is started up and temperature status during time-lapse experiment can be checked.		
Chamber	Display area	Temperature detected with the temperature sensor of the culture chamber is displayed.		
	Setting area	Set temperature inside the culture chamber to be suitable for time-lapse experiment.		
Water	Display area	Temperature detected with the temperature sensor of the humidifier water tank is displayed.		
	Setting area	Set water temperature inside the humidifier water tank to be suitable for time-lapse experiment.		
Outside	Current ambient temperature is displayed. If room temperature is below 18°C or above 28°C a warning symbol appears and "POWER" lamp of the LED indicator blinks rapidly.			
Stable/ Unstable	the humidifier w from "Unstable"	When both the temperature inside the culture chamber and the water temperature inside the humidifier water tank reach the set value and stabilize, the status display changes from "Unstable" to "Stable". And, time-lapse experiment becomes possible. Also, the "STABLE" lamp of the LED indicator on the microscope is lit.		

Displaying the logarithmic graph of temperature change

Click the Temp button to display the Temperature dialog box.



Figure 1.2-4 Temperature dialog box

 Table 1.2-3
 Functions on the Temperature dialog box

ltem	Function
Range Select time range (horizontal axis) of temperature graph.	
Temperature graph	Temperature changes inside of the culture chamber and the humidifier water tank, and that of ambient temperature are shown.
Close button	Select to close the Temperature dialog box.

1.3 Context Menu on the Title Bar

Right click on the title bar of this software window displays the context menu.



Figure 1.3-1 Context menu on the title bar

Table 1.3-1	Functions of context menu

ltem	Function		
About Bio Station IM	The About BioStation IM dialog box is displayed. The dialog box showing the version information of this software appears.		
Device information	The Device information dialog box is displayed. The Device information dialog box allows you to check information about the microscope and camera connected to the control PC.		
Error information	The Error information dialog box is displayed. The item is enabled when an error occurs, and the Error information dialog box allows you to check the cause of the error.		
Preferences	The Preferences dialog box is displayed. The dialog box allows you to configure basic settings appropriate for the environment.		
Hide control panel	Only the left side of the software window remains displayed.		

1.3.1 Checking the Software Versions

Click the About Bio Station IM... menu to display the About BioStation IM dialog box. This dialog box allows you to check the version of the software.



Figure 1.3-2 About BioStation IM dialog box

1.3.2 Checking the Device Information

Click the Device information... menu to display the Device information dialog box.

This dialog box allows you to check information about the microscope and camera connected to the control PC.

The screen illustrated below shows the case for using the BS-IM-MC MOT chamber.

Device info	rmation		×
Microscope	Status	Main Controller Ergo Controller Fiber Illuminations Chamber	Connected Not connected Connected BS-IM-MC MOT
		Chamber Cover Filter Cover	Close Close
	Version	Main Controller Temperature Controller Fiber Illuminations	VDEMO VDEMO VDEMO
	Error	None	
Camera	Status	Main Controller Controller Camera Head	Connected DS-U2 DS-Qi1 Mc
	Version	Main Controller	001.006.1700.000DEMO

Figure 1.3-3 Device information dialog box

1.3.3 Checking the Error Information

Click the Error information... menu to display the Error information dialog box.

The item whose checkbox is checked is the cause of the error.

If there is no error, the Error information... menu is disabled thus this dialog box cannot be displayed.

Difference of the error detection scope by the microscope in use

When the BioStation IM is used as a microscope, only the temperature sensor status can be detected. Only the checkbox of "Temperature controller communication error" is displayed on the Error information dialog box.

Error	information 🛛 🔀
Erro	r (Status=0x01)
$\overline{\mathbf{V}}$	Initialize error
Г	Temperature controller communication error
	Internal shutter error
_ Initia	alize error (Status=0x01)
$\overline{\mathbf{v}}$	X motor error
Г	Y motor error
Г	Z motor error
	Objective motor error
Г	Filter cassette motor error
Г	Internal shutter error
Run	ning error (Status=0x00)
	FAN motor error
Г	Heater error
Г	Temperature control error
	I2C bus error
	Mechanical block sensor error



1.3.4 **Basic Settings**

X Preferences Preferences X -General -General-☑ Data input when capture image saved $\overline{\boldsymbol{\checkmark}}$ Data input when capture image saved ✓ Explorer launches when image capture Explorer launches when image capture Explorer launches when file export Explorer launches when file export Viewing --Viewing-Exposure Time C msec Fractional O msec 🛛 💿 Fractional Exposure Time EPI Lamp Transmission rate C Position EPI Lamp C Transmission rate 🛛 💿 Position BS-IM-MC MOT chamber Dish-▼ Enable AF Mode 🔿 Normal 🛛 💿 Hi-Q4 Туре ☑ Launch as Mark window mode ΟK Cancel ОK Cancel Figure 1.3-5 Preferences dialog box Figure 1.3-6 Preferences dialog box

(BS-IM-C chamber)

Click the Preferences... menu to display the Preferences dialog box.

Table 1.3-2 Functions of Preferences dialog boxes (1/2)

(BS-IM-MC MOT chamber)

Item		Function		
General	Data input when capture image saved	Select to save captured images together with information such as observation conditions.		
	Explorer launches when image capture	Select to display the Explorer after captured images are saved.		
	Explorer launches when file export	Select to display the Explorer after images are exported in the general format.		
Viewing	Exposure Time	Select the unit of exposure time displayed on the software (msec/Fractional (Fractional representation in seconds)).		
		(Only the Fractional representation is available when BioStation IM is used.) \vec{Period} \vec{Period}		

	ltem	Function		
Viewing	EPI Lamp	Select the unit of the light intensity of the EPI lamp displayed on the software (Transmission rate/Position representation). (Only the Position representation is available when BioStation IM is used.) Load settings EPI Lamp 25x 4 Figure 1.3-9 Transmission rate representation Figure 1.3-10 Position representation		
Dish	Туре	Select the dish type (Normal/Hi-Q4) used for observation. Selecting Hi-Q4 shows the fields to input and edit the names of the compartments of the film bottom 4-quadrant dish on the Cell name etc tab.		
BS-IM- MC MOT chamber	Enable AF Mode	Select to enable the AF mode and display the Reference mark register screen. Auto-focusing is carried out whenever you switch between the wells on the New time-lapse setting screen.		
	Launch as Mark window mode	Select to display the Reference mark register screen immediately after the software is started. If this is not selected, the Live observation screen appears instead.		

1.3.5 Hide Control Panel

Click the Hide control panel menu to display only the image view on the left side of the window.



Figure 1.3-12 Hide control panel



When the BioStation IM/IMQ is used as a microscope, select this screen.

You can check the specimen is suitable or not for time-lapse experiment by observing cell shape and the amount of fluorophore in cell.



Figure 2.0-1 Live observation screen

Table 2.0-1 Functions on the Live observation screen
--

	ltem		Function	
(1)	Live observation image display		bservation image of the field of view is displayed. Click in this display e stage until the clicked point locates at the center of this display.	
(2)	Observation point display	The field of view is indicated by the red frame and displayed as the live observation image. Click the desired point to move the stage and to display the live image of the clicked point.		
(3)	Image adjustment buttons	Select to set tone adjustment, saturation check, intensity screening, and pseudo color display.		
(4)	Capture button	Select to capture the live observation image and register it to the Captured image display (5).		
(5)	Captured image display	Display area	The images captured with the Capture button are thumbnailed. Any thumbnail image can be enlarged and displayed on another window by clicking on the enlarge button (magnifier icon).	
		Function	To save the captured images, highlight the thumbnail image and click the Image save button (). All highlighted images are saved at the same time. Click the Trash button to delete the selected thumbnail images.	
(6)	Observation condition and observation point	Adjust the stage position, the focusing condition, and the observation conditions such as filters, magnification, light intensity, and exposure time.		

2.1 Image Adjustment Buttons and Captured Image Display



Figure 2.1-1 Image adjustment buttons and captured image display

Table 2 1-1	Functions of the image adjustment buttons and contured image display $(1/2)$
1 able 2.1-1	Functions of the image adjustment buttons and captured image display (1/2)

Item	Function
Automatic tone curve adjustment apply button	Select to automatically set the range. Display range of intensity is automatically set.
	The highest intensity (A) of the displayed image is set as the upper limit (C) and the lowest intensity (B) is set as the lower limit (D). Figure 2.1-2 Intensity graph
Tone curve adjustment setting button	Select to set tone curve adjustment manually. The Tone curve adjustment dialog box appears to set tone curve adjustment.
Tone curve adjustment cancel button	Select to cancel the applied tone curve adjustment and return to the original screen.
Saturation check button (FL1 & FL2 only)	Select to display saturation point in red. And then, adjust the intensity and gain until there is no saturation point. To clear the saturation check condition, clicked this button again. This function can be used only for the fluorescent microscopy.
Intensity screening button (FL1 & FL2 only)	Select to display the Intensity Screening dialog box for setting intensity screening. This function can be used only for the fluorescent microscopy.
Pseudo color button	Select to display the live observation image with the pseudo color of the filter. This function can be used only for the fluorescent microscopy.
Capture button	Select to capture the live observation image and register it to the Captured image display. If intensity screening and image adjustment are performed, the performed image is registered. With the single image capture switch of the ergonomic controller, this operation can be performed.
Image overlay	Select to overlay more than one thumbnail image selected.
button	Select more than one thumbnail image on the Captured image display (5) and click this button to add the overlaid image to the Captured image display (5).
	The Image overlay button is enabled only when the XY coordinates of selected thumbnails match and different filters are used.

ltem	Function		
Trash button	Select to delete only selected thumbnail image. The frame of selected thumbnail image is displayed in blue.		
Image save button	Select to save all thumbnail images in a file. Thumbnail image must be highlighted prior to saving. Select save format from TIFF, JPEG, BMP, or PNG. For TIFF format, image resolution is selectable between 16 bits and 8 bits.		
Captured image display	The images captured with the Capture button are thumbnailed. Click the Enlarge button (magnifier icon) at the bottom right of thumbnail image to display the enlarged image on other screen. If number of thumbnail images becomes five or more, a scroll bar appears at the bottom of thumbnail display.		
Gallery button	Select to display the Captured Image Gallery window and to show the thumbnailed images captured with the Capture button in a list.		
Reference mark button	This button is displayed only for the BS-IM-MC MOT chamber. Select to display the Reference mark register screen to register the AF position of the reference mark.		

2.1.1 Captured Image List View

Click the Gallery button (📄) to display the Captured Image Gallery window and to show the thumbnailed images captured with the Capture button in a list.



Figure 2.1-3 Captured Image Gallery window

ltem	Function		
Enlarge button	0	Select to display the enlarged image on other screen.	
Image save button		Select to save all thumbnail images selected in a file. The thumbnail images must be highlighted prior to saving. Select the save format from TIFF, JPEG, BMP, or PNG. For TIFF format, image resolution is selectable between 16 bits and 8 bits.	
Image overlay		Select to overlay more than one thumbnail image selected.	
button		Select more than one thumbnail image on the Captured image display (5) and click this button to add the overlaid image to the Captured image display (5).	
		The Image overlay button is enabled only when the XY coordinates of selected thumbnails match and different filters are used.	
Trash button		Select to delete only selected thumbnail image. The frame of selected thumbnail image is displayed in blue.	
Close button	×	Select to close the Captured Image Gallery window.	
Right-click menu	Right click the thumbnailed image to display the menu. In the menu, saving and deleting the clicked images or selected images are available.		

2.1.2 Tone Curve Adjustment

Click the Tone curve adjustment setting button (📄) to display the Tone curve adjustment dialog box.



Figure 2.1-4 Tone curve adjustment dialog box

Table 2.1-3 Tone curve adjustment functions

ltem	Function
Enable tone curve checkbox	Select to enable the tone curve adjustment.
Apply button	Select to apply the set tone curve adjustment setting to the Live observation image display.
Close button	Select to close the Tone curve adjustment dialog box without applying the tone curve adjustment setting.

2.1.3 Checking Saturation

Click the Saturation check button () to display saturation point of the live observation image in red. Adjust the intensity and gain until there is no saturation point.



Figure 2.1-5 Displaying saturation point

2.1.4 Intensity Screening

Image select box

Click the Intensity screening button (🔟) to display the Intensity Screening dialog box.

Figure 2.1-6 Intensity screening dialog box

Table 2.1-4	Intensity screening functions
-------------	-------------------------------

ltem	Function
Enable Screening checkbox	Select to enable the intensity screening functions.
Image select box (yellow box)	Drag a marquee (left click and drag) around the area for intensity screening. Enlarge, reduce, or move the area with a mouse pointer if necessary. Right click the box to display the Delete menu to delete the box.
Image display	Screening points (same intensity points) are displayed in blue.
Histogram	Intensity histogram of the image is displayed. To adjust the intensity range, drag the ▼ and ▲ buttons from side to side.
Apply button	Select to apply setting of intensity screening to the Live observation image display.
Close button	Select to close the Intensity Screening dialog box.

2.1.5 Pseudo Color Display

Click the Pseudo color button () to display the live observation image with the pseudo color of the fluorescent filter. You can capture the live observation image displayed with the pseudo color and save it into a file.



Figure 2.1-7 Pseudo color display

2.2 Setting Observation Conditions (Filter, Magnification, Mode, Z position, and Save)

Some observation condition settings vary between the phase contrast microscopy and the fluorescent microscopy.

Also, there are the Manual mode and the Simple mode for the observation condition. Both modes are available for settings in this section.



