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M411EN10

Nikon

Cell Culture Observation System

BioStation CT

Ver. 3.8

Instructions

Introduction

Thank you for purchasing a Nikon product.

This instruction manual is written for users of the Nikon Cell Culture Observation System, BioStation CT.

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

- No part of this manual may be reproduced or transmitted in any form without prior written permission from Nikon.
- The contents of this manual are subject to change without notice.
- 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.
- Some of the equipment described in this manual may not be included in the set you have purchased.
- If you intend to use any other equipment with this product, read the manual for that equipment too.
- If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.
- Microsoft, Windows, and Internet Explorer are registered trademarks of Microsoft Corporation in the U.S. and other countries. Other product and company names mentioned in this manual are trademarks or registered trademarks of their respective owners.
- By reading this manual thoroughly before use, this product can be used without the need for any special training. Please kindly contact the distributor if you have any questions or find any errors etc. in the manual.

Unpacking and installation

Only Nikon service staff or the trained staff of a Nikon representative is permitted to unpack, install, and initialize the product.

Product warranty

Please read the product warranty card carefully and check the warranty information.

Nikon will not be liable for damage of any kind to cultured cell conditions in the product.

Contents

Safety Precautions

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

Warning and Caution Symbols Used in This Manual

Although this product is designed and manufactured to be completely safe during use, incorrect usage or failure to follow the safety instructions provided may cause personal injury or property damage. To ensure correct usage, read this manual carefully before using the product. Do not discard this manual and keep it handy for easy reference.

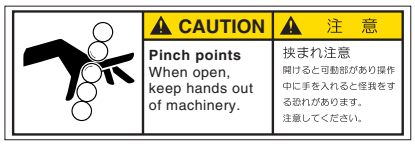
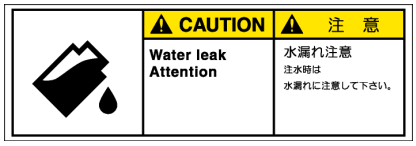
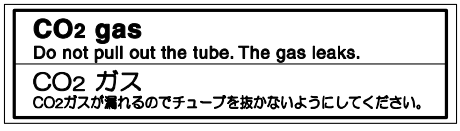
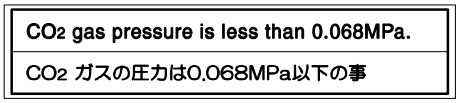
Safety instructions in this manual are marked with the following symbols to highlight their importance. For your safety, always follow the instructions marked with these symbols.

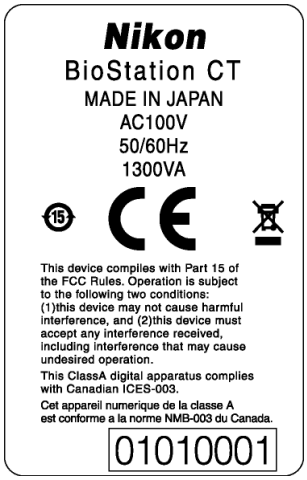
Symbol	Description
⚠ **Warning**	Disregarding instructions marked with this symbol may lead to serious injury or death.
⚠ **Caution**	Disregarding instructions marked with this symbol may lead to injury or property damage.

Warning Labels and Symbols Used on the Product

The symbols on the product mean the need for caution during use. See the instruction manual and read the relevant instructions before operating any part marked with a symbol.

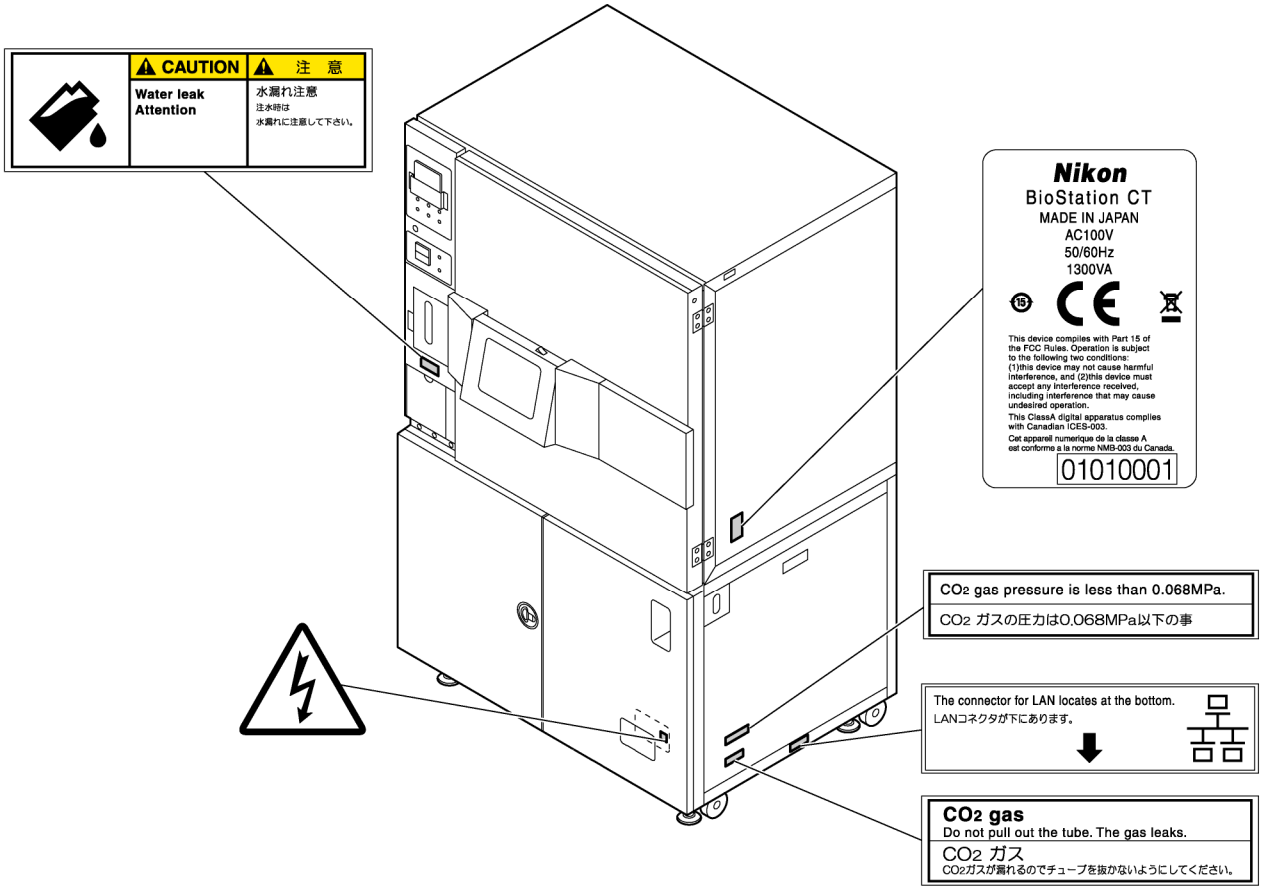
Warning Labels

Warning label/symbol	Detail
	**Risk of electric shock** This symbol is located on the breaker part of the product to call your attention to the following: • High voltage is applied to areas including the breaker terminals and wires. Do not touch these parts to avoid electric shock. • Only trained personnel can operate parts marked with this warning symbol.
	**Risk of pinching hands** This symbol is located on the access gate of the culture chamber calls your attention on the following: • Keep your hands out of the culture chamber during operation to avoid the risk of pinching fingers in movable parts inside the culture chamber.
	**Precautions against heat** This symbol is located on the main glass door of the culture chamber to call your attention to the following: • Parts marked with the symbol become hot during operation. • To prevent burns and fire, do not touch any part marked with this symbol and do not place any flammable material near this part while the product is operating or immediately after it is turned off.
	**Water leak** This symbol is located on the door of the humidifier bottle to call your attention to the following: • Do not pour too much distillated water into the humidifier bottle. • Do not damage the pipe.
	**CO_2 gas** This symbol is located near the bottom on the right side of the product.
	**CO_2 gas supply** Adjust the regulator so that the pressure setting for CO_2 gas supply is 0.068 MPa or lower.

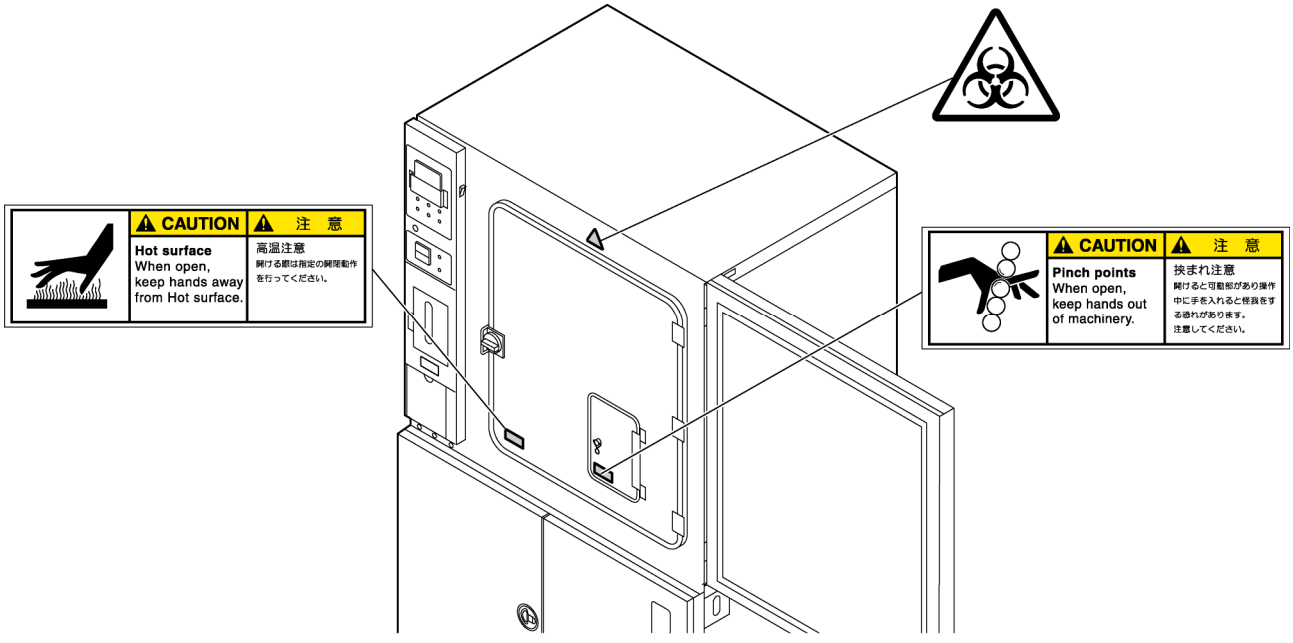
	**General caution** This symbol is located on the side, rear, and top panels of the product to call your attention to the following: • To operate or maintain the product, follow the instructions and cautions shown in the nearby label.
	**Bio-hazard symbol** The product does not have any mechanism or any function against bio-hazard accidents. This symbol is located on the upper part of the main glass door to call your attention to the following: • Hazardous samples may be contained in the CO_2 incubator. Handle the samples with care and follow the safety standards of your facility.
	This symbol is located on the bottom of the right panel and indicates that a LAN connector is provided on the bottom of the product.
	The product nameplate is located on the right panel of the product.

Locations of Warning Labels

Warning labels are affixed to the following locations:



Locations of warning labels



Locations of warning labels (main glass door)

Safety Precautions

Warning

1. Intended application of this product

This product is intended for microscopy and photomicroscopy on cells cultured in cell vessels under conditions suitable for cell culture. Do not use this product for other purpose.

2. Do not disassemble or modify this product.

To prevent electric shock or malfunction, do not disassemble or modify this product. Malfunction and damage due to disassembling or modification are not covered by warranty. Do not disassemble or modify any part other than the parts described in this manual. If you notice any abnormality, contact your nearest Nikon representative.

3. Read the instructions carefully.

To ensure safety, carefully read this manual and the manuals for other equipment used with this product. Be especially sure to follow the warnings and cautions indicated at the start of the manual.

4. Do not use the product in an explosive or combustible gas atmosphere.

Do not use the product in an explosive or combustible gas atmosphere.

An arc may occur in the power on/off operation or in normal operation of the product causing an explosion or fire if used in such atmosphere. This product is not an explosion-proof device.

* For details on combustibles and explosives, see Appendix A, “Hazardous Substances List,” at the end of this manual.

5. Do not use any explosives or inflammables with this product.

Do not use any explosive, inflammable, or substance containing a combustible component with this product.
It may result in explosion or fire.

* For details on combustibles and explosives, see Appendix A, “Hazardous Substances List,” at the end of this manual.

6. Do not use this product in an abnormal state.

If you notice any abnormality such as smoke or an unusual smell, immediately turn off the power and unplug the power cord from the wall outlet. Such an abnormality may result in fire or electric shock.

7. Note on the power supply

The power rating of this product is 100/115/230 VAC and 1300 VA.

Prepare a special wall outlet or a power switch board with enough capacity for this product.

Warning

8. Note on the power cord

- **Use the provided power cord.**
  Be sure to use the provided power cord. Using another power cord may result in malfunction or fire. The product is classified as subject to Class I protection against electrical shock. Make sure the product is connected to an appropriate ground terminal (protective earth terminal).
- **Do not bundle or coil the power cord.**
  Doing so increases the temperature of the cord and may cause a fire.
- **Do not damage the power cord.**
  Do not forcefully bend, pull, or twist the power cord. Doing so damages the power cord and may cause a fire or electric shock.

9. Note on the grounding wire

- **Do not connect the grounding wire to a gas pipe or a water pipe.**
  Doing so may cause a fire.
- **Do not connect the grounding wire to a telephone grounding wire or a lightning conductor.**
  Doing so may cause a fire or electric shock.

10. Note on uninterruptible power supply (UPS)

This product can be used with a UPS. In such a case, do not replace the battery of the UPS by yourself. If the battery needs replacement, contact the supplier of the product.

Additionally, be sure to return the old battery to the supplier of the product. To avoid the risk of explosion, do not discard batteries by yourself. Do not dissolve or cut batteries. Battery electrolyte is hazardous to human health.

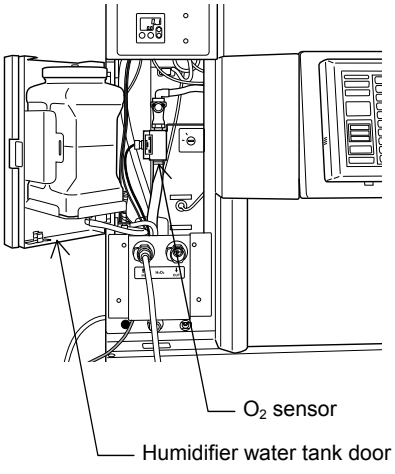
11. Precautions against heat

Do not touch the environmental control units, the humidifier heater, or the illuminator of the observation unit while the product is running. These parts become hot and cause a burn injury if touched. Additionally, to prevent a fire, do not place any liquid or flammable or combustible item near this product.

12. Do not touch the O_2 sensor at high temperatures

The gas sensor becomes hot if the Gas Regulator remains powered on.

Never touch the sensor when opening the humidifier water tank door for the humidifier water tank installed inside the BioStation CT to supply distilled water.



Warning

13. Note on handling CO_2 gas

This product must be connected to a CO_2 cylinder and pressure regulator. Note the following when handling the CO_2 cylinder and pressure regulator:

- **Carefully read the instruction manuals issued by the manufacturers of the CO_2 cylinder and pressure regulator and make sure to follow the instructions.**
- **Install the CO_2 cylinder and pressure regulator and connect the tubes securely so that there is no leakage.**
- **Provide ventilation.**

To use this product safely, make sure to ventilate the room at a flow rate of 3.0m^3/h or more. In addition, in case a problem occurs with the tubes connected to the product to allow use of the recommended regulator, make sure to maintain the CO_2 concentration inside the room at 1000 ppm or less and ventilate the room at a flow rate of 4300 m^3/h or more. Provide sufficient ventilation when placing the product into the room. CO_2 gas flows into the CO_2 incubator to make cell cultures live longer. Ventilate the installation area sufficiently because CO_2 gas flows out from this product.

If sufficient ventilation might not be possible, provide an alarm system that has a CO_2 analyzer or oxygen analyzer.

In addition, CO_2 released in large amounts into the room may cause suffocation. If CO_2 is released, initiate the following safety measures immediately.

- **Leave the room immediately and do not allow others to enter the room.**
- **Inform the security service or fire department.**

14. Note on handling N_2 gas

When this product is used with the optional low-oxygen regulator, an N_2 gas supply, such as a pressurized N_2gas cylinder or an optional N_2 gas generator, must be provided. When the low-oxygen regulator is used, N_2 gas is provided to the incubator of this product. The N_2 gas stored in this product is released into the room at the end. Therefore, a ventilation system with enough capacity must be provided in the room where the product is to be installed. The flow rate for N_2 gas is also applied to the CO_2 gas at this time.

If sufficient ventilation might not be possible, provide an alarm system that has a CO_2 analyzer or oxygen analyzer.

Additionally, in an environment where N_2 gas is used, sufficient caution must be taken to prevent suffocation, such as providing an oxygen analyzer.

Note the following when handling N_2 gas:

- **Carefully read the instructions from the manufacturers of the pressurized N_2 gas cylinder or optional N_2 gas generator supplied with their products, then use their products as instructed with much care.**
- **When installing the pressurized N_2 gas cylinder or optional N_2 gas generator or arranging tubing or piping, take the utmost care to prevent leakage.**
- **Provide ventilation.**

N_2 gas mixes with air easily. If a high concentration of N_2 gas is released into the atmosphere, it reduces the oxygen concentration in the surrounding air. Therefore, if a large amount of N_2 gas is released into the room, it can cause a danger of suffocation. In that case, immediately take the following safety measures:

- **Leave the room immediately and do not allow others to enter the room.**
- **Inform security service or fire department.**

Warning

15. Note on handing a hazardous sample

Before handling a biological sample, its risk must be checked.

Before using hazardous sample with this product, consult your safety supervisor or the safety standard for your facility. When a potentially infectious sample is used, you must wear rubber gloves to avoid direct touch. If such a sample is spilled onto this product, the affected area must be decontaminated in a safe manner. Consult your safety supervisor or the safety standard for your facility.

16. Note on storing and disposing of a hazardous sample

When storing, transporting, or disposing of a hazardous sample, comply with the laws, ordinances, and regulations of international, national, and local governments.

Caution

1. **Isolate this product from the power source when installing, assembling, cabling, and maintaining this product.**

   To prevent electric shock and malfunction, be sure to turn off the main breaker switch of this product and unplug the power cord from the wall outlet before installing, assembling, cabling, or maintaining this product.

2. **Do not wet the product.**

   Do not wet the product. And do not use the product in circumstances where the product is splashed with water. If the product becomes wet, a short circuit may occur resulting damage to the product or causing an abnormal heating. Turn off the main breaker of the product and power switches of peripheral devices immediately. Unplug the power cords from the wall outlet. Then, wipe off the water with a piece of dry cloth. If water enters, stop the use of the product and contact your nearest Nikon representative.

3. **Do not place any object on top of the product.**

   Do not place any object on top of the product or cover it with a piece of cloth or so on. The system temperature will rise, resulting in a malfunction.

4. **Do not block the opening of cooling fans and air vents on the side and the bottom of the product.**

   This product is provided with cooling fans on the side and the bottom and with air vents on the side to cool down the inside of the product. Do not cover the product with a piece of cloth or so on. The system temperature will rise, resulting in a malfunction.

   For installation location of this product, see “2. Installation location” on the next page.

5. **Remove any covers from the product before turning on the product.**

   Do not use the product while covered with a piece of cloth or so on. This will result in an abnormal heating or a fire hazard.

Notes on Handling the Product

1. Handling the product carefully

This product is a precision optical instrument. Handle the product with care to avoid physical shocks and vibrations.
In particular, optical lenses may lose accuracy when even a weak physical shock is applied to them.

Transporting, unpacking, installing, or initializing the product

- Contact your nearest Nikon representative with regard to transporting, unpacking, installing, or initializing the product.

Maintenance

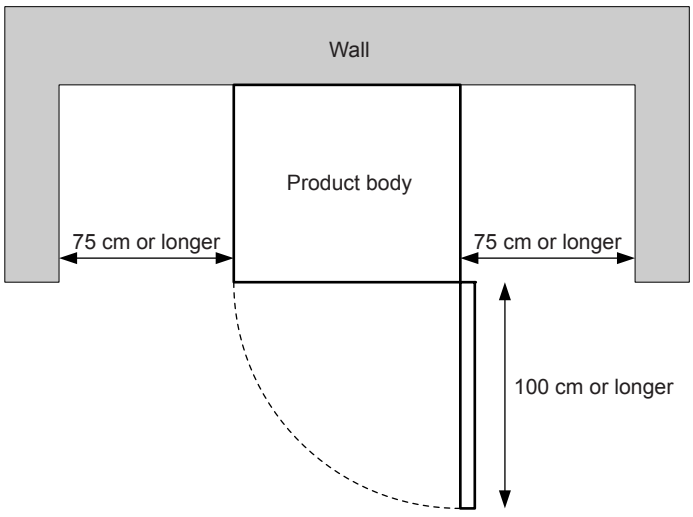
- You must perform the only maintenance items described in Chapter 6, “Daily Maintenance.”
- Take care to avoid pinching your fingers or hand.
- Be careful to avoid scratches or direct contact with the lenses and filters when performing maintenance on observation parts. Scratches or fouling such as fingerprints on optical components such as lenses and filters will degrade images.

2. Installation location

This product is equipped with precision optical devices. Using or storing the product in unsuitable conditions may damage it or may have an adverse effect on its performance. The temperature and humidity of the installation location must be controlled carefully to culture cells properly. Arrange the installation or storage location considering the following conditions:

- Arrange a location where the temperature is from +15 to +28 °C and the relative humidity is 60% or less (no condensation).
- Prepare a sturdy, stable, and level place and install the product there. A maintenance area around the product must be provided as shown in the figure below.

  Note that the product has air vents on both sides. If any other product is placed beside the product, take care that the air vents are not blocked.



- Do not install the product in a location where the product is exposed to direct sunlight, where the product is exposed to direct air from a duct of an air conditioner or so on, or where the temperature changes widely.
- The product must be set level using the four adjustable legs at the four corners of the product bottom. The product must have enough maintenance space above and around the product.
- The product must be installed in an area or a laboratory which has suitable facilities for the risk grade of the samples.

- Avoid areas where many people pass by. Ensure that people are safe even in an emergency.
- Do not install on a dirty floor.
- Do not install in a humid area.
- Do not install in a dusty area.
- Install in a vibration-free area.
- Do not install the product in a narrow space such as a locker or a cabinet.
- Do not install in an area where combustible gas or corrosive gas exists.
- Do not place anything on the product.
- Do not place anything under the product.

* For details on the environmental conditions of the installation and storage location, see Chapter 9, “Specifications.”

3. Thunder

When it starts thundering, immediately turn off the power of the product to prevent malfunction, fire, or electric shock.

4. Handling the optical parts

Scratches or fouling such as fingerprints on optical components (lenses and filters) will degrade microscope images.

Carefully use the optical parts so as not to damage them.

5. Setting the stockers and the carriers

Use only the specified stocker and carrier. Using another stocker or carrier may cause misoperation of the temperature control device. Use the specified stockers and carriers and set them in the proper positions.

6. Cabling the network cable

To prevent electric shock or fire, note the following with regard to cabling the network cable:

- Do not bundle or coil the network cable.
- Do not modify the network cable. Do not bend, twist, or pull the cable with excessive force.
- Do not damage the network cable by placing it under a desk or chair or pinching it with something.
- To prevent fire or electric shock due to a burned cable sheath, do not place the network cable near a thermal appliance such as a heater.
- If the network cable is damaged and the inner wire comes out or the wire is broken, immediately turn off the power to the product and unplug the cable from the cable connector and then contact your nearest Nikon representative to request the cable be replaced.
- Connect the network cable to a connector suitable for the cable.

1 Overview

1.1 Overview of the Product

The Nikon BioStation CT is a cell culture observation system that can observe or photograph cells with an inverted phase contrast microscope built in the CO_2 incubator while culturing cells.

The CO_2 incubator is equipped with a sample loading system and a microscope system to observe cells without unloading them from the CO_2 incubator and to observe them under stable conditions suitable for cell culture.

The microscope system is motorized and can capture cell images with the digital camera built into the system. The motorized stage moves cells, and the autofocus function is used to focus on cells. Therefore, multiple observation points can be set for cells, and culture cell images can be automatically captured in accordance with a specified schedule.

A touch panel LCD is located on the front of the product so that various operations can be performed: cell culture settings, live observation settings, scheduled observation settings, and captured image viewing.

BioStation CT

1.2 Main Features

Basic Function of the CO_2 Incubator

- The incubator maintains the temperature, humidity, and CO_2 concentration at a constant condition to provide appropriate cell culture conditions.
- The CO_2 incubator can incubate up to 30 sample holders set in various vessels such as flasks, dishes, and well plates.
- Samples are loaded through the access gate into the culture chamber in the CO_2 incubator with a special carrier to minimize the environmental change in the CO_2 incubator.

Basic Function of the Microscope

- The product is equipped with an inverted microscope unit. The microscope can perform various microscopies such as APC[*1] phase contrast microscopy.
- Photomicroscopy can be performed under a magnification of 2x, 4x, 10x, 20x, or 40x by switching two objectives and setting an intermediate magnification changer lens.
- The microscope is equipped with a motorized observation stage, which can move in a range of 120 × 86 mm for the microscopic observation area of the microscope.
- The microscope has an autofocus mechanism and a Z-axis drive mechanism to capture multiple images for an observation point by raising or lowering the objective in the Z-axis direction near the focal point.

Scheduled Observation

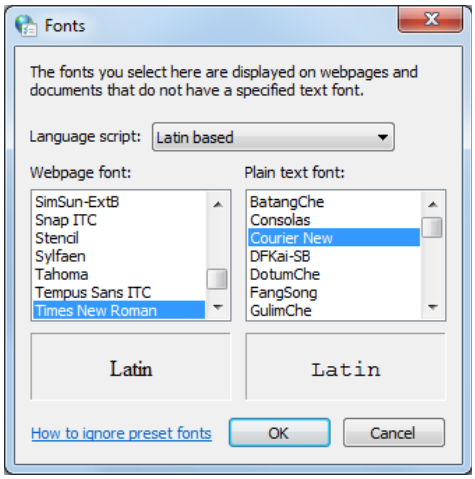
- An observation schedule can be set for each sample. In accordance with the schedule, images at multiple observation points can be captured automatically. Therefore, changes in cells over time can be recorded.

Easy Operation with the Touch Panel

- The touch panel on the front of the product is used to manage samples and set up the product.
- Multiple users can properly manage their samples using the login system. The login system prevents samples from being used mistakenly by other users, as the usage of the sample is limited to the logged in user.

Network Function

- The product can be operated via a network. Samples and the system can be managed, the schedule can be set up, and previously observed images of samples can be viewed from an external PC in the facility.
- Operations can be performed using the Internet browser, Internet Explorer 8 or Internet Explorer 9. Therefore, no special application software is required. However, when the display screen is enlarged, such as with the zoom button in Internet Explorer, the screen layout may collapse. In that case, change the magnification to 100%.
  The screen layout may collapse also depending on the fonts used in Internet Explorer. In that case, change the fonts. It has been confirmed that the screen layout is displayed properly when the default fonts, “Times New Roman” (Web page font) or “Courier New” (Plain text font), are specified for the “Latin based” language script in the font settings of the Internet Options.



- Captured images are recorded with various information, therefore detailed data can be obtained for the images.
- The captured images are downloaded and copied from the product to an external PC. Therefore the original image data remains untouched. This feature is very useful for preventing data tampering.

*1: Apodized phase contrast (APC) microscopy is one type of phase contrast microscopy. It decreases bright circles around the outline of a high phase-contrast object, or halos, and improves the resolution.

Fluorescence Unit (Option)

- The fluorescence unit is equipped with three types of filter cubes that allow the user to observe three different fluorescent molecules, including CFP, GFP, and RFP, and they can be observed in fluorescence observation at the same time. Optionally, up to two types of fluorescence filter cube specified at the time of purchase can be mounted and observation of other fluorescent molecules can be supported.
- The fluorescence unit is equipped with three types of LED as excitation light sources so that three different conditional LED excitation light source settings can be used during observation. Additionally, this product enables you to capture fluorescence images and phase contrast images.
- In the BioStation CT, fluorescence images and phase contrast images can be automatically captured. In this way, the expressions of the fluorescent molecules of the cells will be viewed under more natural conditions.

1.2.1 Overview of the Fluorescence Unit

The fluorescence unit is equipped with the following filters for fluorescence observation and LEDs for excitation light sources.

- Fluorescence observation filters

  The following three types of fluorescence observation filters are provided as standard. Each filter is compatible with one of these channels: Ch1, Ch2 or Ch3.

Fluorescence filter cubes

Channels	Fluorescence Filter Cubes	Model Number	Excitation(nm)		Emission(nm)		Dichroic (nm)
			Center value	Half-value width	Center value	Half-value width	
Ch1	CFP-compatible	CFP-2432C (Semrock)	438	24	483	32	458
Ch2	GFP-compatible	GFP-3035D (Semrock)	472	30	520	35	495
Ch3	RFP-compatible	DsRed2 42005 (Chroma)	540	40	600	50	570

  In addition, up to two types of fluorescence filter cube other than the above can be mounted.

  For details on adding filter cubes, contact your nearest Nikon representative.

- Excitation light sources

  The following three types of LED are equipped as excitation light sources.

Excitation light source (LED)

	Peak Wavelength (nm)
Light source (1)	458
Light source (2)	475
Light source (3)	To 620

In fluorescence image observation, images are captured while the filter is changed for each channel in the same way as in normal epi-fluorescence microscopy.
The optimal excitation wavelength for each filter is set by default in the device as an observation condition per channel.

The following shows the observation conditions for three types of fluorescence filter cubes provided as standard.

2x FL

	Exposure time (Exp time) Unit [100 msec][*1]	Intensity of excitation light source (Maximum value:240)[*2]		
		Luminance (for Ch1)	Luminance (for Ch2)	Luminance (for Ch3)
Ch1	20	200	0	0
Ch2	20	0	200	0
Ch3	20	0	0	200

4x FL

	Exposure time (Exp time) Unit [100 msec][*1]	Intensity of excitation light source (Maximum value:240)[*2]		
		Luminance (for Ch1)	Luminance (for Ch2)	Luminance (for Ch3)
Ch1	4	200	0	0
Ch2	4	0	200	0
Ch3	4	0	0	200

10x FL

	Exposure time (Exp time) Unit [100 msec][*1]	Intensity of excitation light source (Maximum value:240)[*2]		
		Luminance (for Ch1)	Luminance (for Ch2)	Luminance (for Ch3)
Ch1	4	200	0	0
Ch2	4	0	200	0
Ch3	4	0	0	200

20x FL

	Exposure time (Exp time) Unit [100 msec][*1]	Intensity of excitation light source (Maximum value:240)[*2]		
		Luminance (for Ch1)	Luminance (for Ch2)	Luminance (for Ch3)
Ch1	20	200	0	0
Ch2	20	0	200	0
Ch3	20	0	0	200

40x FL

	Exposure time (Exp time) Unit [100 msec][*1]	Intensity of excitation light source (Maximum value:240)[*2]		
		Luminance (for Ch1)	Luminance (for Ch2)	Luminance (for Ch3)
Ch1	40	200	0	0
Ch2	40	0	200	0
Ch3	40	0	0	200

*1 When the exposure time is set as above-mentioned, the calculation becomes 40 × 100[ms] = 4000[ms] = 4[s]. The exposure time becomes (entered value) × 100[ms].

*2 The unit of the excitation light source intensity is non-dimensional, and the maximum value is 240.

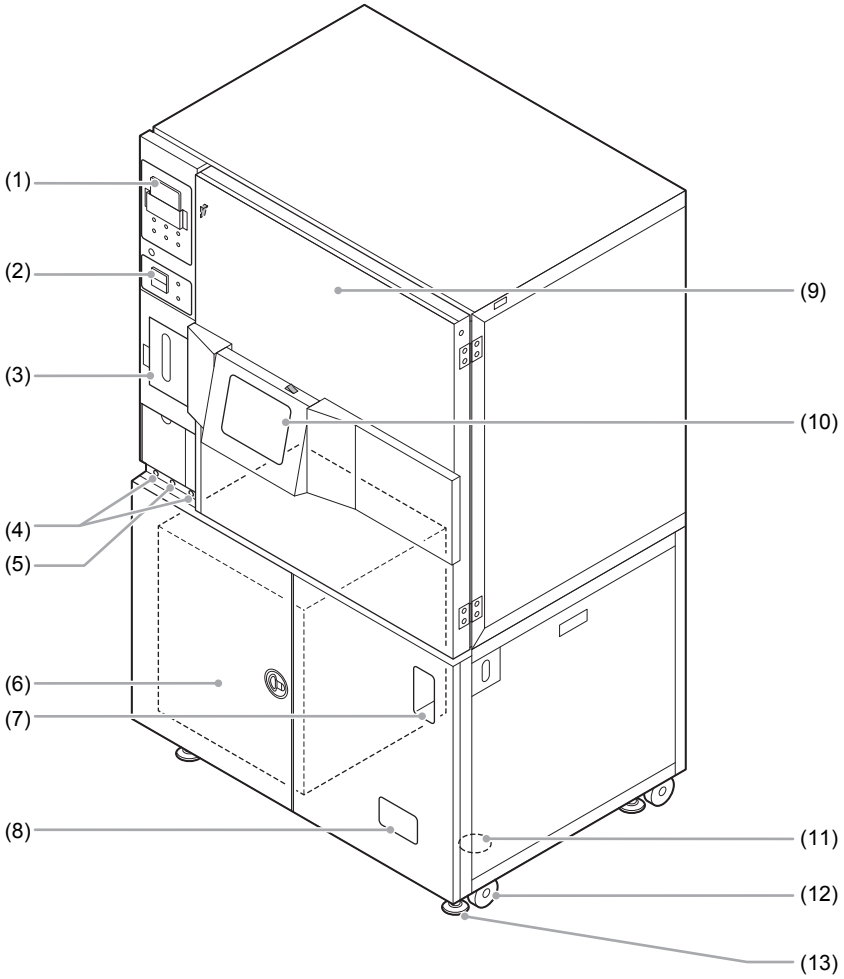
The default observation conditions can be changed in the environmental settings managed by each user (in the User settings window), and settings other than the default luminance can also be added.

For details on the environmental settings, see Chapter 5, “Environmental Settings.”

The conditions set in the environmental settings can also be changed during live observation, if necessary.

2 Name and Function of Each Part

2.1 Front View



Front view

No.	Name	Function
(1)	Temperature/humidity controller	This is the operation panel for controlling the temperature and humidity in the CO_2 incubator, indicating the current values and the setting values. The power switch for the temperature/humidity controller is located on the right of the controller.
(2)	CO_2 concentration controller	This is the operation panel for controlling the concentration of CO_2 in the CO_2 incubator, indicating the current value and the setting value.
(3)	Humidifier water tank door	This door is used to supply distilled water to the humidifier water tank. On the door, a confirmation window is provided for checking the remaining water amount. It is illuminated with a backlight for easy observation.
(4)	Gas In port and Gas Out ports	These ports are used to connect a supply pipe and an exhaust pipe to circulate H_2O_2 gas for sterilization in the CO_2 incubator.

No.	Name	Function
(5)	Drain (coupler)	This is used to purge water from the humidifier before an extended disuse of this product.
(6)	Digital storage part	This part contains the control system. Do not open the digital storage part except when performing maintenance.
(7)	Main power switch	This is a power switch for the movable part.
(8)	Breaker part	A main breaker, a controller breaker, and a CO_2 incubator breaker are provided to this part.
(9)	CO_2 incubator outer door	This is an outer door of the CO_2 incubator, equipped with the touch panel LCD.
(10)	Touch panel LCD	This is a touch panel LCD for controlling the entire system, tiltable by three different angles for ease of operation. The power switch for the LCD is located on the upper side of the display.
(11)	Cable port	This is provided at the bottom of the system and used to pass the power cable, the LAN cable, and the CO_2 pipe.
(12)	Casters	The casters are used to move the product.
(13)	Legs	The legs are used to stabilize the product. They must be securely screwed to hold the product horizontally.

2.2 Inside of the CO_2 Incubator

Main glass door

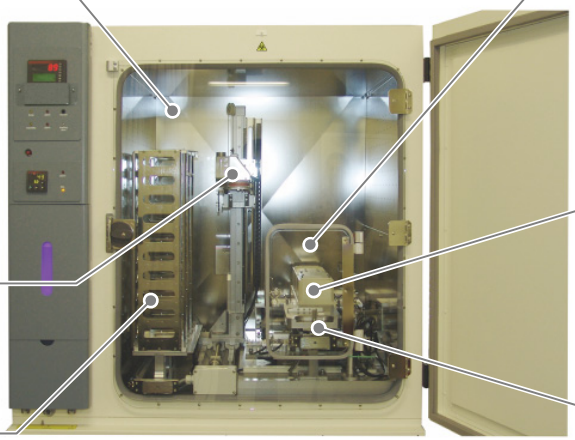
The culture chamber main glass door of strengthened glass is inside the CO_2 incubator outer door. The main glass door must be opened with a special key used only for maintenance work.

Loading unit

This unit loads a sample between the stocker and the observation unit or between the stocker and the carrier.

Stocker

These are three-row and ten-column shelves for storing culture vessels.



Access gate

This door is used for loading/unloading samples. To minimize the environmental change inside the culture chamber caused by opening or closing the door, place a sample on a special holder and load/unload it with a special carrier through the access gate.

Observation part (microscope unit)

This part is composed of a phase contrast microscope, an XY stage, and an illumination unit. The microscope can be used to observe a sample on the XY stage and take pictures.

Carrier slider

This slider transports a carrier to load or unload a sample to/from the culture chamber.

Inside of the CO_2 incubator

If the culture chamber main glass door is left open while cells are culturing, the conditions in the chamber will change and the cells or tissues in the chamber may be adversely affected.

Do not open the door when culturing cells. Usually the door cannot be opened except for maintenance work.

2.2.1 Stockers

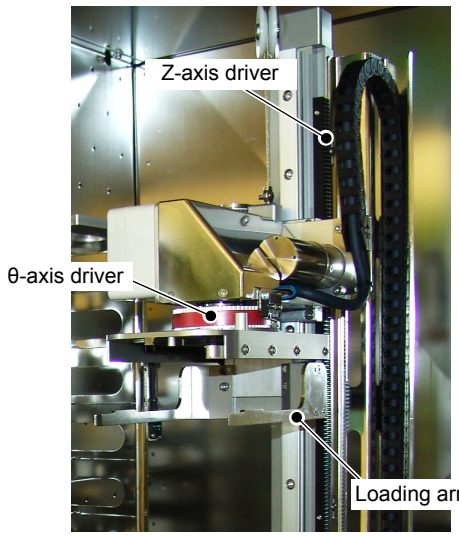
These are three-row and ten-column shelves for storing samples in culture flasks, dishes, and well plates.

Stockers

2.2.2 Loading Unit

The loading unit slowly transports a holder, which contains a sample, between the carrier, a stocker, and the observation part.

> For safety during the transportation, the door cannot be opened or closed using the touch panel display.