Figure 2.2-1 Setting observation conditions (for fluorescent filter; Left: Manual mode, right: Simple mode)

Item		Function
Filter button		for observation. The blue frame appears on the selected filter button. s also available with the Observation method switches of the ergonomic
	Ph	Select to perform phase contrast microscopy with the diascopic illumination of the red LED illuminator built in the microscope. Fluorescent filter is not used.
	FI1 FI2	Select to perform the fluorescent microscopy using the selected filter with the illumination of external mercury lamp. This button does not appear unless the HG precentered fiber illuminator is connected and turned the power on.
Magnification button	20× 414x311um 207x156um 104x78um	Select the magnification for observation. This operation is also available with the Magnification adjustment selector switch (UP/DOWN) of the ergonomic controller.
Simple button		Select the Simple mode. Set the observation condition selecting from the following three given exposure conditions:
	Simple	 Bright: to brighten dark images Middle: to observe half-bright images Dark: to darken too bright images
		Set a condition for each combination of filters (Ph, FI1, and FI2) and magnifications (three types).
Manual button	Manual	Select the Manual mode. Set all observation conditions manually or load the already registered condition file. Set a condition for each combination of filters (Ph, FI1, and FI2) and magnifications (three types).

 Table 2.2-1
 Functions for setting observation conditions (1/2)

Table 2.2-1	Functions for setting observation conditions (2/	2)
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Item		Function
Z Position	For example display the i Even if the f registered v Click the Fix Additionally position. To	appropriate Z position (focus position) for each selected filter. e, set the Z position for the fluorescent filter 1 (FI1) and click the Fix button to input value in red and register it. fluorescent filter 2 (FI2) is selected and the Z position is changed, the alue appears when the fluorescent filter 1 (FI1) is selected again. c button again to clear the registered value. , changing the Z position is available even after once registering the Z display the registered Z position again, click the Go button. ngs can also be performed on the Simple mode.
Save button		Select to save observation condition setting in a setting file or in a file for setting on the Simple mode.

2.2.1 Saving Observation Conditions

Click the Save button () in the observation condition setting area to display the Save setting dialog box. And then, save observation condition setting in a setting file or the Bright, Middle, or Dark setting file on the Simple mode.

Setting file compatibility

Files created by the software of Ver. 2.1 or later cannot be read into the software of Ver. 1.*.

Save settings	×		
Objective	20×		
Filter	Ph		
DIA	150		
Exposure time	5ms		
Gain	1.00		
Resolution	640 × 480 Binning		
Save into file			
C Save as "Simple Setting" for			
🙆 Bright Sample			
🧔 Middle Sample			
🙆 Dark S	ample		
Save	Close		

Figure 2.2-2 Save settings dialog box (Save into file)

Table 2.2-2	Functions for saving observation conditions (Save in	nto file)

Item	Function
Save into file	Select to save the observation condition as a setting file.
Save button	Select to display the Windows Save As dialog box. Input a file name and save the observation condition as a setting file.
Close button	Select to close the dialog box without saving the observation condition.

Figure 2.2-3 Save settings dialog box (Save as "Simple Setting" for)

or)
)

Item	Function				
Save as "Simple Setting"for	Select to save the observation condition as the setting file in the Simple mode. Specify the exposure condition for which the observation condition is saved.				
Save button	Select to display the save confirmation dialog box. To save setting, click the OK button. To return to the Save settings dialog box without saving, click the Cancel button. Figure 2.2-4 Save confirmation dialog box				
Close button	Select to close the dialog box without saving observation condition setting.				

2.3 Setting Observation Conditions (Focus Mode, Automatic Exposure, Condition File Loading, Light Intensity, Exposure Time, Gain, and Resolution)

Each setting in this section is available only on the Manual mode.



Figure 2.3-1 Setting observation conditions on the Manual mode (left: fluorescent filter, right: phase contrast filter)

ltem		Function					
AE (focus)			Select only for	Select only for the Manual mode.			
button	AE(focus))	The exposure adjustment is performed with a focus priority mode. It is useful for the focus adjustment because the exposure time for the dark specimen is 1/6 second maximum. However, noise of the image increases because this operation increases gain. In this case, exposure time and gain are adjusted automatically. These conditions cannot be adjusted manually. Click this button again to clear focus priority mode.				
AE button			Select only for the Manual mode.				
	Automatic exposure is performed once. Exposure time and gused for the automatic exposure are displayed in each item observation condition.						
Load settings	Load settin	ad settings Click here to load the already registered observation condition file.					
Intensity	Adjust intensity of each lamp with the slider and the right and left triangle buttons.						
setting	Display cha	splay changes depending on selected filter.					
		his operation is also available with the Illumination intensity adjustment selector JP/DOWN) switch of the ergonomic controller.					
			fluorescent filt cury lamp).	er: set the intensity of episcopic illumination (external			
	EPI Lamp		Close	Select to open or close the shutter for excitation light. They are enabled only when fluorescent filter is selected.			
			Open	This operation is also available with the Shutter open/close switch of the ergonomic controller.			
			For phase contrast filter: set the intensity of diascopic illumination (ILED illuminator).				
	DIA Lamp	<u>Å</u>	Turn on or off the built-in LED illuminator.				
		₿	Turned off				

 Table 2.3-1
 Functions for setting observation conditions (1/2)

ltem		Function					
Exposure time	Select exposure time from the pull-down menu. Setting value can be increased or decreased by "1" with the ▼ and ▲ buttons.						
Gain	Click the value displayed next to the Gain to display the Edit detailed gain dialog box. In the dialog box, set the compensation value with the keyboard. The compensation value can also be set with the slider and the right and left triangle buttons displayed next to the value.						
Resolution	Select resolution from the Resolution can be set for shown as follows:	or each channel sep		combinations are			
	Resolution 640x480 640x480 Binning 1280x960						
	640x480	✓	✓				
	640x480 Binning	✓	✓				
	1280x960 ✓						
	Resolution combinations for BioStation IM						
	Resolution	800x600	800x600 Binning	1600x1200			
	800x600	✓	✓				
	800x600 Binning	✓	✓				
	1600x1200			\checkmark			
	✓: applicable None: not applicable						

Table 2.3-1	Functions for setting	observation conditions	(2/2)
10010 2.0-1	i unctions for setting	observation contaitions	(2)2)

2.3.1 Gain Compensation Value Fine Adjustment

Click the compensation value displayed next to "Gain" to display the Edit detailed gain dialog box. Gain compensation value can be adjusted finely.

Edit detailed gain		×
1.68	•	
Apply	Close	

Figure 2.3-2 Edit detailed gain dialog box

 Table 2.3-2
 Functions of gain compensation value fine adjustment

Item	Function
Compensation value setting	Set the compensation value with the keyboard or the $ earrow$ and
Apply button	Select to apply selected compensation value and close the dialog box.
Close button	Select to cancel compensation value setting and close the dialog box.

2.4 Observation Point and Focus Adjustment



Figure 2.4-1 Setting observation point



Item		Function		
Jog dial	Coarse focus	Click each direction button of the jog dial to move stage. At the center part of the jog dial, X and Y coordinates of current stage position are displayed.		
	Fine focus	This operation is also available with the X stage knob or the Y stage knob of the ergonomic controller.		
Focus button	Coarse focus	Click the up-arrow button or down-arrow button to move objective in the Z direction.		
	Fine focus	This operation is also available with the Focus knob of the ergonomic controller.		
Undo button	Undo	Select to return observation point to the previous focus position.		
Focus slide bar	Slide up or down	the slider to adjust focus.		
Well switch button	Well	This button is displayed only for the BS-IM-MC MOT chamber. Select to switch the well to observe in the glass bottom 4-well dish. $\int \frac{1}{4} \int \frac{1}{\sqrt{2}} \int \frac{1}$		
		Figure 2.4-2 Position of the well number		



New Time-Iapse Setting Screen

You can set and save observation points and observation conditions, and perform time-lapse experiment on this screen.

Two screens, "Live" screen and "Wide field" screen, are provided to set observation points and observation conditions for time-lapse observation.

On the Live screen, the live image is used for the settings. On the Wide field screen, a captured tiled image is used for the settings.

When using the BS-IM-MC MOT chamber with the AF mode enabled, the New time-lapse setting screen cannot be displayed unless the AF position of the reference mark is registered. To carry out time-lapse observation on the BS-IM-MC MOT chamber, be sure to register the AF position of the reference mark. For details on registering AF position of the reference mark, see Section 1.1.2, "Registering AF Position of the Reference Mark (BS-IM-MC MOT Chamber)".

Do not have an access to the time-lapse result file for the target specimen during time-lapse experiment. Otherwise, experiment data obtained while the file is being accessed may not be saved on the Time-lapse result file.

(Ex.) If you copy the time-lapse result file for backup from Explorer during the time-lapse experiment, the experiment data obtained while it is being copied will not be saved on the file.

3.1 Live Screen

Set observation points and observation conditions checking the live image on this screen.

For the BS-IM-MC MOT chamber with the AF mode enabled, AF is automatically performed for the currently selected well and the well moves to the X-Y position that is previously displayed.



: Displayed only for the BS-IM-MC MOT chamber.



	ltem		Function			
(1)	Screen switch buttons	Select to switch between the Live screen and the Wide field screen.				
(2)	Observation point verification display	The field of view is indicated by the red frame and displayed as the live observation image. The registered observation point is indicated by the blue frame. Click the desired point to move the stage and to display the live image of the clicked point. For the BS-IM-MC MOT chamber, the Well switch tabs are displayed. Click the Well				
			show its observation points.			
(3)	Live observation image display		rvation image of the field of view is displayed. creen to move the stage until the clicked point locates at the center of this			
(4)	lmage adjustment buttons	display.	tone adjustment, saturation check, intensity screening, and pseudo color each function is the same as that for the Live observation screen.			
(5)	Start time-lapse button	Select to start time-lapse experiment. Time-lapse start confirmation dialog box (Confirmation window) appears. If the Pause button (Time-lapse images in process screen) is used to suspend the time-lapse experiment that is running, the Start time-lapse button changes from <u>Start time-lapse</u> to <u>Restart time-lapse</u> for restart.				
(6)	Time-lapse experiment scheme	Points tab	Select to display the observation conditions (filter name, exposure time, and magnification) for each observation point. Click an observation point to display the Point information dialog box. In the dialog box, checking, changing, and deleting the condition are available.			
		Time tab	Select to display the capturing interval time, total observation time, and number of rounds for time-lapse experiment. Click time-lapse experiment time display to display the Timelapse dialog box. In the dialog box, changing and deleting time-lapse experiment time setting are available. Click the New button to add new time-lapse experiment time.			
		Cell name etc tab	Select to input subordinate information of time-lapse experiment. "Sample name", "Cell name", or "User name" can be input. Click any displayed name to display the Cell name etc dialog box. In the dialog box, inputting, changing, and deleting the name are available.			
		Zstack tab	To specify multiple observation points in the Z-direction for an observation point, use this function. Up to 99 observation points can be set by specifying the travel amount and travel count along Z-axis. However, the setting that exceeds the maximum stroke in the Z direction is not available.			
(7)	Observation condition and observation point	Set observation conditions (filter selection, magnification selection, intensity adjustment, and exposure time setting) and move stage and perform focusing with the Jog dial. For the BS-IM-MC MOT chamber, the Well switch button is displayed. Clicking the Well switch button displays the live observation image of the selected well.				
(8)	Time-lapse experiment point registration button	Register After specifying all observation conditions, click the Time-lapse experiment point registration button to register the specified conditions to the Time-lapse experiment scheme. If registered observation condition setting is changed, the setting is overwritten. Up to 99 points can be registered.				

Table 3.1-1 Functions on the New time-lapse setting screen (Live screen)

3.1.1 Time-lapse Confirmation Window

lick the Start time-lapse	e button (S	tart time-lapse	e) to di	splay the	following window.
	Confirmation w	in do w					X
	Tot	al rounds 🖡	2	Tota	ltime Oh	01 m 00s	
	Point X(um)	Y(um)	Z(um)		Filter	Objective	
	1-1 3079.51 1-2 4080.26 1-3 1639.93	3079.51 2159.86 3960.05	150.00 150.00 150.00		Ph Ph Ph	20x 20x 20x	
		_					
	Start time-lapse	•	Prospective file s	size : 3.51 MB		Back to setting	

Figure 3.1-2 Confirmation window

Item	Function
Start time-lapse button	Select to display the Windows Save As dialog box. Input a file name for the time-lapse result file and click the Save button. And then, the Time-lapse image in process screen automatically appears and time-lapse experiment starts.
Back to setting button	Select to cancel time-lapse experiment and return to the New time-lapse setting screen.

3.1.2 Observation Point Verification Display

Checking the registered observation points and current view point are available in the entire observation area. Click in the following screen to move the stage to the clicked point.

For the BS-IM-MC MOT chamber, the Well switch tabs are displayed on the right side of the display. Click the Well switch tab to see the observation points registered to each well.



Figure 3.1-3 Observation point verification display

Table 3.1-3 Functions of observation point vernication display	Table 3.1-3	Functions of observation point verification display
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Item	Function
Registered observation point	The registered observation point is indicated by the blue frame. The number over the blue frame is the same as the point number shown on the Point tab.
Live image display range	The field of view is indicated by the red frame and displayed as the live observation image. X and Y coordinates of the center are displayed.

3.1.3 Time-lapse Experiment Scheme (Points Tab)



Figure 3.1-4 Points tab

Table 3.1-4 Functions on the points tab

Item	Function	
Observation point	Select the Points tab to display observation conditions (filter name, exposure time, and magnification) for each observation point. The number next to the checkbox is the point number indicating the sequence of registration. For the BS-IM-MC MOT chamber, the well number is prefixed to the number. When time-lapse experiment is performed, only point selected with a check mark is observed. Click an observation point to display the Point information dialog box. In the dialog box, checking, changing, and deleting the condition are available.	
Delete button	Select to delete the selected observation point. The Delete confirmation dialog box appears. To delete the selected observation point, click the OK button. To return to the Points tab without deleting, click the Cancel button. Figure 3.1-5 Delete confirmation dialog box	
Clear button	Select to delete all selected observation points. The Delete all confirmation dialog box appears. To delete all selected observation points, click the OK button. To return to the Points tab without deleting, click the Cancel button.	
Load button	Select to load the registered time-lapse experiment condition file.	
Save button	Select to save the registered observation point, observation condition, and time-lapse experiment time to a time-lapse experiment condition file.	
Warning mark	If time-lapse experiment time setting is disabled or hard disk space of control PC is insufficient, this mark appears.	

Checking or deleting observation condition

Click an observation point on the Point tab to display the Point information dialog box. In the dialog box, check observation condition setting for each combination of filters and magnifications.

Point information	X
Ph	20× 414×311 um
DIA Lamp	150
Exposure time	5ms
Gain	1.00
Resolution	640 × 480 Binning
Dish	1
x	3079.51 um
Y	3079.51 um
z	150.00 um
Comment	test
Go	Delete Close

Figure 3.1-7 Point information dialog box

Table 3.1-5	Functions for checking, changing or deleting observation condition
-------------	--

Item	Function
Go button	Select to change the displayed settings. To change displayed observation condition settings to new settings, click the Go button.
Delete button	Select to delete the selected observation point. The Delete confirmation dialog box appears. To delete the selected observation point, click the OK button. To return to the Point information dialog box without deleting, click the Cancel button. Figure 3.1-8 Delete confirmation dialog box
Close button	Select to close the Point information dialog box.
Edit comment button	Select to add or edit a comment per observation point. Click the Edit comment button to display Edit comment dialog box for adding or editing comments.
	Click the Apply button to add the comment after creating it. Click the Close button without clicking the Apply button to ignore the entered comment and close the Edit comment dialog box. Added comment pops up when you double-click the observation point.

3.1.4 Time-lapse Experiment Scheme (Time Tab)

When the stream setting is configured during the time-lapse experiment time setting, the display of Time tab is replaced with Stream.



Figure 3.1-10 Time tab

Table 3.1-6 Functions on the Time tab

Item	Function	
Time-lapse experiment time display	Select to display the capturing interval time, total observation time, and number of rounds for time-lapse experiment. Click time-lapse experiment time display to display the Timelapse dialog box (for setting change). In the dialog box, changing and deleting capturing interval time and total observation time are available.	
Delete button	Select to delete the selected time-lapse experiment time. The Delete confirmation dialog box appears. To delete the selected time-lapse experiment time, click the OK button. To return to the Time tab without deleting, click the Cancel button. Figure 3.1-11 Delete confirmation dialog box	
New button	Select to set new time-lapse experiment time. The Timelapse dialog box (for new registration) appears.	
Clear button	Select to delete all settings of time-lapse experiment time. The Delete all confirmation dialog box appears. To delete all settings of time-lapse experiment time, click the OK button. To return to the Time tab without deleting, click the Cancel button. Figure 3.1-12 Delete all confirmation dialog box	
Load button	Select to load the registered time-lapse experiment condition file.	
Save button	Select to save the registered observation point, observation condition, and time-lapse experiment time to a time-lapse experiment condition file.	

Registering multiple time-lapse experiment times is available.

This software can register multiple time-lapse experiment times, which are composed of different total observation time and capturing interval time.

When a time-lapse experiment starts, the multiple time-lapse experiment times registered with the Time tab are automatically applied in descending order.

Additionally, even during the time-lapse experiment, adding a new time-lapse experiment time and changing or deleting unperformed time-lapse experiment time are available.

,	Acquisition cycle	Total time	Rounds
0	0h 20m 00s	10h 00m 00s	31 Delete
• 0	0h 00m 10s	0h 10m 00s	61 Deleto
0	0h 10m 00s	20h 00m 00s	121 Delete
Nev	N		Clear

Figure 3.1-13 Time tab
Changing or deleting time-lapse experiment time

Click a time-lapse experiment time on the Time tab to display the Timelapse dialog box (for setting change).

Timelapse	X
Acquisition cycle	10 minutes 💌
Total time	20 hours
Rounds	121
	As soon as possible 0h 00m 28s.
Apply	Delete Close

Figure 3.1-14 Timelapse dialog box (for setting change)

Table 3.1-7	Functions for changing or deleting time-lapse experiment time	
-------------	---	--

ltem	Function	
Acquisition cycle	Input capturing interval time for time-lapse experiment. Select a unit from the pull-down menu next to the input box.	
Total time	Input total observation time of time-lapse experiment. Select a unit from the pull-down menu next to the input box.	
Rounds	Round value is displayed. This value is automatically calculated from total observation time and capturing interval time.	
Apply button	Select to apply the input values to time-lapse experiment time setting.	
Delete button	Select to delete the highlighted time-lapse experiment time. The Delete confirmation dialog box appears. To delete the selected time-lapse experiment time, click the OK button. To return to the Timelapse dialog box without deleting, click the Cancel button. BioStation IM Delete from timelapse list Cancel Figure 3.1-15 Delete confirmation dialog box	
Close button	Select to cancel the Time-lapse experiment time setting and close the dialog box.	

Registering new time-lapse experiment time

Click the New button of the Time tab to display the Timelapse dialog box (for new registration). Setting values of capturing interval time and total observation time are the same as those of the Timelapse dialog box (for setting change).

After setting time-lapse experiment time, click the Add button to register the setting.

Timelapse	×
Acquisition cycle	1 minutes 💌
Total time	2 minutes
Rounds	2
	As soon as possible 0h 00m 28s.
Add	Close

Figure 3.1-16 Timelapse dialog box (for new registration)

Registering a new time-lapse experiment time for stream capturing

When the conditions for the stream setting are fulfilled, the Stream setting box appears on the Timelapse dialog box (for new registration).

After configuring the time-lapse experiment time for stream capturing, click the Add button to register the configured settings.

Stream setting limitations

The following observation conditions must be applied for the stream setting. The Stream setting box does not appear on the Timelapse dialog box unless all of the following conditions are met.

- The exposure time must be less than a second.
- One type each for the filter and the objective must be selected.
- A single observation point (Z-stack) must be applied in the Z direction.

Acquisition cycle	15	minutes 💌
Total time	60	minutes 💌
Stream blocks	4	
🔽 Stream setting 🛛 -		
Stream time	10	sec
	As soon a	s possible Oh OOm 19s.

Figure 3.1-17 Timelapse dialog box (for stream capturing)

ltem	Function
Acquisition cycle	Input the capturing interval time for the time-lapse experiment. Select a unit from the pull-down menu next to the input box.
Total time	Input the total observation time of the time-lapse experiment. Select a unit from the pull-down menu next to the input box.
Stream blocks	The number of stream blocks is displayed. The value is automatically calculated from the streaming time, total observation time, and capturing interval time. * The unit of stream data captured with the stream setting is called the "Stream
	block" in this manual.
Stream setting	Select to enable the stream setting.
Stream time	Set the stream capturing time (setting range: 1 to 3600 sec).
Add button	Select to configure the set values as the time-lapse experiment time
Close button	Select to cancel the Time-lapse experiment time setting and to close the dialog box.

Caution on stream capturing

- After the time-lapse experiment for the stream capturing is performed, the volume of the file data becomes heavy. Be sure to keep enough blank space in the hard disk on the control PC before performing the time-lapse experiment.
- For the stream capturing, the number of frames varies among the stream blocks.

3.1.5 Time-lapse Experiment Scheme (Cell Name etc... Tab)



Figure 3.1-18 Cell name etc... tab

Table 3.1-9	Functions on the Cell name etc tab

ltem	Function
Sample name	The Sample name, Cell name, and User name are displayed. Click any displayed name to display the Cell name etc dialog box. In the dialog box, inputting, changing, and deleting the name are available. The time-lapse experiment can be performed even if none of them is input. The input information in the Cell name etc dialog box can be displayed on saved images or Live images.
Cell name	
User name	
Dimension	This field appears when Hi-Q4 (film bottom 4-quadrant dish) is set on the Preferences dialog box. Since the BS-IM-MC MOT chamber does not support the Hi-Q4 (film bottom 4-quadrant dish), the Dimension field does not appear. (See Section 1.3.4, "Basic Settings" as a reference) Click the Dimension field to display the Dimension dialog box. In the dialog box, inputting, changing, and deleting the names of the first to fourth quadrants are available.
Load button	Select to load the saved time-lapse experiment condition file.
Save button	Select to save the registered observation point, observation condition, and time-lapse experiment time as a time-lapse experiment condition file.

Inputting subordinate information of time-lapse experiment

Click Sample name/Cell name/User name on the Cell name etc... tab to display the Cell name etc... dialog box.

Cell name etc		×
Sample name Cell name User name		
Apply	Close	

Figure 3.1-19 Cell name etc... dialog box

Table 3.1-10) Functions for inputting time-lapse experiment subordinate information
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Item	Function
Sample name	
Cell name	Input names (arbitrary letter strings) for the Sample name, Cell name, and User name.
User name	
Apply button	Select to register the input name.
Close button	Select to close the dialog box without registering the input name.

Inputting name of film bottom 4-quadrant dish

Click Dimension field on the Cell name etc... tab to displays the Dimension dialog box.

Dimension		×
First quadrant Second quadrant Third quadrant	test1 test2	
Fourth quadrant	test4	
Apply	Close	





ltem	Function
First quadrant	
Second quadrant	Input names (arbitrary letter strings) for the respective compartments of the film
Third quadrant	bottom 4-quadrant dish.
Fourth quadrant	
Apply button	Select to register the input name.
Close button	Select to close the dialog box without registering the input name.

3.1.6 Time-lapse Experiment Scheme (Zstack Tab)

Use this function to set up multiple observation points in the Z-direction at a same X-Y coordinate. When the Z-stack function is used, the setting information is saved and will be restored at the next time. When the stream setting is configured on the Time tab, the Zstack tab is not displayed.



Figure 3.1-21 Zstack tab

 Table 3.1-12
 Functions on the Zstack tab

Item		Function			
Register points with Z-stack		Select to enable the Z-stack function. On the Time-lapse images in process screen and Time-lapse images Acquired screen, the Z-stack display control is displayed.			
Step	Enter a value w	Specify the travel amount (step width) in the Z direction. Enter a value with the keyboard. (Minimum step: 0.05 μ m) The value can be changed with the arrow buttons in 0.1 μ m steps.			
Independent steps	Select to enable	Select to enable the UP steps function.			
Down/Up	Specify the travel count (step count) in the Z direction. Enter a value with the keyboard. The value can be changed with the arrow buttons. (Acceptable range: 1 to 49 steps) Up to 99 steps can be set for the Z-stack (current observation point + 49 steps for Down/Up).				
From/To	Total travel amounts in the Z direction (plus and minus directions) appear. These values are calculated automatically from the step width and the step count.				
Z-stack sequence	Z-Mch Z	Captures images with all selected filters by Z position of an identical observation point. Select to capture images by filters at an identical observation point in a short time. The filters are switched whenever an image is captured, thus it takes longer time to make observations.			
	Mch-Z	Captures observation images in the Z direction together by selected filters. Select to make observations in a short time with less filter switchovers.			
Load button	Select to load th	ne saved time-lapse experiment condition file.			
Save button		Select to save the registered observation point, observation condition, and time-lapse experiment time as a time-lapse experiment condition file.			

Caution on the Z-stack sequence

- When switching the settings for (Z-Moh Z-y) and (Moh-Z Y-y) after registering the observation points, the Z-stack sequence of all observation points is changed.
- The image of an observation point registered with the Z-stack function cannot be reproduced successively with this application software.

Z position display examples for observation points registered with the Z stack function

The Point information dialog box for an observation point configured with Z stack shows the Z position as shown below.

Reference value (negative-side absolute value – positive-side absolute value, number of steps)

Point information		×
Ph	20× 414x311 um	
DIA Lamp	150	-
Exposure time	5ms	
Gain	1.00	-
Resolution	640 x 480 Binning	
Dish	1	
x	3079.51 um	
Y	3079.51 um	
z	150.00 um	
Comment	test	
Go	Delete Close	

Figure 3.1-22 Z position examples

3.1.7 Setting and Editing Observation Conditions (Setting for Observation Filter and Magnification/Editing Z-stack)



Figure 3.1-23 Setting or changing observation conditions

Table 3.1-13	Functions for setting or changing observation conditions

Item		Function	
Time-lapse experiment point registration button	Register Register the observation points and conditions to the Time-lapse experiment scheme. If registered observation condition setting is changed, the setting is overwrited and the set		
New registration	New point	Select to register observation condition for a new observation point.	
/setting change select menu	For point	Select to change observation condition of the existing observation point.	
Filter checkbox	Select the filter used for the time-lapse experiment.		
Filter button	Select the filter button to display the image with the selected filter. The blue frame appears on the selected filter button. This operation is also available with the Observation method switches of the ergonomic controller.		
	Ph	Select to perform phase contrast microscopy with the diascopic illumination of the red LED illuminator built in the microscope. Fluorescent filter is not used.	
	F11 F12	Select to perform the fluorescent microscopy using the selected filter with the illumination of external mercury lamp. This button does not appear unless the HG precentered fiber illuminator is connected and turned the power on.	
Magnification checkbox	Select the magnification for the time-lapse experiment.		
Magnification button	20× 414x311um 207x156um 104x78u		

Details about setting observation condition, moving observation point, and focusing

The following operations are the same as those of the Live observation screen: setting observation condition, selecting observation point with the Jog dial, and focusing.

Reference: Chapter 2, "Live Observation Screen"

- Section 2.2, "Setting Observation Conditions (Filter, Magnification, Mode, Z position, and Save)"
- Section 2.3, "Setting Observation Conditions (Focus Mode, Automatic Exposure, Condition File Loading, Light Intensity, Exposure Time, Gain, and Resolution)"
- Section 2.4, "Observation Point and Focus Adjustment"

Ph Fit	F12 20× 414×311um	40× 207x156um 104x78un
Simple Man	ual AE(foo	us) AE
Load settings		
EPI Lamp 🍈	100X 4	
Exposure time	300ms 💌	÷
Gain	1.00 ┥ 🔟	Þ
Resolution	640 × 480 Binning 💌	
Z Position	150.00 um	Fix Go
Well		Focus Undo
1		
2	\sim	
4	P A	
-	< (×=3000.33 Y=3000.33 ► ►	- 150.00 um
	V	•

Editing Z-stack

Follow the procedure below to edit the Z-stack of an existing observation point.