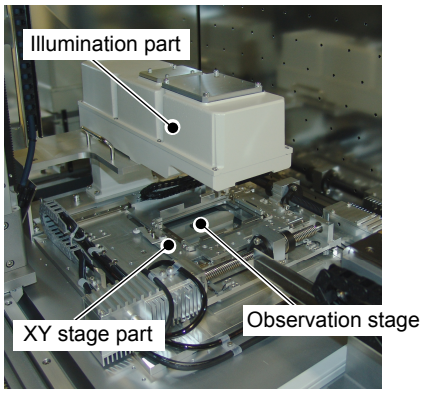
Loading unit

2.2.3 Observation Part

This part is composed of the XY stage, the illumination part, and the microscope part. The microscope part is located under the culture chamber.

The microscope part is provided with an inverted type phase contrast microscope. By changing two objectives and an intermediate variable magnification lens, photomicrography can be performed with a magnification between 2x to 40x. The microscope part is also provided with an autofocus function and an automatic illumination brightness control function.

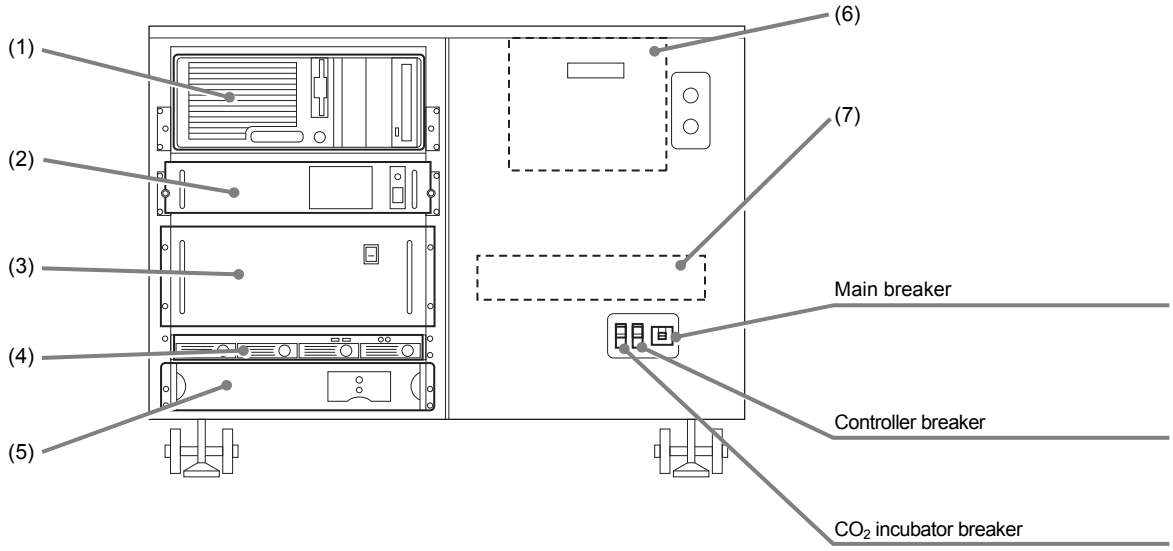
The XY stage part has motors to move the stage in a range of 120 × 86 mm for microscopic observations.



Observation part

2.3 Digital Storage Part

The following units are stored inside the lower front door of the product.



Digital storage part

* The units marked with dotted lines are located inside the product.

No.	Name	Function
(1)	Control PC	The PC controls the entire system in accordance with the operation on the touch panel or network. The PC controls the product in accordance with the specified schedule and take photographs of culture cells automatically.
(2)	Observation part control box	The control box controls the observation part including the microscope unit.
(3)	Loading unit control box	The control box controls the loading unit.
(4)	File server	This file server stores data such as images taken in photomicrography.
(5)	Uninterruptible power supply (UPS)	The UPS supplies power to the control PC and other devices. If the usual power supply to the product stops, the UPS temporarily supplies power from the battery to the product. In such cases, the PC will be shut down automatically.
(6)	Microscope part	The Microscope part is contained in a cover to prevent dust from adhering to the part. To maintain the performance of the microscope, do not open the cover.
(7)	Camera control box	The control box controls the digital camera attached to the microscope.

3 Basic Operation

This chapter describes safety precautions and basic operation of this system.

3.1 Safety Precautions

Warning

Do not use the product in an explosive or combustible gas atmosphere.

An arc may occur in the power on/off operation or in normal operation of the system and an explosion or fire may be caused if used in such atmosphere. This product is not an explosion-proof device.

* **For details on combustibles and explosives, see Appendix A, "Hazardous Substances List," at the end of this manual.**

Warning

Keep your hands out of the culture chamber except when operating the carrier slider to avoid the risk of pinching fingers with the movable part inside the culture chamber.

Caution

This system does not adjust for daylight savings time. As such, depending on the location where this product is used, you may notice a difference between the actual local time and the time shown on the product. Pay attention when setting the schedule observation settings and viewing the observation history.

3.2 Starting and Ending the System

This system is designed under the assumption that the power to this system is always on. If the power is turned off, the CO_2 incubator stops and cells being cultured will be adversely affected. Do not turn off the power to the system in normal use.
Should a power outage cause this system to stop, see Section 8.8, “Power Outage Response and Recovery” for how to perform recovery.

A control PC installed in the product will automatically restart for maintenance at 0:30 a.m. on the 2nd day of every month (midnight on the 1st day).
If observation is scheduled during the automatic restart, the PC will not restart and the scheduled observation will take precedence over the restart.

In addition, the culture conditions are not affected because only the control PC will restart.

3.2.1 Startup Procedure for the First Time

Follow the procedure as described below to start up the system for the first time:

Caution

If a holder is located on the stage at the startup procedure, scheduled observations and live observations cannot be performed. If a holder is found on the stage, open the outer door and the main glass door of the incubator, take out the holder before starting up the system.

(1) Check the connection of the power cord.

Make sure that the power cord of this system is securely plugged in to the wall outlet.

(2) Make sure that the CO_2 cylinder is securely connected.

Make sure that the CO_2 pipe routing from the bottom of the product is securely connected to the CO_2 cylinder. (For details on connecting the CO_2 cylinder, see Section 6.2, “Replacing/Connecting CO_2 Cylinders.”)

(3) Supply distilled water into the humidifier water tank.

(4) Turn on the main breaker.

(5) Turn on the controller breaker.

(6) Turn on the CO_2 incubator breaker.

(7) Turn on the UPS.

(8) Press the ON button for the Main power switch.

Power is supplied throughout the system, and the system starts operation.

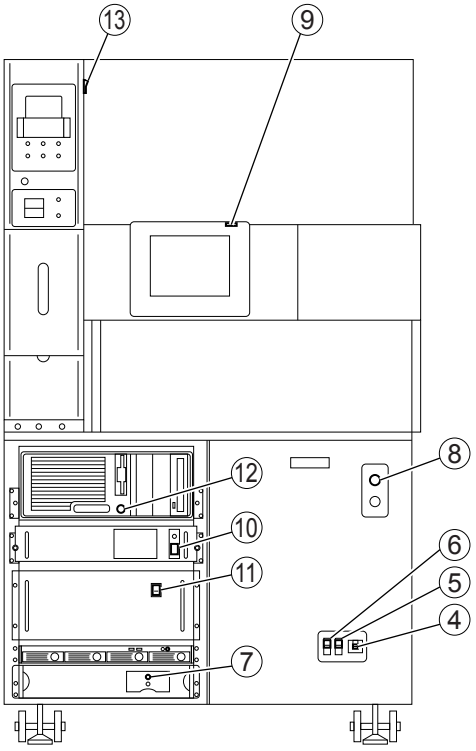
(9) Turn on the touch panel display.

(10) Turn on the observation part control box.

(11) Turn on the loading unit control box.

(12) Turn on the control PC.

(13) Turn on the CO_2 Incubator.



Turning on the system for the first time

(14) Set the CO_2 Incubator.

1. **After setting the temperature to 37°C, set the humidity to OFF.**
2. **Wait for more than 8 hours after the temperature inside the CO_2 incubator is stabilized at 37°C, and then set the humidity inside the CO_2 incubator to 90%RH.**
3. **Wait until the temperature and the humidity in the CO_2 Incubator is stabilized, and then start supplying CO_2.**

   Turn the secondary pressure regulator valve of the CO_2 cylinder in the OPEN direction.

   Check that the CO_2 concentration inside the CO_2 Incubator has reached the set value, and then load the samples.

   For details on setting the temperature and the humidity, see Section 3.3.2, “Setting the Temperature and the Humidity.”

3.2.2 Startup Procedure in Normal Case

To turn on the system again after turning it off for maintenance activities and so on, follow the procedure as described below.

Caution

If a holder is located on the stage at the startup procedure, scheduled observations and live observations cannot be performed. Open the outer door and the main glass door of the CO_2 Incubator, and then move the holder back to the previous position of the stockers before starting up the system.

(1) Check the connection of the power cord.

Make sure that the power cord is securely plugged to the wall outlet.

(2) Turn on the main breaker.

(3) Turn on the controller breaker.

(4) Turn on the CO_2 incubator breaker.

(5) Turn on the UPS.

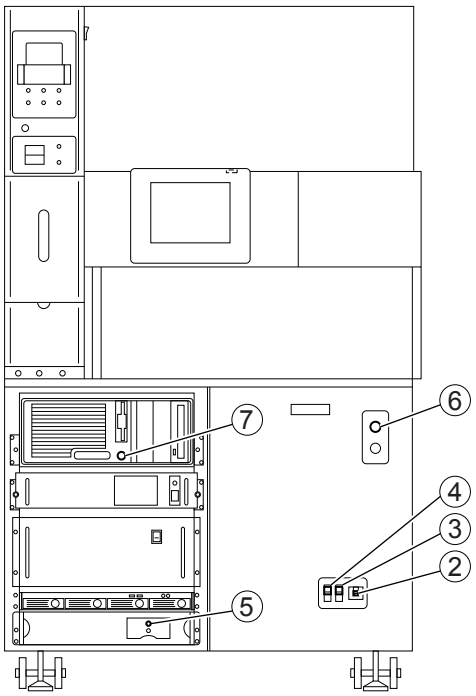
(6) Press the ON button for the main power switch.

Power is supplied throughout the system, and the system starts operation.
The power to the file server is automatically turned on.

(7) Turn on the control PC.

(8) Set the CO_2 Incubator.

For details, see Section 3.2.1 (14), “Startup Procedure for the First Time.”



Turning on the system

3.2.3 Shutdown Procedure in Normal Case

This section describes the procedure for turning off the power to this system. To perform maintenance activities and so on, turn off the system in accordance with the following procedure.

(1) Stop supplying CO_2.

Turn the secondary pressure regulator valve of CO_2 cylinder in the SHUT direction.

(2) Open the Functions window of the Touch panel display and press the Shutdown button.

The Shutdown dialog is displayed. Press the OK button to turn off the control PC. Wait until the file server powers down. (This can take up to several minutes).

> The Shutdown button in the Functions window is not displayed unless an administrator user logs in with the touch panel.

(3) Press the OFF button for the main power switch.

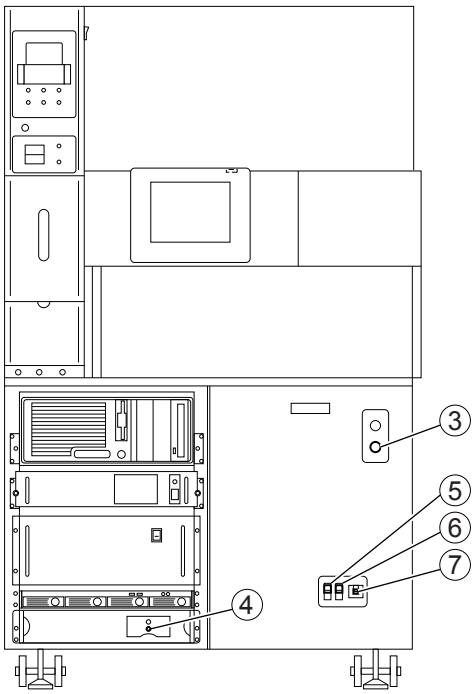
(4) Turn off the UPS.

(5) Turn off the CO_2 incubator breaker.

(6) Turn off the controller breaker.

(7) Turn off the main breaker.

(8) Unplug the power cord from the wall outlet.



Turning off the system

In case of thunder

When it starts thundering, immediately turn off the power to the system and unplug the power cord from the wall outlet.

3.2.4 Shutdown Procedure before Extended Disuse

Before extended disuse, turn off all power switches in accordance with the following procedure.

(1) Stop supplying CO_2.

Turn the secondary pressure regulator valve of CO_2 cylinder in the SHUT direction.

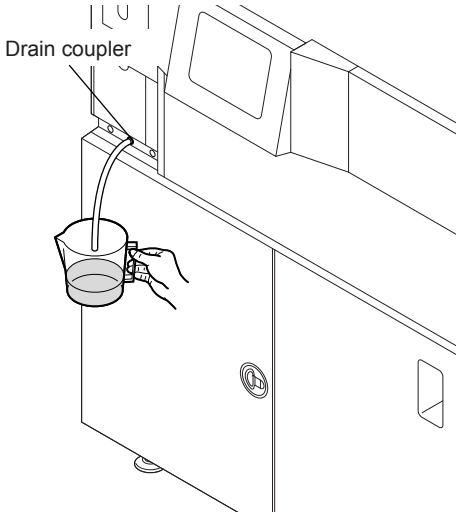
(2) Make sure that all schedules are completed.

If there is an uncompleted scheduled task, wait until it finishes or forcibly finish it.

(3) Open the Functions window of the touch panel display and press the Shutdown button.

The Shutdown dialog box is displayed. Press the OK button to turn off the control PC. Wait until the file server powers down. (This can take up to several minutes.)

> The Shutdown button in the Functions window is not displayed unless an administrator user logs in with the touch panel.



Draining water from the humidifier

(4) Insert the provided tube into the drain coupler on the front of the system, and then purge water from the humidifier.

(5) Press the OFF button of the main power switch.

(6) Open the door of the digital storage unit and turn off the loading unit control box.

(7) Turn off the observation part control box.

(8) Turn off the touch panel display.

(9) Turn off the UPS.

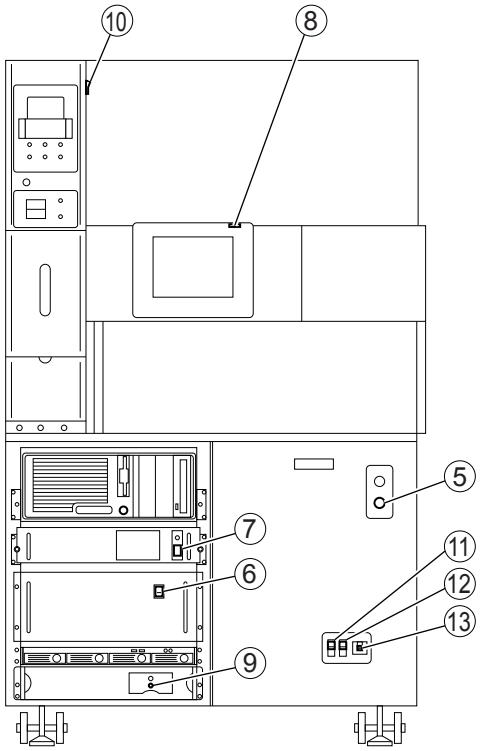
(10) Turn off the CO_2 incubator.

(11) Turn off the CO_2 incubator breaker.

(12) Turn off the controller breaker.

(13) Turn off the main breaker.

(14) Unplug the power cord from the wall outlet.



Turning off the system

3.3 Operating the CO_2 Incubator

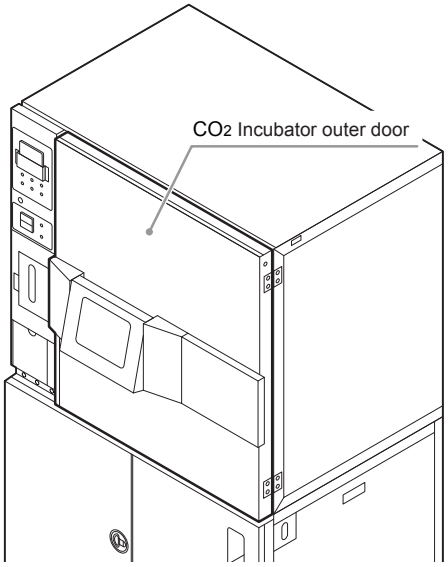
3.3.1 Opening and Closing the Door

Warning

Keep your hands out of the CO_2 incubator during operation to avoid the risk of pinching fingers with the movable part inside the CO_2 incubator.

CO_2 incubator outer door

The CO_2 incubator outer door has an embedded heater to keep the temperature constant. The CO_2 incubator outer door should be closed to minimize the environmental changes inside the incubator when culturing cells.

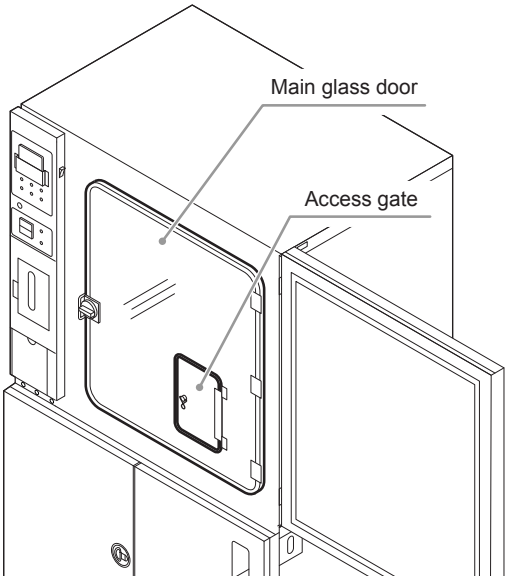


Access gate

When loading/unloading samples into/from the culture chamber in the incubator, use the access gate.

To ensure safety, the access gate is equipped with a lock mechanism so that the door cannot be opened without operating the touch panel display.

For details on how to use the access gate, see Chapter 4, “Operation.”



Main glass door

When performing maintenance work in the culture chamber, open the main glass door of the culture chamber in the incubator. To ensure safety, the main glass door is equipped with a lock mechanism so that the door cannot be opened without turning off all power switches for the product.

For details on the shutdown procedure, see Section 3.2, “Starting and Ending the System.”

3.3.2 Setting the Temperature and the Humidity

Use the Temperature/humidity controller on the left front of the system to set the temperature and humidity in the CO_2 incubator. The current temperature and humidity in the CO_2 incubator are alternately displayed on the display unit on the upper part of the Temperature/humidity controller. The Temperature/humidity settings are displayed in the lower display. Set the temperature and humidity in the CO_2 incubator in accordance with the following procedure.

Setting the temperature

(1) Remove the cover of the Temperature/humidity controller.

(2) Press the ▼ button on the Temperature/humidity controller to move the ▶ mark on the setting value display unit to "SP1."

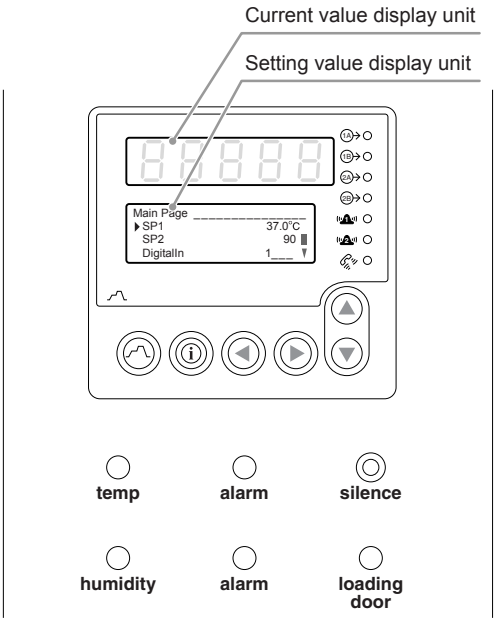
(3) Press the ▶ button to move the cursor to the Temperature value to be changed.

The selected temperature value blinks.

(4) Press the ▲ button or the ▼ button to display the desired temperature.

The temperature setting can be changed by 0.1°C.

(5) Press the ▶ button to set the temperature.



Temperature/humidity controller

Setting the humidity

(1) Remove the cover of the Temperature/humidity controller.

(2) Press the ▼ button on the Temperature/humidity controller to move the ▶ mark on the setting value display unit to "SP2."

(3) Press the ▶ button to move the cursor to the humidity value to be changed.

The selected humidity value blinks.

(4) Press the ▲ button or the ▼ button to display the desired humidity.

The humidity setting can be changed by 1%.

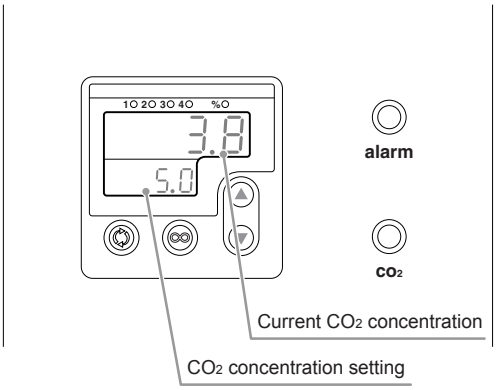
(5) Press the ▶ button to set the humidity.

3.3.3 Setting the CO_2 Concentration

Use the CO_2 concentration controller on the left front of this system to set the CO_2 concentration in the CO_2 incubator. The current CO_2 concentration is displayed in the upper part of the display unit of the CO_2 concentration controller, and the CO_2 concentration setting is displayed in the lower part. Set the CO_2 concentration in the CO_2 incubator in accordance with the following procedure.

(1) Press the ▲ button or the ▼ button to set a CO_2 concentration value.

The CO_2 concentration setting can be changed by 0.1%.



CO_2 concentration controller

For details on how to change the temperature and humidity values, and CO_2 alarm settings, contact your nearest Nikon representative.

Default values of alarm settings

Alarm setting item	Default values	
Temperature	Lower limit: 30°C	Upper limit: 40°C
Humidity	Lower limit: 75%RH	Upper limit: 100%RH
CO_2	Lower limit: 4.0%	Upper limit: 5.5%

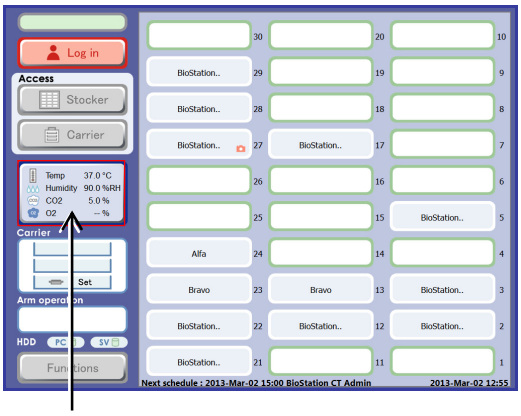
3.3.4 Checking the Environmental Changes

This system records changes in the temperature, humidity, CO_2 concentration and O_2 concentration in the CO_2 incubator. The changes can be shown on the touch panel display in graph format. Perform the following procedure to check environmental changes in the Environmental factor graph window.

Environmental factor graph window

Press the Status button on the touch panel display to display the Environmental factor graph window.

The temperature, humidity, CO_2 concentration and O_2 concentration in the CO_2 incubator are visible on the Status button. The O_2 concentration is displayed as -9.9% when an Oxygen Control unit is not connected, and --% when the option is set to OFF even though an Oxygen Control unit is connected.

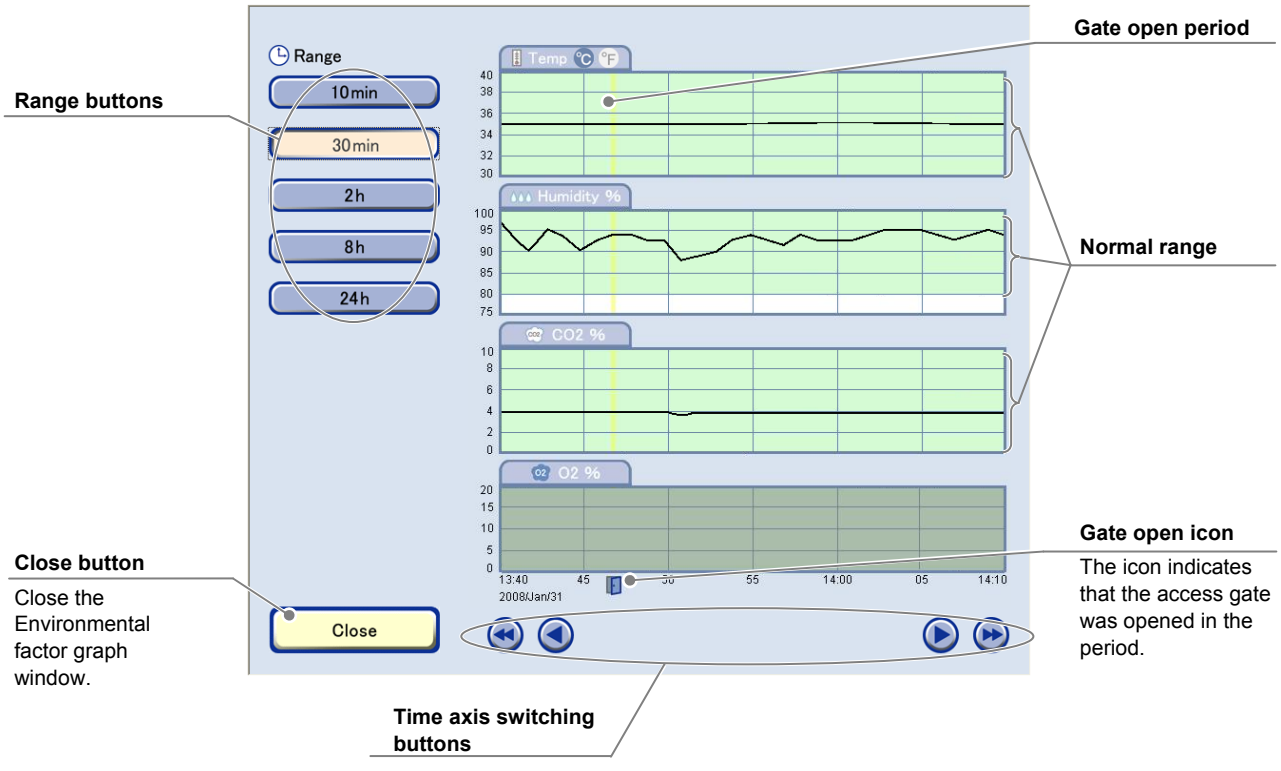


Initial screen (System status screen)

The four graphs for temperature, humidity, CO_2 concentration and O_2 concentration are displayed from the top of the Environmental factor graph window. The O_2 concentration graph is grayed out when an Oxygen Control unit is not connected.

Normal ranges (set in the Alarm settings) are displayed in green on the graphs. If a value is outside the normal range, the corresponding alarm lamp will turn on the temperature/humidity controller and the CO_2 concentration controller. The Alarm lamp can be checked on the BioStation CT.

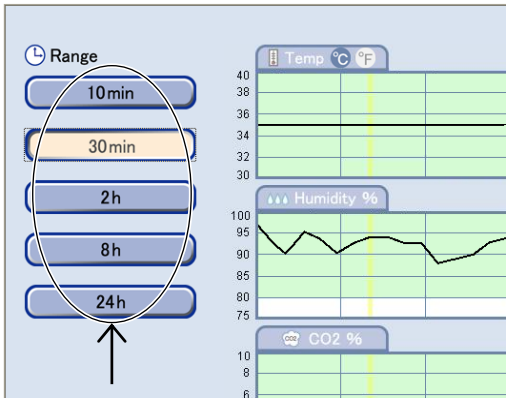
When the access gate is opened, a gate open icon appears below the graph. The gate open period is displayed in yellow in the graphs. The system shutdown period is displayed in gray.



Environmental factor graph window

Changing the range

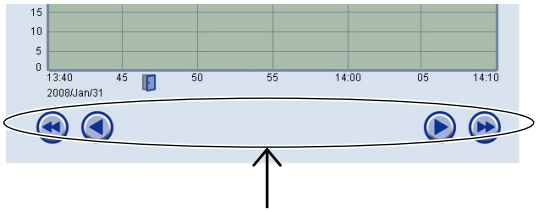
Press the Range button on the left side of the window to display graphs for the time range displayed on the Range button. When the 30 min button is pressed, the environmental changes from 30 minutes ago to the present are displayed in the graphs.



Shifting the time axis

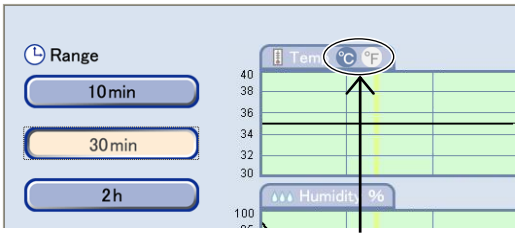
Press the arrow button below the graphs to shift the time axis of the graphs and change the display of the graphs.

- : Shift back the time axis by one grid.
- : Shift forward the time axis by one grid.
- : Shift back the time axis by one window.
- : Shift forward the time axis by one window.



Changing the temperature unit

Press "°C" or "°F" on the tab to change the unit of the temperature graph to Celsius or Fahrenheit.



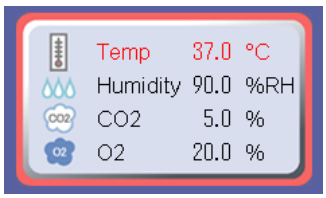
3.3.5 Status Button Color Change

If there is a change in the temperature, humidity, CO_2 concentration, or O_2 concentration due to a control error in the system, the color of the frame and characters of the Status button change to red to indicate an abnormality. If the O_2 concentration value deviates from the preset upper-limit and lower-limit values, the color of the Status button and the character display area changes to yellow. When you find any abnormality, take proper measures in accordance with the description in 8.2 “Troubleshooting on the CO_2 Incubator” and the instruction manual of the optional Oxygen Control unit.

Display indicating controller errors

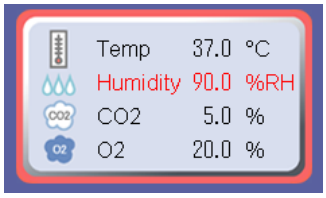
♦ **When temperature control is abnormal**

The button frame and the text in the Temp display area are displayed in red.



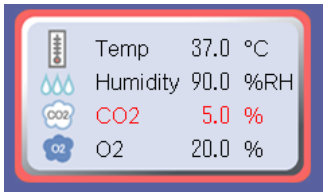
♦ **When humidity control is abnormal**

The button frame and the text in the Humidity display area are displayed in red.



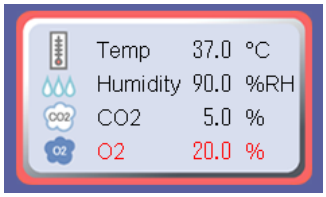
♦ **When CO_2 concentration control is abnormal**

The button frame and the text in the CO_2 display area are displayed in red.



♦ **When O_2 concentration control is abnormal**

The button frame and the text in the O_2 display area are displayed in red.



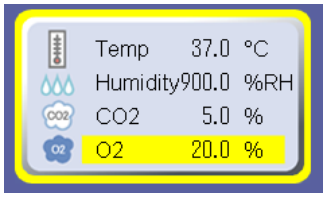
Display indicating that the value deviates form the preset range

♦ **When O_2 concentration value deviates from the preset range**

The button frame and the O_2 display area are displayed in yellow.

If a control error occurs at the same time, the button frame is displayed in red.

For details on setting the O_2 concentration value, see the instruction manual for the optional Oxygen Control unit.

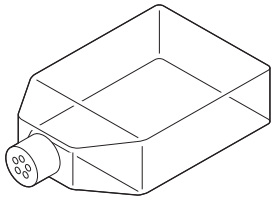


3.4 Holders and Vessels

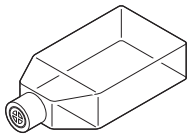
This system utilizes holders as adapters to handle various culture vessels. The holders enable culture vessels to be inserted in the correct direction and position. This mechanism clearly defines the orientation and position of a culture vessel and offers very good repeatability for the observation position even though the culture vessel is loaded and unloaded repeatedly.

3.4.1 Available Culture Vessels

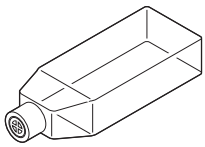
The culture vessels that can be used with this system are shown below. Do not use other culture vessels. Note that there are some exceptions. (See the next section.)



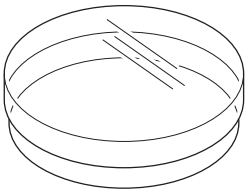
75 cm^2 culture flask



25 cm^2 culture flask
(Wide type)



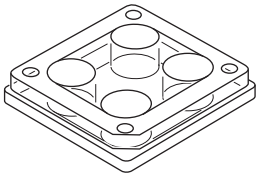
25 cm^2 culture flask
(Oblong type)



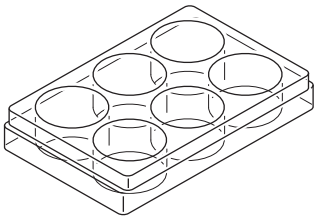
100 mm dish

60 mm dish

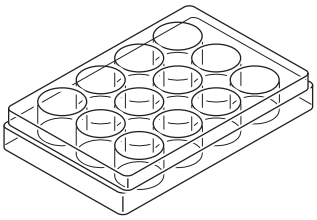
35 mm dish



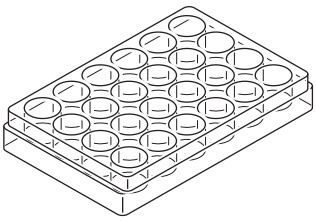
4-well multidish [*1]
(Nunc products only)



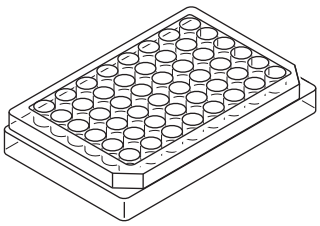
6-well plate



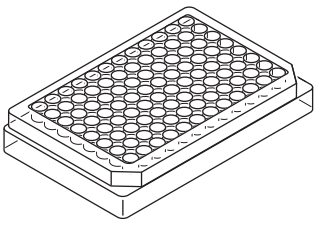
12-well plate



24-well plate



48-well plate



96-well plate

***1: There is no corresponding vessel selection in the Vessel selection window for a Nunc 4-well multidish, so load it as a 100 mm dish. Note that in this case the default observation position for a 100 mm dish cannot be used. With the Nunc 4-well multidish, only the Point observation with custom observation points registered in the Ph live observation window can be scheduled for observation.**

3.4.1.1 Unavailable culture vessels

The culture vessels that cannot be used with this system are shown below. (Those confirmed as of 2013.)

- TPP 100 mm dish
  TPP 100 mm dishes that are larger than normal 100 mm dishes interfere in the loading arm of this system. If used, the vessel may drop inside the system resulting in contamination.
- TPP well plate
  TPP well plates cannot be secured with the “BS-H/WP Well plate holder” due to its edge shape. If used, it may cause problems during timelapse observation.
- Round-bottom 96-well plate
  Some round-bottom 96-well plates have a culture plane outside the AF range of this system, resulting in defocusing.
  Contact your nearest Nikon representative for details.

3.4.2 Available Holders

Special holders are available for the culture vessels in the previous section. Use the holder suitable for each culture vessel.
Holders used in this system are designed for culture vessels manufactured by Corning Incorporated. Culture vessels manufactured by Bacto (Falcon Labware), Greiner Bio One International, Asahi Techno Glass Corporation (Iwaki), and Nalge Nunc International are also available for this product. If you wish to use holders of other brands, contact your nearest Nikon representative.

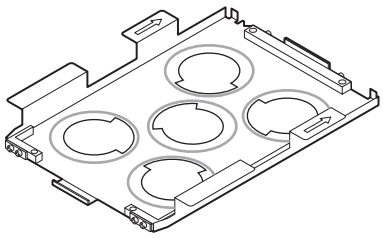
The 96-well plate is compatible with the products of several companies listed in the table below.

Manufacturer	Product Name	Product No./Catalog No./Product Code
Corning	96 Well Cell Culture Plates	3595
BD Falcon	96-well Microplate	353072
Greiner Bio-One	96 Well Polystyrene Cell Culture Microplates	655180
Iwaki	Multiple Well Plates	3865-096
Nunc	F96 MicroWell™ Plates	167008

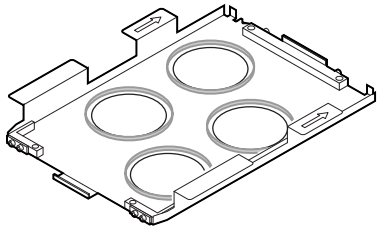
Arrow symbols are located on both guides of each holder that indicate the insertion direction into the carrier.

Caution

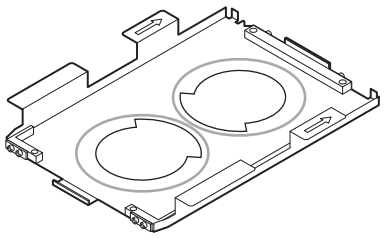
- **Holders are not sterilized when shipped. If necessary, sterilize them before use.**
- **Do not disassemble or modify holders.**
- **Handle holders with care.**
- **Holders are precision products. Do not put anything on them or drop them to avoid pressure and impact.**
- **When storing holders, do not stack them. Instead, place them individually on a level surface. (Stacking can cause the holders to become deformed.)**



BS-H/D35
35 mm dish holder

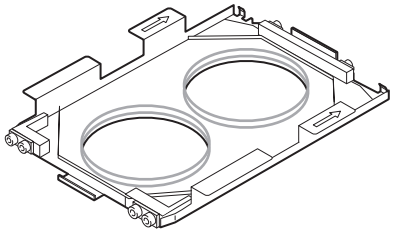


BS-H/D35-F [*1]
35 mm dish special holder
(manufactured by BD Falcon)

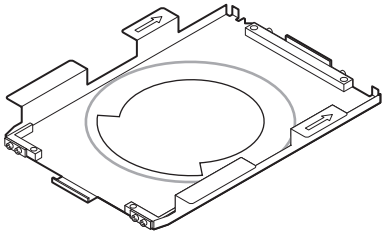


BS-H/D60
60 mm dish holder

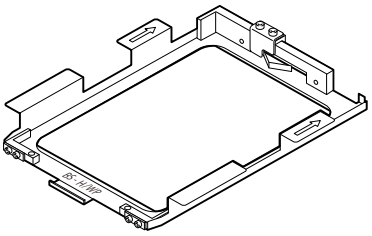
***1: The BS-H/D35-F 35 mm dish holder is designed by BD Falcon for use with 35 mm culture dishes. Use only with "BD Primaria™ 35 mm Cell Culture Dish with Easy-Grip™ (353801)." This culture dish can be used for standard observations, as well as for embryo automatic observations. For details on how to use the holder for embryo automatic observation, see the "BioStation CT Ver. 3.4 Embryo Automatic Observation Function Instructions."**



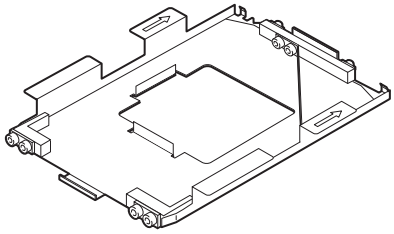
BS-H/D60-N [*2]
60 mm dish special holder
(manufactured by Nunc)



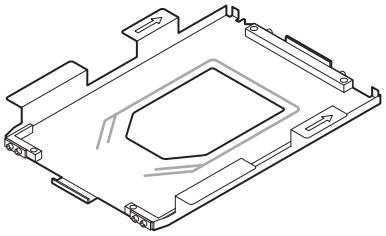
BS-H/D100
100 mm dish holder



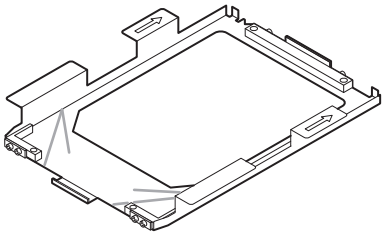
BS-H/WP
Well plate holder
(6-well plate, 12-well plate,
24-well plate, 48-well plate
and 96-well plate)



BS-H/WP4-N [*3]
4-well plate special holder
(manufactured by Nunc)



BS-H/F25
25 cm^2 flask holder



BS-H/F75
75 cm^2 flask holder

***2: The BS-H/D60-N 60 mm dish holder is designed by Nunc for use with 60 mm culture dishes. Use only with "Nunc 60 mm dish (150270)."**

***3: BS-H/WP4-N 4-well plate holder is designed by Nunc for use with 4-well multidishes. Use only with "Nunc 4-well multidish (144444)."**

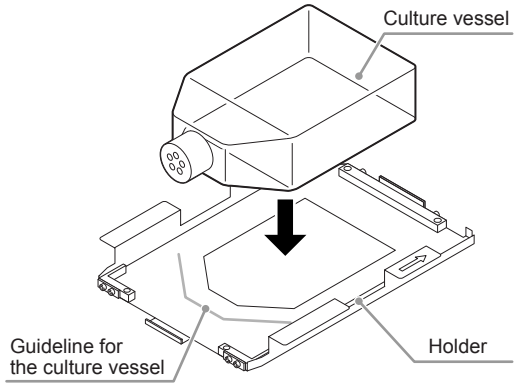
3.4.3 Setting the Culture Vessel onto the Holder

Setting the dish/flask

The size of culture vessels varies depending on the manufacturer. The guideline on the holder shows the minimum size.