Note that editing is not available after the start of time-lapse experiment.

(1) Display the observation point of which you want to edit Z-stack.

Select an observation point to edit on the New registration/setting change select menu.

(2) Open the Zstack tab and edit Z-stack.

(3) Click the Time-lapse experiment point registration button to confirm updated setting conditions.



Figure 3.1-24 Selecting observation point

Points Time Ce	ell nam	e etc Zst	ack		
🔽 Register points with	Z-stack				
Step: 0.50	um	🔽 Indep	endent steps		
Down: 2	steps	Up: 2	steps		
From: -1.00	um	To: +1.00	 um		
Z-stack sequence Z-Mch Z					
Load			Save		
-	Regis	ter			

Figure 3.1-25 Zstack tab

Points Time	Cell name	etc Zst	ack	
Register points w	ith Z-stack			
Step: 0.50	🕂 um	🔽 Indep	endent steps	
Down: 3	steps	Up: 4	steps	
From: -1.50	 um	To: +2.00	um	
Z-stack sequence Z-Mch Z				
Load	Regist		Save	

Figure 3.1-26 Confirming setting conditions

3.2 Wide Field Screen

If a specimen is exposed to an excitation light during the observation point settings and the observation condition settings, the deterioration speed of the specimen becomes faster. To avoid it, use a captured tiled image to set up observation points and conditions.

On the Wide field screen, you cannot adjust the light intensity and the focusing condition. Therefore, adjust intensity and focus on the Live screen in advance.



Figure 3.2-1 New time-lapse setting screen (Wide field screen)

Table 3.2-1 Functions on the New time-lapse setting screen (Wide field screen) (1/2)

	ltem	Function
(1)	Screen switch buttons	Select to switch between the Live screen and the Wide field screen.
(2)	Observation point verification display	The current pointer is indicated by the red frame. The registered observation point is indicated by the blue frame.
(3)	Browse/clear buttons	Select to set and clear a browse area of a tiled image.
(4)	Tiled image display	The images browsed as a tiled image are displayed. The current pointer is indicated by the yellow frame. The registered observation point is indicated by the blue frame.
(5)	Image adjustment buttons	Select to set up tone curve adjustment. Operation of these buttons is the same as that of the Live observation screen.
(6)	Partial enlargement	Click the Magnifier button and drag the mouse pointer on the tiled image to enlarge the area according to the magnification selected from the pull-down menu.
(7)	Cross line display checkbox	The Show cross line checkbox appears when Hi-Q4 (film bottom 4-quadrant dish) is selected on the Preferences dialog box. Select to display the crosshair. This software identifies the quadrants 1 to 4 with the crosshair.

	Item	Function			
(8)	Start time-lapse button	Select to start a time-lapse experiment. A time-lapse start confirmation dialog box (Confirmation window) appears.			
(9)	Time-lapse experiment scheme	Displays and functions of the tabs are same as those of the Live screen.			
(10)	Observation condition and observation point settings	Switch filters and a magnification to be used. When an enlarged view of the tiled image is displayed, the display area can be scrolled with the operation of these controls.			
(11)	Save Wide field image button	Select to combine range-selected observation area and save it as one Wide field image.			
		Click this button to show the Save As dialog box. Select the image format from TIFF, JPEG, BMP, or PNG. (The resolution of he image is fixed.)			
		In addition, a combined Wide field image will be reduced so that it can fit a size of one Wide field area or smaller based on a long side.			
		(Ex.: For a 2×2 matrix observation area, a combined image will be scaled down so that one side of the image is equal to the long side of the original size image.)			
		Same length			
		Combined Wide field image			

Table 2.2.4	Eurotions on the No.	v time lenes setting	n aaraan /Mi	de field eereem) (2/	2
1 able 3.2-1	Functions on the Nev	w ume-lapse setting	y screen (wr	ue nelu screen) (Zr.	2)

3.2.1 Observation Point Verification Display

The current pointer location, the registered observation area locations, and the current image display area can be identified on the upper screen.



Figure 3.2-2 Observation point verification display (magnifications; left: Full/middle: 2×/right: 4×)

Table 3.2-2	Functions of observation point verification display
-------------	---

Item	Function
Registered observation point	The registered observation point is indicated by the blue frame. The number on the blue frame corresponds with the point number shown on the Point tab.
Current pointer	The red frame (yellow frame on the tiled image display) indicates the current pointer and provides the X and Y coordinates of the center. Click the desired point to move the current pointer to the position.
Image display area	The area of the tiled image is indicated by the purple large frame. The purple frame does not appear when the magnification is "Full", because the entire tiled image is displayed.

For the BS-IM-MC MOT chamber, the Well switch tabs are displayed on the right side of the display. Click the Well switch tab to see the observation points registered to each well.



Figure 3.2-3 Observation point verification display for the BS-IM-MC MOT chamber (magnification: Full)

3.2.2 Browsing or Clearing the Tiled Image

The observation area is browsed as a tiled image.



Figure 3.2-4 Tiled image display



ltem	Function		
Browse/clear buttons	Clear browse area Select to clear browsing the tiled image.		
	Browse Select to browse the selected area with the mouse pointer a a tiled image. (See the figure at lower left.)		
	Browse all	Select to browse the entire image as a tiled image. (See the figure at lower right.)	
Blue frame	Registered observation point.		
	The number of a registered observation point appears within the frame.		
Yellow frame	Current pointer, which specifies an observation point		
Scroll bar	Use this scroll bar to scroll the display when the tiled image is enlarged.		



Figure 3.2-5 Tiling images (left: Browse/right: Browse all)

Browsing only the image within selected area

(1) Select browsing area.

Drag (left click and drag) a marquee around the area to browse on the tiled image display.

(2) Click the Browse button.

Click the Browse button to start browsing image within the selected area. Display of the Browse button changes to "Browse cancel" while the image is browsed. To stop browsing the image, click the Browse cancel button.

(3) To select multiple areas, repeat selecting the areas to browse and click the Browse button.



Figure 3.2-6 Selecting browsing area

Browsing the entire image of observation area

(1) Click the Browse all button.

Click the Browse all button to start browsing the entire image of observation area.

Display of the Browse all button changes to "Browse cancel" while image is browsed. To stop browsing image, click the Browse cancel button.



Figure 3.2-7 Starting browsing image



3.2.3 Setting Observation Conditions and Moving the Stage

Figure 3.2-8 Setting observation condition

Table 3.2-4	Functions for setting observation conditions and moving the stage (1/2)
-------------	---

ltem		Function		
Time-lapse experiment point registration button	Register	Select to register the observation point data and the observation conditions for the browsing to the Time-lapse experiment scheme.		
Filter setting checkboxes	Select the fi	ilter used for the browsing the image as a tiled image.		
Filter buttons	When selec displayed. Switching di Filter button The blue fra	Iter button to display the image with the selected filter. ting multiple filters, the images overlaid with the selected filters are isplay while browsing an image is not available. Is not selected when browsing the tiled image are disabled to select. The appears on the selected filter button. tion is also available with the Observation method switches of the controller.		
	Ph	Select to perform phase contrast microscopy with the diascopic illumination of the red LED illuminator built in the microscope. Fluorescent filter is not used.		
	FI1 FI2	Select to perform the fluorescent microscopy using the selected filter with the illumination of external mercury lamp. This button does not appear if the HG precentered optical fiber light source is connected and turned the power on.		
Selecting magnification		e field screen, magnification is fixed to 20×. Therefore, changing on is not available.		
Register Wide field image points		Select to register points in an observation area (in blue frame) selected on the Wide field screen in a batch.		
in a batch checkbox	Click the Time-lapse experiment point registration button with the checkbox checked to register the points in the selected area (if frame) in a batch.			
	WideField	The points will be registered from left top -> right top -> left bottom -> right bottom.		
		When the total of registered observation points and points to be registered exceeds 99 points or selected area overlaps a registered Wide field area (that is, the XY coordinates match completely), the batch registration is not available.		

Item	Function		
Image display		Select magnification of tiled image display with these buttons.	
magnification button		Full: Entire observation area is displayed.	
button		2×: Image is enlarged by twice.	
		4×: Image is enlarged by four times.	
	Full 2x 4x 20x 40x 80x	20×: Displayed image size is one sheet of a tiled image.	
		40×: Image is enlarged by 40 times.	
		80×: Image is enlarged by 80 times.	
Jog dial	Coarse focus	Click each direction button of the jog dial to scroll the tiled image when the entire tiled image is not displayed due to partial enlargement.	
	Fine focus	At the center part of the jog dial, X and Y coordinates of current pointer are displayed. This operation is also available with the X stage knob or the Y stage knob of the ergonomic controller.	
Well switch button	Well	/ell	
		This button is displayed only for the BS-IM-MC MOT chamber. Select to switch the well to observe in the glass bottom 4-well dish.	

 Table 3.2-4
 Operations for setting observation conditions and moving the stage (2/2)

Time-lapse Images in Process Screen

This screen shows process of time-lapse experiment. When time-lapse experiment is started, this screen automatically appears.

Therefore, this screen cannot be displayed by operating any buttons.

There are the Channels display and the Points display for the Time-lapse images in process screen.

ltem	Function
Channels	Four display areas are provided for one observation point. Three filter images, filters overlapped image, and Ratio image can be displayed at the same time.
Points	One display area is provided for one observation point. Switch the display area to display each filter image and overlapped image. Therefore, up to four observation points can be observed at the same time.

Table 4.0-1 Channels display and Points display

4.1 Channels Display



Figure 4.1-1 Time-lapse images in process screen (Channels display)

Table 4.1-1	Functions on the	Time-lapse images in process screen	(Channels display) (1/3)
-------------	------------------	-------------------------------------	--------------------------

Item		Function
(1) Screen switch buttons		Select to switch between the Channels screen and the Points screen.
(2)	Image magnification and enlarge/reduce buttons	Magnification of the current image compared to the original image size (resolution) is displayed. Click the magnifier buttons to enlarge or reduce the image.
(3)	Scroll mode button	Scroll the image freely by clicking this button and dragging the mouse pointer on the enlarged image.

I able 4.1-1 Functions on the Time-lapse images in process screen (Channels display) (2/3)				
(4)	Clipping button	Select to per	form c	lipping on the image area. The function is turned on or
(4)		off each time	e the bu	utton is clicked.
(5)	Magnification buttons	Select to sho	ow the	current magnification.
(6)	Z-stack display control	is checked o pull-down m observation	on the Ż enu sh image	v control is displayed when Register points with Z-stack Zstack tab of the New time-lapse setting screen. The ows the Z coordinate of the image taken, and time-lapse display can be switched by making a choice. The slider in the image display along the Z coordinate.
(7)	Image of time-lapse experiment (Ph)		trast fil	roscopy image of time-lapse experiment is displayed. ter is not selected for observation condition, its image is
(8)	Image of time-lapse experiment (FI1)		cent fil	copy image of time-lapse experiment is displayed. ter (FI1) is not selected for observation condition, its yed.
(9)	Image of time-lapse experiment (FI2)	Fluorescent microscopy image of time-lapse experiment is displayed. If the fluorescent filter (Fl2) is not selected for observation condition, its image is not displayed.		
(10)	Image of time-lapse experiment (Multi	Ph FI1	FI2	When two or more filters are selected, their images are overlapped and displayed.
	Ch)	Ratio		Select to display the Ratio image of time-lapse experiment.
		F11./F12		Select to display the Ratio setting dialog box and set the Ratio image.
(11)	Intensity graph	This graph shows changes in intensity during time-lap The color of graph line is the same as that of each filt		
(12)	Time line display and image playback control	Use the slider to check process of time-lapse experiment. The ◀▶ button at the right and left of the slide bar is used to reproduce images step by step or reproduce images step by step in a reverse order. Also, use the image control buttons to pause, playback, forward fast, playback slowly, reverse slowly, repeat partially, and mark a point.		
(13)	Observation point verification display	The observation point of time-lapse experiment is indicated by the yellow frame. The registered observation point is indicated by the blue frame.		
(14)	Time-lapse experiment scheme	Points tabSelect to display the observation conditions (filter name, exposure time, and magnification) for each observation point. Click an observation point to display the Point information dialog box. In the dialog box, checking, changing, and deleting the condition are available. Click the Stop button to stop the running time-lapse experiment.		
		Time tab	time, a Click t Timela time-la	to display the capturing interval time, total observation and number of rounds for time-lapse experiment. ime-lapse experiment time display to display the apse dialog box. In the dialog box, changing and deleting apse experiment time setting are available. he New button to add new time-lapse experiment time.

Table 4.1-1	Functions on the Time-lapse images in process screen (Channels display) (2/3)	1
	anotione on the rand apos in proceed concern (enalited alopia) (are)	,

	Item Function				
(14)	Time-lapse experiment scheme	Cell name etc tab Select to input subordinate information of time-lapse experiment. "Sample name", "Cell name", or "User name" can be input as subordinate information. Click any displayed name to display the Cell name etc dialog box. In the dialog box, inputting, changing, and deleting the name are available.			
(15)	Time-lapse experiment process display	The time-lapse experiment time, start and estimated end time of time-lapse experiment, number of rounds, elapsed time, and logarithmic graph of temperature change are displayed.			
(16)	Saturation alert icon	 If saturation occurs during the time-lapse experiment, this icon appears. (Determined as saturation when adjacent two pixels or more reached the maximum intensity continuously.) Click the icon to display a saturation information dialog. This icon, once appeared, remains displayed after the saturation has been reset. 			
(17)	Print button	Select to print the image captured with time-lapse experiment. The Print dialog box appears.			
(18)	Save button	Select to save images captured with the time-lapse experiment to an image file. When a clipping area is set, the save confirmation dialog box for the clipped image appears. Click the OK button in the dialog box. Then, the Save image dialog box appears. To cancel the save operation and return to the Time-lapse images in process screen, click the Cancel button.			
		When a clipping area is not set, this dialog box does not appear, but the Save image dialog box appears.			

Table 4 1-1	Functions on the Time-I	anse images in proc	ess screen (Channels (display (3/3)
		apoo magoo m proo	000 001 0011 (01141111010 0	

4.1.1 Image Magnification and Enlarge/Reduce



Figure 4.1-3 Image magnification

Table 4.1-2	Functions of image magnification and enlarge/reduce
-------------	---

Item	Function	
Magnification	Magnification of the current image compared to the original image size (resolution) is displayed.	
Enlarge/reduce buttons	\odot	Select to enlarge or reduce the magnification of displayed images by 5%.
Enlarge/reduce menu	38% 💌	Click the down arrow ($\mathbf{\nabla}$) button at the right of the magnification to display the image magnification list box. Click a magnification to enlarge or reduce the image magnification.

4.1.2 Scrolling an Enlarged Image



Figure 4.1-4 Scroll mode button

Table 4.1-3	Eunction for	scrolling an	enlarged image
1 abie 4.1-5	Function for	scroning an	emargeu image

ltem	Function
Scroll mode	Scroll the image freely by clicking this button and dragging the mouse pointer on the enlarged image.
button	When the image is dragged without clicking the scroll mode button or clipping button, a region of interest (ROI) area is drawn.

4.1.3 Setting a Clipping Range of the Displayed Image



Figure 4.1-5 Clipping button

Table 4.1-4 Function for setting a clipping range of the displayed image
--

Item	Function	
Clipping	Select to set a clipping range of the displayed image.	
button	Select (left click and drag) an area of the displayed image. A clipping range of the image can be set. If this operation is performed for one image, the same clipping area is set for other three images. However, when a clipping area is set for an image, only the set clipping area (clipping image) can be saved.	
	Click the clipping button again to turn off the clipping function. To delete the set clipping area, display the delete confirmation dialog box by right clicking the clipping area and selecting Delete from the submenu, or selecting the clipping area and pressing the Delete key on the keyboard, then click the OK button in the dialog box.	Bio Station IM Delete clipping? OK Cancel Figure 4.1-6 Delete confirmation dialog box

Saving an image when a clipping area is set with the clipping function

If a clipping area is set, click the Save button to display the save confirmation dialog box for the clipping image and click the OK button to save the clipping image. If a clipping area is not set, the displayed image is saved.



4.1.4 Magnification Button



	Table 4.1-5 Magnification function
ltem	Function
Magnification button	Magnification used for running time-lapse experiment is displayed.

4.1.5 Z-stack Display Control



Figure 4.1-9 Z-stack display

 Table 4.1-6
 Functions on the Z-stack display

Item	Function		
Z-stack sequence icon	The means to capture observation image in the Z direction during the time-lapse experiment is indicated.		
Pull-down menu	of the cap	The Z coordinate of the image currently displayed is displayed. Select the Z coordinate of the captured image displayed in the pull-down menu to switch the time-lapse observation image display.	
Slider	Switch the Z coordinate to the positive or negative side from the reference observation point for the image display.		
Z focus mark button		Select to register the optimum image displayed by switching the slider bar as the focus point.	
Z focus graph button	F	Select to display the Z focus graph dialog. This allows you to check the points registered by using the Z focus mark button on a graph.	

Graphical display of focus positions

Clicking the Z focus graph button (🖂) shows the Z focus graph dialog box.



Figure 4.1-10 Z focus graph dialog box

Table 4.1-7	Functions on g	raphical display	of focus positions
-------------	----------------	------------------	--------------------

Item	Function
Target	Click the ▼ button on the right side of the Target item to show the selection list of the observation points. Selecting an observation point shows a graph of the focus positions registered for the observation point.
Close button	Select to closes the Z focus graph dialog box.

4.1.6 Time-lapse Observation Images Display (Ph/FI1/FI2/Multi)

Images of time-lapse experiment are displayed.

If a filter (Ph/Fl) is not selected for time-lapse experiment scheme, its image cannot be displayed.



Figure 4.1-11 Time-lapse observation images display

ltem	Function			
Observation image display	Each obse	rvation images are displayed respectively on each display area.		
Filter button	FI1Select to change the filter name and color of each filter button. Right click the filter button to open the sub-menu. Select the sub-menu to display the Filter settings dialog box. You can change the filter name and select an image color in this dialog 			
Scale bar button		Select to display a scale bar on the bottom left of observation image. The scale length is 10 μ m and has three ticks at 0 μ m, 5 μ m, and 10 μ m.		
Automatic tone curve adjustment apply button		Select to automatically set the range. The range of the intensity to be displayed is automatically set. The highest intensity (A) of the displayed image is set as the upper limit (C) and the lowest intensity (B) is set as the lower limit (D). (D) (B) (A) $(C)Figure 4.1-12 Intensity graph$		
Tone curve adjustment setting button	F	Select to set tone curve adjustment manually. The Tone curve adjustment dialog box appears to set tone curve adjustment.		
Tone curve adjustment cancel button	L+	Select to cancel tone curve adjustment and return to the original screen.		

Item	Function			
Saturation check button		Select to display saturation point in red. To clear the saturation check condition, clicked this button again. This function can be used only for the fluorescent microscopy.		
Intensity screening button		Select to display the Intensity Screening dialog box for setting intensity screening. This function can be used only for the fluorescent microscopy.		
Pseudo color button	8	Select to assign a pseudo color for fluorescence images. For overlaid images, this button is available only when a fluorescence image is used. When this button is clicked, the saturation check button and the intensity screening button are disabled. On the other hand, when the saturation check button or the intensity screening button is clicked, the pseudo color button is disabled.		
Enlarge/reduce buttons		Enlarge button Reduce button	Select to enlarge the image. The enlarge button changes to the reduce button after the image is enlarged. Click the reduce button to reduce the image to the original size.	
Overlapping buttons	Ph FI1	Select to display selected filters overlapped image or display Ratio image.		
Ratio setting button	FI1/FI2		Select to set Ratio image. The Ratio setting dialog box appears.	

Changing filter name and display color

Right click the FI1 or FI2 filter button to open the sub-menu (Filter settings). Select the sub-menu to display the Filter settings dialog box.

Filter settings	
Filter FI1 Name FL1 EX/DM/BA 535 575 590 5	
Apply Close	Custom color setting button

Figure 4.1-14 Filter settings dialog box

Table 4.1-10	Functions for	changing filter nar	ne, display color, a	and overlaid image color
--------------	---------------	---------------------	----------------------	--------------------------

ltem	Function		
Filter	The selected filter name is displayed.		
Name	Enter a filter name (up to four alphanumeric characters).		
EX/DM/BA	Set the wavelength for the three filters. Selecting the file name from the drop-down list box of the Name box automatically apply the default wavelength and color to these three filters. To change the wavelength, adjust the value clicking the up or down arrow (\blacktriangle or \blacktriangledown) buttons or entering values.		
Color	Select a color from these buttons to apply its color to filter button displayed image color and graph line. Click the desired color to display a check symbol.		
Use the custom color to overlap the image	Select to enable the custom color function with which you can set up the pseudo color for fluorescence images and the image color for overlaid image.		
Custom color setting button	Select to display the Color dialog box. You can create an arbitrary color. The color can be used for the pseudo color of fluorescence images and for the display color of overlaid images.		
Apply button	Select to close the dialog box applying the settings of file name and color.		
Close button	Select to close the dialog box without applying settings.		

Setting ratio

Ratio Setting Ratio Channel F11/F12	٥
Ratio Range 0.25 .	4.00 Fit
Background Threshold	
FI1	FI2
Apply	Close

Click the Ratio setting button (_______) of the time-lapse experiment image (Multi Ch) to display the Ratio Setting dialog box.

In this area, set the lower limit of the intensity for ratio setting.

Figure 4.1-16 Ratio Setting dialog box

 Table 4.1-11
 Functions for setting ratio

Item	Function
Ratio Channel	Select "Ratio Channel" from the pull-down menu.
Ratio Range	Input "Ratio Range".
Apply button	Select to register Ratio settings.
Close button	Select to close the dialog box without applying Ratio settings.

4.1.7 Intensity Graph





Table 4.1-12	Functions	of intensity	araph
	i unotiono	or micenony	grupn

ltem	Function		
Time Range	Select the time range (horizontal axis) of one-page graph from the pull-down menu.		
	Fit: total observation time fits in one-page graph.		
	 1×, 2×, 3×, and 4×: calculated from minimum interval time, value of Time Range, and coefficient. Time per scale = (4)/(value of Time Range) × (minimum interval time) Time range of one-page graph = (time per scale) × 7 		
	Example: when value of Time Range is 2× and minimum interval time is 10 minutes: (4/2) × 10 = 20 minutes (time per scale) 20 × 7 = 140 minutes (time range of one-page graph)		
	If total observation time cannot fit in one page, scroll bar appears at the bottom of the graph.		
Intensity Range	Select the intensity range (vertical axis) of one-page graph from the pull-down menu. Fit: full range of measured intensity fits in one-page graph.		
	 1×, 2×, 3×, and 4×: calculated from the following equations when one unit is the range of 0 to the maximum intensity (measurement result)/4. Intensity range of one-page graph= (4/value of Intensity Range) × one unit 		
	Example: when value of Intensity Range is 2× and one unit is 600 (Intensity: 0 to 2400): (4/2) × 600 = 1200 (intensity range for one-page graph)		
	When value of Intensity Range is 2× or more, a scroll bar appears at the right side of the graph.		
Grid button	Select to display or hide grid line on graph.		
Calculate button	Select to calculate intensity value.		
	If this button is clicked after an area of an observation image is selected, the intensity graph for the selected area is displayed.		
	See Section 4.1.8, "Intensity Graph Displaying Method".		

4.1.8 Intensity Graph Displaying Method

Intensity graph of an image can be displayed during a time-lapse experiment or by selecting an area (by specifying an area using the ROI mark) in the display area of a time-lapse result file.

The procedure to show the intensity graph for the Channels display is different from that for the Points display.

Channels display

1. Select the area of image to display the intensity graph.

Drag (left click and drag) a marquee around the area for the intensity graph display.

When performed in one display area, the same areas in other three displays are selected at the same time.

Image area setting for an intensity graph

When only the phase contrast (Ph) filter is selected for the microscopy, no image area can be set for an intensity graph because the intensity analysis is not necessary.



Figure 4.1-18 Selecting the area of image to display an intensity graph

Note on setting the ROI mark

• In the scroll mode or the clipping mode, no ROI mark can be drawn on the image area. Therefore, cancel the scroll mode or the clipping mode to draw an ROI mark.

2. Display the intensity graph.

Click the Calculate button. The intensity graph of the selected area is displayed in the intensity graph display area.

When the intensity graph is displayed, the selected area frame changes from yellow to red.

Display area of an intensity graph

For information on the time (horizontal axis) and the intensity (vertical axis) of the intensity graph, see Section 4.1.7, "Intensity Graph".



Figure 4.1-20 Displaying the intensity graph

Points view

1. Select the area of image to display intensity graph.

Drag (left click and drag) a marquee around the area for intensity graph display.

Different points and range for intensity graph display can be set to each observation point.

Image area setting for an intensity graph

When only the phase contrast (Ph) filter is selected for the microscopy, no image area can be set for an intensity graph because the intensity analysis is not necessary.



Figure 4.1-21 Selecting the area of image to display an intensity graph

Note on setting the ROI mark

• In the scroll mode or the clipping mode, no ROI mark can be drawn on the image area. Therefore, cancel the scroll mode or the clipping mode to draw an ROI mark.

2. Select the observation point to display the intensity graph.

Click an observation point of an image to display its intensity graph.

A blue frame appears on the point number of the selected observation image.



Figure 4.1-22 Selecting the area of image to display the intensity graph

3. Display the intensity graph.

Click the Calculate button.

The intensity graph of the selected area is displayed in the intensity graph display area.

When the intensity graph of a selected area is displayed, its frame color changes from yellow to red The frame color of other selected areas remains yellow.

Display area of an intensity graph

For information on the time (horizontal axis) and the intensity (vertical axis) of the intensity graph, see Section 4.1.7, "Intensity Graph".