(1) To fix the culture vessel onto the holder, affix commercially available double-faced tape to the holder.

> If the culture vessel is not fixed onto the holder, the performance of the product might be affected. Be sure to place the culture vessel so it is level (e.g., not tilted) when placing it on the double-faced tape on the holder. Use double-faced tape that is 0.5 mm thick or less.



Placing a culture vessel onto a holder

(2) Set the culture vessel onto the holder.
For details on culture vessel positions, see “Culture vessel positions on each holder” in this section.

> A flask has a specific orientation and must be placed in accordance with that orientation. (See the figure on the right.) Read the manual for the holders for details.

Setting the culture vessels onto a holder with silicon guides

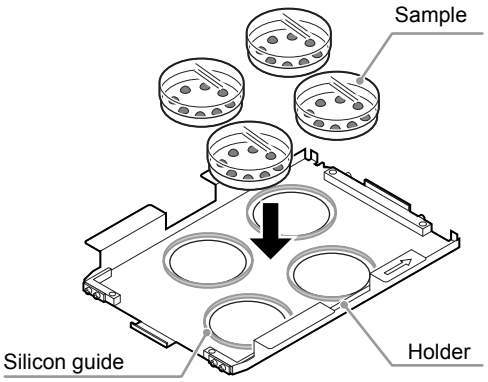
When using a BS-H/D35-F 35 mm dish special holder manufactured by BD Falcon or BS-H/D60-N 60 mm dish special holder manufactured by Nunc, set the culture vessels onto the holder as described below.

(1) Set the culture vessel onto the special holder.
For details on culture vessel positions, see “Culture vessel positions on each holder” in this section.

Set and tuck sample dishes into the silicon guide by turning them.

> Do not sway samples while setting them onto the holder.

Restore samples which are reloaded for culture medium replacement or etc, to the same position on the same holder in the same direction.

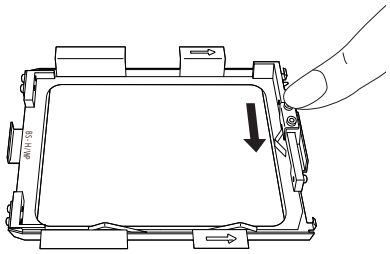


Setting the culture vessels onto a holder with silicon guides

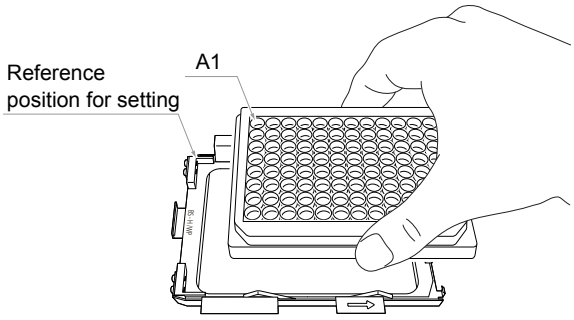
Setting the well plate

Place the well plate on the holder with a spring.

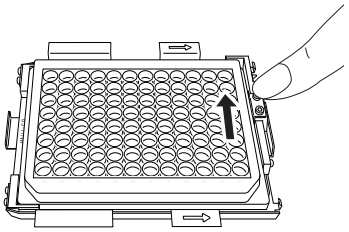
(1) Slide the lever in the direction of the arrow to release the spring.



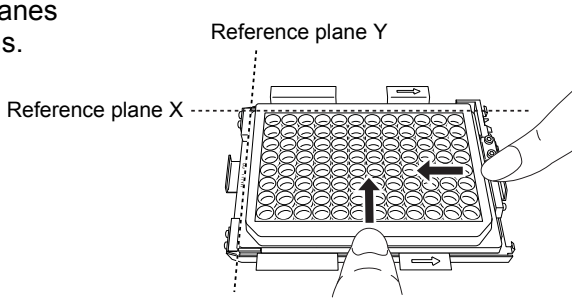
(2) Set the well plate on the holder while matching the upper-left reference position (A1) of the well plate with the upper-left reference position (for setting) of the holder.



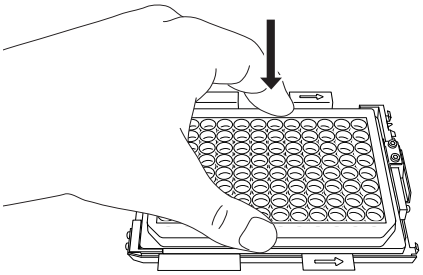
(3) Slide the lever in the direction of the arrow to tension the spring.



(4) Press the well plate against the reference planes of the holder to match the reference positions.

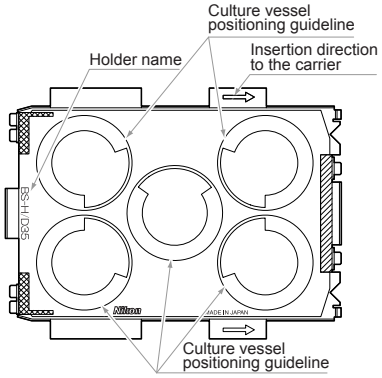


(5) Press the edges of the well plate outside the observation area to make sure that the well plate is placed on the inner bottom surface of the holder.

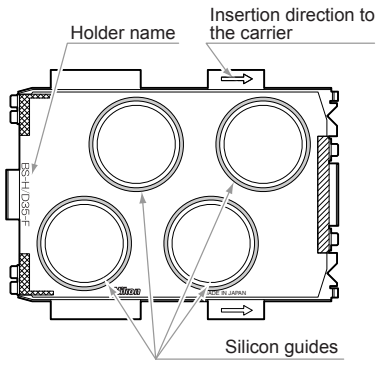


Culture vessel positions on each holder

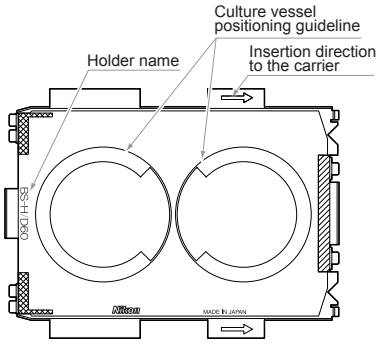
For details on culture vessel positions, see the culture vessel positioning guidelines or silicon guides in the following figures.



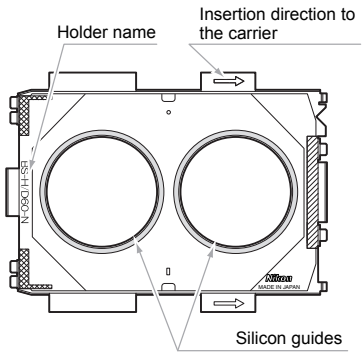
BS-H/D35
35 mm dish holder



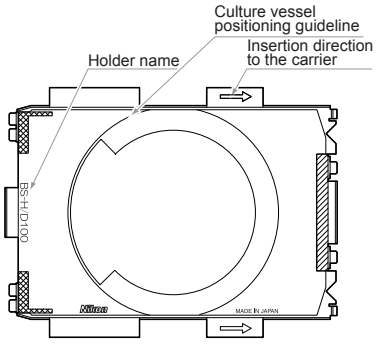
BS-H/D35-F *1
35 mm dish special holder
(manufactured by BD Falcon)



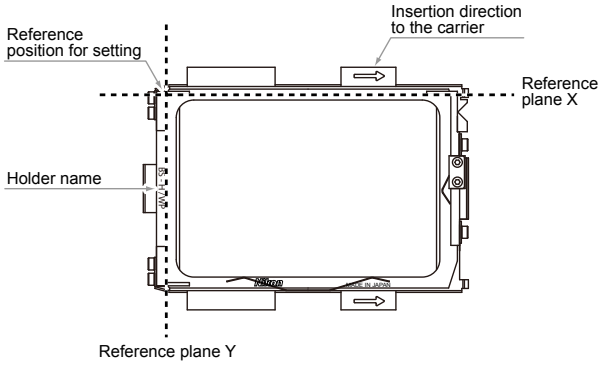
BS-H/D60
60 mm dish holder



BS-H/D60-N *2
60 mm dish special holder
(manufactured by Nunc)



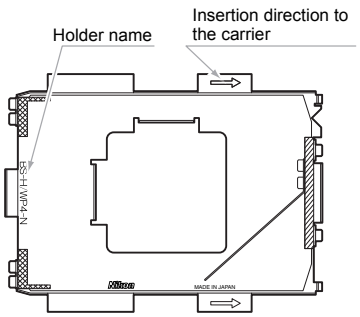
BS-H/D100
100 mm dish holder



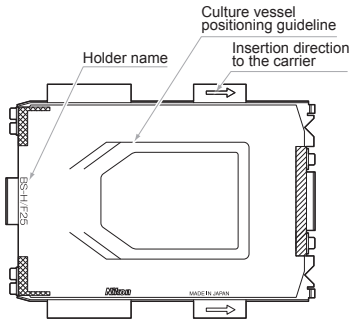
BS-H/WP
Well plate holder
(6-well plate, 12-well plate 24-well plate,
48-well plate and 96-well plate)

***1: The BS-H/D35-F 35 mm dish holder is designed by BD Falcon for use with 35 mm culture dishes. Use only with "BD Primaria™ 35 mm Cell Culture Dish with Easy-Grip™ (353801)."**

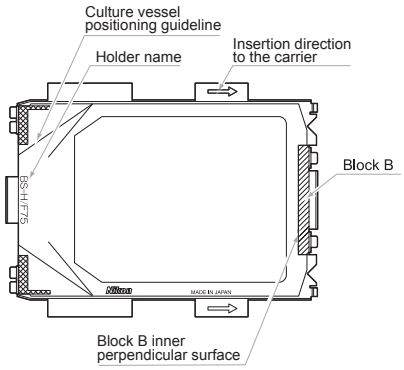
***2: The BS-H/D60-N 60 mm dish holder is designed by Nunc for use with 60 mm culture dishes. Use only with "Nunc 60 mm dish (150270)."**



BS-H/WP4-N *3
4-well plate special holder
(manufactured by Nunc)



BS-H/F25
25 cm^2 flask holder



BS-H/F75
75 cm^2 flask holder

***3: BS-H/WP4-N 4-well plate holder is designed by Nunc for use with 4-well multidishes. Use only with "Nunc 4-well multidish (144444)."**

Dish: Place the culture vessel so that it is located at the center of the outline of the guideline.

BS-H/F25: Place the culture vessel so that its right side aligns with the culture vessel positioning guideline on the right side of the holder.

BS-H/F75: Place the culture vessel so that its right side is against the perpendicular surface inside Block B.

Well plate: Place the well plate by setting its upper-left reference position at a position where reference plane X, reference plane Y, and the inner bottom surface of the holder cross each other as shown in the figure.

Holder with silicon guides:
Push the dishes into the silicon guides properly so that the bottom of the culture dishes touches the end.

3.4.4 Writing Letters on the Culture Vessels

To read the letters written on the top of culture vessels using a macro image, adhere to the following:

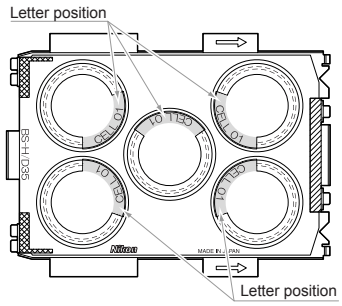
- Write letters on the periphery on each vessel.
- See the letter position figure of each vessel for the letter size and position.

Caution

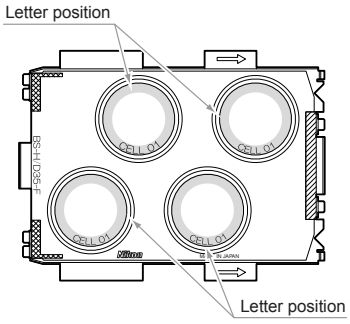
Use a permanent marker. Do not use a marker which causes feathering under high humidity circumstances. The feathering may cause contamination.

Letter position

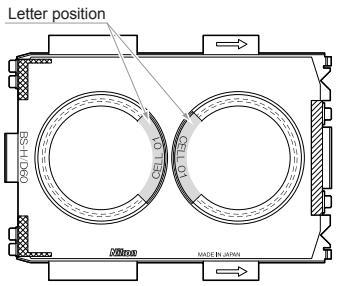
See the following figures for letter positions.



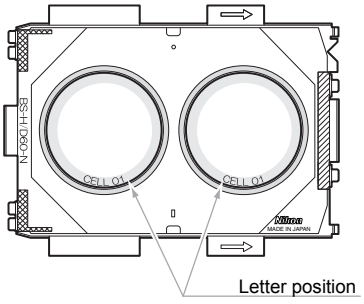
BS-H/D35
35 mm dish holder



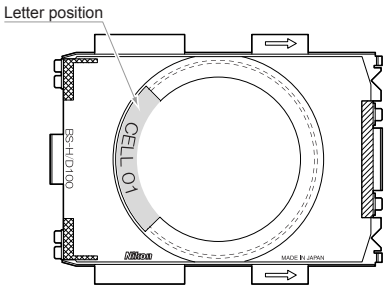
BS-H/D35-F *1
35 mm dish special holder
(manufactured by BD Falcon)



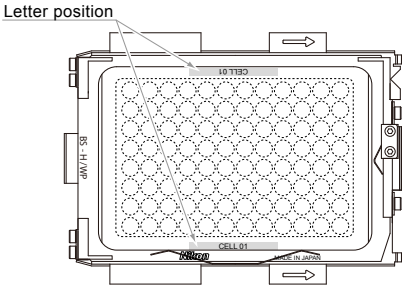
BS-H/D60
60 mm dish holder



BS-H/D60-N *2
60 mm dish special holder
(manufactured by Nunc)



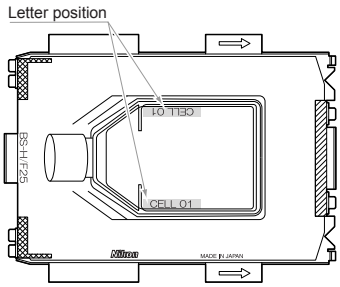
BS-H/D100
100 mm dish holder



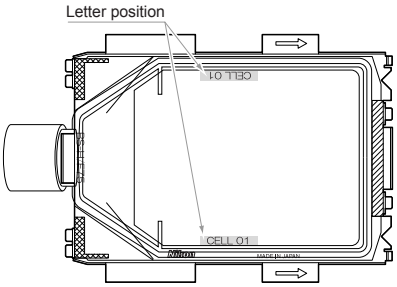
BS-H/WP
Well plate holder
(6-well plate,12-well plate, 24-well plate, 48-well plate and 96-well plate)

***1: The BS-H/D35-F 35 mm dish holder is designed by BD Falcon for use with 35 mm culture dishes. Use only with "BD Primaria™ 35 mm Cell Culture Dish with Easy-Grip™ (353801)." For details on letter positions when using this holder for embryo automatic observation, see the "BioStation CT Ver. 3.4 Embryo Automatic Observation Function Instructions."**

***2: The BS-H/D60-N 60 mm dish holder is designed by Nunc for use with 60 mm culture dishes. Use only with "Nunc 60 mm dish (150270)."**



BS-H/F25
25 cm^2 flask holder



BS-H/F75
75 cm^2 flask holder

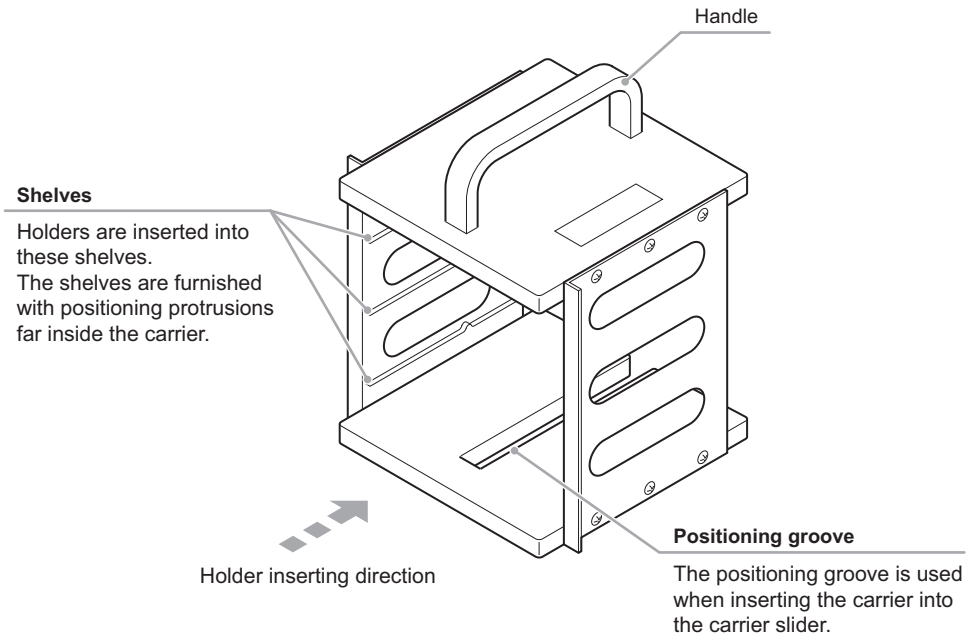
3.5 Carrier

3.5.1 Carrier

The carrier is a case for loading/unloading samples to/from the product. The carrier can transport up to three holders in which culture vessels are installed. Use a specialized holder for insertion.

Caution

- **Carriers are not sterilized when shipped. If necessary, sterilize them before use.**
- **Do not disassemble or modify carriers.**
- **Handle carriers with care.**



Carrier

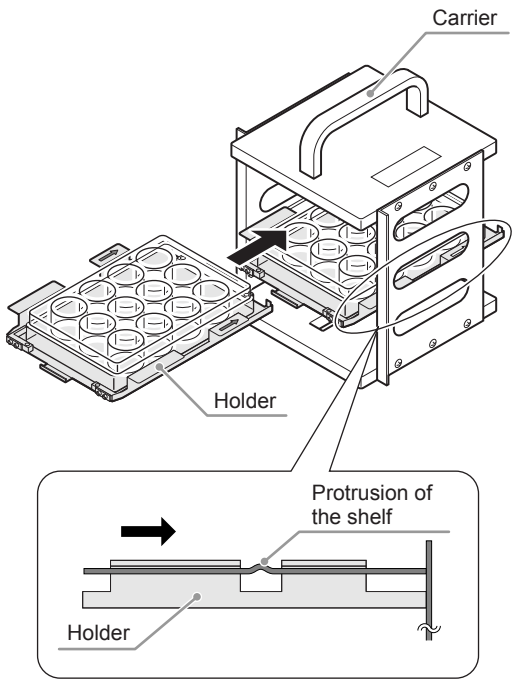
3.5.2 Inserting the Holder into the Carrier

Slowly insert the holder into the carrier in the direction of the arrow.

Check the arrow symbols on both guides of the holder. Insert the holder carefully into the carrier in the direction of the arrow.

Make sure that the holder is located in the position where the protrusion of the shelf is located in the cutaway part of the guide of the holder.

Check the insertion direction of the holder when inserting the holder into the carrier. If the holder is inserted in the wrong way, the holder extends off the carrier.



Inserting the holder to the carrier

3.6 Stockers

Caution

- **Stockers are not sterilized when shipped. If necessary, sterilize them before use.**
- **Stockers must be attached during product installation. Do not detach them except for maintenance work.**
- **Do not disassemble or modify stockers.**
- **Handle stockers with care.**

3.7 Connecting to a Network

3.7.1 Network Connection

This product can be connected to a network via a LAN cable. When this product is connected to a network, cultured sample images observed using this system can be viewed and observation schedules can be set using a PC connected to the network.

3.7.2 Connection

Connecting to a LAN system

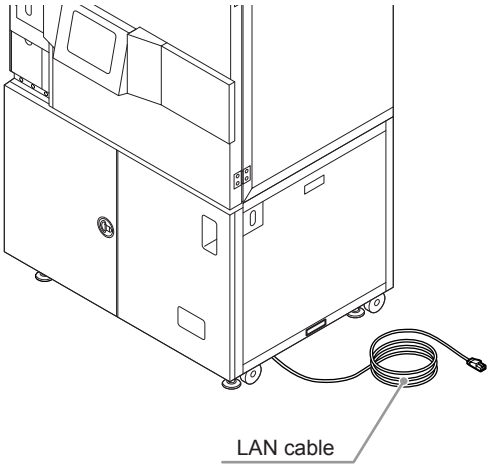
Use a LAN cable (category 5E or higher, straight cable) to connect the product with the network hub for the facility's LAN.

> The internal components of this product are designed for Gigabit Ethernet connection. When connecting this device to a PC, there are occasions when this product may not perform due to network problems.

Connecting to a PC directly

Use the LAN cable (category 5E or higher, cross-wired cable) to connect the product to a PC directly.

> For details on the procedure for setting the Network, contact your nearest Nikon representative.



Connecting to a network

4 Operation

This chapter describes the operation of this product.

4.1 Operating the Touch Panel Display

The touch panel display can be operated by lightly pressing the displayed buttons with the ball of your finger.

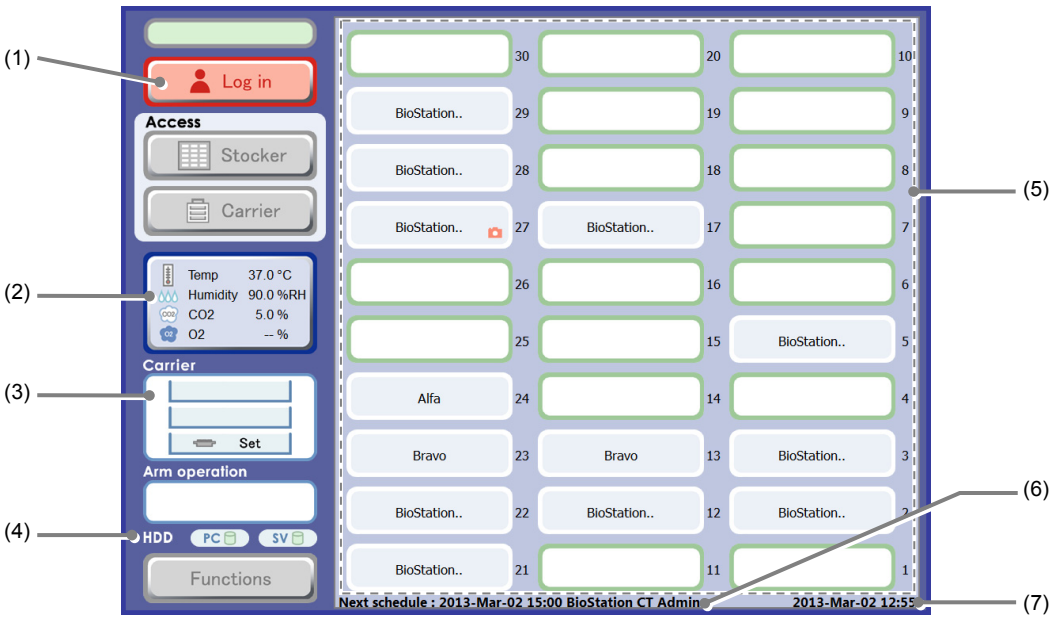
Caution

The touch panel display may be damaged if pressed using something hard such as a ballpoint pen, tool, or a finger nail. For information on the handling of the touch panel, see the manual provided with the touch panel.

4.1.1 Initial Screen

When this product is started, the System status screen is displayed first.
The operation status can be checked on the System status screen.

Before login



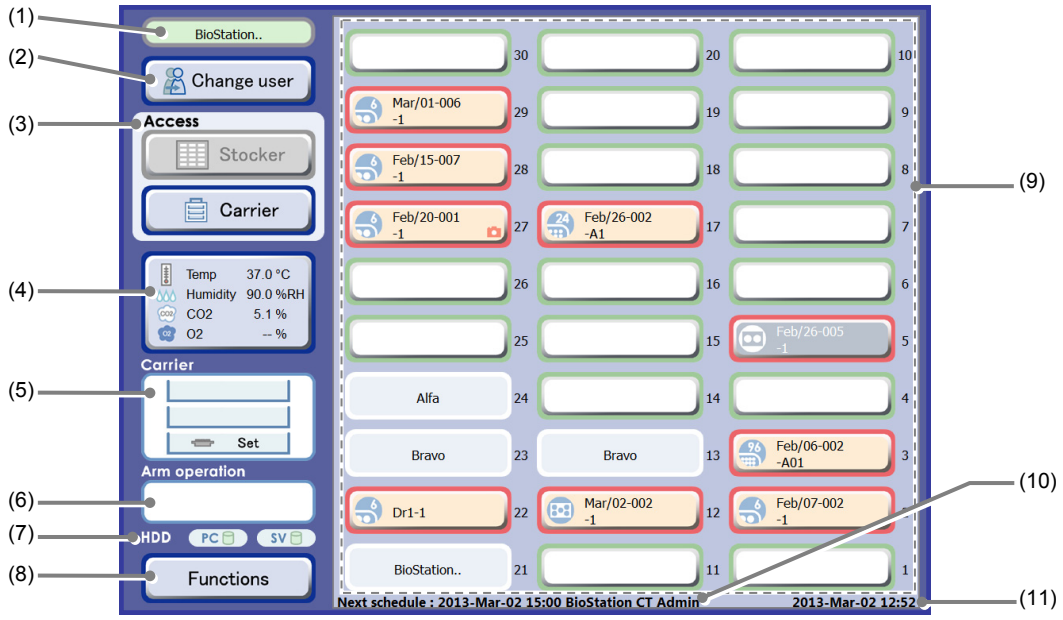
System status screen (before login)

No.	Name	Function
(1)	Log in button	Press this button to display the Select User window. Log in to the system.
(2)	Status button	The temperature, humidity, CO_2 concentration and O_2 concentration in the CO_2 incubator are visible on the Status button. Press this button to display the Environmental factor graph window. See Section 3.3.4, “Checking the Environmental Changes.”
(3)	Carrier area	This area displays the status of the 3-column carrier.
(4)	HDD area	This area displays the status of the control PC and file server hard disk drive.

No.	Name	Function
(5)	Stocker display area	This area displays the status of the three-row and ten-column stocker. A user name or a sample name is displayed on the stockers in use. Nothing is displayed on an empty stocker. The stocker numbers are displayed next to the stocker buttons. The stockers in three rows and ten columns are numbered from 1 to 30. Press the Stocker button whose stocker contains a sample to display the Select function window.
(6)	Information on the most recent scheduled observation	This area displays the date & time and user name on the most recent scheduled observation.
(7)	Date and time	This area displays the current date & time.

After login

When a user follows the procedure in Section 4.2.1, “Login” to log in to this system, the stocker display area of the System status screen changes as follows:



System status screen (after login)

No.	Name	Function
(1)	User name display area	This area displays the user name specified during the login procedure.
(2)	Change user button	Press this button to display the Select User window. A logged in user can be switched or logged out.
(3)	Access area	Select the sample load/reload method. **Stocker button:** (Currently not available.) **Carrier button:** Unlock the access gate to allow the carrier to be loaded from the access gate into the culture chamber or unloaded.

No.	Name	Function
(4)	Status button	The temperature, humidity, CO_2 concentration and O_2 concentration in the CO_2 incubator are visible on the Status button. Press this button to display the Environmental factor graph window. See Section 3.3.4, “Checking the Environmental Changes.”
(5)	Carrier area	This area displays the status of the 3-column carrier.
(6)	Arm operation area	This area displays the transport status of the sample by icons.
(7)	HDD area	This area displays the status of the control PC and file server hard disk drive.
(8)	Functions button	Press this button to display the Function window. This window is used for operations such as schedule confirmation, search, latest photo listing, user environmental settings, observation data deletion and master maintenance.
(9)	Stocker display area	This area displays the status of the three-row and ten-column stocker. A user name or a sample name is displayed on the stockers in use. Nothing is displayed on an empty stocker. The sample cultures of the login user are framed in red. The stocker numbers are displayed next to the stocker buttons. The stockers in three rows and ten columns are numbered from 1 to 30. Press the Stocker button whose stocker contains a sample to display the Select function window.
(10)	Information on the most recent scheduled observation	This area displays the date & time and user name on the most recent scheduled observation.
(11)	Date and time	This area displays the current date & time.

4.1.2 Stocker Display Area

The stocker display area displays the status of samples stored in the stockers. Press a button in the stocker display area to open the Select function window and operate the corresponding sample.

Button appearance

Button appearance in the stocker display area

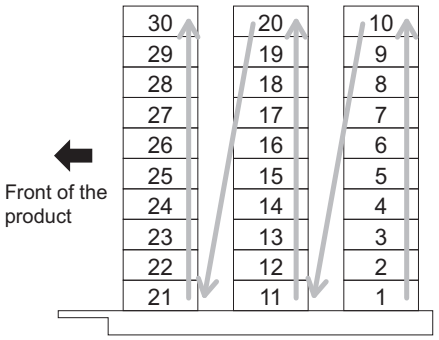
Appearance	Frame color	Detail
	Red	This is a sample which belongs to the user as specified during the login procedure. Only this type of sample can be operated by the user.
	Red	This is a holder that is being used for scheduled observation.
	Red	This is a stocker that is temporarily unloading a sample so the medium can be changed or the sample can be checked.
	Green	This is an empty stocker.
	White	This is an empty stocker that can be used by any user.
	White	This is a sample that does not belong to the user specified in the login procedure. Samples indicated by this mark cannot be operated by the logged in user. All stockers are displayed in this manner when users are logged out.

Stocker number

The stockers in three rows and ten columns are numbered from 1 to 30 as shown in the figure on the right.

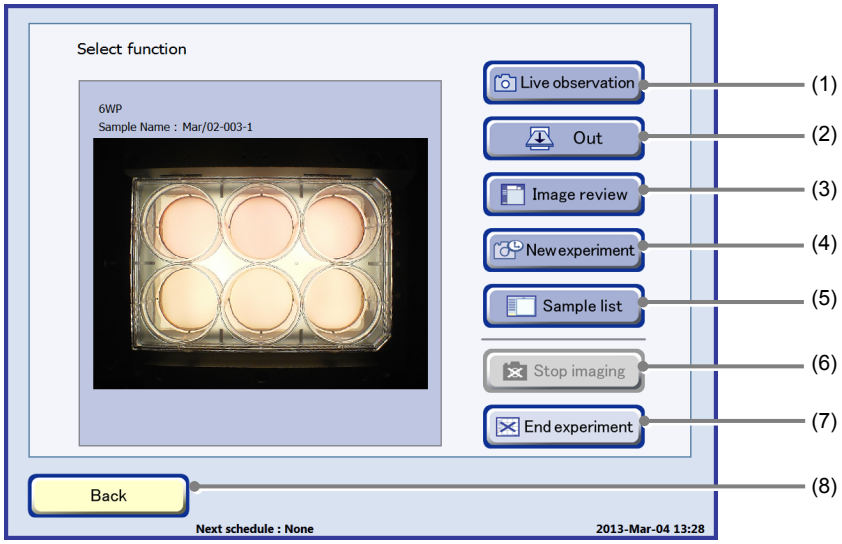
The rear stockers are numbered from 1 to 10, the center stockers are numbered from 11 to 20, and the front stockers are numbered from 21 to 30.

These stocker numbers are displayed next to the stocker buttons in the stocker display area.



Stocker number

Select function window displayed by the Stocker button



Select function window

No.	Name	Function
(1)	Live observation button	Press this button to display the Ph live observation window and perform live image observation.
(2)	Out button	Press this button to display the Unload purpose selection window and unload the sample to the carrier.
(3)	Image review button	Press this button to display the Image review window and view the observation data.
(4)	New experiment button	Press this button to display the Observation condition setting window and set the schedule observation.
(5)	Sample list button	Press this button to display the Sample list window.
(6)	Stop imaging button	Press this button to cancel capturing in stage exclusive mode or cancel stage standby in stage holding on mode. Enabled only when stage exclusive mode or stage holding on mode is set for a scheduled observation.
(7)	End experiment button	Press this button to delete all scheduled observations scheduled for the samples in the selected holder.
(8)	Back button	Press this button to display the System status screen.

Icon

The Icons in the stocker display area indicate the type of culture vessel used for samples and the status of the stocker. The design of the light blue icon indicates the type and size of the culture vessel.

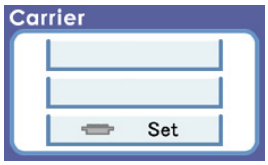
Icons in the stocker display area

Appearance	Color	Detail
	Blue	A 75 cm^2 culture flask or the 25 cm^2 culture flask (wide type)
	Blue	A 25 cm^2 culture flask (oblong type)
	Blue	A 100 mm dish
	Blue	A 60 mm dish
	Blue	A BD Falcon 35 mm dish. For details on using it for embryo automatic observation (optional), see the “Embryo Automatic Observation Function Instructions.”
	Blue	A 35 mm dish
	Blue	A 6-well plate
	Blue	A 12-well plate
	Blue	A 24-well plate
	Blue	A 48-well plate
	Blue	A 96-well plate
 Log out	Blue	A sample currently being observed. The culture vessel icon changes to this camera icon during observation.
 Log in	Red	
	Pink	A sample temporarily stored in a stocker
	Orange	A sample scheduled for observation

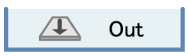
4.1.3 Carrier Area

The Carrier area displays the three carrier shelf columns. The following icons show the status of the holder set in each column of the carrier.

Icons for displaying the setting status of the carrier

Icon	Detail
	The carrier is not set in the carrier slider.
	The carrier is set in the carrier slider.

Icons in the Carrier area

Icon	Detail
	A holder is not set in the carrier.
	A sample to be loaded is set in the carrier.
	If the door is opened during live observation, the icon turns red after live observation is finished. The color returns to normal if a sample is loaded or the door is opened again.
	A sample for loading is being loaded from the carrier to the stocker or the observation part.
	A sample for unloading is being unloaded from the stocker or the observation unit to the carrier.
	A sample for unloading has been returned to the carrier.

4.1.4 Arm Operation Area

The Arm operation area shows the transport status using the following icons. When any of these icons is displayed, the door cannot be opened or closed for safety.

Icons on the Arm operation area

Icon	Detail
	A sample is being transported from the carrier to the stocker.
	A sample is being transported from the stocker to the carrier.
	A sample is being transported from the stocker to the XY stage.
	A sample is being transported from the XY stage to the stocker.
	A sample is being transported from the carrier to the observation unit's XY stage.
	A sample is being transported from the observation unit's XY stage to the carrier.

4.1.5 HDD Area

When an error occurs on the file server's hard disk drive or the control PC's hard disk drive, or when the file server for storing image data is running out of space, the appearance of the HDD areas on the System status screen changes.

If an error occurs on the hard disk drive, immediately contact your nearest Nikon representative.

If the file server's hard disk drive is not recognized, observation data and image data cannot be saved. Be sure that the file server is switched on, and if not, turn on the power switch.

When the free space is insufficient for saving new observation data, old observation data is automatically deleted in the order it was recorded. When one of the following alert icons is displayed, back up any necessary observation data and image data to an external PC. If the free space of the file server is increased to 200 gigabytes or more by deleting unnecessary old observation data, the alert icon disappears.

For the procedure for downloading the observation data or image data to an external PC, see the "BioStation CT Ver. 3.8 Instructions (PC Operations)." For the procedure for deleting the Observation Data, see Section 7.2, "Deleting the Observation Data."

HDD area

Appearance	Color	Detail
	White	The control PC hard disk drive is running normally.
	White	The file server hard disk drive is running normally.
	Blue	An error occurred on the hard disk drive of the control PC.
	Blue	An error occurred on the hard disk drive of the file server.
	White	Unable to recognize the hard disk drive of the file server. This icon disappears when connection is confirmed.
	Red	Unable to recognize the hard disk drive of the file server after a predetermined time.
	Yellow	The free space on the file server is 200 gigabytes or less.
	Red	The free space on the file server is 100 gigabytes or less.

4.1.6 Checking the Usage Status of the Stocker

This section describes the procedure for viewing the usage status of the stocker.

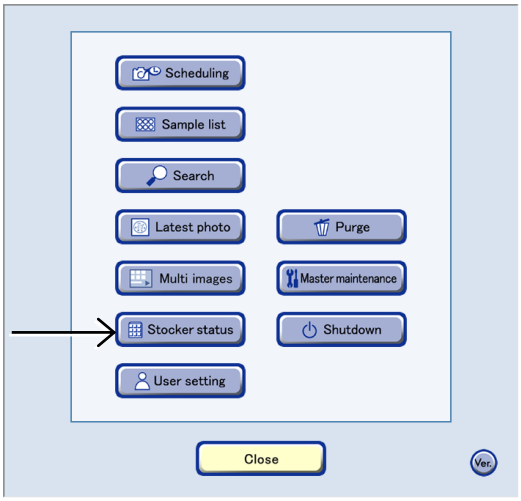
(1) Press the Functions button on the System status screen.

The Functions window appears.

System status screen

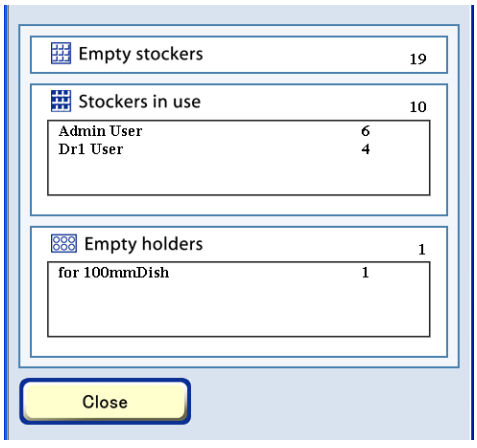
(2) Press the Stocker status button.

The Stocker usage status window appears.



Functions window

In the Stocker usage status window, the number of empty stockers, the number of used stockers, and the number of empty holders can be checked.