Figure 4.1-24 Displaying the intensity graph

4.1.9 Time Line Display and Image Playback Operation



Figure 4.1-25 Time line display and image playback control

Table 4.1-13	Functions or	n time line d	lisplay and	image pla	yback control	(1/2)
--------------	--------------	---------------	-------------	-----------	---------------	-------

ltem	Function		
A to B repeating mark	A8	The repeating section set with the Repeat partially button is indicated.	
Number of stream block	Stream block: 3	The number of the stream block of the playback slider's current position is displayed. This field is displayed when playingback of the image captured by stream.	
Number of rounds	Round: 5	The number of rounds of the playback slider's current position is displayed.	
Playback slider	 ▲ Oh 11m 49s	This slider shows the current playback point and elapsed time, and moves in synchronization with the playback image. To play back the image from the point, move this slider to a point with a mouse pointer or the right and left arrow buttons.	
Step buttons	• •	Select to play back the image forward or backward frame by frame.	
Stream block forward buttons	↔ >>	Select to forward or backward to the start position of the stream block. These buttons appear when the image captured by stream capturing is played back.	
Pause button		Select to pause playback of the image.	
Slow playback buttons		Select to slowly playback the image forward or backward. (One to three images per second. The speed changes each time the button is clicked. The number of dots blinking of the button indicates the speed.)	
Playback button		Select to play back the image. (Five images per second)	
Fast-forward button		Select to fast-forward the image. (Six settings with skip intervals of 2, 3, 5, 10, 20, and 30 are available. The reproduction speed changes every time the button is clicked. The circle symbol of the button changes in accordance with the reproduction speed.)	
Marked image playback button		Select to play back ten or more images set before and after the user mark or the cell stimulus mark. When the playback slider is on either mark, images set before and after the mark are played back. When the playback slider is not on either mark, images set before and after the next mark are played back.	
Repeat partially button	A-B	Select to set or clear a repeating section and to repeat the selected repeating section. The A to B repeating mark appears on the selected repeating section. Each time this button is clicked, the function changes as follows: set A point -> set B point -> repeat from A to B -> clear repeating section.	

ltom	Table 4.1-13 Functions on time line display and image playback control (2/2) Euroction			
Item		Function		
User mark setting button	$\overline{\mathbb{V}}$	Select to set the user mark. The user mark is displayed at the current playback point or observation point.		
Cell stimulus mark setting button		Select to set the cell stimulus mark. The cell stimulus mark is displayed at the current playback point or observation point.		
	•	Right click this button to display the submenu for assigning keys. Assignable keys are "Space", "Return", "Shift", and "Tab". Select one from these four keys. And then, the selected key can be used instead of the cell stimulus mark setting button. If the None key is selected, no key is assigned.		
User mark		This mark is displayed at the point set with the user mark setting button. Right click the user mark to display the submenu.Edit DeleteFigure 4.1-27submenu		
		Select Delete from the submenu to delete comments. Select Edit to display the Mark Comment Input dialog box with which you can input, confirm, or change comments.		
	×	Click the Apply button in the Mark Comment Input dialog box to apply the edited comments.		
		Left click the user mark to move the playback slider to the user mark position. Apply Close		
		Figure 4.1-28 Mark Comment Input dialog box		
Cell stimulus mark		This mark is displayed at the point set with the cell stimulus mark setting button. Edit Right click the cell stimulus mark to display the submenu. Figure 4.1-29 submenu		
		Select Delete from the submenu to delete comments. Select Edit to display the Mark Comment Input dialog box with which you can input, confirm, or change comments.		
	Ţ	Click the Apply button in the Mark Comment Input dialog box to apply the edited comments.		
		Left click the cell stimulus mark to move the playback slider to the cell stimulus mark position.		
		Figure 4.1-30 Mark Comment Input dialog box		

Table 4 1 12	Eurotions on t	timo lino dienla	and image n	avback control (2/	21
Table 4.1-13	Functions on i	unie nne uispia	y anu imaye pi	ayback control (2/2	<u>~</u>)

4.1.10 Time-lapse Experiment Scheme (Points Tab)





```
Table 4.1-14 Functions on the Points tab
```

Item	Function		
Observation point	Select to display the observation conditions (filter name, exposure time, and magnification) for each observation point. When time-lapse experiment is performed, only the point selected with a check mark is observed. Click an observation point to display the Point information dialog box. In the dialog box, checking, changing, and deleting detailed condition are available.		
Stop button	Select to stop the running time-lapse experiment. The stop confirmation dialog box appears. To stop time-lapse experiment, click the OK button. Figure 4.1-32 Stop confirmation dialog box		
Resume button	When time-lapse experiment is stopped, the Stop button is changed to the Resume button. Click the Resume button to restart time-lapse experiment.		

Checking observation condition

Only checking the observation condition is available in the Point information dialog box displayed this time.

Point information		×
Ph FII	FI2 20× 414×311 um	
DIA Lamp	150	_
Exposure time	5ms	
Gain	1.00	-
Resolution	640 × 480 Binning	
Dish	1	
x	1344.19 um	
Y	5021.86 um	
z	150.00 um (148.50um - 151.50um, 7steps)	
Comment	test g	
	Close	

Figure 4.1-33 Point information dialog box

Table 4.1-15 Function for checking the observation condition

Item	Function
Close button Select to close the Point information dialog box.	

4.1.11 Time-lapse Experiment Scheme (Time Tab)

When the stream setting is configured during the time-lapse experiment time setting, the display of Time tab is replaced with Stream.

	Points Time Cell name etc				
I		Acquisition cycle	Total time	Rounds	
		0h 20m 00s	10h 00m 00s	31 🕕	elete
	•	0h 00m 10s	0h 10m 00s	61 🕕	elete
		0h 10m 00s	20h 00m 00s	121 🕕	elete
ļ					
	New				

Figure 4.1-34 Time tab

 Table 4.1-16
 Functions on the Time tab

Item	Function		
Time-lapse experiment time	Capturing interval time, total observation time, and rounds for time-lapse experiment are displayed. Click a time-lapse experiment time to display the Timelapse dialog box. In the dialog box, changing and deleting capturing interval time setting and total observation time setting are available.		
Delete button	Select to delete the selected time-lapse experiment time. The Delete confirmation dialog box appears. To delete the selected time-lapse experiment time, click the OK button. To return to the Time tab without deleting, click the Cancel button. However, currently running and already done time-lapse experiment times cannot be deleted as the Delete button becomes disabled.	BioStation Pelete from timelapse list Cancel Cancel Figure 4.1-35 Delete confirmation dialog box	
Red dot	Indicates the time-lapse experiment is running.		
New button	Select to set new time-lapse experiment time. The Timelapse dialog box appears for new registratio time.	n of time-lapse experiment	

Details on the Timelapse dialog box imelanse 10 minutes -Acquisition cycle Operation of the Timelapse dialog box is the same as that of the Total time 20 hours • New time-lapse setting screen. 121 Rounds As soon as possible 0h 00m 28s Reference: Chapter 3 "New Time-lapse Setting Screen" Apply Delete Close Section 3.1.4, "Time-lapse Experiment Scheme (Time Tab)" Figure 4.1-36 For setting change Changing or deleting time-lapse experiment time Registering new time-lapse experiment time minutes Acquisition cycle Total time 2 minutes • Rounds 2 As soon as possible 0h 00m 28s Add Close Figure 4.1-37 For new registration



Figure 4.1-38 Time-lapse experiment process display

Table 4.1-17	Functions on the time-lapse experiment process display
	i anotiono on the time tapoe experiment proceede alopiay

Item	Function	
Start time/ estimated end time	Start time and estimated end time of time-lapse experiment are displayed. (month/date/time)	
Time line	Process of time-lapse experiment is displayed with the bar graph.	
Number of rounds	Number of rounds of running time-lapse experiment is displayed. (current number of rounds/total number of rounds)	
Passage of time	Passage of time-lapse experiment time is displayed (current passage of time/total observation time).	
Temperature log button	The logarithmic graph of temperature change since the software is started (the Temperature dialog box) is displayed. This dialog box is the same as the one that is displayed by clicking the Temp button shown in upper right of the window.	
Pause button	Select to pause the running time-lapse experiment.	
	If suspended, the Start time-lapse button on the New time-lapse setting screen changes from Start time-lapse to Restart time-lapse for restart.	
Stop button	Select to stop the running time-lapse experiment.	

Displaying the logarithmic graph of temperature change

Click the Temperature log button (w)) in the time-lapse experiment process area to display the following dialog box.



time-lapse experiment.

Figure 4.1-39 Temperature dialog box

Item	Function
Range Select time range (horizontal axis) of temperature graph.	
Temperature graph	Temperature changes inside of the culture chamber and the humidifier water tank, and that of ambient temperature are shown.
Close button	Select to close the Temperature dialog box.

4.1.13 Captured Image Printing

Click the Print button (📄) to display the Print dialog box.

The Print dialog box changes depending on the observation image conditions (Channels display or Points display).

Use this dialog box to print the images captured with time-lapse experiment.

Print		Print
Select channel		Sele
Ph		V
🔽 FI1		V
🔽 F12		V
🔽 Multi ch		V
🔽 Intensity Gr	aph	v
Print	Cancel	

Print dialog box for Channels display

Print dialog box for Points display

Cancel

Select channel

 Image: Point 1

 Image: Point 2

 Image: Point 3

 Image: Point 4

 Image: Intensity Graph

Print

Figure 4.1-40 Print dialog boxes

Table 4.1-19 Functions of captured image printing

ltem	Function	
Select channel	Select the image to be printed. For Channels display, select a filter. For Points display, select an observation point. The filters that are not specified for the time-lapse experiment condition are disabled.	
Print button	Select to print an image.	
Cancel button	Select to stop printing and close the dialog box.	

4.1.14 Captured Image Saving

Click the Save button (📄) to display the Save image dialog box.

If a clipping area is set, the save confirmation dialog box for the clipped image appears. Click the OK button in the dialog box. Then, the Save image dialog box appears.



Figure 4.1-41 Save image dialog box

Item	Function
Save as(BioStation IM format) button	During time-lapse experiment, this button is disabled because the file is already saved at the beginning of the experiment.
Export images and data (Single point) button	Select to save the time-lapse images captured at a single observation point as image file(s). The Export images and data (Single point) dialog box appears.
Export images and data (Multi points) button	Select to save the time-lapse images captured at multiple observation points as image files. The Export images and data (Multi points) dialog box appears.
Export large images (Multi points) button	Select to save the time-lapse images (Wide field image) as image file(s). The Export large images (Multi points) dialog box appears.
Cancel button	Select to close the Save image dialog box.
Image data save settings for a single observation point (1/2)

Click the Export images and data (Single point) button of the Save image dialog box to display the Export images and data (Single point) dialog box.

Export images and data (Single point)	×
Export images and data of Point 1 and 20x	•
Save time range	
O All	
C One shot of current time	
C Neighbors of current time	
Concatenate all marks	
Input time range	
Round 1 to 25	
Time 0:00:00 to 2:47:59	
Save channel	
I▼ Ph	
🔽 FI1 (🗖 use pseudo color)	
✓ FI2 (□ use pseudo color)	
📕 Multi ch	
🔲 Intensity data	
✓ Time-lapse-log	
Mark comment	
Next Back Cance	1

-+ i, 1 1-12 E d data (Sinala int) dial . . -:

Table	-		ges and data (Single point) dialog box save settings for a single observation point (1/2)	
Item	Function			
Export images and data of	Select the observation point and magnification to save the image from the pull-down menu.			
Save time range	All		Select to save all images of the selected observation point under the selected magnification in a file.	
	One shot of current time		Select to save only the currently displayed image in a file.	
	Neighbors of current time Concatenate all marks		Select to save the currently displayed image, the five preceding images, and the five following images (total of 11 images) into a file.	
			Select to save all concatenated images into a file. The concatenated images consist of images of all marks including user marks and cell stimulate marks added in the Time Line, five preceding images of each mark, and five following images of each mark. If neither user mark nor cell stimulate mark is set, this item is disabled.	
	Input time range	Round	Saves the image in the specified round range as a file. According to the round count specified in these fields, the time of the Time field is automatically set.	
	Time		Saves the image within the specified shooting time as a file. According to the time specified in these fields, the count of the Round field is automatically set.	

67

ltem	Function			
Save channel	Ph	Select to save the Ph channel data into a file.		
	FI1	Select to save the FI1 channel data into a file. To save the image with the pseudo color, check the use pseudo color checkbox.		
	FI2	Select to save the FI2 channel data into a file. To save the image with the pseudo color, check the use pseudo color checkbox.		
	Multi ch	Select to save overlaid image composed of any of the Ph, FI1, or FI2 channel.		
	Intensity data	Select to save the intensity data into a file. This option is enabled only when the intensity is calculated.		
	Time-lapse-log	Select to save the time-lapse experiment log into a file.		
	Mark comment	Select to save the comments of the user mark and cell stimulus marks. This option is enabled only when the user mark or the cell stimulus mark is used.		
Next button	Select to display the Save images (Single point) dialog box. In the dialog box, specify the destination to save image and input its file name and file format.			
Back button	Select to return to the Save image dialog box.			
Cancel button	Select to close the Exp	Select to close the Export images and data (Single point) dialog box.		

Table 4.1-21 Functions of image data save settings for a single observation point (2/2)

Image data save settings for a single observation point (2/2)

Click the Next button of the Export images and data (Single point) dialog box to display the Save images (Single point) dialog box.

Save images (Single point)			
Folder is and Settings\Cimon\My Documents\			
Save image			
Format AVI(Gray:8bit Color:24bit)			
AVI settings			
🔿 Interval Time 👖 🚔 fps			
Total Time 25 ÷ sec			
Frequency every 2 frames			
Filename			
For Ph test_sach080512_ch1			
For FI1 test_sach080512_ch2			
For FI2 test_sach080512_ch3			
For Multi ch			
⊢ 🔽 Save z-stack images			
According focus setting			
C According range setting			
From -0.50 💌 To +0.50 💌			
Advanced settings			
└────────────────────────────────────			
Format CSV			
Filename			
└── Time-lapse-log			
Filename test_sach080512_timelapse .csv			
Mark comment			
Filename .csv			
Save Back Cancel			

Figure 4.1-43 Save images (Single point) dialog box

Table 4.1-22 Functions of image data save settings for a single observation point (1/2)

Item	Function		
Folder	Select a folder to sav Click the button For Folder dialog box destination to save.	to display the Browse	
Save image	Format	Select a format from six formats to save images. TIFF (Gray: 16 bits/Color: 24 bits), TIFF (Gray: 8 bits/Color: 24 bits), BMP (Gray: 8 bits/Color: 24 bits), PNG (Gray: 8 bits /Color: 24 bits), JPEG (Gray: 8 bits/Color: 24 bits), or AVI	

Item	Function			
Save image	AVI settings	Enabled when "AVI" is selected in the Format field.		
		Interval Time:	Specifies the frame rate (number of frames displayed per second) at which pictures taken are saved into an AVI file (maximum frames: 60).	
		Total Time:	Specifies the time during which pictures taken are saved into an AVI file. The number of frames and total time are calculated, and if the frame rate is above 33 fps, an error is indicated.	
		Frequency every:	•	
	Filename	Input a file name to save images. Channel names will be added to the end of file names automatically. Ph: _ch1, FI1: _ch2, FI2: _ch2, Multi: _ch4		
	Save z-stack images	Enables only the Z-stack images. Select to enable the Z-stack save settings.		
		 According focus setting: Saves images at the Z position registered by the Z focus mark button. According range setting: Saves images including Z-stack data. By changing the values of the From/To fields, the Z-stack range to save the image can be specified. Displays the Save images (Advanced settings) dialog. This allows you to configure, for example, information to be written into images to be saved. 		
	Advanced settings button			
Save Intensity data	Format	Select a format from the pull-down menu to save the intensity graph.		
	Filename Input a file name to save the The suffix, "_intensity", is add automatically.		to save the intensity graph. sity", is added to the end of the file name	
Time-lapse log	Input a file name to save a log file (text file) of time-lapse experiment. The suffix, "_timelapse", is added to the end of the file name automatically.			
Mark comment	Input a file name to save comments of the user marks and cell stimulus marks. The suffix, "_mark", is added to the end of the file name automatically.			
Save button	Select to save the image into a file according to setting. After saving is succeeded, the dialog box appears. Click the OK button to close the dialog box.			
Back button	Select to return to the	Export images and	Figure 4.1-45 Save succeeded dialog box d data (Single point) dialog box.	
Cancel button				
	Select to close the Save images (Single point) dialog box.			

How to write information into images:

Use the Save images (Advanced settings) dialog box that can be displayed with the advanced settings... button.

If "TIFF (Gray: 16 bits/Color: 24 bits)" is selected in the Format field, writing information is disabled. To enter the information on the images to be saved, select "Gray: 8 bits/Color: 24 bits" in the Format field.

Save images (Advanced set	tings) 🛛 🔀
Image processing	
Apply tone curve proces	sing
🔽 Write current ROI into in	ages
🔽 Add ratio color bar (only	ratio image)
└ ─ ─ Overlay text ─────	
Observation settings	left-upper 💌
🔽 Scale & User Mark	left-lower 💌
🔽 Cell name etc	right-upper 💌
Round information	right-lower 💌
Use font setting	
Name	Size
Arial	9
Arial	9
Arial Black Arial Narrow	
Arial Rounded MT Bo	
Batang BatangChe	14
Plackaddar TC	10
Color	Set Default
ОК	Cancel

Figure 4.1-46 Save images (Advanced settings) dialog box

Table 4.1-23 Settings for writing information into images (1/2)

Item	Function		
Image processing	Apply tone curve processing	Select to save the image that underwent tone curve adjustment. Not available when saving a Wide field image.	
	Write current ROI into images	Select to write the ROI mark into images for the intensity graph. Not available when saving a Wide field image.	
	Add ratio color bar	Select to add a color bar to an image for which the ratio is set. The color bar appears on the right side of the image. Not available when saving a Wide field image.	
Overlay text	Check this checkbox to enable the following checkboxes to specify the write position for each item to be written into the image. For the image in which the information is written, see Section 4.1.15, "Example of a Saved Image".Observation settingsSelect to write the observation conditions such as the exposure time, gain, and resolution and select their position of display.		
	Scale & User Mark	Select to write the scale and user mark, and select their position of display. Not available when saving a Wide field image.	
	Cell name etc	Select to write the cell name and etc, and select their position of display.	
	Round information	Select to write the number of round, capturing date, and passage of capturing time, and select their position of display.	

Item	Function			
Use font setting	Select to enable the following checkboxes to specify the font, size, and color of the characters for the image to be written.			
	Name	Specifies font of the character written into the image.		
	Size	Size Specifies size of the character written into the image.		
	Color Specifies color of the character written into the image.			
	Set Default	Restores the character settings written into the image to the default settings.		
OK button	Select to save the settings and to return to the Save images (Single point) dialog.			
Cancel button	Select to close the Save images (Advanced settings) dialog box.			

 Table 4.1-23
 Settings for writing information into images (2/2)

Image data save settings for multiple observation points (1/2)

Click the Export images and data (Multi points) button of the Save image dialog box to display the Export images and data (Multi points) dialog box.

Figure 4.1-47 Export images and data (Multi points) dialog box

Table 4404 Functions of ima		into a has a mustic manager (4/0)
Table 4.1-24 Functions of ima	ge data save settings for mult	iple observation points (1/2)

Item	Function			
Select export data	Select ALL Points	Select to highlight all observation points.		
	Point 1 to Point n	All registered observation points appear here. To select desired observation points, uncheck the Select ALL Points checkbox and check desired observation points.		
Save time range	range All Select to save all images of the selected point under the selected magnification in			
	One shot of current time	Select to save only the currently displayed image in a file.		
	Neighbors of current time	Select to save the currently displayed image, the five preceding images, and the five following images (total of 11 images) into a file.		
	Concatenate all marks	Select to save all concatenated images into a file. The concatenated images consist of images of all marks including user marks and cell stimulate marks added in the Time Line, five preceding images of each mark, and five following images of each mark. If neither user mark nor cell stimulate mark is set, this item is disabled.		

Item		Function		
Save time range	Input time range	Round	Saves the image in the specified round range as a file. According to the round count specified in these fields, the time of the Time field is automatically set.	
		Time	Saves the image within the specified shooting time as a file. According to the time specified in these fields, the count of the Round field is automatically set.	
Save channel	Ph	Select to	o save the Ph channel data into a file.	
	FI1	Select to save the FI1 channel data into a file. To save the image with the pseudo color, check the use pseudo color checkbox.		
	FI2	Select to save the FI2 channel data into a file. To save the image with the pseudo color, check the use ps color checkbox. Select to save overlaid image into a file with the displayed condition.		
	Multi ch			
Next button		Select to display the Save images (Multi points) dialog box. In the dialog box, specify the destination to save image and input its file name and file format.		
Back button	Select to return to	Select to return to the Save image dialog box.		
Cancel button	Select to close the Export images and data (Multi point) dialog box.			

Table 4.1-24	Functions of image data save settings for multiple observation points (2/2)
--------------	---

Image data save settings for multiple observation points (2/2)

Click the Next button of the Export images and data (Multi points) dialog box to display the Save images (Multi points) dialog box.

Save images	(Multi points)
Folder	and Settings¥takas¥My Documents¥
_Save image –	
Format	AVI(Gray:8bit Color:24bit)
	AVI settings
	💿 Interval Time 👖 🖶 fps
	🔿 Total Time 100 🗮 sec
	Frequency every 2 frames
Filename	test_time080807_PhFI1FI2_5P
Г	🗖 Save z-stack images —
	According focus setting
	According range setting
	From 🔽 To 🔽
	Advanced settings
Save	Back Cancel

Figure 4.1-48 Save images (Multi points) dialog box

Table 4.1-25 Functions of image data save settings for multiple observation points (1/2)

Item	Function			
Folder	Select a folder to save images. Click the button to display the Browse For Folder dialog box and specify the destination to save.		Browse For Folder	
Save image	Format	Select a format from six formats to save images. TIFF (Gray: 16 bits/Color: 24 bits) TIFF (Gray: 8 bits/Color: 24 bits) BMP (Gray: 8 bits/Color: 24 bits) PNG (Gray: 8 bits/Color: 24 bits) JPEG (Gray: 8 bits/Color: 24 bits) AVI		

Item		Fun	ction	
Save image	AVI setting	Enabled when "AVI" is selected in the Format field.		
		Interval Time:	Specifies the frame rate (number of frames displayed per second) at which pictures taken are saved into an AVI file (maximum frames: 60).	
		Total Time:	Specifies the time during which pictures taken are saved into an AVI file. The number of frames and total time are calculated, and if the frame rate is above 33 fps, an error is indicated.	
		Frequency every:	 Enabled when this checkbox is enabled. (Allowable range: 2 to 99) The thinning-out setting is valid for both Interval Time/Total Time. 	
	Filename	Input a file name to save images. Channel names are added to the end of file names automatically. Ph: _ch1, FI1: _ch2, FI2: _ch2, Multi: _ch4		
	Advanced settings button	This allows you to written into image	(Advanced settings) dialog box appears. configure, for example, information to be to be saved. ow to write information into images" on	
Save button	Select to save the ima according to setting. After saving is succee appears. Click the OK button to	ded, the dialog box		
		Figure 4.1-50 Save succeeded dialog box		
Back button	Select to return to the Export images and data (Multi points) dialog box.			
Cancel button	Select to close the Save images (Multi point) dialog box.			

Table 4.1-25 Functions of image data save settings for multiple observation points (2/2)

Image data save settings for Wide field image (1/2)

Click the Export large images (Multi points) button of the Save image dialog box to display the Export large images (Multi points) dialog box.

Export large images (Multi points)					
_Select exp	ort data				
Target	Point 1-1 and 2	0x	•		
Order	Type1	▼ X 3 ▼	Y 3 🔽		
Preview	1	2	3		
	4	5	6		
	7	8	9		
Save time	range				
💿 All					
O One	shot of current t	ime			
O Neigl	hbors of current	time			
O Conc	atenate all mark:	s			
C Input	time range				
Rou	Round 1 to 4				
Ti	Time 0:00:00 to 0:03:01				
Save channel					
✓ Ph					
🔽 Fl1 (🔲 use pseudo color)					
🔽 FI2 (🔲 use pseudo color)					
🗖 Multi ch					
Next		Back	Cancel		

Figure 4.1-49 Export large images (Multi points) dialog box

Table 4.1-26 Functions of image data save settings for Wide field image (1/2)

Item	Function		
Select export data	Target	Select the first observation point of a Wide field image to save.	
	Order	Select the registration order of observation points in a Wide field image to save.	
		Usually use Type1 when the Register Wide field image points in a batch checkbox is enabled.	
		Use Type2 to Type4 when the points are registered manually one by one.	
		Type1: Left top -> right top -> left bottom -> right bottom	
		Type2: Left top -> right top -> left bottom -> right bottom, turn back on even lines	
		Type3: Left bottom -> right bottom -> left top -> right top	
		Type4: Left bottom -> right bottom -> left top -> right top, turn back on even lines	

Item		Functions of image data save settings for wide field image (2/2)			
	X/Y combo	Solor	t (motrix lil		
Select export data	box	Select (matrix-like) selection area of a Wide field image. It allows you to select any number of images when you want to save a combined image of observation points that are not adjacent.			
		check	Usually, when using the Register Wide field image points in a batch checkbox to register an area, the number of images is automatically selected for registration at the time the Target is selected.		
	Preview	Displa	ays the arra	ay of observation points.	
Save time range	All Select to save all images of the selected observa under the selected magnification in a file.		save all images of the selected observation point esclected magnification in a file.		
	One shot of current time		Select to	save only the currently displayed image in a file.	
	Neighbors of current time		preceding	save the currently displayed image, the five g images, and the five following images (total of 11 nto a file.	
	Concatenate all marks	 Select to save all concatenated images into a file. The concatenated images consist of images of all marks including user marks and cell stimulate marks added in the Time Line, five preceding images of each mark, and five following images of each mark. If neither user mark nor cell stimulate mark is set, this item i disabled. 		eatenated images consist of images of all marks user marks and cell stimulate marks added in the e, five preceding images of each mark, and five images of each mark. user mark nor cell stimulate mark is set, this item is	
	Input time range		Round	Saves the image in the specified round range as a file. According to the round count specified in these fields, the time of the Time field is automatically set.	
		a file.		According to the time specified in these fields, the	
Save channel	Ph		Select to	save the Ph channel data into a file.	
	FI1		To save t	save the FI1 channel data into a file. the image with the pseudo color, check the use olor checkbox.	
	FI2	Select to save the Fl2 channel data into a file. To save the image with the pseudo color, check the use pseudo color checkbox.		the image with the pseudo color, check the use	
	Multi ch	Select to save overlaid image into a file with the displayed condition.			
Next button	Select to display the Save large images (Multi points) dialog box. In the dialog box, specify the destination to save image and input its file name and file format.				
Back button	Select to return to the Save image dialog box.				
Cancel button	Select to close	e the E>	ort large	images (Multi points) dialog box.	

Table 4 1-26	Functions of imag	a data sava sattings	for Wide field image	(2/2)
Table 4.1-20	Functions of imag	e uala save sellings	s for white held inhage	\$ (2/2)

Image data save settings for Wide field image (2/2)

Click the Next button of the Export large images (Multi points) dialog box to display the Save large images (Multi points) dialog box.

Save large i	mages (Multi points) 🛛 🗙
Folder	C:¥Documents and Settings¥0000¥My
_Save image-	·
Format	Tiff(Gray:8bit Color:24bit)
Filename	AVI settings Total Time T fps Total Time 2 sec Frequency every 2 frames test Save z-stack images
	According focus setting According range setting From -1.50 To +1.50
	Advanced settings
Save	Back Cancel

Figure 4.1-50 Save large images (Multi points) dialog box

Table 4.1-27 Functions of image data save settings for Wide field image (1/2)

Item	Function		
Folder	Select a folder to save images. Click the button to display the Browse For Folder dialog box and specify the destination to save.		Browse For Folder Image Ima
Save image	Format	Select a format from six formats to save images. TIFF (Gray:16bit Color:24bit) TIFF (Gray:8bit Color:24bit) BMP (Gray:8bit Color:24bit) PNG (Gray:8bit Color:24bit) JPEG (Gray:8bit Color:24bit) AVI	

Item	Function				
Save image	AVI settings	Enabled when "AVI" is selected in the Format field. Interval Time: Specifies the frame rate (number of frames displayed per second) at which pictures taken are saved into an AVI file (maximum frames: 60).			
		 Total Time: Specifies the time during which pictures are saved into an AVI file. The number of frames and total time are calculated, and if the frame rate is above fps, an error is indicated. Frequency every: Enabled when this checkbox is enabled. (Allowable range: 2 to 99) The thinning-out setting is valid for both Interval Time/Total Time. 			
	FilenameInput a file name to save images. Channel names are added to the end o Ph: _ch1, Fl1: _ch2, Fl2: _ch2, Multi: _ch2		re added to the end of file names automatically.		
	Advanced settingsbutton	This allows you to into images to be	(Advanced settings) dialog box appears. configure, for example, information to be written saved. bw to write information into images", on page 71.		
Save button	to setting. After saving is suc appears.	e image into a file according cceeded, the dialog box on to close the dialog box. Figure 4.1-52 Save succeeded dialog box			
Back button	Select to return to	to the Export large images (Multi points) dialog box.			
Cancel button		se the Save large images (Multi points) dialog box.			