Stocker usage status window

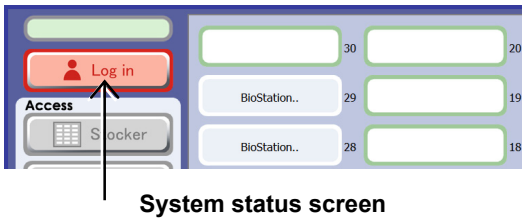
4.2 Login and Logout

4.2.1 Login

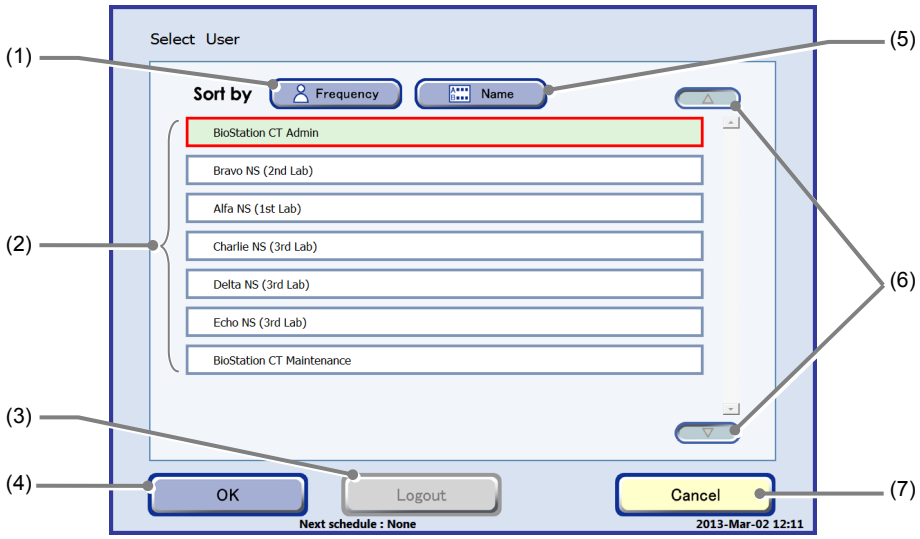
To operate the culture vessel using this system, log in with the touch panel display. To log in to this system, follow the procedure below.

(1) Press the Log in button on the System status screen.

The Select User window appears.



System status screen



Select User window

No.	Name	Function
(1)	Frequency button	Press this button to sort the user names in descending order of frequency of login.
(2)	User list	Select the log in user.
(3)	Logout button	Press this button to log out and display the System status screen.
(4)	OK button	Press this button to log in as the selected user.
(5)	Name button	Press this button to sort the user names in alphabetical order.
(6)	Up and down buttons	Press this button to scroll the list upward or downward.
(7)	Cancel button	Press this button to return to the System status screen without changing the user.

User

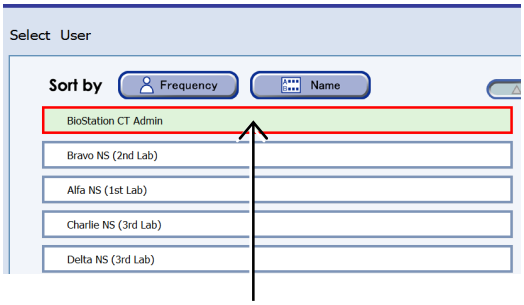
A person who performs operations on samples using this system.
The user name and the name of the sample to be operated on can be recorded by specifying a user name when logging in to this system.

For details on registering a login name, see Chapter 7, “CT Administrative Functions.”

(2) Select the user name (the user's registered name) from the user list.

With this operation, the selected user name is saved in this product's operation history. This information is used if it is necessary to track operations later.

In the user list, names of the users that have been registered in the user master data are displayed.



> Login names for users that have not been registered in the user master data are not displayed in the user list. Be sure to register a login name for users when registering them. For details on registering user names, see Section 7.3.2, "User Master Data."

(3) Press the OK button.

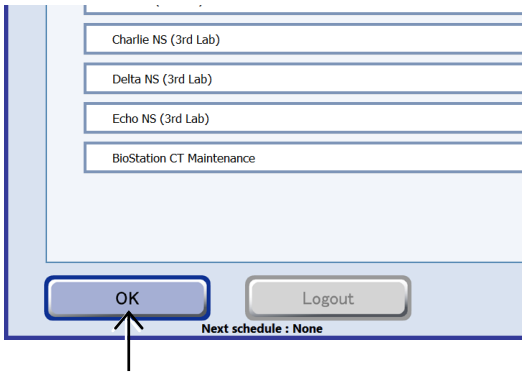
Without a password

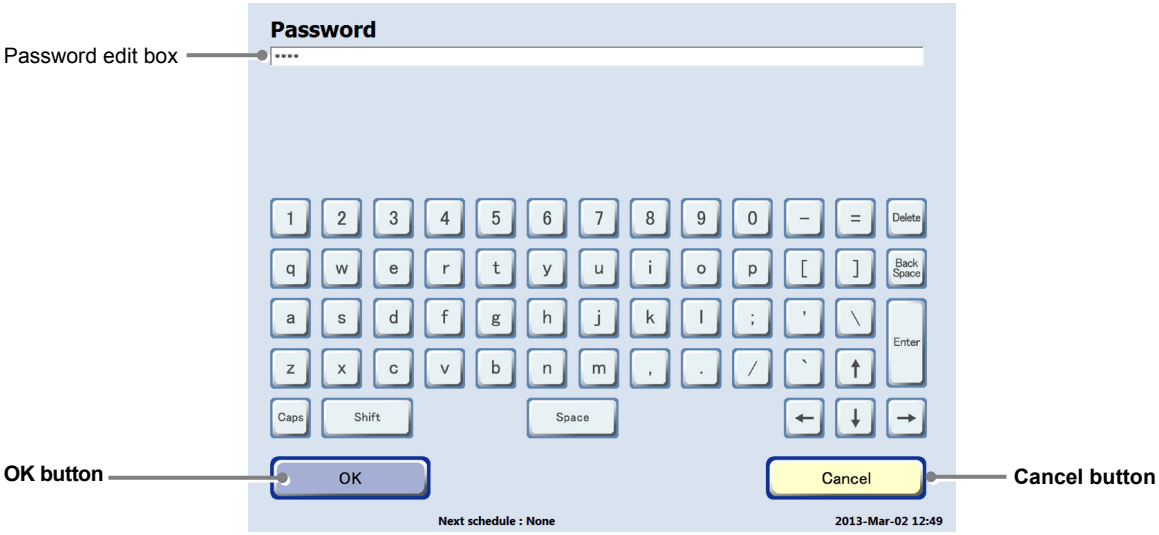
If a login password has not been set, log in is automatically performed without a password being entered and the System status screen appears.

With a password

If a login password has been set, a Keyboard window for entering the password appears.

For details on setting a password, see Chapter 5, "Environmental Settings" or Section 7.3.2, "User Master Data."



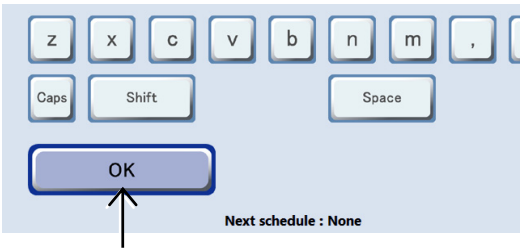


Keyboard window

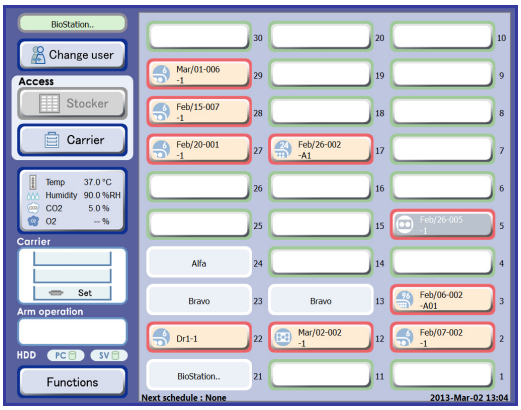
1. **Enter the password in the Keyboard window.**
2. **Press the OK button.**

   Log in is performed and the System status screen appears.

   If the entered password is wrong, the Select User window appears.



The operable samples are displayed with a red frame in the stocker display area of the System status screen after log in.



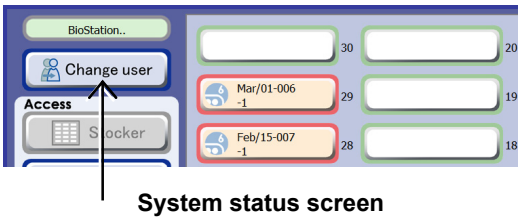
System status screen

4.2.2 Logout

To log out to this product, follow the procedure below.

(1) Press the Chagne user button on the System status screen.

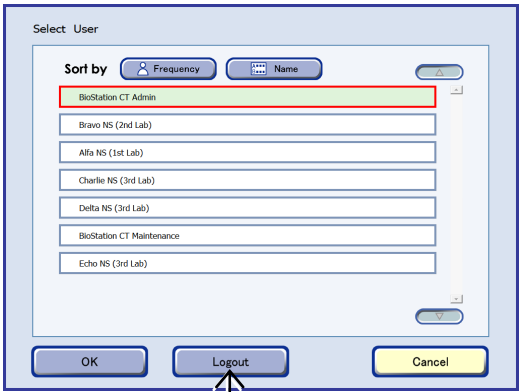
The Select User window appears.



System status screen

(2) Press the Logout button in the Select User window.

The System status screen before login is displayed.



Select User window

4.3 Loading and Unloading Samples

This section describes the procedure for loading/unloading samples to/from the access gate using a carrier.

4.3.1 Precautions on Samples

- Do not use unspecified culture vessels. For details on the culture vessels that can be used, see Section 3.4.1, “Available Culture Vessels.”
- Perform operations using clean culture vessels, holders, and carriers.
- If more than the specified amount of culture fluid is used, the culture fluid or the sample may spill out.
- Perform operations for filling a culture vessel with culture fluid or placing a sample into the culture vessel inside a safety cabinet appropriate for the risk grade of the sample.
- Be careful not to spill samples when carrying culture vessels that contain samples.

4.3.2 Loading Samples

This section describes the procedure for loading or unloading a carrier and setting a sample in the carrier.

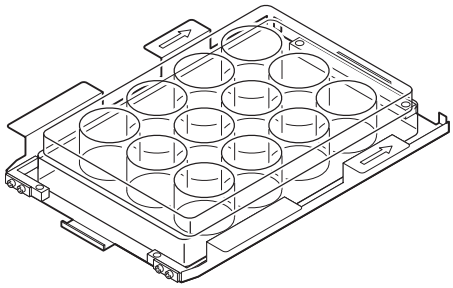
4.3.2.1 Preparing samples

This section describes the procedure for setting the culture vessels in the holder and the carrier. Perform the following procedure inside a safety cabinet.

(1) Set the culture vessel in the holder.

Use a holder appropriate for the culture vessel.

> If the culture vessel is not fixed on the holder, the performance of the product may be degraded. If a vessel other than a well plate is used, use double-sided tape to attach the holder to the culture vessel. Make sure that the culture vessel is placed level.



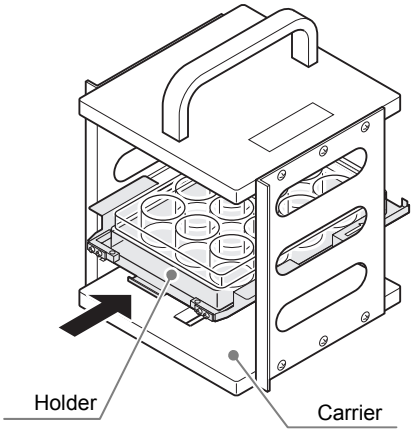
Setting the 12-well plate to the holder

(2) Set the holder in the carrier.

Slowly slide the holder into the shelf of the carrier as far as it goes.

Up to three carriers can be set in a holder.

> The holder can also be inserted into the carrier that has been installed on a carrier slider.



Setting a holder in a carrier

4.3.2.2 Loading the carrier

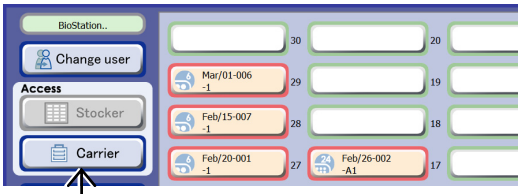
This section describes the procedure for loading the carrier into the culture chamber through the access gate.

There are two types of loading procedures: loading the carrier after specifying the stocker to be loaded and loading the carrier before specifying the stocker to be loaded. To load the carrier after specifying the stocker, see Section 4.3.2.3, “Loading a new sample.”

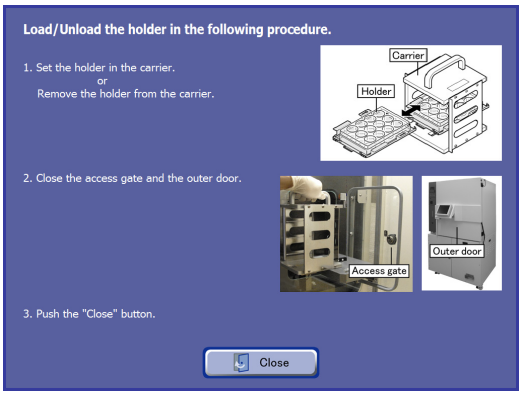
(1) Press the Carrier button in the Access area of the System status screen on the touch panel display.

The Load/Unload procedure window is displayed and the access gate is unlocked. When the access gate is unlocked, the loading door lamp on the temperature/humidity controller area lights.

If the user has not logged in yet or a sample is being transported in the CO_2 incubator, the Carrier button is disabled.



System status screen



Load/Unload procedure window

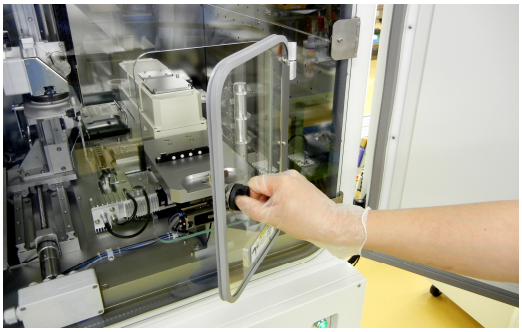
(2) Open the CO_2 incubator outer door.

Open the CO_2 incubator outer door by pulling the handle at the bottom of the touch panel display.

CO_2 incubator outer door

(3) Open the access gate.

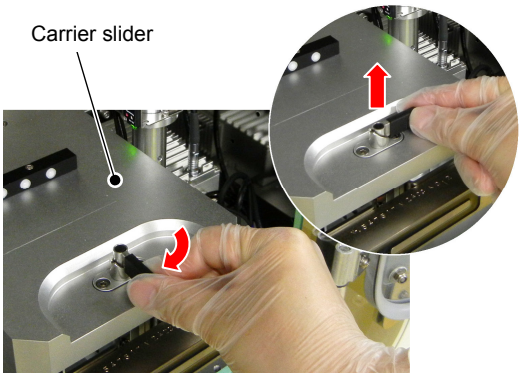
Open the access gate by turning the knob vertically and pulling the knob.



Access gate

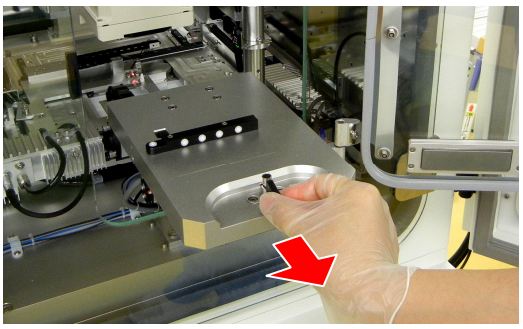
(4) Pull up the knob on the carrier slider and turn it clockwise 90 degrees.

The carrier slider unlocks.



(5) Pull out the carrier slider.

The carrier may have been loaded into the system in advance to maintain the temperature of the carrier.

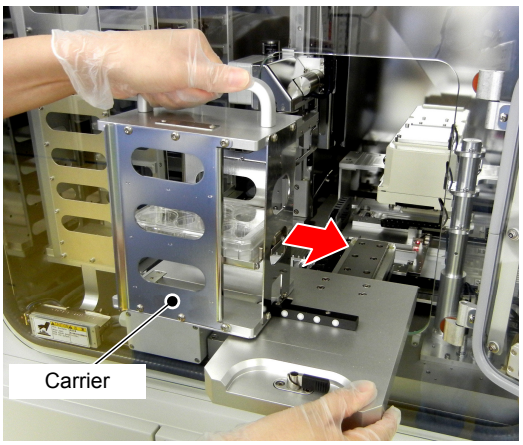


Carrier slider

(6) Put the carrier onto the carrier slider.

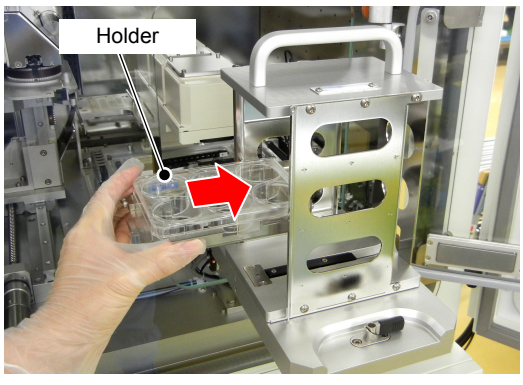
Align the groove at the bottom of the carrier with the protrusion on the carrier slider and set the carrier by sliding it from left to the right.

When attaching the carrier, fully insert the carrier into the end of the carrier slider.
The system will not recognize the carrier after loading if it is even 1 or 2 mm off the carrier slider.



Loading the carrier

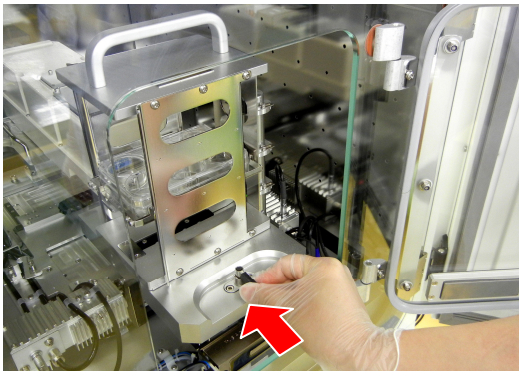
If the carrier has been set to the carrier slider already, insert the holder that contains the samples to load into the carrier.



Loading the carrier

(7) Slowly push the carrier slider into the culture chamber.

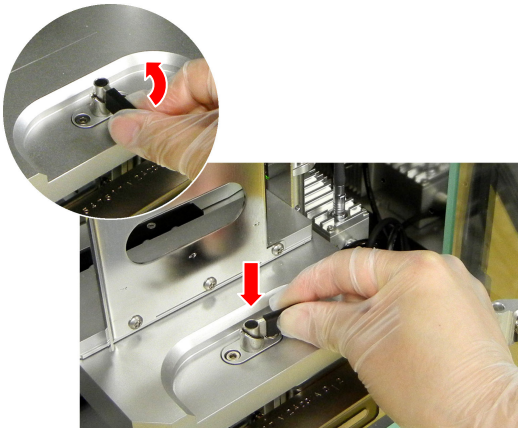
When culture vessels are set in the carrier, move the carrier slider slowly so that the culture fluid remains still.



Carrier slider

(8) Turn the knob on the carrier slider counterclockwise 90 degrees and push it down.

The carrier slider locks.



(9) Close the access gate.

Close the access gate, and turn the knob horizontally to lock the access gate.

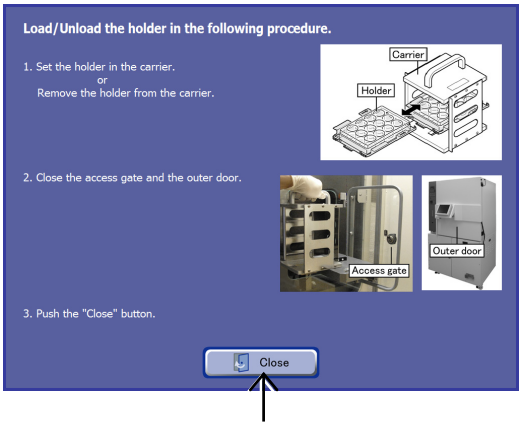
Access gate

(10) Close the CO_2 incubator outer door.

CO_2 incubator outer door

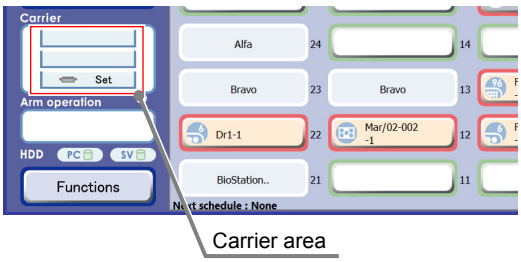
(11) Press the Close button on the touch panel display.

The Load/Unload procedure window closes and the System status screen appears.



Load/Unload procedure window

In the Carrier area of the System status screen, “Set” is displayed at the position of the carrier on which the holder is set.



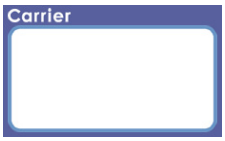
System status screen

Carrier installation check

If a sample cannot be observed or the holder cannot be moved to the stocker even though the carrier is loaded into the system, the system may not recognize the carrier as the carrier has not been installed correctly.

Check the carrier status in the Carrier area on the System status screen to see if the carrier has been installed properly.

If the Carrier area is blank as shown on the right, the carrier has not yet been recognized. (See Section 4.1.3, “Carrier Area.”)



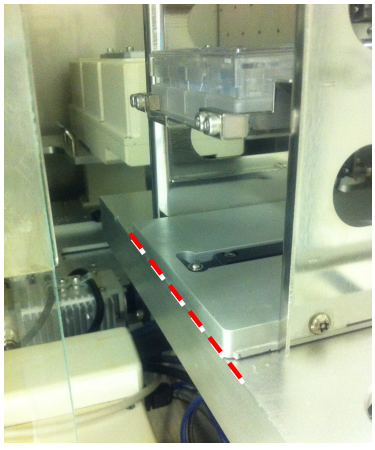
The carrier has not yet been set or recognized

If the carrier has not been recognized, the carrier may not have been inserted into the carrier slider completely.

Even a misalignment of 1 or 2 mm affects the recognition of the carrier. When the carrier is installed correctly, the base plate of the carrier should fit the carrier slider completely as shown in the picture on the right.

If the carrier is misaligned, open the access gate and fully insert the carrier into the end of the carrier slider, and then check the carrier installation status in the Carrier area again.

If the problem still occurs after the above action, contact your nearest Nikon representative.



Installing a carrier

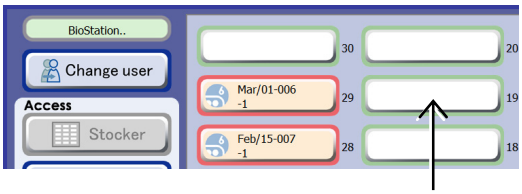
4.3.2.3 Loading a new sample

This section describes the procedure for loading a new sample to the stocker for the first time.

For the procedure for reloading an unloaded sample for the purpose of changing the culture medium and so on, see Section 4.3.2.4, “Reloading samples.”

(1) Press a vacant stocker button in the stocker display area of the System status screen.

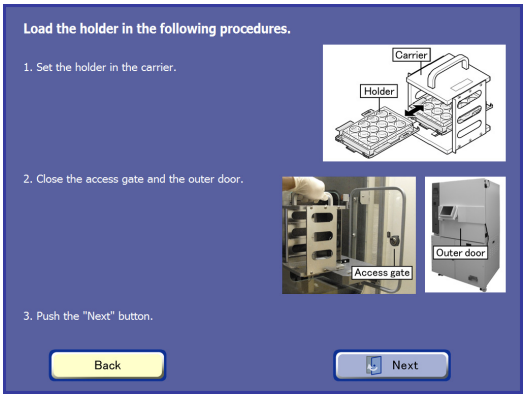
The Loading procedure window appears.



System status screen

(2) If the carrier has not yet been loaded into the system, follow steps (3) to (11) to load the carrier.

Proceed to step (12) if the carrier that contains samples has already been loaded into the system.



Loading procedure window

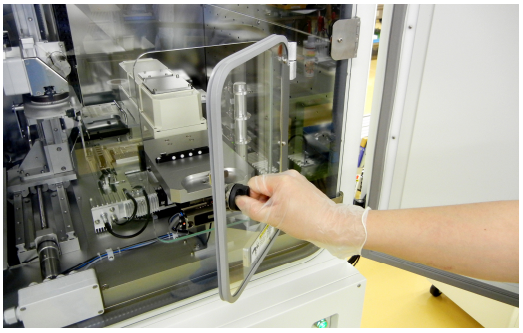
(3) Open the CO_2 incubator outer door.

Open the CO_2 incubator outer door by pulling the handle at the bottom of the touch panel display.

CO_2 incubator outer door

(4) Open the access gate.

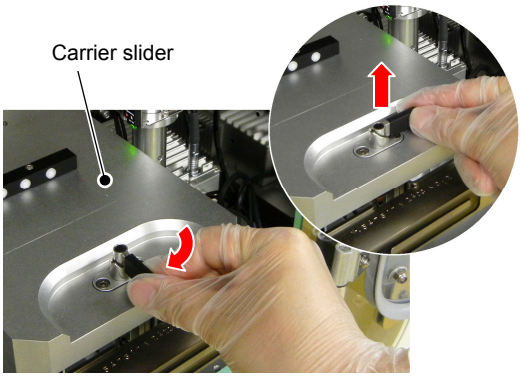
Open the access gate by turning the knob vertically and pulling the knob.



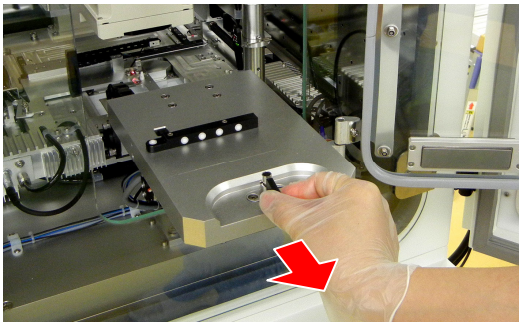
Access gate

(5) Pull up the knob on the carrier slider and turn it clockwise 90 degrees.

The carrier slider unlocks.



(6) Pull out the carrier slider.

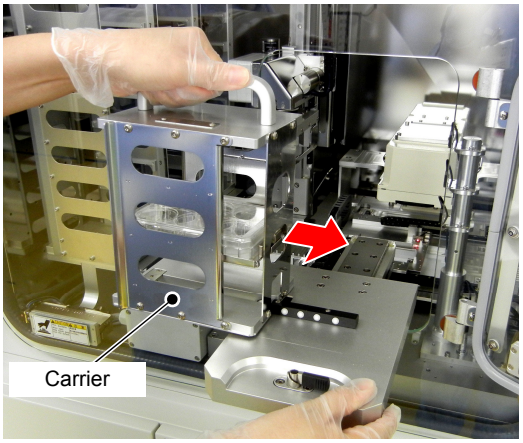


Carrier slider

(7) Put the carrier onto the carrier slider.

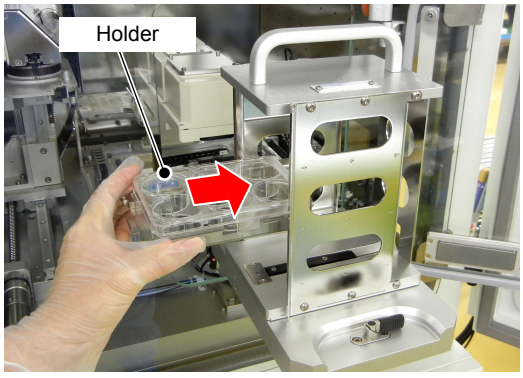
Align the groove at the bottom of the carrier with the protrusion on the carrier slider and set the carrier by sliding it from left to the right.

When attaching the carrier, fully insert the carrier into the end of the carrier slider.
The system will not recognize the carrier after loading if it is even 1 or 2 mm off the carrier slider.



Loading the carrier

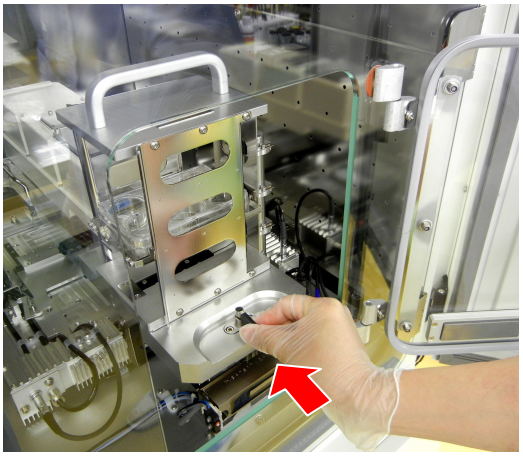
If the carrier has been set to the carrier slider already, insert the holder that contains the samples to load into the carrier.



Loading the holder

(8) Slowly push the carrier slider into the culture chamber.

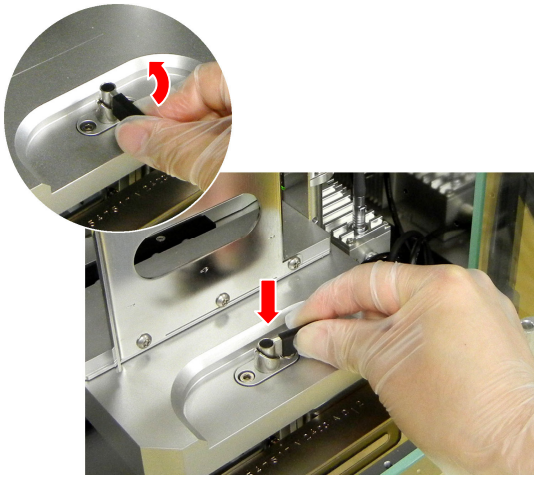
When culture vessels are set in the carrier, move the carrier slider slowly so that the culture fluid remains still.



Carrier slider

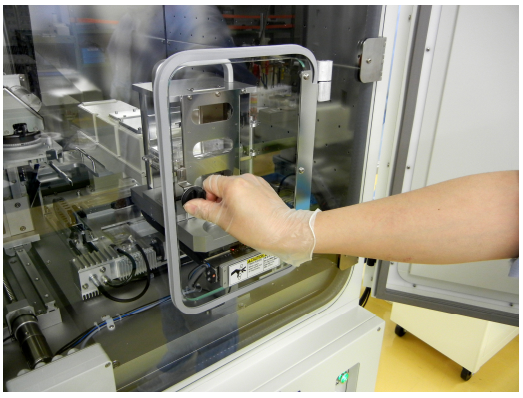
(9) Turn the knob on the carrier slider counterclockwise 90 degrees and push it down.

The carrier slider locks.



(10) Close the access gate.

Close the access gate, and turn the knob horizontally to lock the access gate.



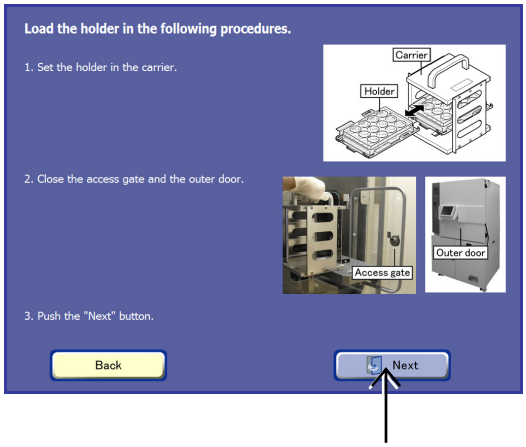
Access gate

(11) Close the CO_2 incubator outer door.

CO_2 incubator outer door

(12) Press the Next button on the touch panel display.

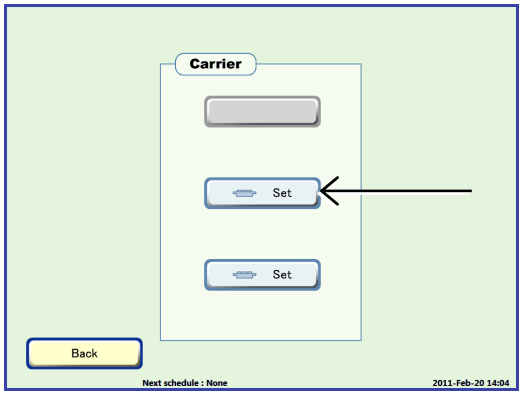
The Load holder selection window appears.



Loading procedure window

(13) Press the Set button for the carrier on which the holder to be loaded is set.

The Vessel type selection window appears.

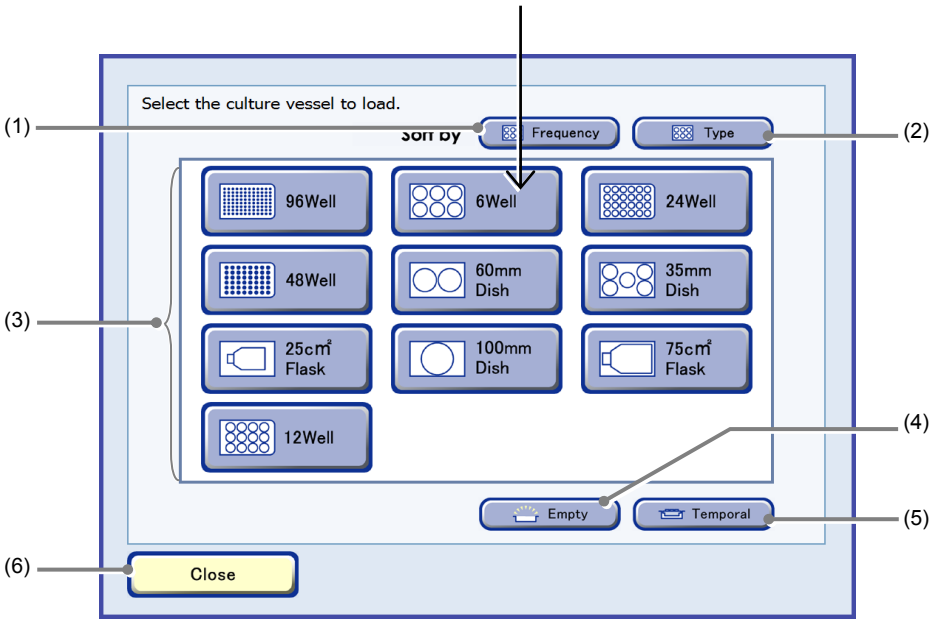


Load holder selection window

(14) Select the culture vessel used for the sample to be loaded.

1. Press one of the vessel type selection buttons to select the type of the vessel to be loaded.

The Vessel selection window appears and a list of each manufacturer's vessels included in the selected vessel type is displayed.



Vessel type selection window

No.	Name	Function
(1)	Frequency button	Press this button to sort the list in descending order of frequency of usage.
(2)	Type button	Press this button to sort the vessel types in alphabetical order.
(3)	Vessel type selection buttons	Select the type of the vessel to be loaded. When a vessel type is selected, a product list of the selected vessel type for each manufacturer is displayed.
(4)	Empty	Press this button to load an empty holder. A window in which the vessel type of the empty holder can be selected is displayed.
(5)	Temporal	Press this button to load a temporarily stored holder into the selected stocker.
(6)	Close button	Press this button to close the vessel type selection window.

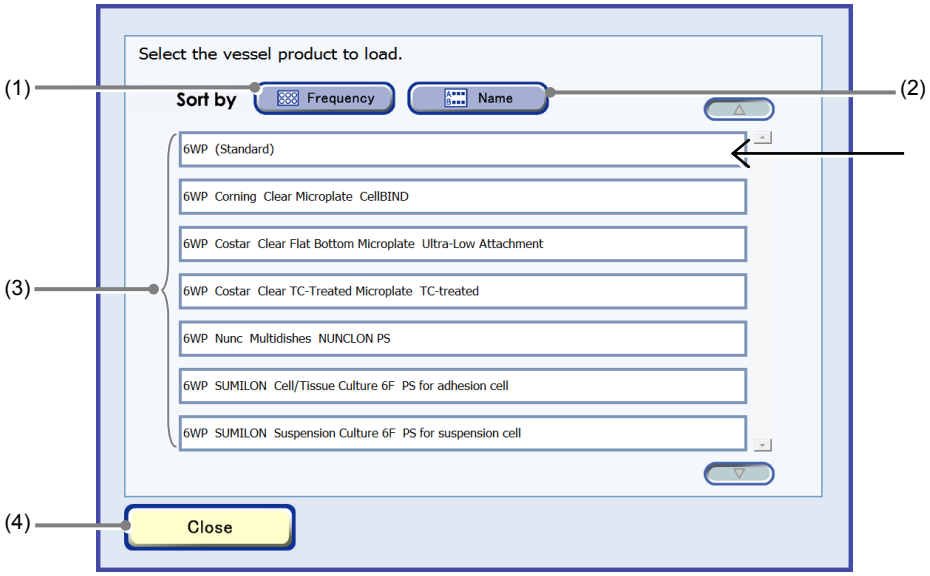
When using Nunc 4-well multi dish

When using a Nunc 4-well multi dish, load it as a 100 mm dish because there are no corresponding vessel selection buttons in the Vessel selection window when loading.

Note that in this case, the default observation position for the 100 mm dish cannot be used. With the Nunc 4-well multi dish, only the Point observation with custom observation points registered in the Ph live observation window can be scheduled for observation.

2. From the Culture vessel list, select the culture vessel used for the sample to be loaded.

Select the vessel. The Load information entry window appears.



Vessel selection window

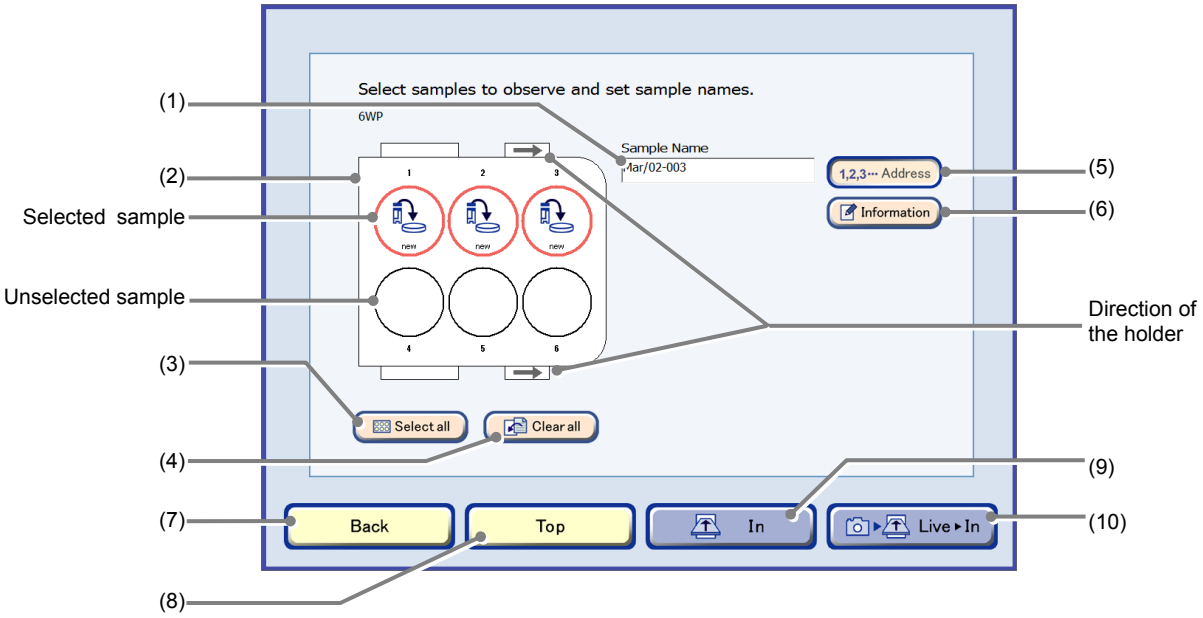
No.	Name	Function
(1)	Frequency button	Press this button to sort the list in descending order of frequency of the usage.
(2)	Name button	Press this button to sort the vessel names in alphabetical order.
(3)	Vessel type list	Select the culture vessel used for the sample to be loaded. If there is no need to specify the manufacturer of the culture vessels, select (Standard). When using a 96- or 48-well plate, check the name of the product in use and select the corresponding vessel from the Vessel selection list.
(4)	Close button	Press this button to close the vessel selection window.