Table 4 1-27	Functions of image data save settings for Wide field image (2/2)	١
	anotione et inlage data ouve oottinge for that inlage (ini	,

4.1.15 Example of a Saved Image



Scale

Displaying the number of rounds, photo date, and passage of photo time

4.2 Points Display

This screen shows process of time-lapse experiment.

One display area is provided for one observation point. Switch the display area to display each filter image and overlapped image. Therefore, up to four observation points can be observed at the same time. Functions other than the way to display images are the same as those for the Time-lapse images in process Screen (Channels).



Figure 4.2-1 Time-lapse images in process screen (Points display)

 Table 4.2-1
 Functions on the Time-lapse images in process screen (Points display)

Item		Function			
(1) Overlapping buttons		Select to highlight filters for references. The selected filter is checked and the image is displayed.			
		Multiple filters can be selected. When multiple filters are selected, the selected filters overlapped image is displayed.			
(2)	Observation	Images at different points can be displayed on the four observation image displays.			
	image display	Additionally, the ROI for intensity analysis can be set for each observation point.			
		Click in the observation image display to select an observation point. The point number at the upper left of the observation image display is marked with a blue box.			
(3)	Selecting observation	Only the image at the checked observation point is displayed on the observation image display.			
	points	Up to four observation points can be displayed at a time.			
(4)	Calculate button	Select to calculate intensity value. If this button is clicked after an area of an observation image is selected, the intensity graph for the selected area is displayed.			
(5)	ROI mark	The ROI mark is normally marked with a yellow frame, and the ROI mark when an intensity graph is displayed is marked with a red frame.			

5 Time-lapse Images Acquired Screen

This screen automatically appears at the end of time-lapse experiment. On this screen, loading and reproducing the saved time-lapse result files are available. There are the Channels display and the Points display for the Time-lapse images Acquired screen. A part of functions is different from those of the Time-lapse images in process screen. This section indicates only the different part.

File compatibility of the saved file of time-lapse result files

Files created by the software of Ver. 1.* to Ver. 2.0 can be read into the software of Ver. 2.1 or later, whereas those created by the software of Ver. 2.1 or later cannot be read into the software of Ver. 1.*.

Additionally, if a file created by the software of Ver. 1.* to Ver. 2.0 is read into the software of Ver. 2.1 or later, it is automatically upgraded to the file format of the version currently used. Once upgraded, it cannot be read into the software of the version by which it was originally created.

5.1 Channels Display



Figure 5.1-1 Time-lapse images Acquired screen (Channels display)

Table 5.1-1 Functions on the Time-lapse images Acquired screen (Channels display) (1/2)

Item		Function		
(1) File load button Select to load the saved time-lapse result file.				
(2)	Observation point and file information	Position of observation point, its X and Y coordinates, loaded file name of time-lapse result file, sample name, and cell name are displayed.		

	Item	Function			
(3)	Time-lapse experiment result	Total time/start time/end time of time-lapse experiment, number of rounds, logarithmic graph of temperature changes, and maximum and minimum values of temperature change are displayed.			
(4)	Temperature log button	Select to display the logarithmic graph of temperature change (the Temperature dialog box) during time-lapse experiment. For functions of the Temperature dialog box, see "Displaying the logarithmic graph of temperature change" on page 64.			
		Clicking the Temperature log button on the Time-lapse images in process screen displays the current temperature status during time-lapse experiment; however, clicking the Temperature log button on the Time-lapse images Acquired screen displays the temperature status during time-lapse experiment result.			
(5)	Save button	Select to save a time-lapse result file as an alias file, or to save an image captured with the time-lapse experiment into an image file. The Save button on the Time-lapse images Acquired screen has a shortcut key setting (Ctrl+S key on the keyboard). If a clipping area is set, the save confirmation dialog box for the clipped image appears. Click the OK button in the dialog box. Then, the Save image dialog box appears. To cancel the save operation and return to the Time-lapse images Acquired screen, click the Cancel button.			
		If a clipping area is not set, this dialog box does not appear, but the Save image dialog box appears.			
(6)	Fluctuation correction button	elect to measure the fluctuation amount of the stage from a captured image nd correct the image by shifting it in the X and Y directions. In principle, the nage size is reduced by the shift amount. The area shifted is marked with a ack frame.			
		Corrected image data is saved in a new file.			

Table 5.1-1 Functions on the Time-lapse images Acquired screen (Channels display) (2/2)

5.1.1 Saving Time-lapse Result File as a New File and Saving a Captured Image

Click the Save button (📄) or press the Ctrl+S key on the keyboard shows the Save image dialog box.

When a clipping area is set and the OK button is clicked on the save confirmation dialog box for a clipping image, the Save image dialog box appears.



Figure 5.1-3 Save image dialog box

Table 5.1-2	Functions for saving time-lapse result file as a new file and saving a captured image	
-------------	---	--

ltem	Function		
Save as	Select to save the time-lapse result file as a new file.		
(BioStation IM format) button	The Save into Bio Station IM format (ics/ids) dialog box appears.		
Export images and data (Single point) button	Select to save the time-lapse images captured at a single observation point as image file(s). The Export images and data (Single point) dialog box appears.		
Export images and data	Select to save the time-lapse images captured at multiple observation points as image files.		
(Multi points) button	The Export images and data (Multi points) dialog box appears.		
Export large images	Select to save the time-lapse images (Wide field image) as image file(s).		
(Multi points) button	The Export large images (Multi points) dialog box appears.		
Cancel button	Select to close the dialog box.		

Export images and data (Single point) dialog box and Export images and data (Multi points) dialog box

Operations of the two Export images and data dialog boxes are the same as the Time-lapse images in process screen.

Refer to the following sections: Chapter 4, "Time-lapse Images in Process Screen"

Section 4.1.14, "Captured Image Saving" Image data save settings for a single observation point (1/2) and (2/2) Image data save settings for multiple observation points (1/2) and (2/2)

Export images and data of	Point 1 and 20x
Save time range	
O AI	
O One shot of current t	time
C Neighbors of current	time
Concatenate all mark	ks
Input time range	
Round 1	▲ to 25 ▲
Time 0:00:00	to 2:47:59
Save channel	
🔽 Ph	
🔽 Fl1 (🗖 use ps	eudo color)
🔽 FI2 (🗖 use ps	eudo color)
📕 Multi ch	
🔲 Intensity data	
✓ Time-lapse-log	
Mark comment	
Next	Back Cancel

Saving into the Bio Station IM format

Click the Save into Bio Station IM format (ics/ids) button of the Save image dialog box to display the Save into Bio Station IM format (ics/ids) dialog box.

Save into Bio Station IM format (ics/ids) 🛛 🛛 🔀				
Folder F:\lmage\				
Filename test				
Save time range				
C All				
C One shot of current time				
C Neighbors of current time				
Concatenate all marks				
Input time range				
Round 1 🔶 to 25				
Time 0:00:00 to 2:47:59				
Save points				
🔽 Select ALL Points				
Point 1				
Point 2 Point 3				
Point 4				
Save tone curve processing parameter				
Save BOI information				
Save Back Cancel				

Figure 5.1-5 Save into Bio Station IM format (ics/ids) dialog box

Table 5.1-3 Functions on the Save into Bio Station IM format dialog box (1/2)

ltem	Function
Folder	Select a folder to save an image. Click the button to display the Browse For Folder dialog box and specify the destination to save.
Filename	Input a file name for the time-lapse result file.

Item	Function			
Save time range	All		Select to save all images of the selected observation point under the selected magnification into a file.	
	One shot of current time		Select to save only the currently displayed image in a file.	
	Neighbors of curre	ent time	Select to save the currently displayed image, the five preceding images, and the five following images (total of 11 images) into a file.	
	The ma ma ima eao If n		Select to save all concatenated images into a file. The concatenated images consist of images of all marks including user marks and cell stimulate marks added in the Time Line, five preceding images of each mark, and five following images of each mark. If neither user mark nor cell stimulate mark is set, this item is disabled.	
	Input time range	Round	Saves the image in the specified round range as a file. According to the round count specified in these fields, the time of the Time field is automatically set.	
		Time	Saves the image within the specified shooting time as a file. According to the time specified in these fields, the count of the Round field is automatically set.	
Save points	Select an observa	tion point	to save.	
Save tone curve processing parameter	Select to save the tone curve adjustment parameter. Only tone curve adjustment parameter is saved. The image to which the tone curve adjustment applied is not saved.			
Save ROI information	Select to save positions of the ROI set for each observation point.			
Save button	Select to save images into a file according to settings. When the file is saved successfully, a dialog box appears. Click the OK button to close the dialog box.			
Back button	Select to return to the Save image dialog box.			
Cancel button	Select to cancel saving and close the Save into Bio Station IM format (ics/ids) dialog box.			

Table 5.1-3 Functions on the Save into Bio Station IM format dialog box (2/2)

5.2 Points Display

On this screen, one observation point occupies one display area. A filtered image or an overlapped filtered image of the observation point can be displayed on the area when specified. Up to four observation points can be observed at the same time.

Functions other than the way to display images are the same as those for the Time-lapse images in process Screen (Channels).



Figure 5.2-1 Time-lapse images Acquired screen (Points display)

Table 5.2-1	Functions on the	Time-lapse images	Acquired screen (Points display)
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Item		Function
(1)	Screen switch button	Select to highlight filters for references. The selected filter is checked and the image is displayed.
		Multiple filters can be selected. When multiple filters are selected, the selected filters overlapped image is displayed.
(2)	Observation image display	Images at different points can be displayed on the four observation image displays.
		Additionally, the ROI for intensity analysis can be set for each observation point.
		Click in the observation image display to select an observation point. The point number at the upper left of the observation image display is marked with a blue box.
(3)	Selecting observation points	Only the image at the checked observation point is displayed on the observation image display.
		Up to four observation points can be displayed at a time.
(4)	Calculate button	Click this button to calculate intensity value. If this button is clicked after an area of an observation image is selected, the intensity graph for the selected area is displayed.
(5)	ROI mark	The ROI mark is normally marked with a yellow frame, and the ROI mark when a intensity graph is displayed is marked with a red frame.

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株式会社ニコンインステック 本 社 〒100-0006 東京都千代田区有楽町 1-12-1(新有楽町ビル 4F) 電話: (03)3216-9171 (産来機器) 電話: (03)3216-9171 (産来機器)

札幌営業所 〒060-0051 札幌市中央区南1条東2-8-2(SRビル8F) 電話: (011)281-2535(パイオサイエンス・産業機器)

名古屋営業所 〒465-0093 名古屋市名東区一社 3-86 (クエストビル 2F) 電話: (052) 709-6851 (パイオサイエンス・産業機器)

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NIIKON INSTRUMENTS INC. 1300 Walt Whitman Road, Melvile, N.Y. 11747-3064, U.S.A. tel. +1-631-547-8500

NIKON INSTRUMENTS EUROPE B.V. Laan van Kronenburg 2, 1183 AS Amstelveen, The Netherlands tel. +31-20-44-96-300

NIKON INSTRUMENTS(SHANGHAI)CO.,LTD. tel. +86-21-6841-2050 NIKON SINGAPORE PTE LTD tel. +65-6559-3618

NIKON MALAYSIA SDN BHD tel. +60-3-7809-3688

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NIKON INDIA PRIVATE LIMITED tel. +91-124-4688500

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NIKON AG tel. +41-43 277-28-67 NIKON UK LTD. tel. +44-208-247-1717

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NIKON BELUX tel. +32-2-705-56-65

NIKON METROLOGY, INC. 12701 Grand River Avenue, Brighton, MI 48116 U.S.A. tel. +1-810-220-4360 sales_us@nikonmetrology.com

NIKON METROLOGY EUROPE NV Geldemaaksebaan 329, 3001 Leuven, Belgium tel. +32-16-74-01-00 sales_europe@nikonmetrology.com

NIKON METROLOGY GMBH tel. +49-6023-91733-0 sales_germany@nikonmetrology.com

NIKON METROLOGY SARL tel. +33-1-60-86-09-76 sales_france@nikonmetrology.com

NIKON METROLOGY UK LTD. tel. +44-1332-811-349 sales_uk@nikonmetrology.com

バイオサイエンス / Bio Science

株式会社ニコン 〒100-8331 実京都干代田区有楽町1-12-1 新有楽町ビル アシストルメンツカンバニー 営業統括部 営業部 パイオサイエンス営業課 電話:(33)326-2375 インストルメンツカンパニー 営業統括部 営業戦略部 パイオサイエンス課 電話:(33)326-2360

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札幌営業所 〒060-0051 札幌市中央区南 1 条東 2-8-2(SR ビル 8F) 電話: (011)281-2535(バイオサイエンス・産業機器) 名古屋営業所 〒465-0093 名古屋市名東区一社 3-86 (クエストビル 2F) 電話:(052)709-6851 (バイオサイエンス・産業機器) 関西支店 〒532-0003 大阪市淀川区宮原3-3-31(上村ニッセイビル) 電話:(06)6394-8801(バイオサイエンス)

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NIIKON INSTRUMENTS INC. 1300 Walt Whitman Road, Melvile, N.Y. 11747-3064, U.S.A. tel. +1-631-547-8500 NIKON INSTRUMENTS EUROPE B.V. Laan van Kronenburg 2, 1183 AS Amstelveen, The Netherlands tel. +31-20-44-96-300 NIKON INSTRUMENTS(SHANGHAI)CO.,LTD. tel. +86-21-6841-2050 NIKON SINGAPORE PTE LTD tel. +65-6559-3618 NIKON MALAYSIA SDN BHD tel. +60-3-7809-3688

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