Abbreviation and description for vessels

Vessel type	Abbreviation in the list	Description
Petri Dish	35PD	35 mm dish
	60PD	60 mm dish
	100PD	100 mm dish
Culture Flask Suffix “A”: flat-bottom type. Without suffix “A”: partially-slanted (cant) bottom type. Suffix “(ob)”: oblong type.	25CF(ob)	25 cm^2 culture flask(ob)
	25CF_A(ob)	25 cm^2 culture flask A(ob)
	25CF	25 cm^2 culture flask
	25CF_A	25 cm^2 culture flask A
	75CF	75 cm^2 culture flask
	75CF_A	75 cm^2 culture flask A
Well Plate	6WP	6-well plate
	12WP	12-well plate
	24WP	24-well plate
	48WP	48-well plate
	96WP	96-well plate

(15) Select samples to load and set the samples name.

The sample name can be changed later.



Load information entry window

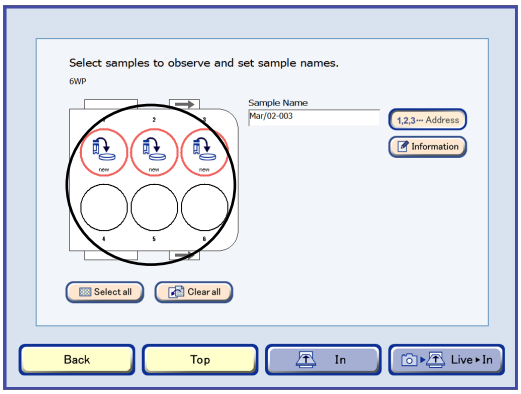
No.	Name	Function
(1)	Sample Name field	This field displays and/or enter the sample name. Press the Sample Name field to display a keyboard window to enter the sample name.
(2)	Sample registration area	Select the sample for load. When using a 96-, 48-well plate, press the sample registration area to display the 96-well or 48-well plate sample selection window.
(3)	Select all button	Press this button to select all samples.
(4)	Clear all button	Press this button to deselect all samples.
(5)	1,2,3... Address button	An address number is automatically added after a sample name. This function is set to ON by default. To disable this function so that the address number is not added after the sample name, press the 1,2,3... Address button to make it convex.
(6)	Information button	Press this button to display the Sample information batch entry window.
(7)	Back button	Press this button to return to the previous window.
(8)	Top button	Press this button to display the System status screen.
(9)	In button	Press this button to register sample information and start loading.
(10)	Live → In button	Press this button to observe the live image of the sample during loading.

a. When a vessel other than 96-, 48-well plate is used

a-1. Press the sample to be loaded in the sample registration area.

The frame color of the selected sample turns red. To deselect the sample, press the sample again.

Press the Select all button to select all samples.

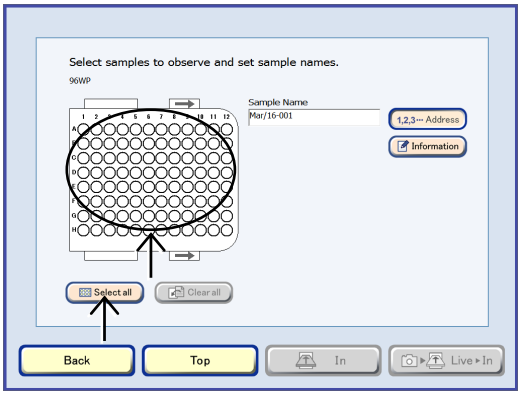


Load information entry window

b. When using a 96-, 48-well plate

Press the Select all button to select all samples.

b-1. To select a sample to load individually, press the sample registration area in the Load information entry window.



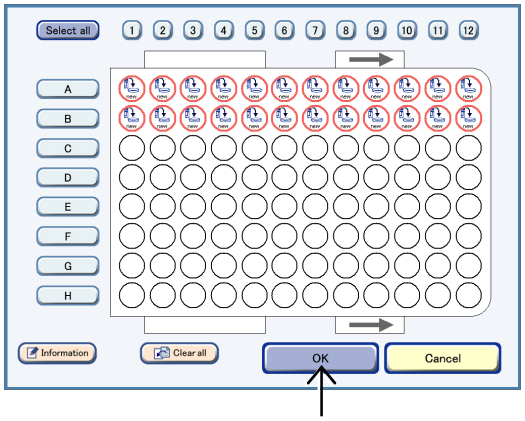
Well plate sample selection window (96-well)

b-2. Press the sample to be loaded. The frame color of the selected sample turns red.

Press the Select all button to select all samples.

Press one of the vertical alphabet buttons to select all samples in the corresponding row. Press one of the horizontal number buttons to select all samples in the corresponding column. When the selected sample is pressed again, the selection is canceled.

Press the OK button to apply the selections and reopen the Load information entry window.

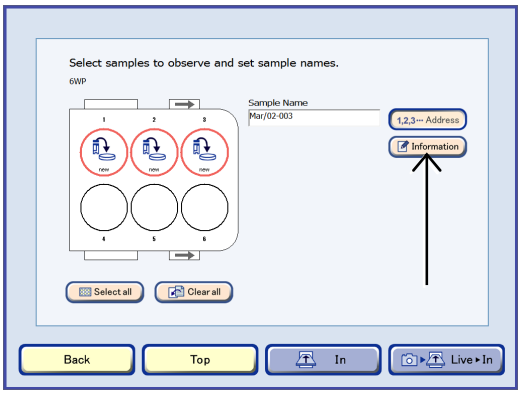


Well plate sample selection window (96-well)

If an empty well on which cells are not seeded is also registered when registering samples, autofocus on other wells may not work correctly during observation. Images may be defocused. Nikon recommends not registering an empty well on which cells are not seeded, if possible.
If an empty well is registered, do not select it in the Observation condition setting window.

(16) Press the Information button to display the Sample information entry window.

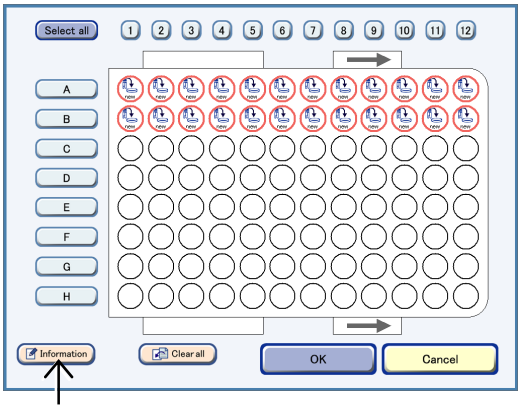
The Sample information batch entry window appears.



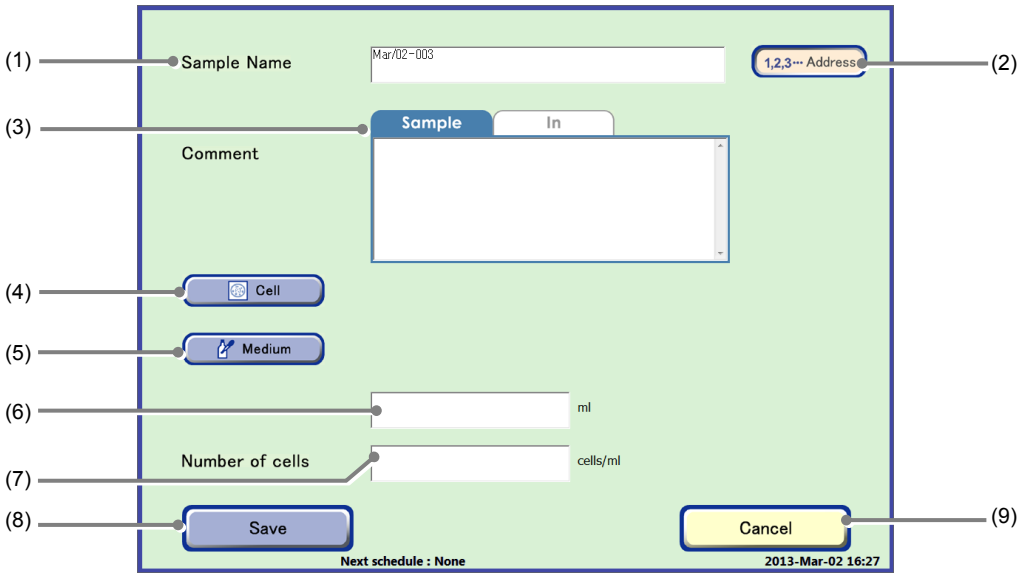
Load information entry window

When using a 96-, 48-well plate

Alternatively, on each Well plate sample selection window for 96-well and 48-well, press the Information button to display the Sample information batch entry window.



Well plate sample selection window (96-well)



Sample information batch entry window

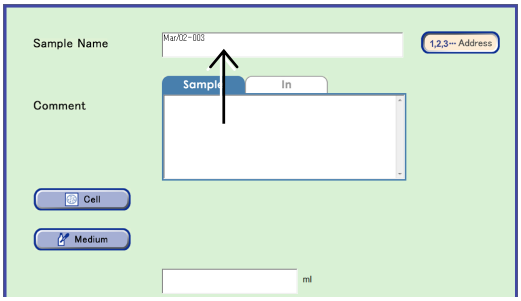
No.	Name	Function
(1)	Sample Name field	Enter a sample name.
(2)	1,2,3... Address button	The address number is appended to the end of the file name in the order of the well number of the culture vessel.
(3)	Comment field	Enter a sample comment on the Sample tab and a loading comment on the In tab.

No.	Name	Function
(4)	Cell button	Press this button to display the Cell list window.
(5)	Medium button	Press this button to display the Medium list window.
(6)	Culture media quantity field	Enter the culture media quantity.
(7)	Number of cells field	Enter the number of cells.
(8)	Save button	Press this button to save the entered sample information and display the Load information entry window.
(9)	Cancel button	Press this button to return to the Load information entry window.

(17) Enter a sample name.

1. Press the Sample Name field to display the Sample Name Keyboard window.

The keyboard window for entering the sample name is displayed.



Sample information batch entry window

2. Enter the sample name in the Keyboard window and then press the OK button.

The sample information batch entry window appears again and the entered sample name is displayed in the Sample Name field.

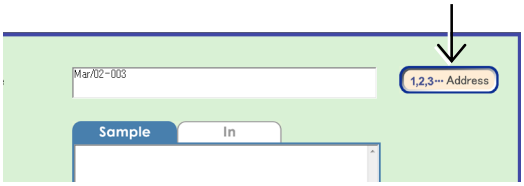
If a sample name is not entered by the user, “(date) – (serial number)” is automatically assigned as the sample name

(example: Apr/01-001).

Keyboard window

3. To not have the address number added after the sample name, press the 1,2,3... Address button to make it convex.

The 1,2,3… Address button is set to ON (convex) by default. When the 1,2,3... Address button is pressed, an address number is appended to the end of the file name in the order of the well number of the culture vessel.



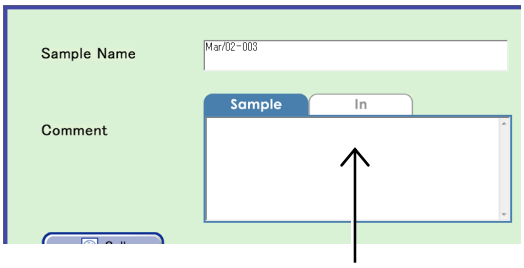
Sample information batch entry window

(18) Enter a comment.

A sample comment can be entered in the Sample tab and a loading comment can be entered in the In tab.

1. Press the Comment field to display the Keyboard window.

A comment of up to 128 characters excluding a carriage return character can be entered.



Sample information batch entry window

2. **Enter a comment and then press the OK button.**

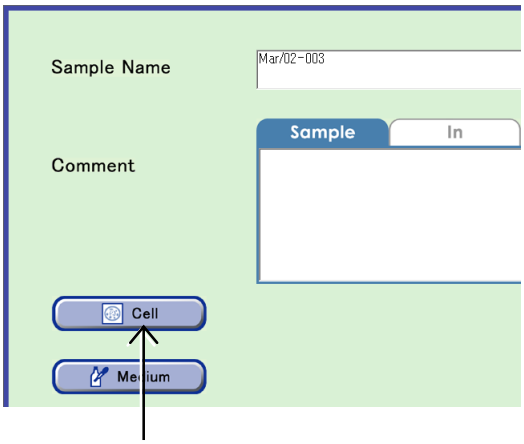
   The Sample information batch entry window appears again and the comment is displayed.

Keyboard window

(19) Select a cell name.

1. **Press the Cell button.**

   The Cell list window appears.

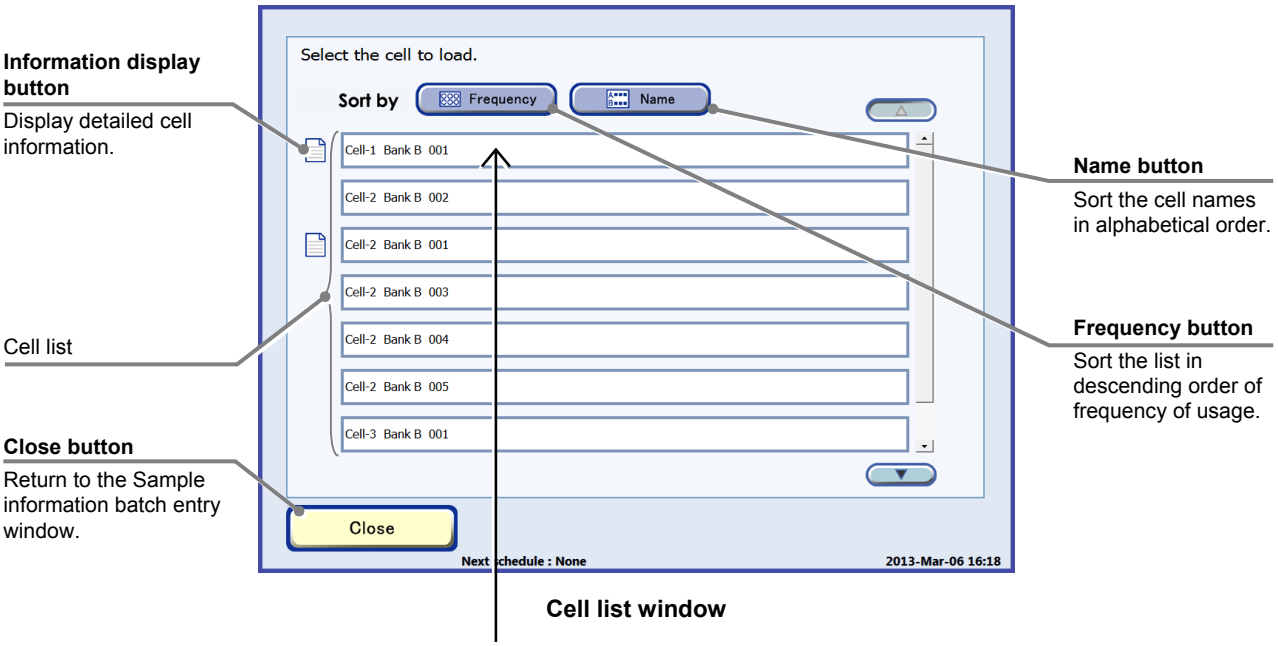


Sample information batch entry window

2. **From the cell list, select a cell in the sample.**

   The Sample information batch entry window is displayed and the selected cell name appears.

> The cell names displayed in the Cell list window must be registered in advance in the Cell master data window of the Master data maintenance window. For details on the procedure for registering cell master data, see Section 7.3.4, “Cell Master Data.”

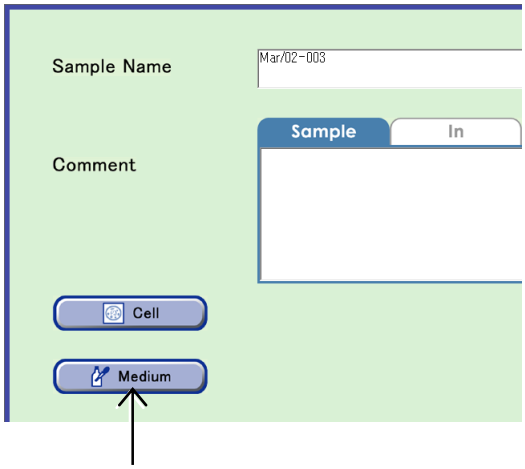


Cell list window

(20) Select the prepared medium of the sample.

1. Press the Medium button.

The Prepared medium list window is displayed.

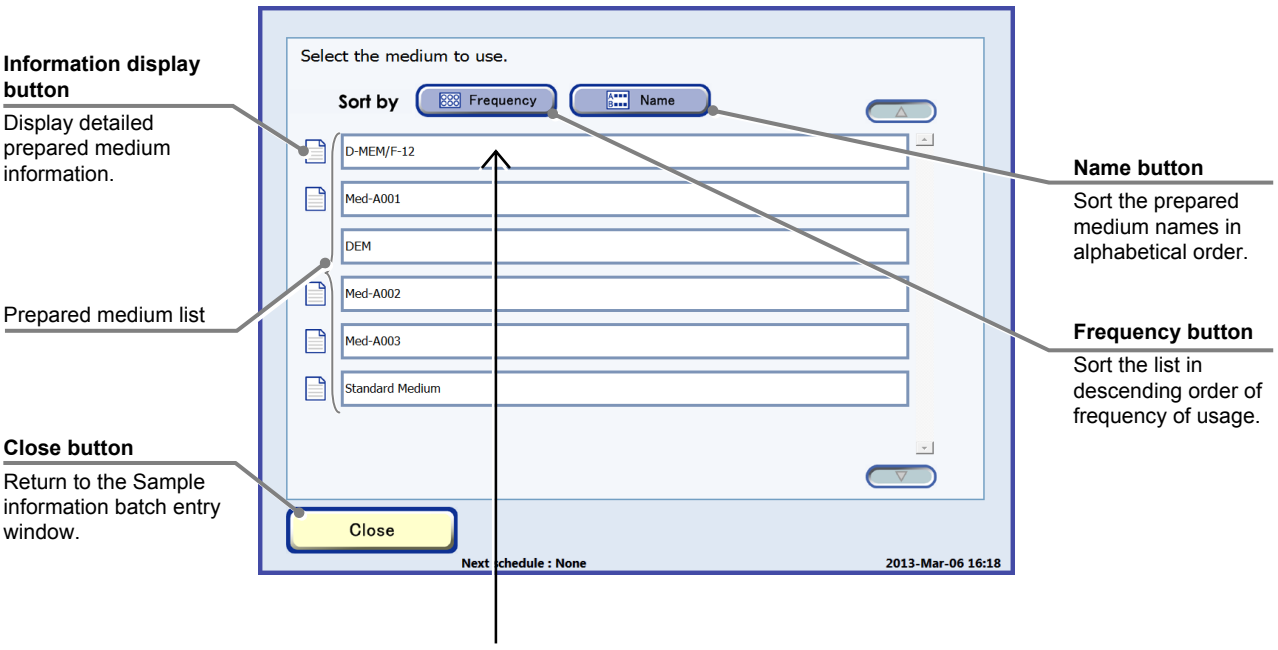


Sample information batch entry window

2. From the list, select the prepared medium of the sample.

The Sample information batch entry window is displayed and the selected prepared medium name appears.

> The prepared medium names displayed in the Prepared medium list window must be registered in advance in the Prepared medium master window of the Master data maintenance window. For details on the procedure for registering prepared medium master data, see Section 7.3.5, “Prepared Medium Master Data.”

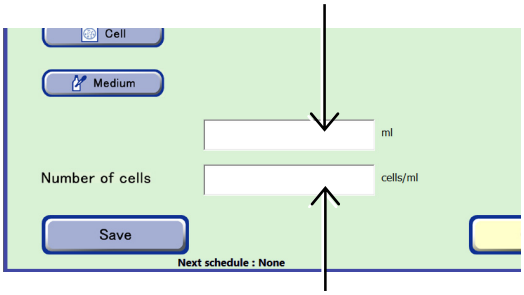


Prepared medium list window

(21) Enter the medium quantity and the number of cells.

1. **Press the Medium quantity field and the Number of cells field to display the Keyboard window.**
2. **Enter the medium quantity and the number of cells and then press the OK button.**

   The Sample information batch entry window is displayed and the medium quantity and the number of cells appear in each field.

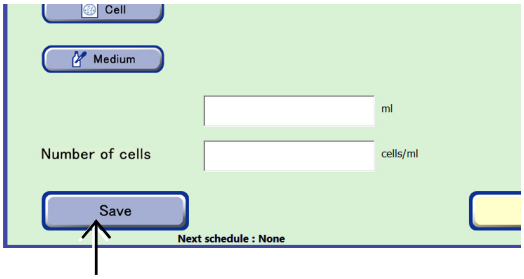


Sample information batch entry window

Keyboard window

(22) Press the Save button.

The entered sample information is saved and then the Load information entry window appears.

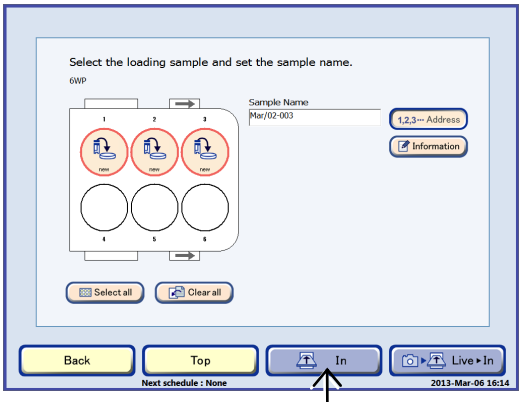


Sample information batch entry window

(23) Press the In button in the Load information entry window.

The color of the sample for which sample information is registered turns orange.

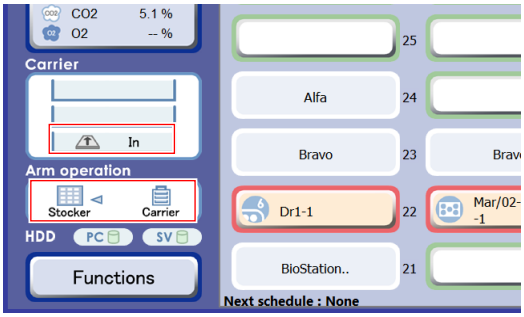
Transport of the sample is started and the System status screen appears again.



Load information entry window

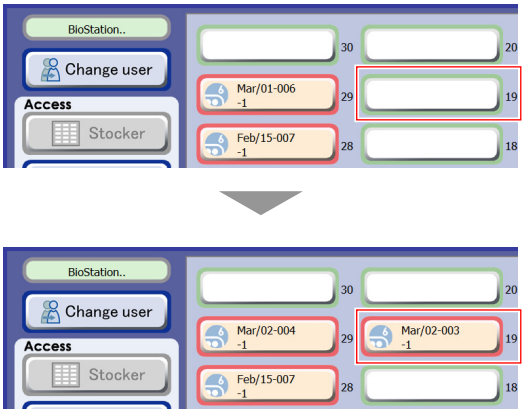
In the Carrier area of the System status screen, “In” is displayed to indicate that the holder is being loaded.

The Arm operation area indicates that a holder is being transported from the carrier to the stocker.



System status screen

When transport to the stocker is completed, the display of the vacant stocker is turned to the occupied state.

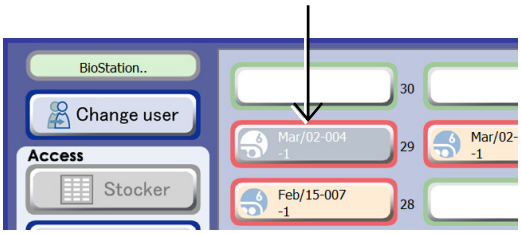


4.3.2.4 Reloading samples

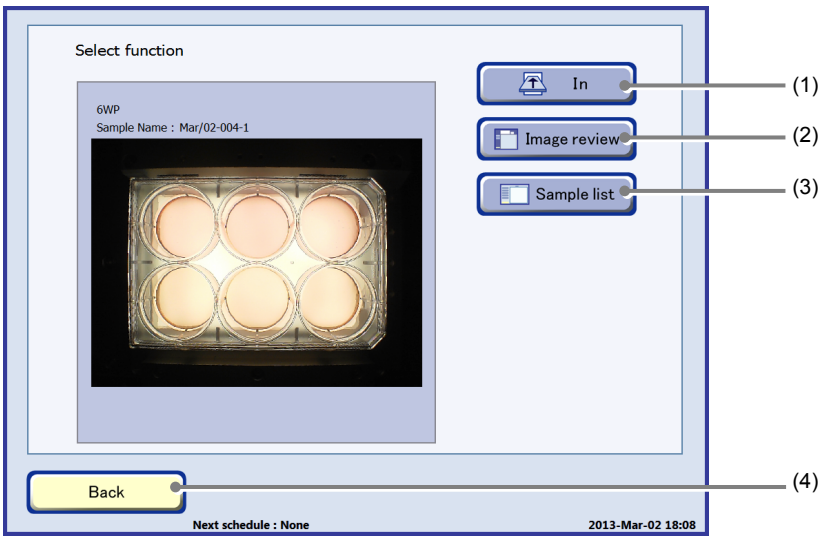
This section describes the procedure for reloading a sample that was unloaded for the purpose of changing the medium into the culture chamber.

(1) Press the stocker button that indicates a sample is temporarily unloaded (same location as before unloading).

The Select function window (Reload) appears.



System status screen



Select function window (Reload)

No.	Name	Function
(1)	In button	Press this button to start loading.
(2)	Image review button	Press this button to display the Image review window.
(3)	Sample list button	Press this button to display the Sample list window.
(4)	Back button	Press this button to display the System status screen.

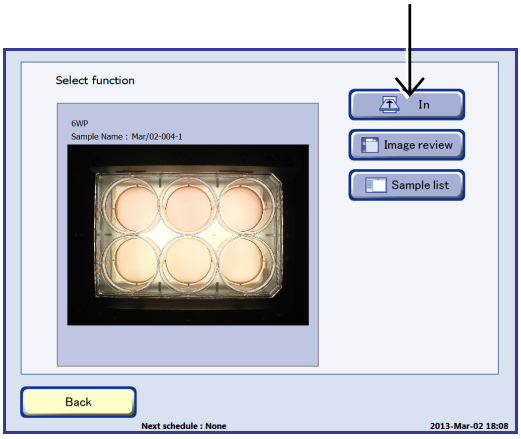
When the sample no longer needs to be reloaded

Since the status of the stocker indicates that the sample has been temporarily unloaded, load a (dummy) sample temporarily, select “No return” in the Unload purpose selection window and then unload the sample again.
(When “Medium change” is selected as the purpose of unloading, the status of the stocker that contains the sample is unchanged until it is reloaded.)

For details on the procedure for reloading a sample, see this section.
For details on the procedure for unloading a sample, see Section 4.3.3, “Unloading Samples.”

(2) Press the In button.

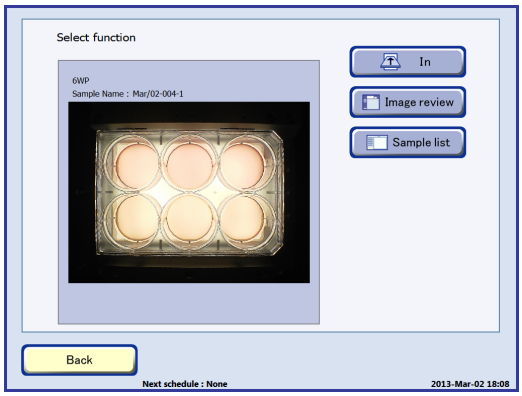
The Loading procedure window appears.



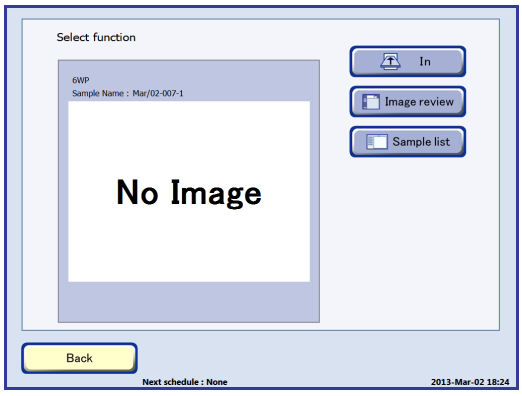
Select function window (Reload)

When a sample that has its macro image captured is reloaded, the Macro image is displayed in the Select function window (Reload) by default.

When a sample that does not have its macro image captured is reloaded, “No Image” appears in the image display area.



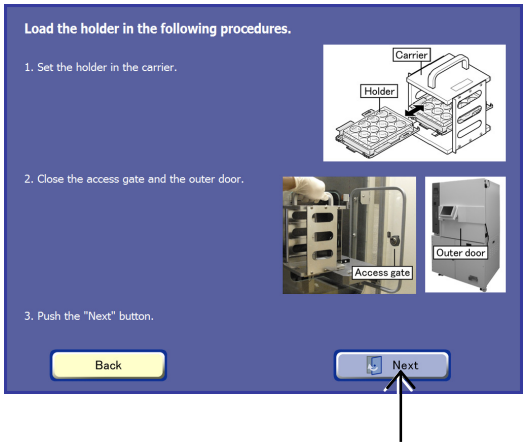
Select function window (Reload)
(with Macro image)



Select function window (Reload)
(without Macro image)

(3) If the carrier has already been set in the system, press the Next button.

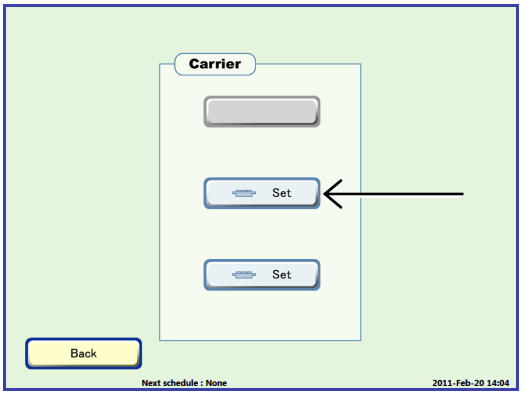
The Load holder selection window appears.



Loading procedure window

(4) Press the Set button of the carrier on which the holder to be loaded is set.

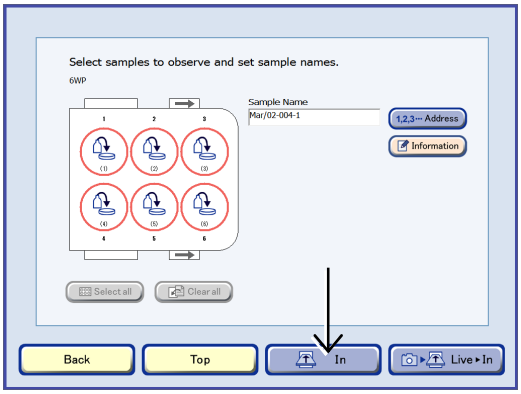
The Load information entry window appears.



Load holder selection window

(5) Press the In button to load the sample directly to the stocker.

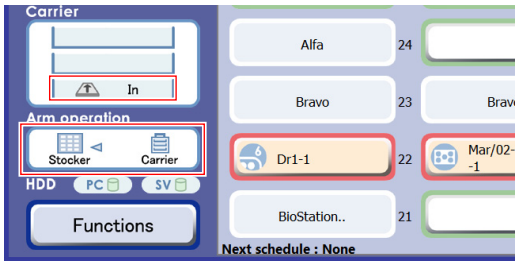
Transport of the sample is started and the System status screen appears again.



Load information entry window

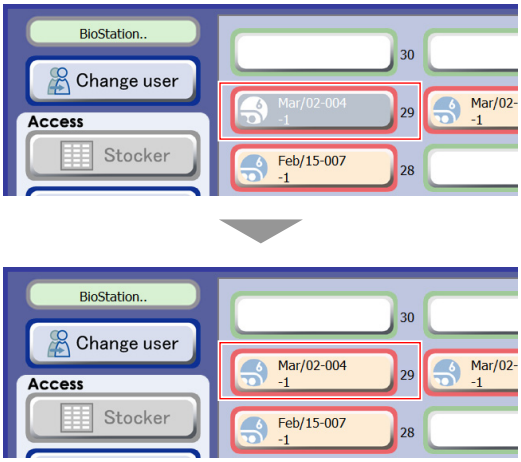
In the Carrier area of the System status screen, “In” is displayed to indicate that the holder is being loaded.

The Arm operation area indicates that a holder is being transported from the carrier to the stocker.



System status screen

When transport to the stocker is completed, the display of the vacant stocker changes to the occupied state.



4.3.3 Unloading Samples

To unload the sample from the culture chamber for the purpose of changing the medium and so on, follow the procedure below.

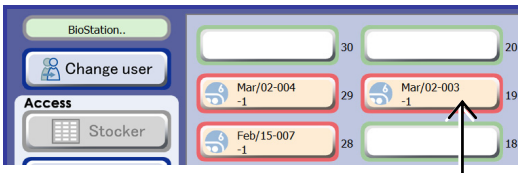
> When a sample is unloaded with “No return” selected as the unloading purpose, the scheduled observation settings are deleted. To reload the sample, register the schedule after loading the sample again. For the procedure for setting a scheduled observation, see Section 4.6, “Scheduled Observation (Automatic Observation).”

4.3.3.1 Unloading samples

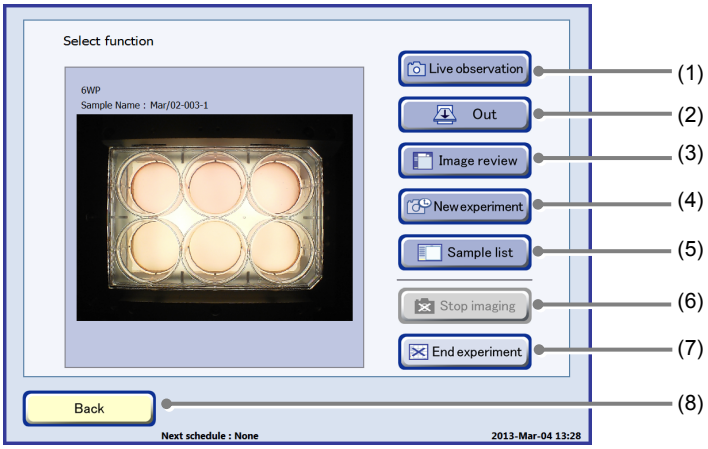
This section describes the procedure for unloading the sample from the stocker to the carrier.

(1) Press the stocker to be unloaded in the Stocker display area on the System status screen.

The Select function window appears.



System status screen



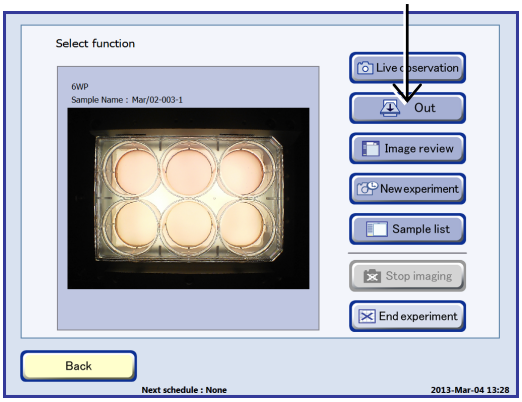
Select function window

No.	Name	Function
(1)	Live observation button	Press this button to display the Ph live observation window and perform live image observation.
(2)	Out button	Press this button to display the Unload purpose selection window and unload the sample to the carrier.
(3)	Image review button	Press this button to display the Image review window and view the observation data.
(4)	New experiment button	Press this button to display the Observation condition setting window and set the schedule observation.
(5)	Sample list button	Press this button to display the Sample list window.
(6)	Stop imaging button	Press this button to cancel capturing in stage exclusive mode or cancel stage standby in stage holding on mode. Enabled only when stage exclusive mode or stage holding on mode is set for a scheduled observation.

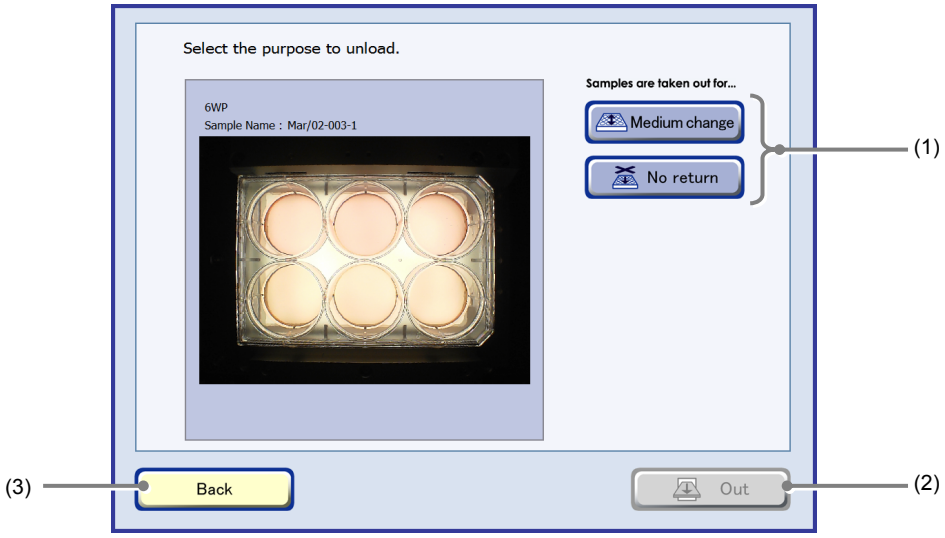
No.	Name	Function
(7)	End experiment button	Press this button to delete all scheduled observations scheduled for the samples in the selected holder.
(8)	Back button	Press this button to display the System status screen.

(2) Press the Out button.

The Unload purpose selection window appears.



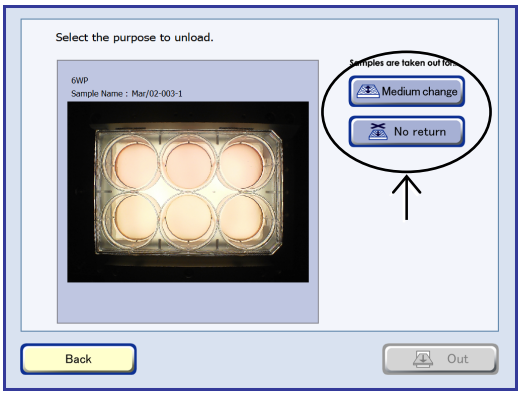
Select function window



Unload purpose selection window

No.	Name	Function
(1)	Unload purpose selection	Select the unloading purpose. **Medium change button:** Medium change **No return button:** The sample will not be reloaded
(2)	Out button	Press this button to display the Unload holder selection window. This button is enabled when an unloading purpose is selected.
(3)	Back button	Press this button to display the Select function window.

(3) Select the purpose of unloading.



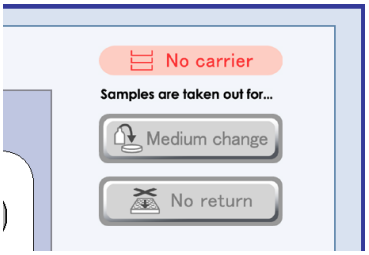
Unload purpose selection window

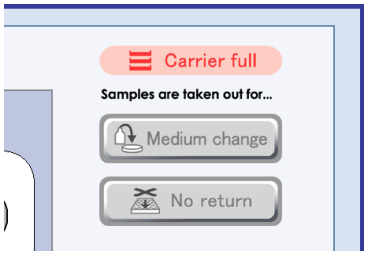
If a sample is reloaded after it is unloaded for the purpose of “Medium change”, the custom observation points and scheduled observation settings registered before unloading are preserved. Scheduled observation is not performed while unloading, however, it can be performed after reloading. For details, see Section 4.6, “Scheduled Observation (Automatic observation).”

When a sample is unloaded with “No return” selected as the unloading purpose, the registered custom observation points and schedule settings are deleted.

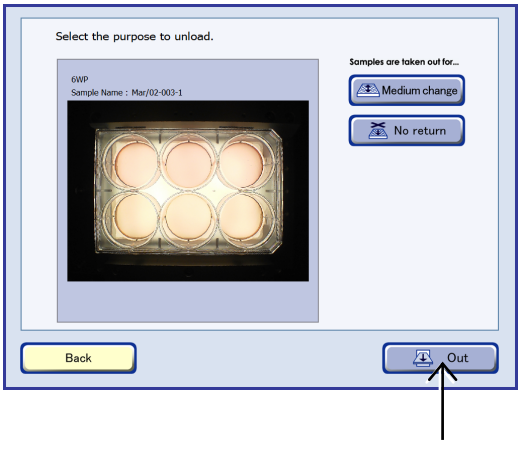
If the Unload purpose button can no longer be pressed (i.e., is disabled), the carrier is either not set on the carrier slider, or there are no vacant positions in the carrier. The corresponding reason (No carrier, or Carrier full) is displayed in the top right of the screen.

Place a carrier that has a vacant position on the carrier slider and start the procedure again.





(4) When the Out button is pressed after the purpose of unloading is selected, the Unload holder selection window appears.

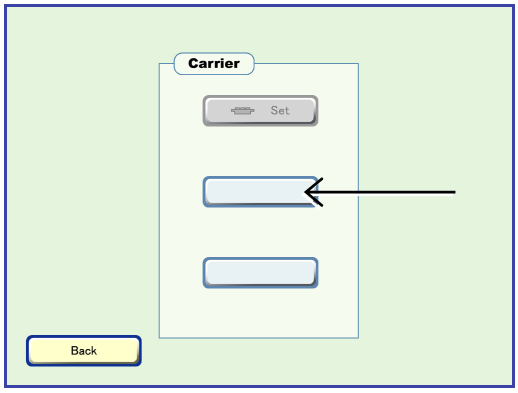


Unload purpose selection window

(5) Select the position of the carrier to be unloaded.

If a carrier position is occupied, it cannot be selected.

When the button for the vacant carrier is pressed, the System status screen appears again and the sample is unloaded.

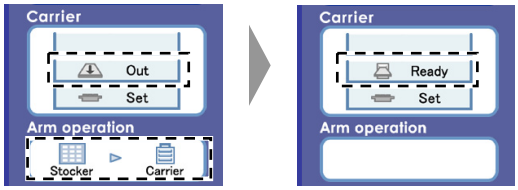


Unload holder selection window

In the Carrier area of the System status screen, “Out” is displayed to indicate that the holder is being unloaded.

The Arm operation area indicates that a holder is being transported from the stocker to the carrier.

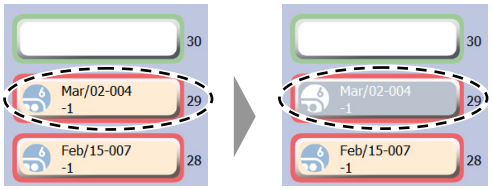
When transport is completed, the icon in the Carrier area changes to the Ready icon.



Carrier area

If “Medium change” is selected as the purpose of unloading in the Select function window, the sample button in the stocker display area turns gray to indicate that it is temporarily unloaded.

If a scheduled observation has been registered, that schedule setting is retained. Scheduled observation is not performed while unloading, however, it can be performed after reloading.

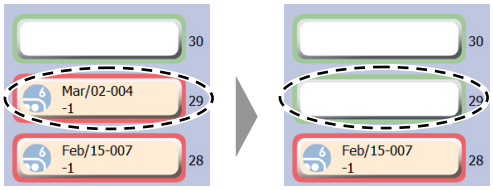


Stocker display area

(when the purpose of unloading is “Medium change”)

If “No return” is selected as the purpose of unloading in the Select function window, the sample button in the stocker display area changes to indicate that the stocker is vacant.

If a scheduled observation has been registered, the schedule setting is deleted.



Stocker display area

(when the purpose of unloading is “No return”)

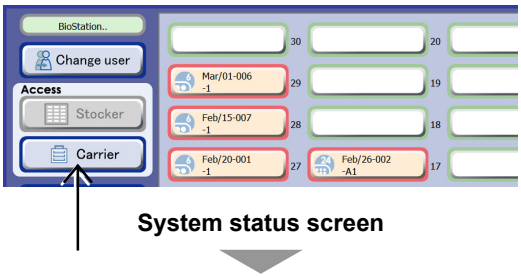
4.3.3.2 Unloading the carrier

This section describes the procedure for unloading a carrier through the access gate.

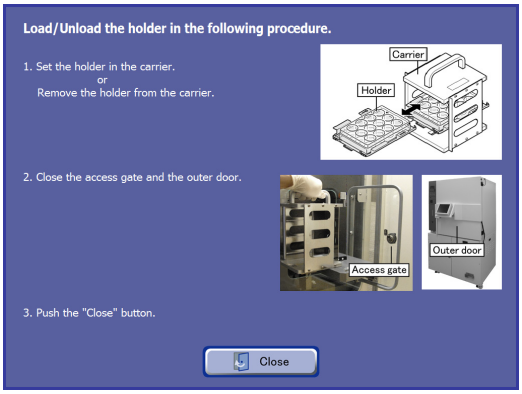
(1) Press the Carrier button in the Access area of the System status screen on the touch panel display.

The Load/Unload procedure window is displayed and the access gate is unlocked. When the access gate is unlocked, the loading door lamp on the temperature/humidity controller area lights.

The Carrier button is disabled while samples are transferred into the CO_2 incubator.



System status screen



Load/Unload procedure window

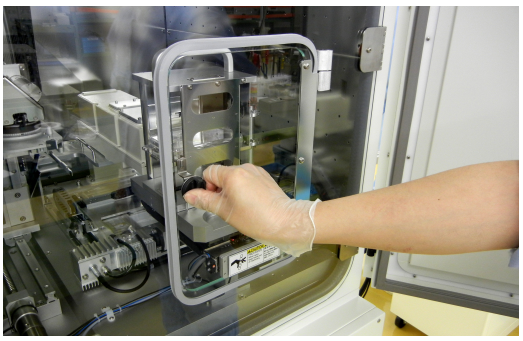
(2) Open the CO_2 incubator outer door.

Open the CO_2 incubator outer door by pulling the handle at the bottom of the touch panel display.

CO_2 incubator outer door

(3) Open the access gate.

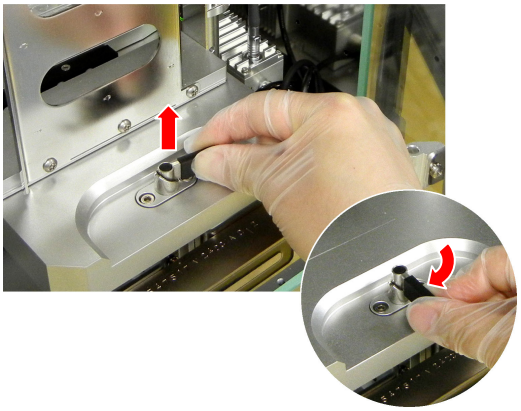
Open the Access gate by turning the knob vertically and pulling the knob.



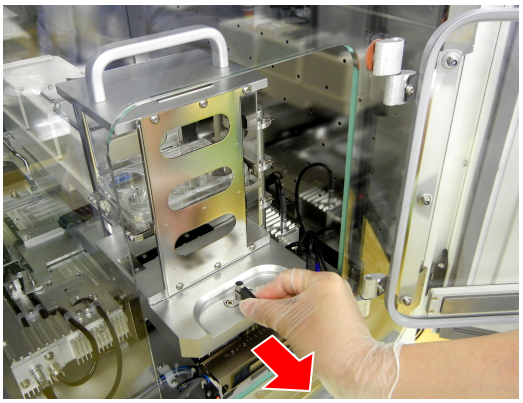
Access gate

(4) Pull up the knob of the carrier slider and turn it clockwise 90 degrees.

The carrier slider is unlocked.



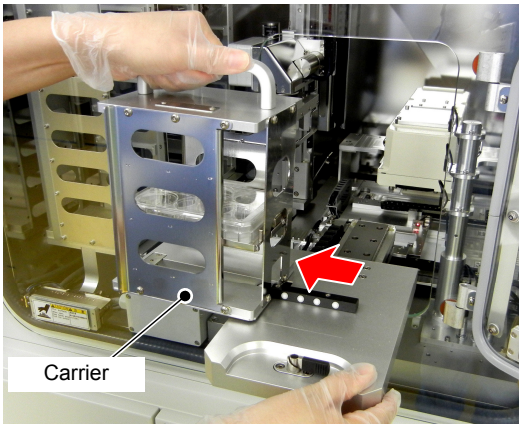
(5) Slowly pull out the carrier slider.



Carrier slider

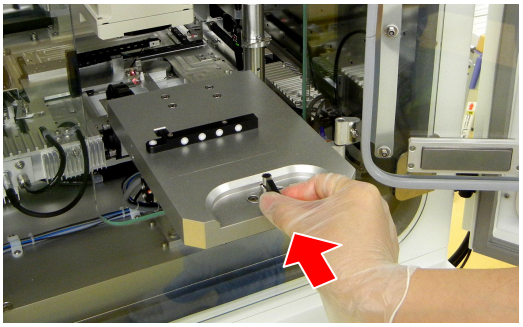
(6) Remove the carrier.

Remove the carrier from the carrier slider by sliding it leftward.



Unloading the carrier

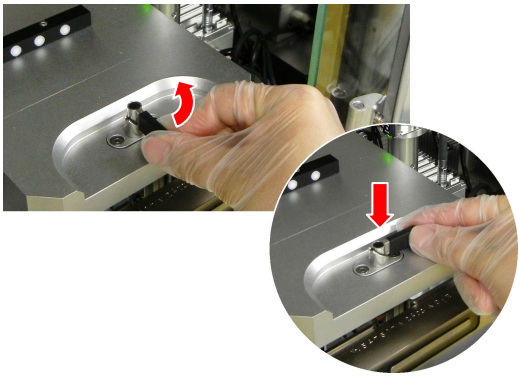
(7) Push the carrier slider into the culture chamber.



Carrier slider

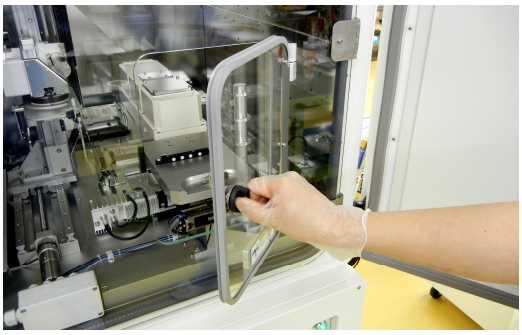
(8) Turn the knob on the carrier slider counterclockwise 90 degrees and push it down.

The carrier slider is locked.



(9) Close the access gate.

Close the access gate and turn the knob horizontally to lock the access gate.



Access gate

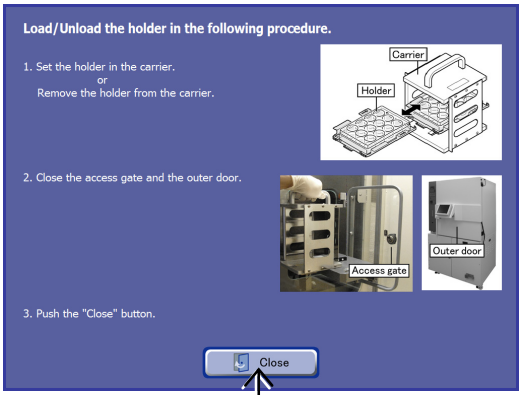
(10) Close the CO_2 incubator outer door.

Put the removed carrier into a safety cabinet immediately after removal.

CO_2 incubator outer door

(11) Press the Close button on the touch panel display.

The Load/Unload procedure window closes and the System status screen appears.



Load/Unload procedure window

4.4 Observing and Capturing Samples

This product can be used to observe and capture a sample loaded into the culture chamber or being cultured in the culture chamber, with the built-in microscope in the observation unit. There are two observation methods as follows:

Live observation (manual observation):

The sample in the stocker or the carrier is observed by operating the touch panel display manually.
For the live observation procedure, see Section 4.5, “Live Observation (Manual Observation).”

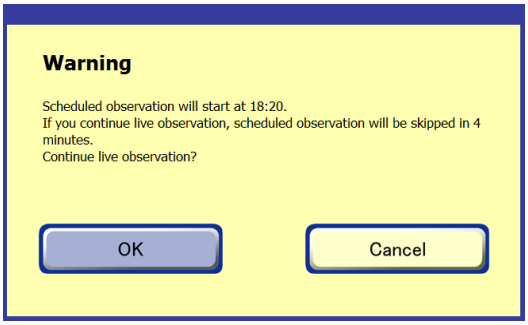
Scheduled observation (automatic observation):

By setting the observation schedule for each sample in the system, culture images are automatically captured at the specified times.
For the scheduled observation procedure, see Section 4.6, “Scheduled Observation (Automatic Observation).”

Alarm window indicating the start time of a scheduled observation

If the start time of a scheduled observation occurs during live observation, an alarm window appears. In the window, live observation can be stopped or scheduled observation can be canceled.

If the alarm window is left displayed, the sample under live observation automatically moves to the original position and the scheduled observation starts in accordance with the settings.



Caution

When observing a sample immediately after it is loaded, be very careful with condensation on the culture vessel lid. Condensation on the lid may degrade autofocus performance.

If the temperature of a culture vessel is significantly lower than that of the interior of this product, condensation may occur on the lid. Make sure that there is no condensation causing temperature difference before loading the culture vessel, or wait until the temperature difference is minimized before performing observation.

4.4.1 Observation Area

Caution

The observation image may be unclear when certain types or shapes of culture vessel are used, or some observation point is observed in phase contrast microscopy. This phenomenon may also occur on the BioStation CT because the product performs phase contrast microscopy.

This phenomenon occurs frequently at low magnification (2x or 4x) observations, especially with a small area culture vessel such as a 96-well plate, a 48-well plate, or 24-well plate.
This phenomenon occurs in the peripheral areas of a culture vessel at high magnification (10x, 20x, or 40x) observations.

Consider these conditions before setting observation conditions for live observation and scheduled observations. For details on desirable observation areas, see the following tables.

Desirable observation areas (6-well plate)

Magnification 2x	Within 4 mm radius of the well center
Magnification 4x	Within 6.5 mm radius of the well center
Magnification 10x	Within 12.5 mm radius of the well center
Magnification 20x	Within 12.5 mm radius of the well center
Magnification 40x	Within 12.5 mm radius of the well center

Desirable observation areas (96-well plate)

Magnification 2x	No good observation area
Magnification 4x	No good observation area
Magnification 10x	Within 0.8 mm radius of the well center
Magnification 20x	Within 0.8 mm radius of the well center
Magnification 40x	Within 0.8 mm radius of the well center

Unclear image area near the peripheral of a culture vessel

Magnification 2x	13.5 mm or near from the peripheral area of a culture vessel
Magnification 4x	10.5 mm or near from the peripheral area of a culture vessel
Magnification 10x	5.0 mm or near from the peripheral area of a culture vessel
Magnification 20x	5.0 mm or near from the peripheral area of a culture vessel
Magnification 40x	5.0 mm or near from the peripheral area of a culture vessel

4.5 Live Observation (Manual Observation)

This section describes the procedure for manually observing a sample in the stocker or the carrier.

Caution

During live image operation, handle the touch panel in a manner that does not cause vibration to the product. The observation image may be disturbed by vibration.

4.5.1 Ph live observation window

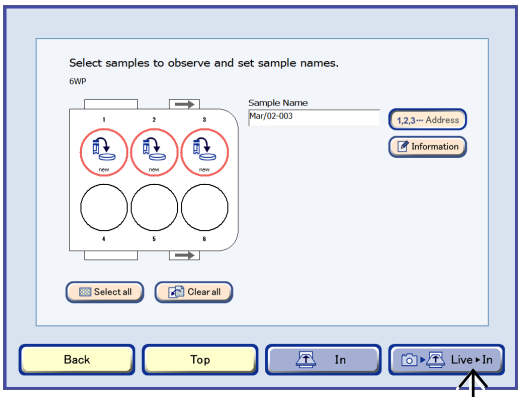
4.5.1.1 Displaying the Ph live observation window

(1) Display the Ph live observation window.

To observe a live image of the sample during loading

1. **Press the Live In button in the Load information entry window.**

   The sample is transported from the carrier to the observation stage and the Ph live observation window appears.

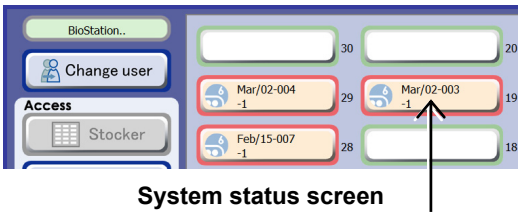


Load information entry window

To observe the live image of the sample being cultured

1. **On the System status screen, press the button of the stocker where the sample is stored.**

   The Unload window appears.

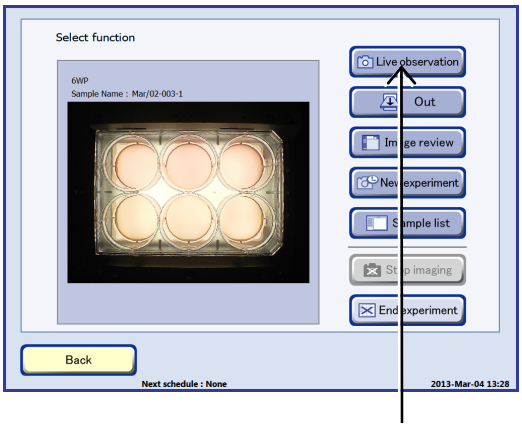


System status screen

2. **Press the Live observation button in the Select function window.**

   A live observation start confirmation dialog box appears. Press the OK button to close the dialog box.

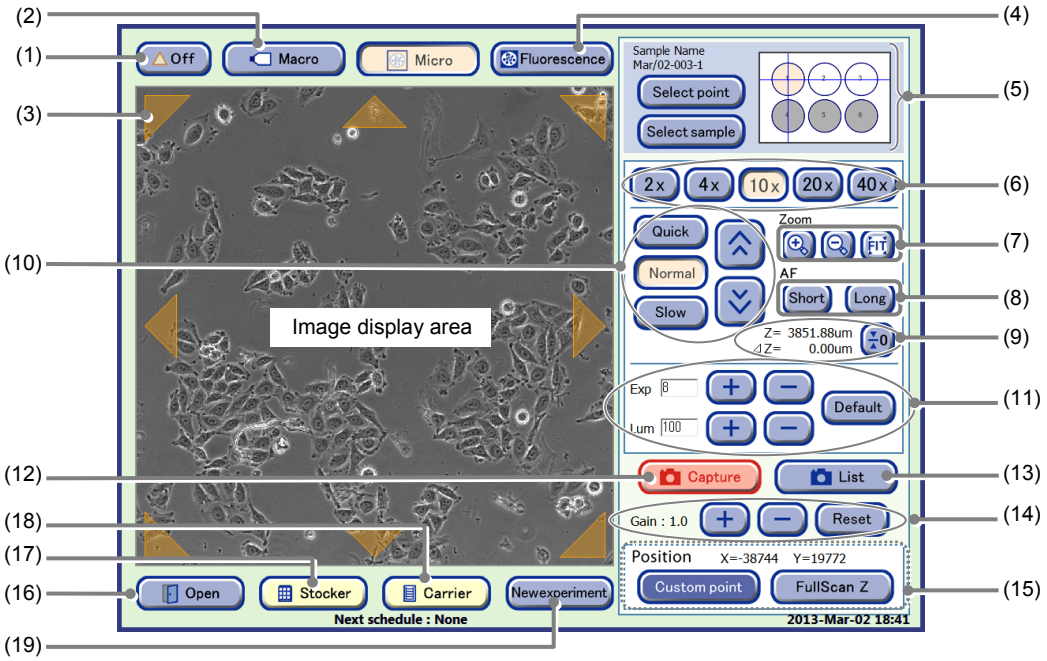
   The sample is transported from the stocker to the observation stage and the Ph live observation window appears.



Select function window

> During a scheduled observation, live observation cannot be performed. If the start time of a scheduled observation comes, a message appears. (See “Alarm window indicating the start time of a scheduled observation” in Section 4.4, “Observing and Capturing Samples.”)

When the window is switched to the Ph live observation window, autofocus is performed automatically. If autofocus is not performed, set the function in the user setting window. For details, see Chapter 5, “Environmental Settings.”



Ph live observation window

No.	Name	Function
(1)	△Off button	Press this button to display or hide the observation field shift mark.
(2)	Macro button	Press this button to display the Macro live observation window.
(3)	Observation field shift mark	Press the marks to shift the observation field up/down, left/right, or diagonally, by one frame at a time.
(4)	Fluorescence button	Press this button to display the FL image capture window. When the Fluorescence unit is not connected, the Fluorescence button is disabled.
(5)	Holder map	This map displays the current observation point. **Select point button:** Display the Select point window. The observation point used for the most recent scheduled observation and custom observation point can be redisplayed to check the current status of that observation point. **Select sample button:** Display the Select sample window. Samples for observation in the holder can be switched.
(6)	Magnification select button	Press this button to select the observation magnification.
(7)	Zoom area	Change the display size of the image. **+, - buttons:** Enlarge or reduce the image. **FIT button:** Display the image on the full screen.
(8)	AF area	Perform autofocus. **Short button:** Find the appropriate focal point near the current Z position. **Long button:** Long button to find the focal point from a wide range. (Auto focusing takes time.)

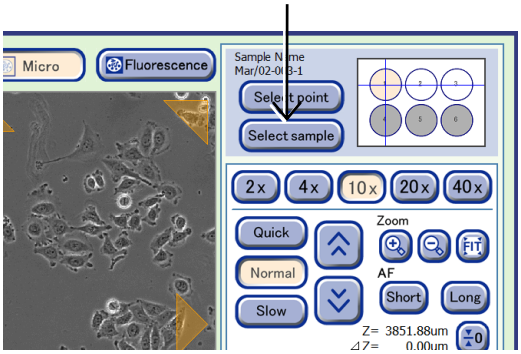
No.	Name	Function
(9)	Z position	**Z=:** (Absolute value of Z) Display the value of the actual Z position. **ΔZ=:** (Relative value of Z) Display the Z position value relative to the Z position set using the Calibration button. **:** (Calibration button) Set the current Z position as the origin (0). When selected, ΔZ becomes 0.
(10)	Focus adjustment area	Adjust the focus of the micro image. : Move the focus position in the Z direction (manual focus). Keep pressed for continuous movement. **Quick button:** Speed up movement in the Z direction (coarse). **Normal button:** Move in the Z direction at normal speed. **Slow button:** Slow down the movement in the Z direction (fine).
(11)	Camera adjustment	Adjust the camera settings. Press the +/- button or enter a numeric value in the entry field for adjustment. **Exp field:** Adjust the exposure time. **Lum field:** Adjust the intensity. **Default button:** Restore the default camera settings.
(12)	Capture button	Press this button to capture and display the image in the Ph image register window.
(13)	Image List button List	Press this button to display the list of the captured images.
(14)	Brightness adjustment of a displayed image	Adjust the brightness of a displayed image. **Gain:** Press the +/- button to adjust the contrast by ±0.1. **Reset button:** Reset the brightness of a displayed image.
(15)	Position area	Register the custom observation point. **Custom point button:** Register the center of field displayed in the Image display area as the custom observation point (X, Y, Z). **FullScan Z button:** Register the center of field displayed in the Image display area as the custom observation point that can be used for custom focus during Full Scan observation.
(16)	Open button	Press this button when opening the door during live observation before loading a sample into the CO_2 incubator. The Ph live observation window can be restored when the Close button is pressed after loading the sample.
(17)	Stocker button	Press this button to finish the live observation and return the holder to the stocker.
(18)	Carrier button	Press this button to finish the live observation and return the holder to the carrier.
(19)	New experiment button	Press this button to display the Observation condition setting window. Scheduled observation can be set for samples under live observation.

4.5.1.2 Capturing a Ph live image and registering a custom observation point

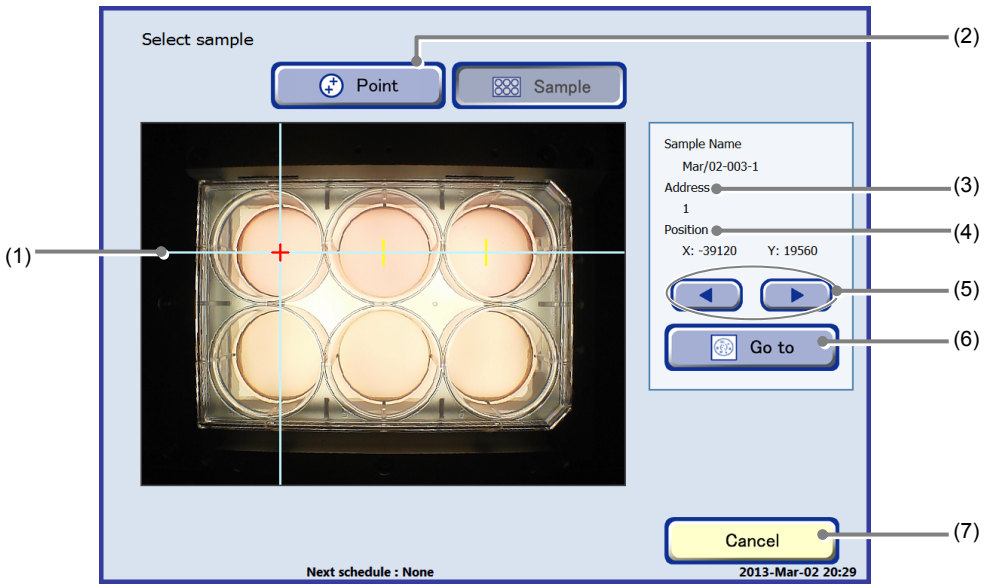
(1) Select a sample for observation.

1. Press the Select sample button.

The Select sample window appears.



Ph live observation window



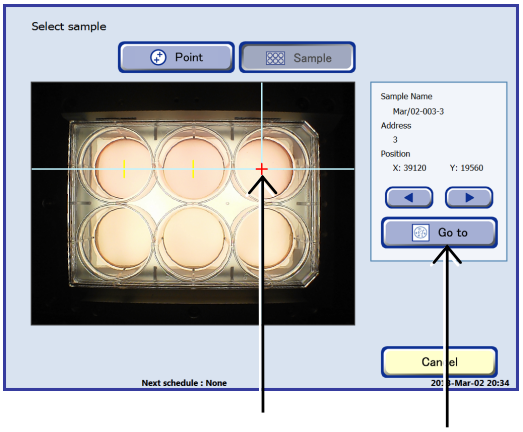
Select sample window

No.	Name	Function
(1)	Holder map display	This area displays the holder map on the macro image. Press the sample on the holder map to select the sample to display micro image.
(2)	Point button	Press this button to switch the window to the Select point window. (See Section 4.5.5.1.)
(3)	Address display	This area displays the address of the sample selected in the holder map.
(4)	Coordinates of the observation point	This area displays the coordinates (x, y) for the observation point, where (0, 0) is the center of the holder.
(5)	◄/► button	Select the sample in numeric order.
(6)	Go to button	Press this button to display the Ph live observation window of the selected sample.
(7)	Cancel button	Press this button to return to the Ph live observation window.

2. **On the holder map display, press the sample to be observed. Alternatively, the sample can be selected by pressing the ◄/► buttons.**

3. **Press the Go to button.**

   The micro image (Ph) of the selected sample appears in the Ph live observation window.



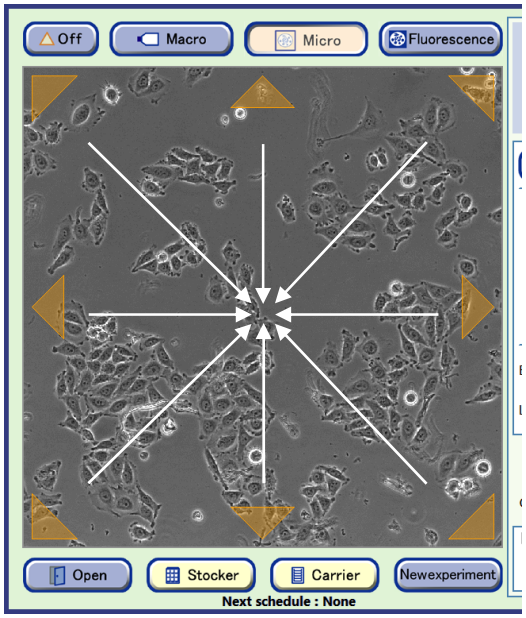
(2) Shift the image display to find the observation point.

Press a position on the Image display area to display that position at the center. The micro image (Ph) is redisplayed with the pressed position located at the center.

Shift the image display by pressing the image display area until the observation point appears.

To shift the observation field by one frame at a time, turn ON the observation field shift marks ON/OFF button and then shift the display position by pressing observation field shift mark displayed around the micro image.

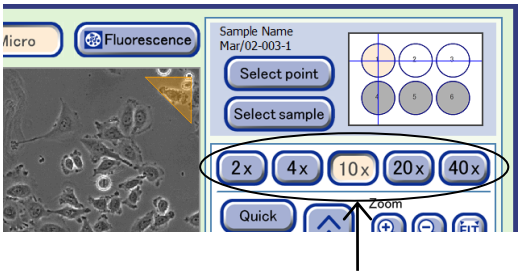
> The previous observation point of captured images or the scheduled observation point can be displayed again.
>
> For the procedure for redisplaying the registered observation point, see Section 4.5.5, “Redisplaying the Registered Observation Point.”



Ph live observation window

(3) Change the image magnification by pressing the magnification selection button.

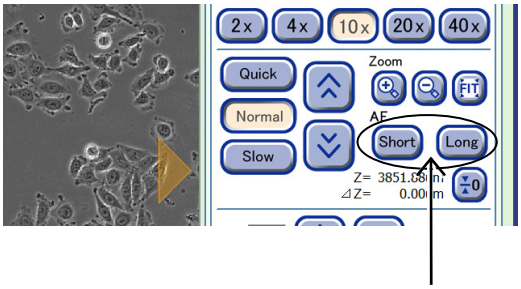
The Ph image or FL image is enlarged or reduced in accordance with the selected observation magnification.



(4) Repeat autofocus as necessary by pressing the Short or Long autofocus range button.

Press the Short button to find the appropriate focal point near the current Z position.

Press the Long button to find the focal point from a wide range. (Auto focusing takes time.)

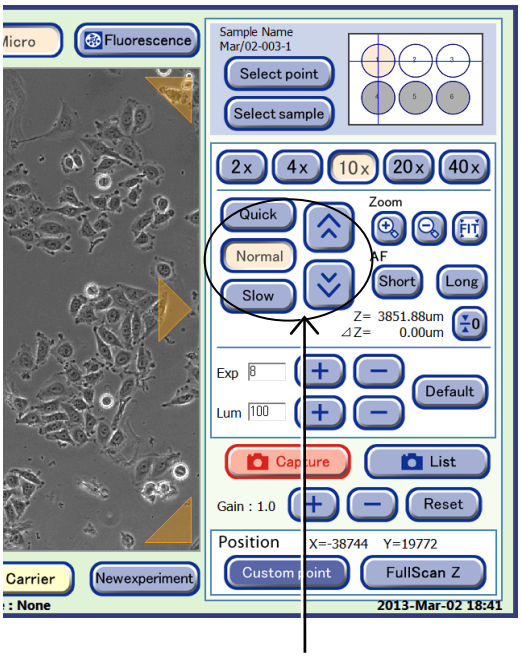


(5) Adjust the focus of the micro image.

1. Press the ⩓ / ⩔ button to adjust the focus of the micro image.

Press the ⩓ / ⩔ button to move the micro image by one unit in the Z axis direction.

Press and hold the ⩓ / ⩔ button to continuously move the micro image in the Z axis direction. To change the speed, switch the setting either to Slow or to Quick.

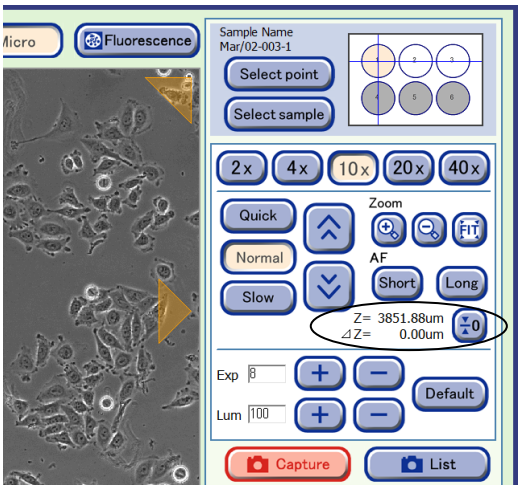


2. To set the current Z position as the origin, press the Calibration button.

ΔZ is displayed as “0” and this position is the origin for the relative position of Z.

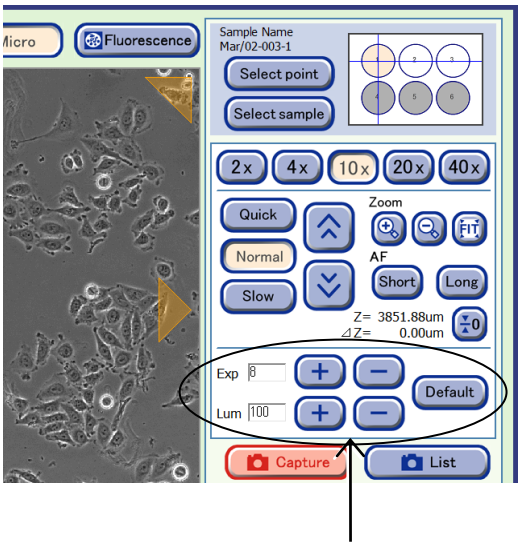
This position will be kept as the origin (0) during live observation even if another sample is loaded.

The absolute value of the actual Z position is displayed above the relative value.



(6) To change the exposure time (msec), use the the +/- button to adjust or enter an exposure time (1 to 20000) using a keyboard displayed by pressing the Exp field, and then press the OK button.

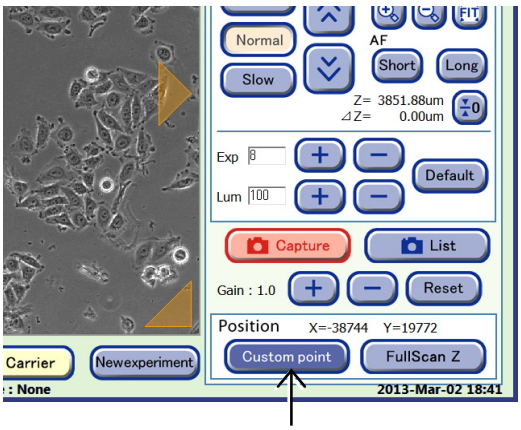
(7) To change the brightness, use the +/- button to adjust or enter a brightness value (1 to 220) using a keyboard displayed by pressing the Lum field, and then press the OK button.



(8) Register the X, Y, Z position as a custom observation point.

1. Press the Custom point button in the Position area.

A custom observation point registration confirmation dialog box appears.

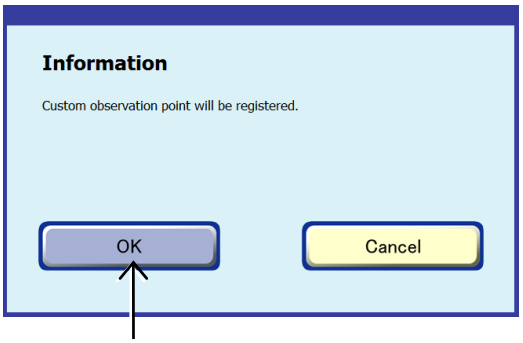


Ph live observation window

2. Press the OK button to close the dialog box.

The currently displayed live observation point (center of the field of view displayed in the Image display area) is registered as a custom observation point.

When the custom observation point is set, the micro image (Ph) is also saved.



Confirmation dialog box

(9) Set the Z position as the custom observation point to be used for Custom focus during Full Scan observation.

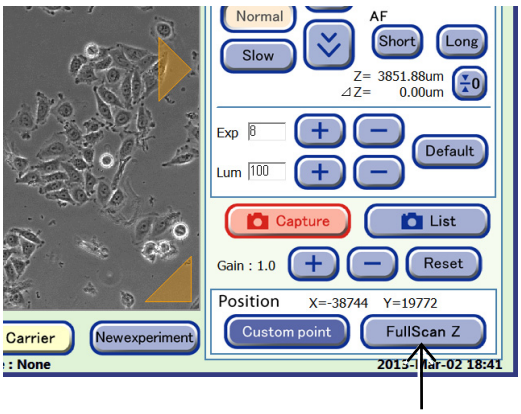
When observing a sample at low density during Full Scan observation, autofocus may fail if there is no cell in the field of view in which focus is to be adjusted. In this case, focus can be adjusted properly for observation by registering the Z position as a custom observation point for Full Scan observation.

1. Press the FullScan Z button in the Position area.

The center of the field of view displayed in the Image display area is registered as a custom observation point.

The color of the FullScan Z button changes to orange.

> Only one FullScan Z position can be set for each sample.
> If the FullScan Z button is pressed again at another position for the same sample, the FullScan Z setting is overwritten.



Ph live observation window

If an observation point is already registered at the same coordinate when setting custom observation point

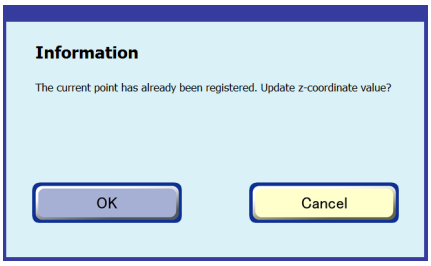
A confirmation dialog box appears if an attempt is made to register the same coordinates as the already registered custom observation point.

When the X, Y coordinates match the already set custom observation point, but the Z coordinate is different

An overwrite existing custom observation point confirmation dialog box appears.

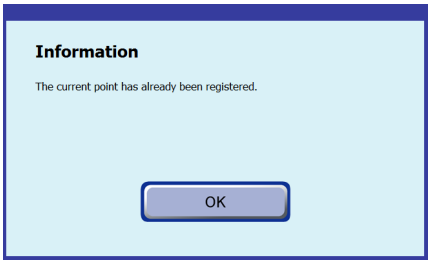
Press OK to overwrite the custom observation point and save the displayed micro image.

Press Cancel to close the conformation dialog box without overwriting the custom observation point.



When the X, Y and Z coordinates match the already set custom observation point

The following confirmation dialog box appears.



Up to 25 custom observation points can be registered per sample.

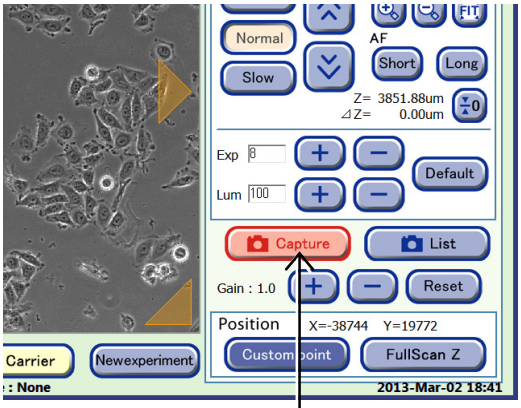
The observation points registered in this step can be checked in the Select point window displayed by pressing the Select point button. For details, see Section 4.5.5, “Redisplaying the Registered Observation Position.”

Additionally, in scheduled observation there is a function for moving to a registered observation point (Z) without using the autofocus function. For details, see Section 4.6.2, “Setting the Schedule Observation Conditions.”

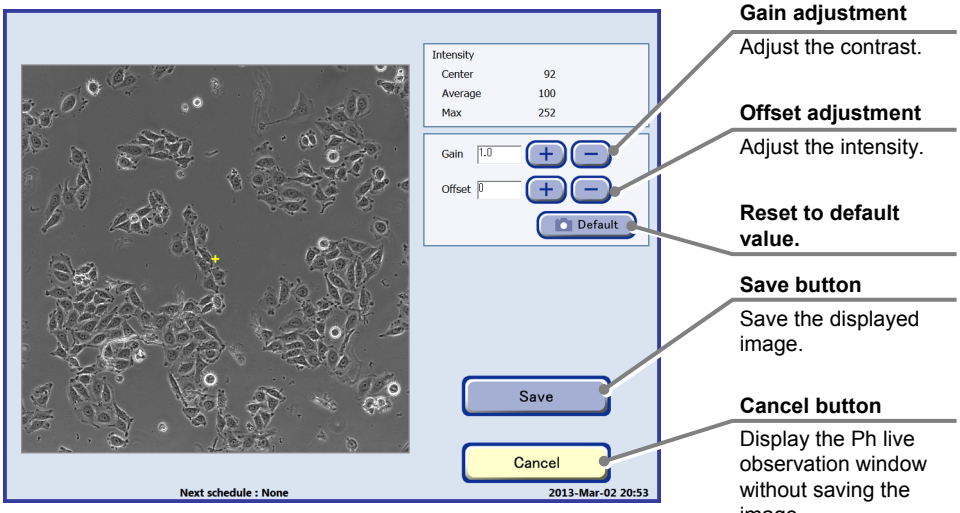
(10) Capturing a micro image (Ph).

1. Press the Capture button.

The currently displayed micro image (Ph) is captured and the Ph image register window appears.



Ph live observation window

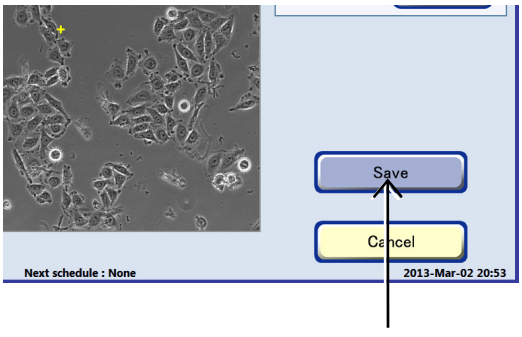


Ph image register window

> Contrast and intensity adjustments only apply to the images being displayed and they are not reflected onto the images saved using the Save button.

2. Press the Save button.

The displayed image is saved and the Ph live observation window appears again.



> **Viewing and download preparation of micro images (Ph image)**
>
> To continue with viewing and download preparation of micro images, see Section 4.7.3.1, “Viewing the micro images (Ph image) and preparing download.”

4.5.2 FL Live Observation Window

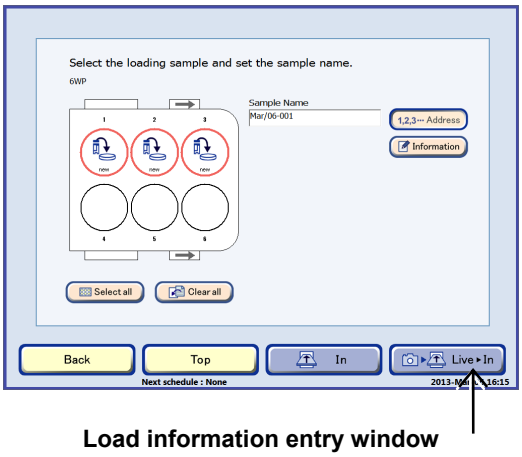
4.5.2.1 Displaying the FL live observation window

(1) Display the Ph live observation window.

To observe a live image of the sample during loading

1. **Press the Live In button in the Load information entry window.**

   The sample is transported from the carrier to the observation stage and the Ph live observation window appears.

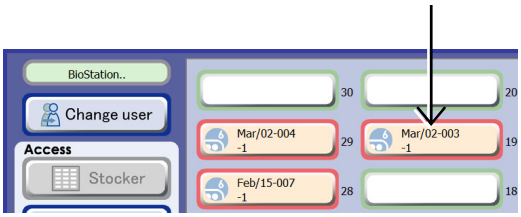


Load information entry window

To observe the live image of the sample being cultured

1. **On the System status screen, press the button of the stocker where the sample is stored.**

   The Unload window appears.

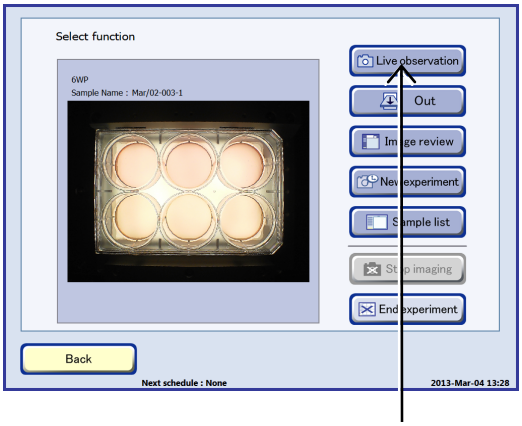


System status screen

2. **Press the Live observation button in the Select function window.**

   A live observation start confirmation dialog box appears. Press the OK button to close the dialog box.

   The sample is transported from the stocker to the observation stage and the Ph live observation window appears.



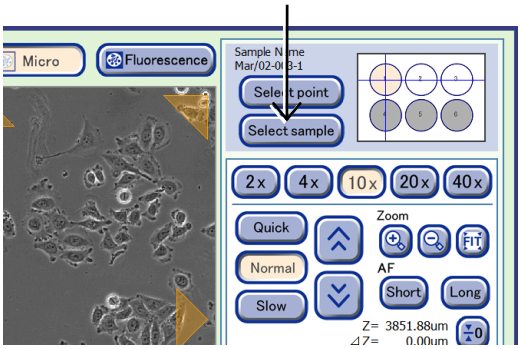
Select function window

> During a scheduled observation, live observation cannot be performed. If the start time of a scheduled observation comes, a message appears. (See “Alarm window indicating the start time of a scheduled observation” in Section 4.4, “Observing and Capturing Samples.”)

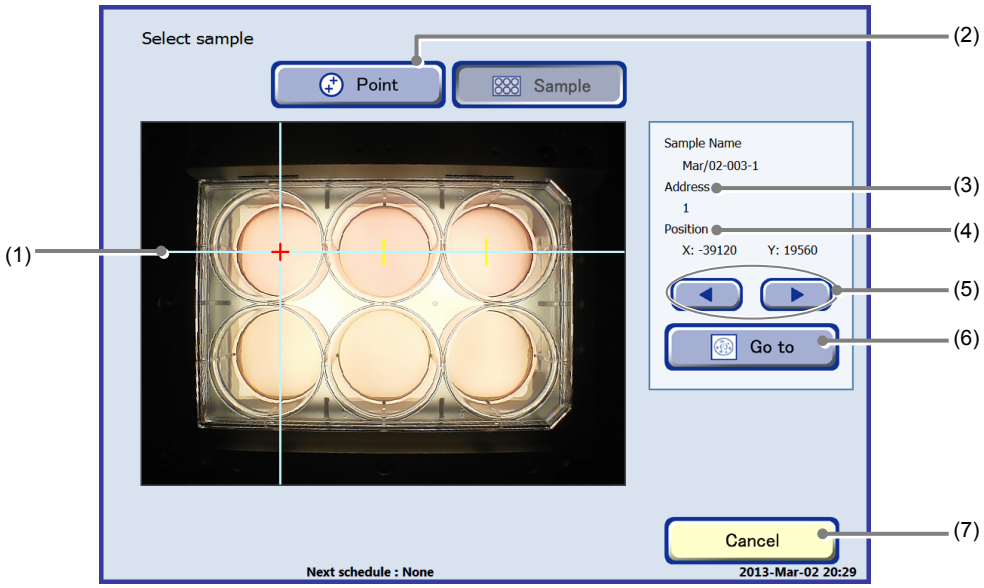
(2) In the Ph live observation window, select a sample for observation in the holder.

1. Press the Select sample button.

The Select sample window appears.



Ph live observation window



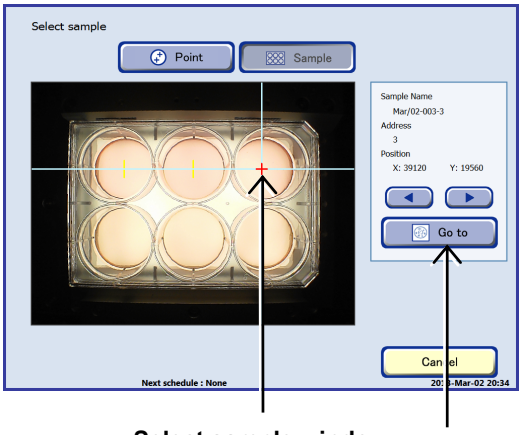
Select sample window

No.	Name	Function
(1)	Holder map display	This area displays the holder map on the macro image. Press the sample on the holder map to select the sample to display micro image.
(2)	Point button	Press this button to switch the window to the Select point window. (See Section 4.5.5.1.)
(3)	Address display	This area displays the address of the sample selected in the holder map.
(4)	Coordinates of the observation point	This area displays the coordinates (x, y) for the observation point, where (0, 0) is the center of the holder.
(5)	◄/► button	Press this button to select the sample in numeric order.
(6)	Go to button	Press this button to display the Ph live observation window of the selected sample.
(7)	Cancel button	Press this button to return to the Ph live observation window.

2. On the holder map display, press the sample to be observed. Alternatively, the sample can be selected by pressing the ◄/► buttons.

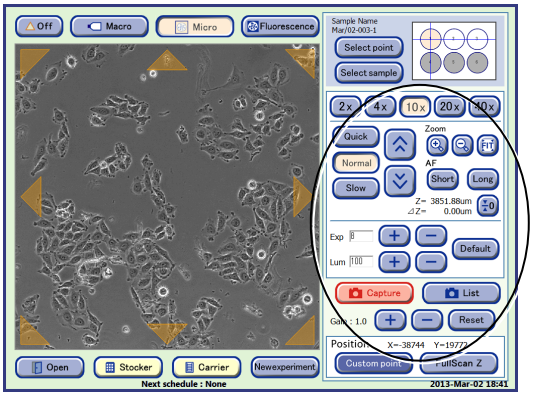
3. Press the Go to button.

The micro image (Ph) of the selected sample appears in the Ph live observation window.



Select sample window

(3) In the Ph live observation window, specify an appropriate observation point, select an observation magnification and adjust focus.



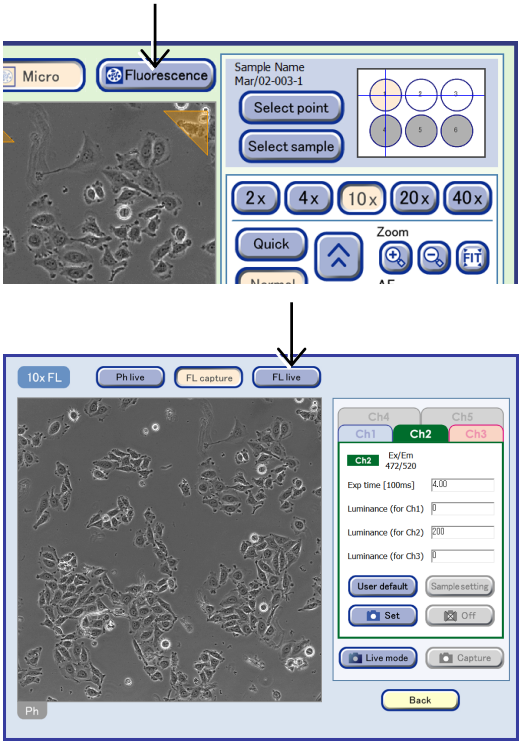
Ph live observation window

(4) Press the Fluorescence button.

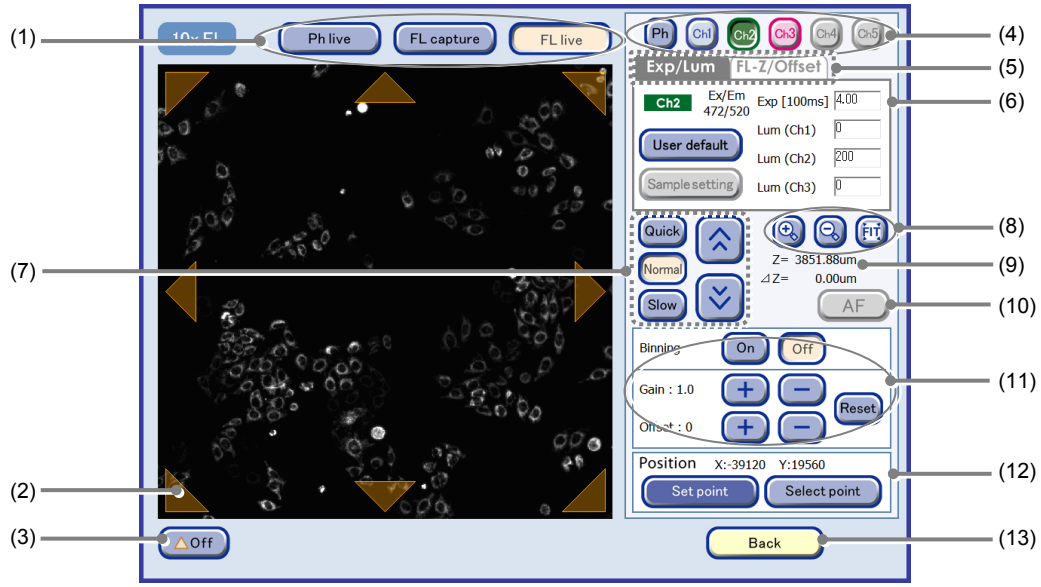
The FL image capture window appears.

(5) In the FL image capture window, press the FL live button.

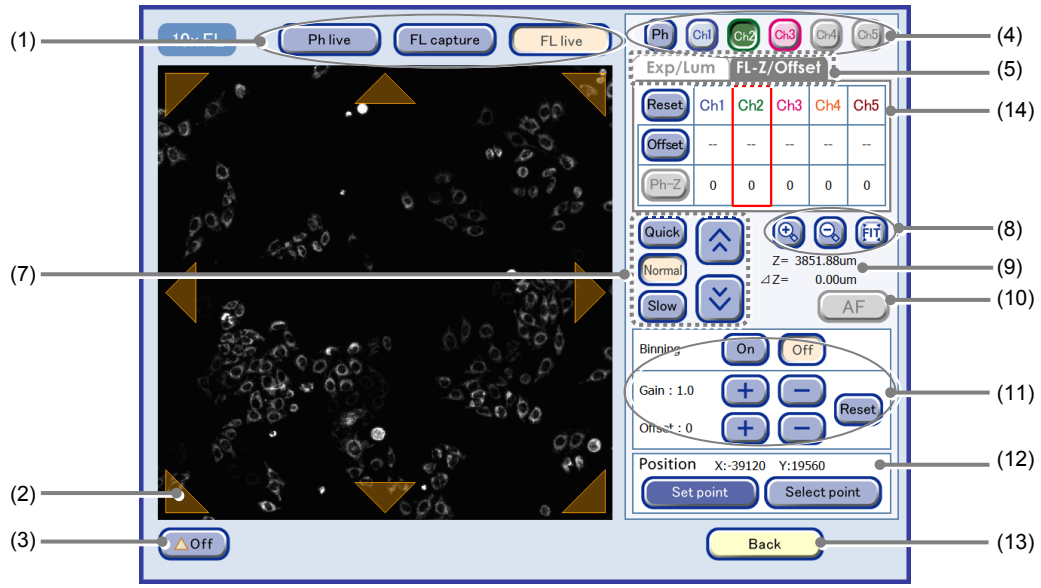
The FL live observation window appears.



FL image capture window



FL live observation window (Exp/Lum tab display)



FL live observation window (FL-Z/Offset tab display)

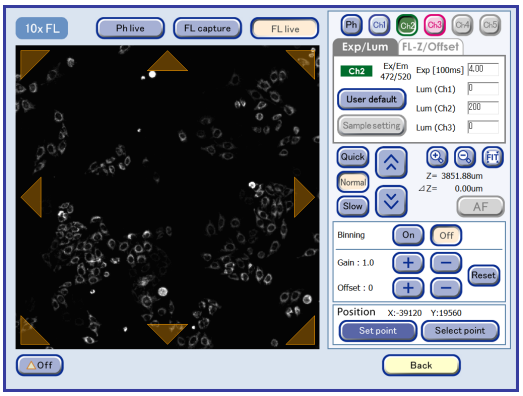
No.	Name	Function
(1)	Window switching button	Switch the window. **Ph live button:** Display the Ph live observation window. **FL capture button:** Display the FL image capture window.
(2)	Observation field shift mark	Press the marks to shift the observation field up/down, left/right, or diagonally, by one frame at a time.
(3)	△Off button	Press this button to display or hide the observation field shift mark.
(4)	Channel switching button	Press this button to switch the channel for display the image. (The Ch4 and Ch5 tabs can be selected only when they are registered.)

No.	Name	Function
(5)	Setting area switching tabs	Press a tab to switch the exposure condition setting area display. **Exp/Lum tab:** Set the exposure time and the intensity of each excitation light source. **FL-Z/Offset tab:** Correct the Z position by chromatic aberration for each channel.
(6)	Exposure condition setting area	Change the exposure conditions (exposure time and the intensity of each excitation light source) for each channel. **Exp field:** Adjust the exposure time. **Lum field:** Adjust the intensity. **User default button:** Restore the image exposure conditions to the default settings. **Sample setting button:** Apply the registered exposure conditions.
(7)	Focus adjustment area	Adjust the focus of the micro image.  : Move the focus position in the Z direction (manual focus). Keep pressed for continuous movement. **Quick button:** Speed up movement in the Z direction (coarse). **Normal button:** Move in the Z direction at normal speed. **Slow button:** Slow down the movement in the Z direction (fine).
(8)	Zoom area	Change the display size of the image. **+, - buttons:** Enlarge or reduce the image. **FIT button:** Display the image on the full screen.
(9)	Z position	**Z=:** (Absolute value of Z) Display the value of the actual Z position. **ΔZ=:** (Relative value of Z) Display the Z position value relative to the Ph Z position set in the FL-Z/Offset tab.
(10)	AF button	Press this button to find the appropriate focal point near the current Z position. This button is enabled only when the Ph button is pressed.
(11)	Brightness adjustment of a displayed image	Adjust the brightness of a displayed image. **Binning:** The ON button displays a binning image. The image is displayed brighter, which makes focusing easier. (Note that this display condition is not reflected onto the saved captured image data.) **Gain:** Press the +/- button to adjust the contrast by ±0.1. **Offset:** Press the +/- button to adjust the intensity by ±10. **Reset button:** Reset the brightness of a displayed image.

No.	Name	Function
(12)	Position area	Register and display the custom observation point. **Custom point button:** Register the center of field displayed in the Image display area as the custom observation point (X, Y, Z). **Select point button:** Display the Select point window. The observation point used for the most recent scheduled observation can be redisplayed to check the current status of that observation point.
(13)	Back button	Press this button to display the FL image capture window.
(14)	Z position correction area	Correct the Z position by chromatic aberration for each channel. **Reset button:** Reset the offset value registered for a fluorescence channel being displayed. **Offset button:** Register the current Z position of a fluorescence channel being displayed as an offset value from a phase contrast image. **Ph-Z button:** Register the Z position of the phase contrast image as the offset reference value.

4.5.2.2 Displaying a FL live image and registering a custom observation point

(1) Display the FL live observation window for an observation sample in the holder by following steps in Section 4.5.2.1, "Displaying the FL live observation window."



FL live observation window

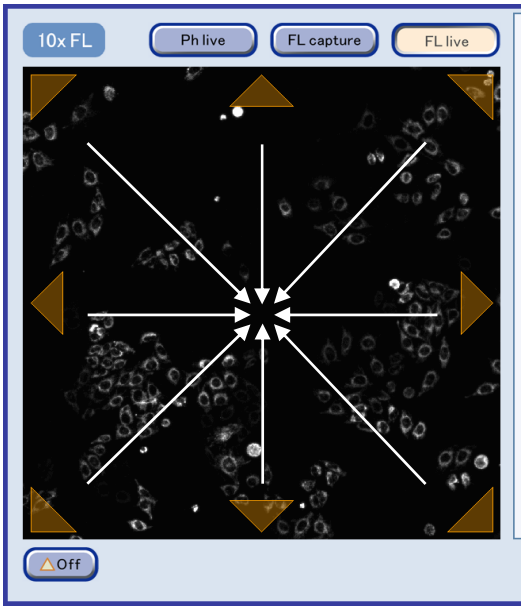
(2) Shift the image display to find the observation point.

Press a position on the Image display area to display that position at the center. The micro image (FL) is redisplayed with the pressed position located at the center.

Shift the image display by pressing the image display area until the observation point appears.

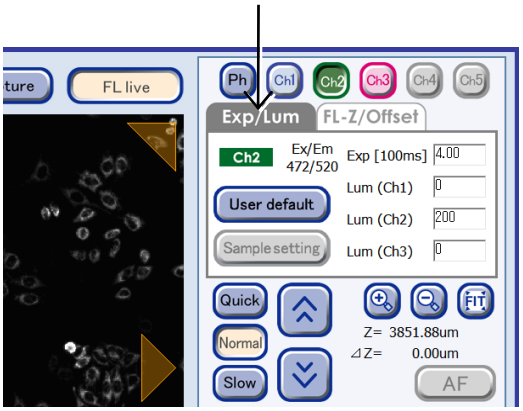
To shift the observation field by one frame at a time, turn ON the observation field shift marks ON/OFF button and then shift the display position by pressing observation field shift mark displayed around the Image display area.

> The previous observation point of captured images or the scheduled observation point can be displayed again.
>
> For the procedure for redisplaying the registered observation point, see Section 4.5.5, "Redisplaying the Registered Observation Point."



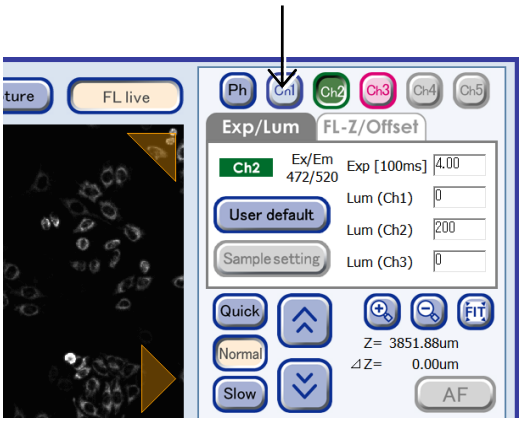
(3) Set the exposure conditions for each channel.

1. Press the Exp/Lum tab.



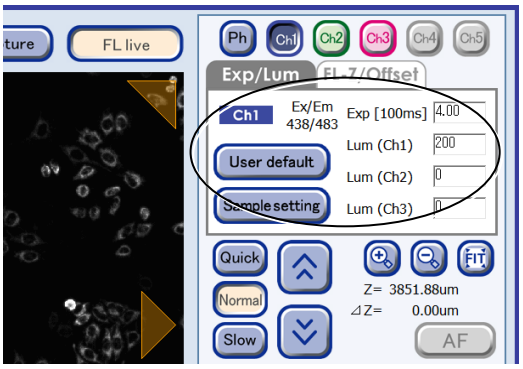
FL live observation window (Exp/Lum tab)

2. **Press the channel button of the desired fluorescence image to be displayed in the image display area to display the corresponding setting area.**



3. **Change the exposure conditions (exposure time and the intensity of each excitation light source) for each channel as necessary.**

   The unit of the exposure time is [100ms], but up to the 2nd decimal digit (i.e. unit: 1 ms) can be entered.
   The maximum value of the intensity of excitation light source is 240.



To enter appropriate exposure conditions

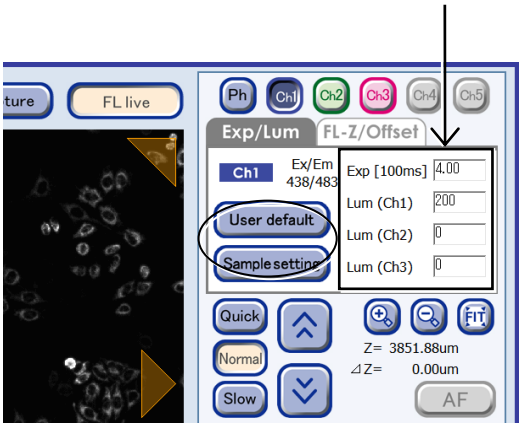
Press the setting area and enter an appropriate value.

To use the default exposure conditions

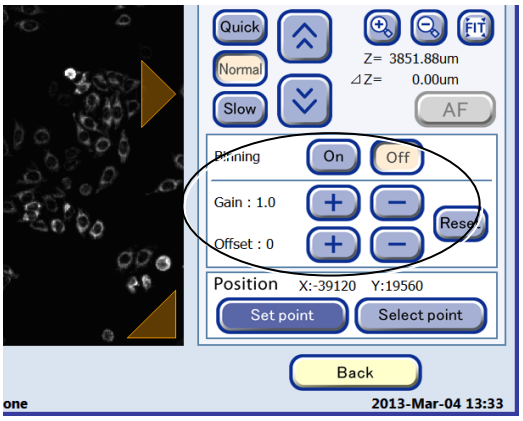
Press the User default button. The default values set for the environmental settings are applied. For details on setting the default values, see Chapter 5, “Environmental Settings.”

To use registered exposure conditions

Press the Sample setting button. The registered exposure conditions are applied.



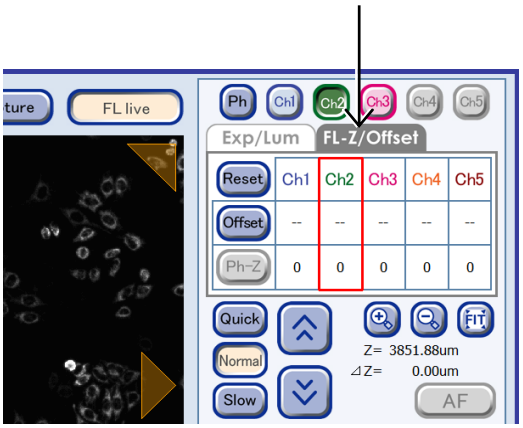
4. **If the display rate becomes slower as the exposure time increases, use Binning, Gain and Offset to adjust the brightness.**



(4) Register the offset value of the Z position for each channel.

The AF Z position and the optimum Z position of a fluorescence image may differ depending on the sample. By registering the difference as an offset value, the value will be applied as the Z position of the fluorescence image during scheduled observation, which enables the acquisition of the fluorescence image at a better focus position.

> This is especially effective for observation of 20x or more.
>
> If no value is registered, the phase contrast image and fluorescence image are acquired at the same Z position (AF position).



FL live observation window (FL-Z/Offset tab)

1. Press the FL-Z/Offset tab.

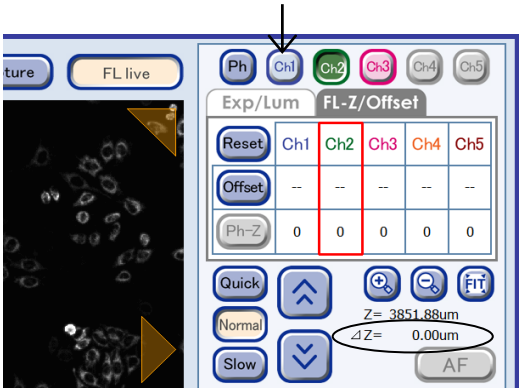
A setting area where the offset value can be registered is displayed.

2. Press the button of the fluorescence channel for which the offset value is to be registered.

ΔZ is displayed as “0” and this position is the origin for the relative position of Z.

The Z position that was adjusted in the Ph live observation window is registered as a value (absolute value) of the actual Z position of the phase contrast image.

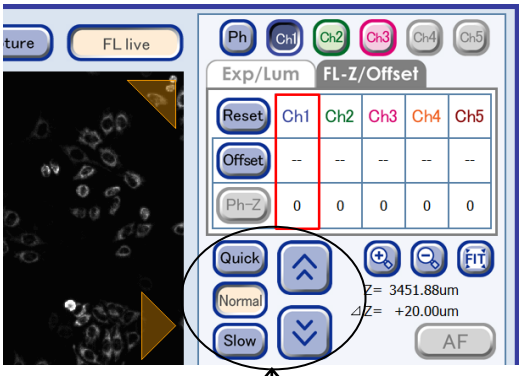
The absolute value of the actual Z position is displayed above the relative value.



3. Press the ⩓ / ⩔ button to adjust the focus of the selected FL channel.

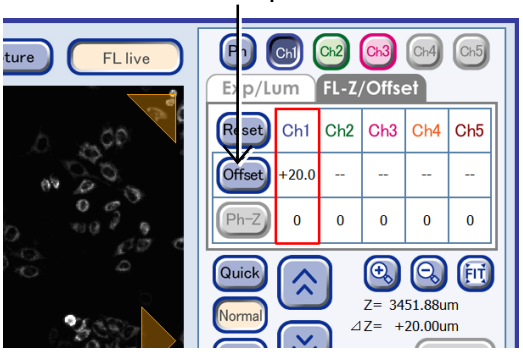
Press the ⩓ / ⩔ button to move the micro image by one unit in the Z axis direction.

Press and hold the ⩓ / ⩔ button to continuously move the micro image in the Z axis direction. To change the speed, switch the setting either to Slow or to Quick.

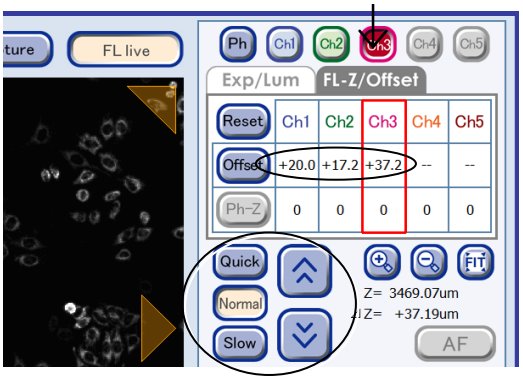


4. Press the Offset button to register the Z position of the selected channel as an offset value.

The registered offset value is displayed in the field of the selected channel.



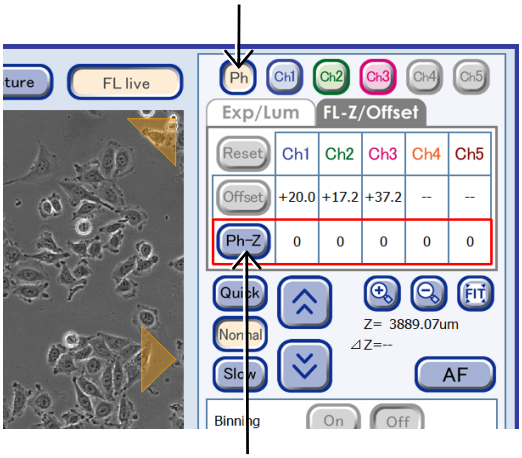
5. Follow Steps 2 to 4 for each channel for which an offset value is to be registered.



The Z position that was adjusted in the Ph live observation window is registered as a value (absolute value) of the actual Z position of the phase contrast image on the FL live observation window. However, the Z position of a phase contrast image can be registered again later.

In this case, use the Ph button to display and focus the phase contrast image. Then, press the Ph-Z button. The Z position of the phase contrast image will be registered again as an absolute value.

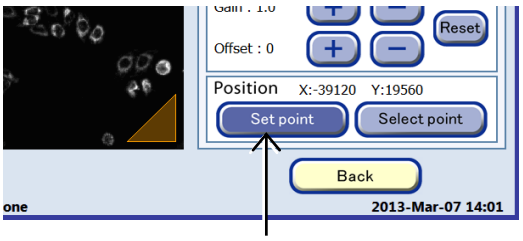
When registering the offset value of the Z position for each fluorescence channel, if the observation field of view is shifted to find a focus position, the Z reference value of the registered phase contrast image will be reset once it is moved a certain amount (10 frames) in XY direction from the point at which the Z reference value of the phase contrast image has been registered. Also in this case, press the Ph-Z button to register the Z position of the phase contrast image as an absolute position again.



(5) Register the X, Y, Z position as a custom observation point.

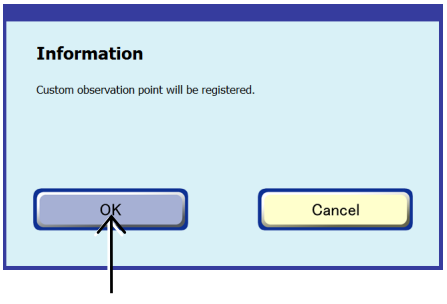
1. Press the Set point button in the Position area.

A custom observation point registration confirmation dialog box appears.



2. Press the OK button to close the dialog box.

The currently displayed live observation point (center of the field of view displayed in the Image display area) is registered as a custom observation point.



Confirmation dialog box

If an observation point is already registered at the same coordinate when setting custom observation point

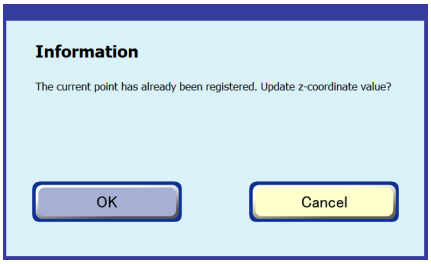
A confirmation dialog box appears if an attempt is made to register the same coordinates as the already registered custom observation point.

When the X, Y coordinates match the already set custom observation point, but the Z coordinate is different

An overwrite existing custom observation point confirmation dialog box appears.

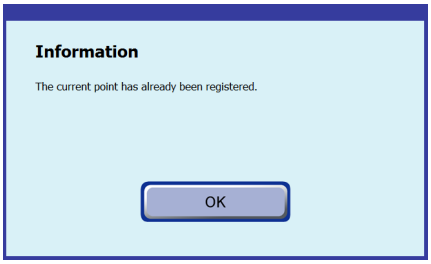
Press OK to overwrite the custom observation point and save the displayed micro image.

Press Cancel to close the conformation dialog box without overwriting the custom observation point.



When the X, Y and Z coordinates match the already set custom observation point

The following confirmation dialog box appears.



Up to 25 custom observation points can be registered per sample.

The observation points registered in this step can be checked in the Select point window displayed by pressing the Select point button. For details, see Section 4.5.5, “Redisplaying the Registered Observation Position.”

Additionally, in scheduled observation there is a function for moving to a registered observation point (Z) without using the autofocus function. For details, see Section 4.6.2, “Setting the Schedule Observation Conditions.”

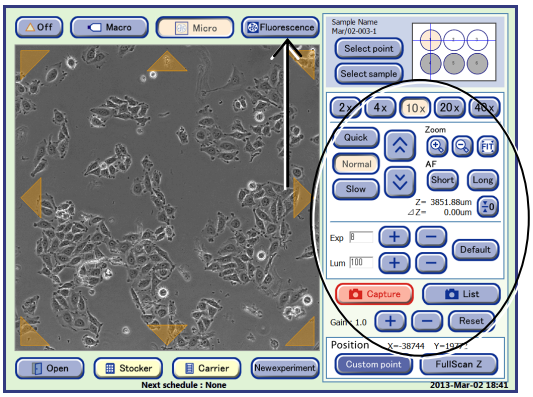
4.5.2.3 Capturing a FL live image

(1) Display the Ph live observation window.

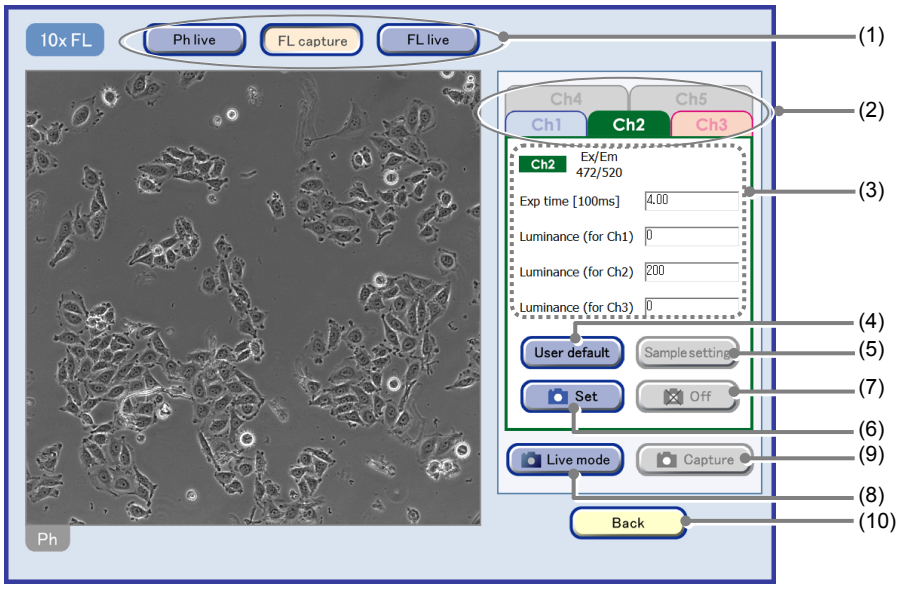
(2) In the Ph live observation window, specify an appropriate observation point, select an observation magnification and adjust focus.

(3) Press the Fluorescence button

The FL image capture window appears.



Ph live observation window

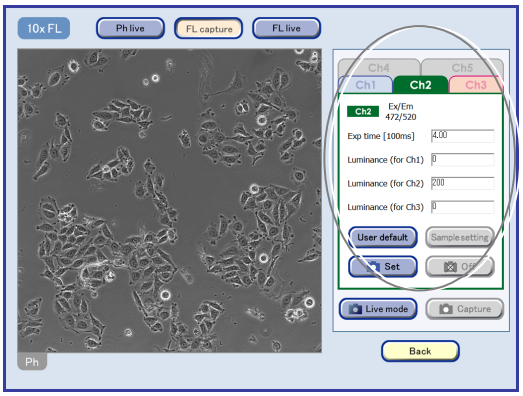


FL image capture window

No.	Name	Function
(1)	Window switching button	Switch the window. **Ph live button:** Display the Ph live observation window. **FL live button:** Display the FL live observation window.
(2)	Channel switch tabs	Press a tab to switch the channel for setting the exposure condition. (The Ch4 and Ch5 tabs can be selected only when they are registered.)
(3)	Exposure condition settings area	Change the exposure conditions (exposure time and the intensity of each excitation light source) for each channel.
(4)	User default button	Press this button to restore the image exposure conditions to the default settings.
(5)	Sample setting button	Press this button to apply the registered exposure conditions.
(6)	Set button	Press this button to apply capturing targets with the selected settings.
(7)	Off button	Press this button to exclude the image from those to be captured.
(8)	Live mode button	Press this button to display the FL live observation window.

No.	Name	Function
(9)	Capture button	Press this button to capture the image of the channel specified by pressing the Set button. The Captured FL image confirmation window is displayed.
(10)	Back button	Press this button to close the FL image capture window.

(4) Set the exposure conditions for each channel in the FL image capture window.



FL image capture window

1. Press a tab for a channel to be captured to display the corresponding setting fields.

2. Change the exposure conditions (exposure time and the intensity of each excitation light source) for each channel as necessary.

The unit of the exposure time is [100ms], but up to the 2nd decimal digit (i.e. unit: 1 ms) can be entered.
The maximum value of the intensity of excitation light source is 240.

To enter appropriate exposure conditions

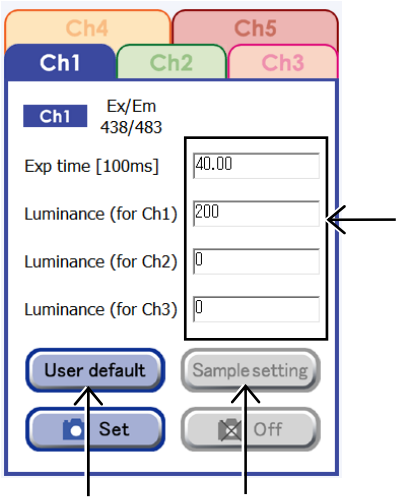
Press the setting area and enter an appropriate value.

To use the default exposure conditions

Press the User default button. The default values set for the environmental settings are applied. For details on setting the default values, see Chapter 5, “Environmental Settings.”

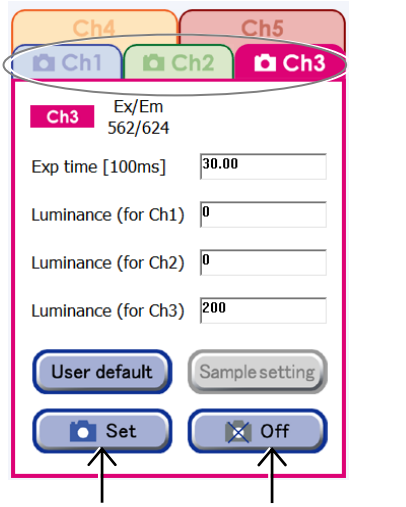
To use registered exposure conditions

Press the Sample setting button. The registered exposure conditions are applied. For details on registering exposure conditions, see step (8).



3. After entering capture conditions, press the Set button.

The settings are applied to the selected channel, and a camera icon which indicates that a capturing target is set appears on the tab.



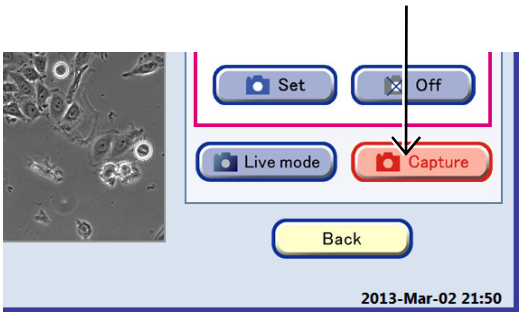
> Press the Off button to disable channels.
> The camera icon disappears from the tab.

4. Repeat steps 1 to 3 for each desired channel to set the exposure conditions.

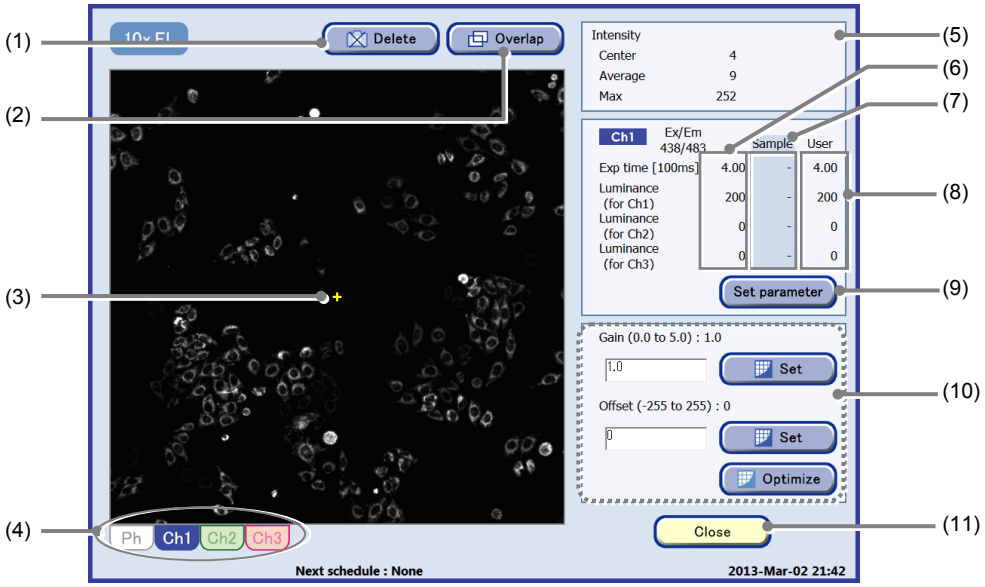
(5) Press the Capture button.

Fluorescence images for channels specified as capturing targets are captured using the specified exposure conditions. A series of images is captured when multiple channels are displayed.

When capturing of the images in all specified channels is completed, the Captured FL image confirmation window appears with the captured images displayed.



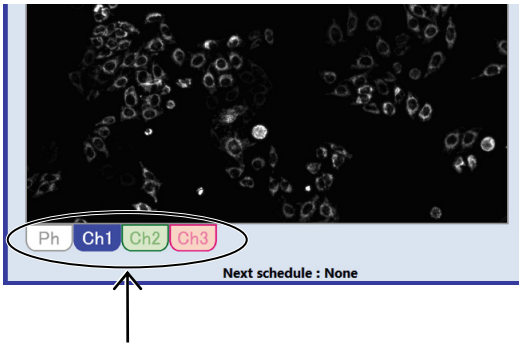
FL image capture window



Captured FL image confirmation window

No.	Name	Function
(1)	Delete button	Press this button to delete the displayed image from the save list.
(2)	Overlap button	Press this button to overlay the displayed fluorescence image for the specified channel and the phase contrast image.
(3)	Image center cross	The symbol indicates the image center.
(4)	Display switch tabs	Select a tab to display the image for the channel.
(5)	Intensity area	This area displays the intensity of the captured image.
(6)	Image exposure conditions	The set exposure conditions are displayed for the captured image.
(7)	Registered exposure conditions	The registered exposure conditions are displayed.
(8)	Default exposure conditions	The default values of the exposure conditions are displayed.
(9)	Set parameter button	Press this button to register the observation conditions at the time when the image was captured.
(10)	Image compensation area	To correct the captured image, enter a gain value and an offset value.
(11)	Close button	Press this button to close the Fluorescence image confirmation window.

(6) Press the desired Display switch tab and check the image of each channel.



(7) Images that do not need to be saved can be excluded from the save list.

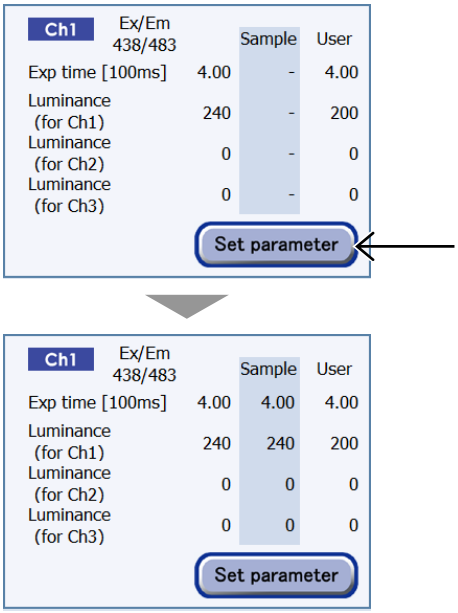
Select the tab and display the image to be excluded, and then select the Delete button. The displayed image is deleted from the save list and the selected tab disappears.

(8) The exposure conditions specified in the FL image capture window can be registered.

Press the Set parameter button. The exposure conditions at the time the displayed image was captured are registered and the registered exposure conditions are displayed in the Sample area. Exposure conditions can be registered for each channel.

Registered exposure conditions can be loaded for use in live observation or scheduled observation.

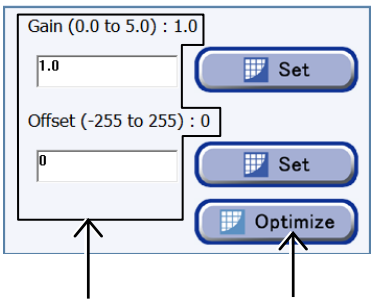
The registered exposure conditions for a sample are used as the default exposure conditions for the scheduled observation of the sample.



(9) To adjust the image contrast to enable easy confirmation of the image displayed in the window, use the image correction field.

<u>Automatic image correction</u>
Press the Optimize button to correct the image contrast automatically.

<u>Manual image correction</u>
Enter a gain value and an offset value into the fields and press the Set button.

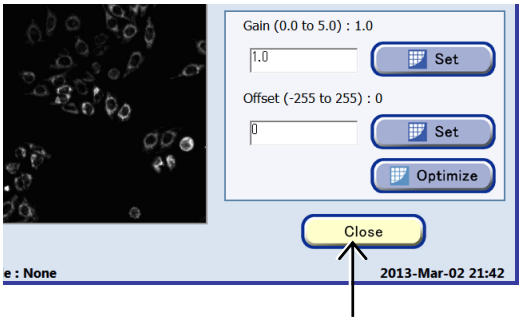


The image correction is applied only to the displayed image and not to the saved image data.

(10) After checking the image, press the Close button.

The FL image capture window appears again.

In the lower part of the FL image capture window, the tabs for the images to be saved are also displayed.



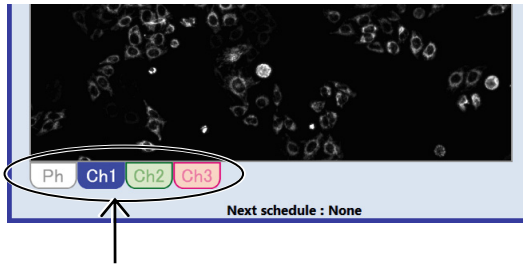
(11) If necessary, repeat steps (4) to (10) to capture fluorescence images.

Up to nine images can be saved at a time.

(12) Check the fluorescence images to be saved.

1. **Press the tab below the image area in the FL image capture window to switch the fluorescence image to be saved.**

   Each image to be saved can be displayed one by one by pressing the tab.

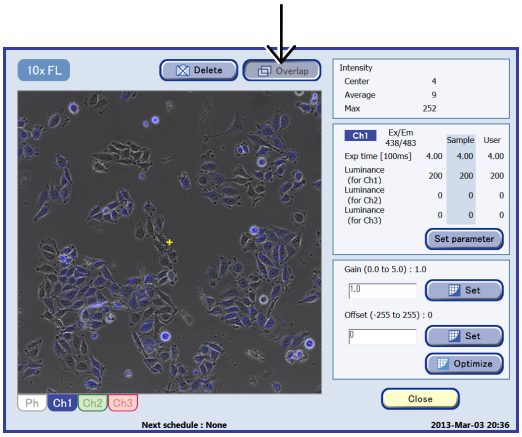


FL image capture window

2. **To overlay the displayed fluorescence image and phase contrast images, select the FL channel tab, and then press the Overlap button.**

   The fluorescence image and the phase contrast image are overlaid.

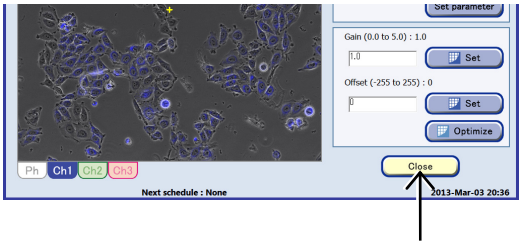
   To cancel the overlaid image, press the Overlap button again.



FL image capture window (overlaid image)

(13) After the fluorescence images are captured, press the Close button.

The image displayed on the tab is saved, and then the FL image capture window appears.



Viewing and download preparation of micro images (FL image)

To continue with viewing and download preparation of micro images, see Section 4.7.3.2, “Viewing and download preparation of micro images (FL image).”

4.5.3 Micro Images Captured during Live Observation

The Ph images and FL images captured and saved during live observation can be checked in the Live observation micro image list window.

The images observed and saved during the operation of the Ph live observation window are listed as thumbnail images at each observation position in the Live observation micro image list window.

The phase contrast images captured by pressing the Capture button or the Set button in the Position area in the Ph live observation window are displayed in the list, however, the phase contrast images automatically captured as a result of the fluorescence image capturing are not displayed in the list .
Additionally, when the Ph live observation window is closed (by pressing the Carrier button or the Stocker button), the list display does not appear. To check live observation results later on, go to the Image review window.

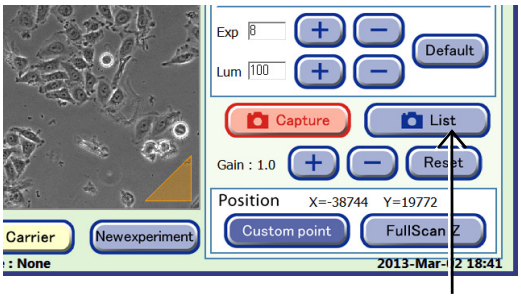
4.5.3.1 Displaying Live observation micro image list

The following describes the procedure for checking fluorescence images in the Live observation micro image list window.

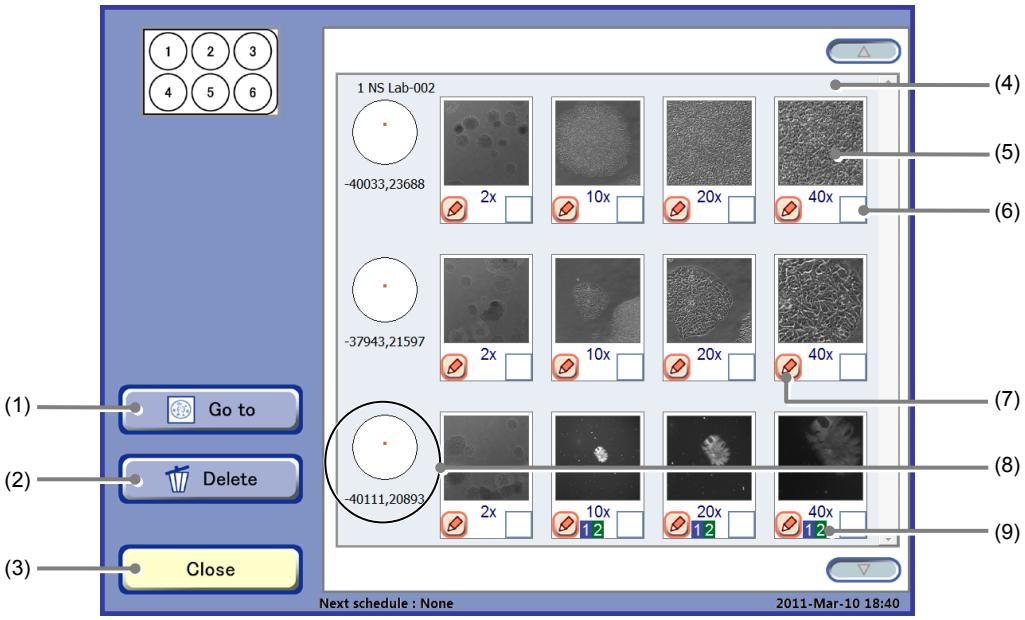
(1) Press the Image List button 

in the Ph live observation window.

The Live observation micro image list window appears.



Ph live observation window



Live observation micro image list window

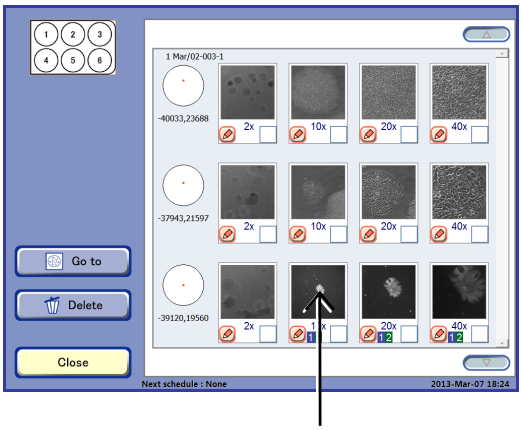
No.	Name	Function
(1)	Go to button	Press this button to display the observation point of the selected image in the Ph live observation window. (See Section 4.5.5.2.)

No.	Name	Function
(2)	Delete button	Press this button to delete the selected micro image. (See Section 4.5.3.2.)
(3)	Close button	Press this button to return to the Ph live observation window.
(4)	Micro thumbnail images	This area displays images in the numerical order and position order of the sample number.
(5)	Thumbnail images	Press an image to display the Point image display window (Ph).
(6)	Image selection button	Press this button to select an image.
(7)	Comment button	Press this button to display the Micro image comment edit window. (See Section 4.5.3.3.)
(8)	Coordinates of the observation point	This area displays the coordinates (x, y) for the observation point on the selected image, where (0, 0) is the center of the holder.
(9)	Observation magnification and FL channel	This area displays the observation magnification used to capture the image between the Enlarge button and Comment button. The FL channel number is displayed for the captured fluorescence images.

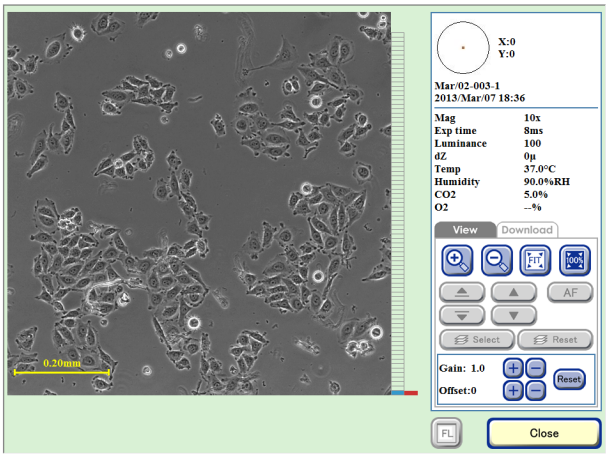
(2) To display the enlarged image, press the thumbnail image.

The Live observation micro image display window appears.

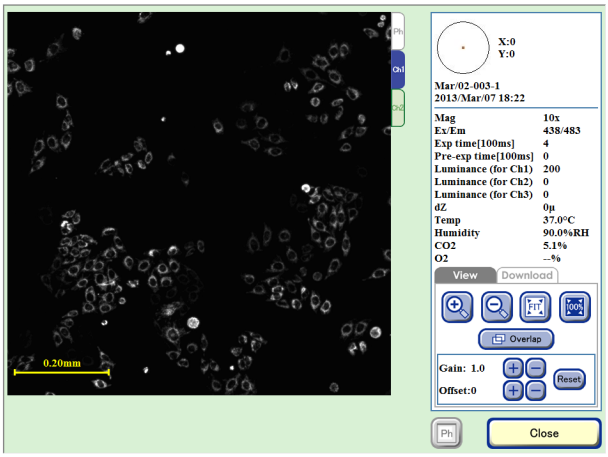
The type of thumbnail image (Ph live/FL live) determines how the Live observation micro image display window is displayed.



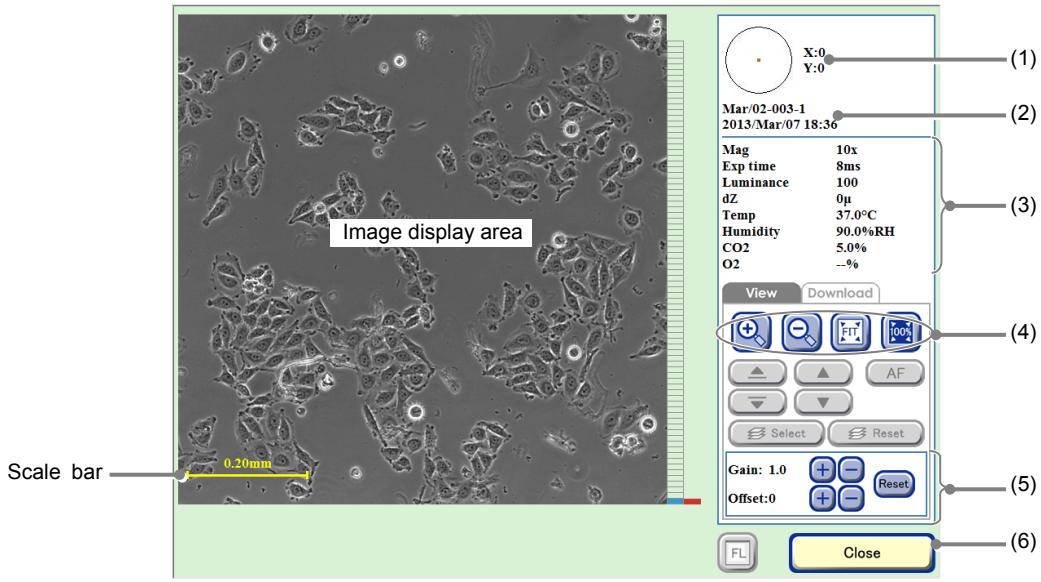
Live observation micro image list window



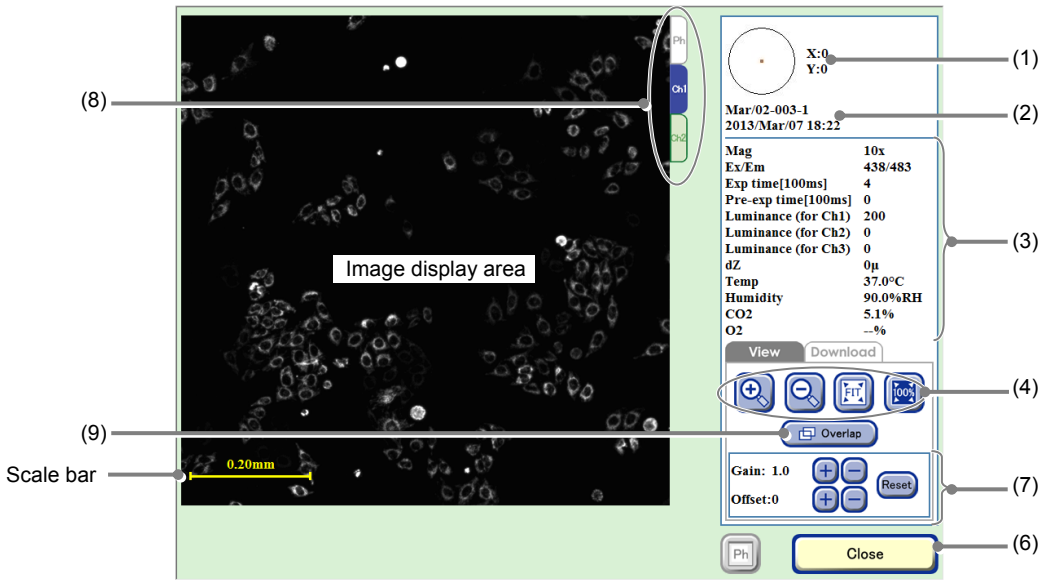
Live observation micro image display window (Ph)



Live observation micro image display window (FL)



Live observation micro image display window (Ph)



Live observation micro image display window (FL)

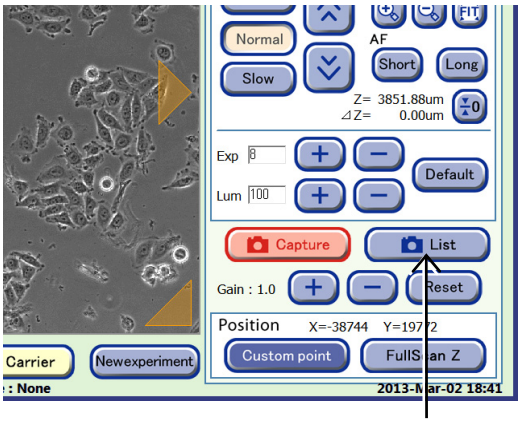
No.	Name	Function
(1)	Coordinates of the observation point	This area displays the coordinates (x,y) for the observation point where (0,0) is the center of the sample.
(2)	Sample information	This area displays the observation point, sample name, and captured date and time.
(3)	Image capture conditions and environmental information	This area displays the Image capture conditions and the temperature, humidity, and CO_2 concentration and O_2 concentration (optional) in the CO_2 incubator.
(4)	Display size change buttons	**+, - buttons:** Enlarge or reduce the image. **FIT button:** Display the image on the full screen. **100% button:** Display the image with the actual size.

No.	Name	Function
(5)	Brightness adjustment of a displayed image (Ph)	Adjust the brightness of a displayed image. **Gain:** Press the +/- button to adjust the contrast by ±0.1. **Offset:** Press the +/- button to adjust the brightness by ±5. **Reset button:** Reset the brightness of a displayed image.
(6)	Close button	Press this button to close the Live observation micro image display window.
(7)	Brightness adjustment of a displayed image (FL)	Adjust the brightness of a displayed image. **Gain:** Press the +/- button to adjust the contrast by ±0.1. **Offset:** Press the +/- button to adjust the brightness by ±10. **Reset button:** Reset the brightness of a displayed image.
(8)	Display switch tabs	Press a tab to switch the channel to check an image. (Enabled only in the Live observation micro image display window (FL))
(9)	Overlap button	Press this button to overlay the fluorescence image and the phase contrast image. (Enabled only in the Live observation micro image display window (FL))

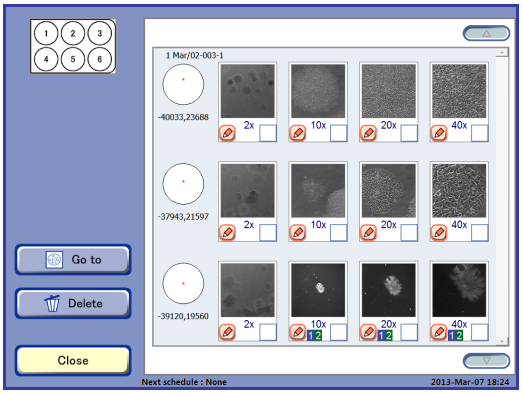
4.5.3.2 Deleting a captured live observation micro image

(1) Press the Image List button [List] in the Ph live observation window.

The Live observation micro image list window appears.



Ph live observation window

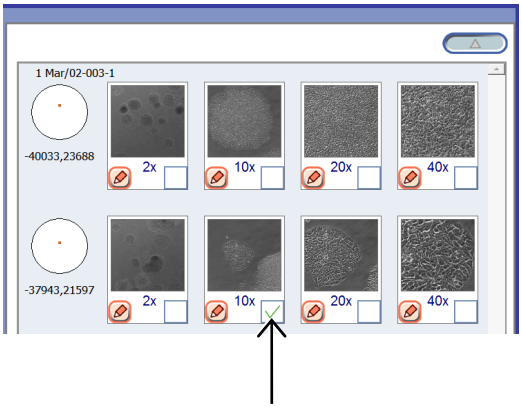


Live observation micro image list window

(2) Press the Image selection button below the image to be deleted.

A checked symbol appears in the check box for the image corresponding to the selected Image selection button.

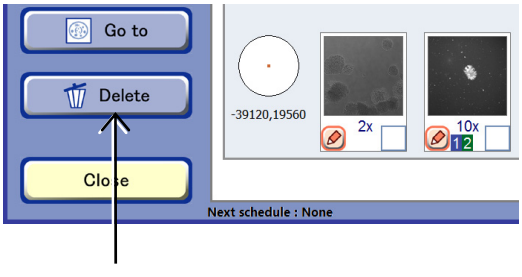
Multiple images can be selected.



(3) Press the Delete button.

The image delete confirmation dialog box appears.

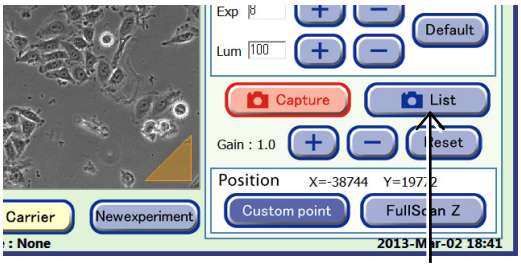
By pressing the OK button, the selected live observation micro image is deleted from the Live observation micro image list window.



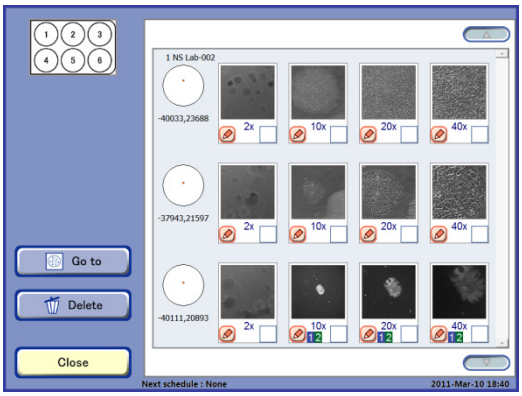
4.5.3.3 Editing a comment for a captured live observation micro image

(1) Press the Image List button [List] in the Ph live observation window.

Live observation micro image list window appear.



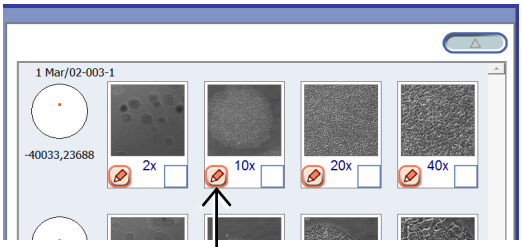
Ph live observation window

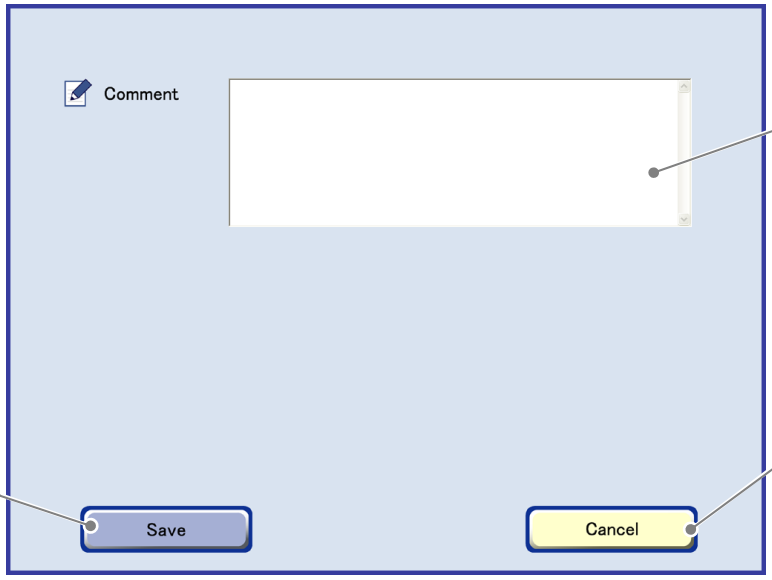


Live observation micro image list window

(2) Press the Comment button at the bottom of a thumbnail image to enter a comment.

The Micro image comment edit window appears.





Comment field

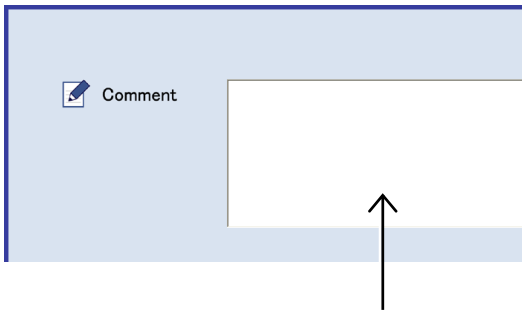
Save button
Save the comment for the micro image.

Cancel button
Close the Micro image comment edit window without saving the comment.

Micro image comment edit window

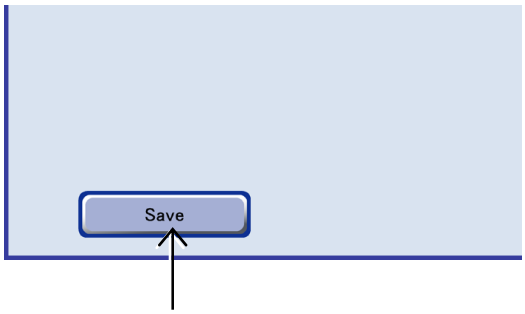
(3) Press the Comment field and enter a comment in the Keyboard window.

A comment up to 128 characters excluding carriage return characters can be entered.



(4) Press the Save button.

The live observation micro image comment is saved and the Micro image comment edit window is closed.

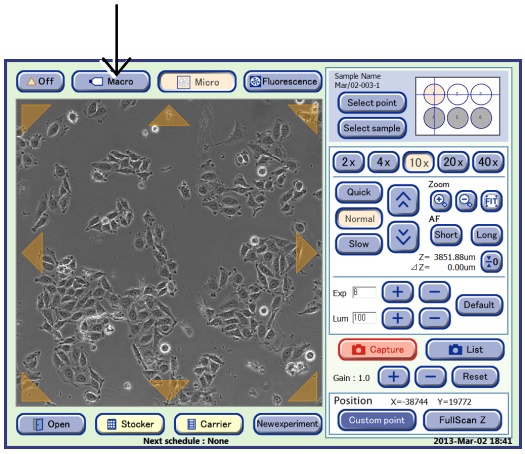


4.5.4 Macro Live Observation Window

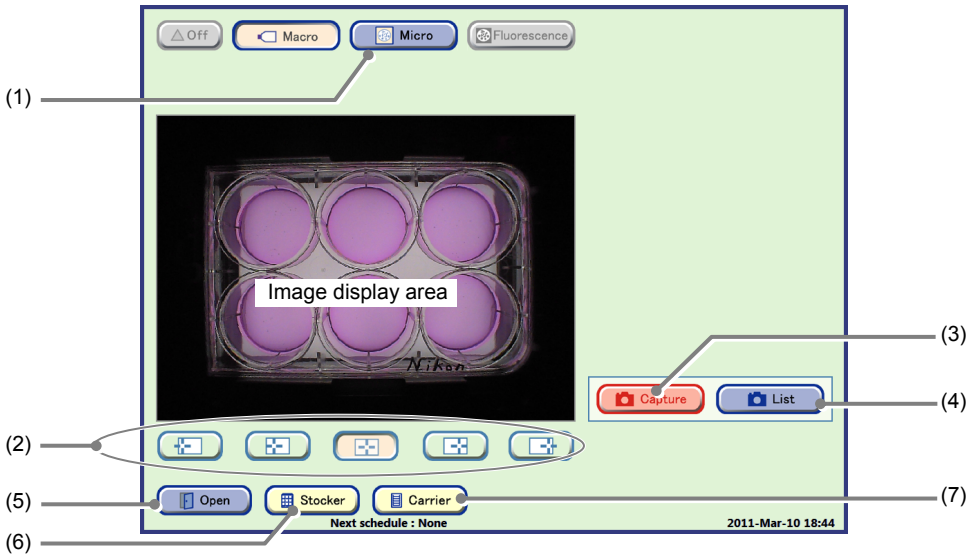
4.5.4.1 Displaying the Macro live observation window and macro image capture

(1) Display the Macro live observation window.

Press the Macro button in the Ph live observation window to switch to the Macro live observation window.



Ph live observation window

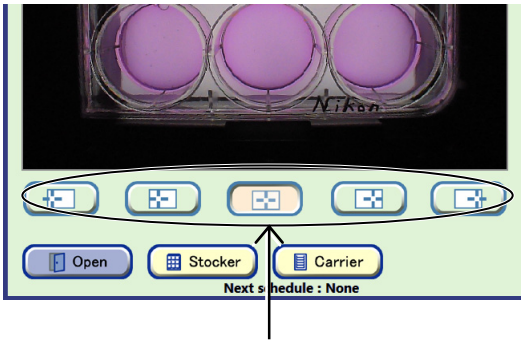


Macro live observation window

No.	Name	Function
(1)	Micro button	Press this button to display the Ph live observation window.
(2)	Five position buttons	Adjust the capturing position of the macro right and left in five points.
(3)	Capture button	Press this button to capture a macro image and display the image in the Macro image register window.
(4)	List button	Press this button to display the Live observation macro image list window. (See Section 4.5.4.2.)
(5)	Open button	Press this button when opening the door during live observation before loading a sample into the CO_2 incubator. This window can be restored when the Close button is pressed after loading the sample.
(6)	Stocker button	Press this button to finish live observation and return the holder to the stocker.
(7)	Carrier button	Press this button to finish live observation and return the holder to the carrier.

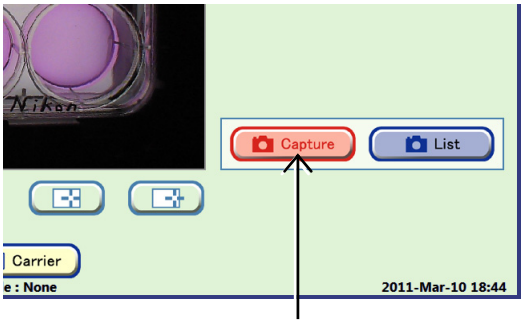
(2) Adjust the position where the macro image is captured.

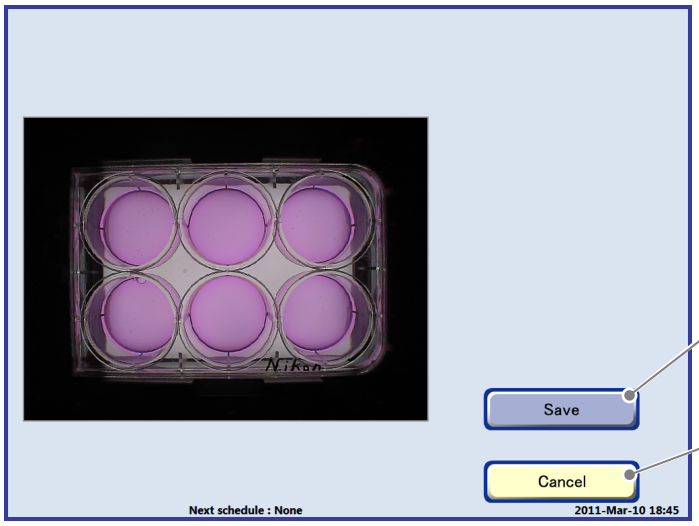
Adjust the position of the macro image to the right or left using the five position buttons.



(3) Press the Capture button to capture a macro image.

A macro image is captured at the currently displayed position and the Macro image register window appears.





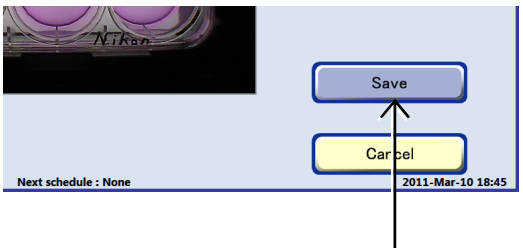
Save button
Save the displayed image.

Cancel button
Display the Macro live observation window without saving the image.

Macro image register window

(4) Press the Save button.

The displayed image is saved and the Macro live observation window appears.



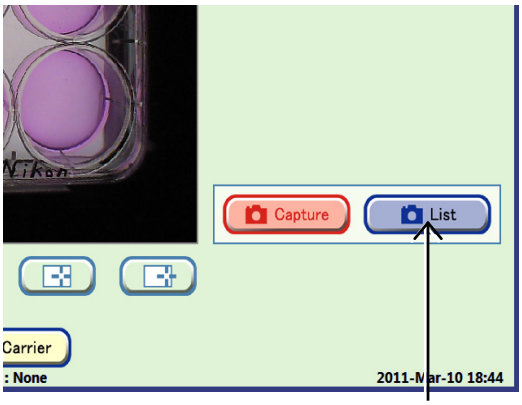
Viewing macro images and preparing download

To continue with viewing macro images and preparing download, see Section 4.7.3.3, “Viewing the macro image and preparing download.”

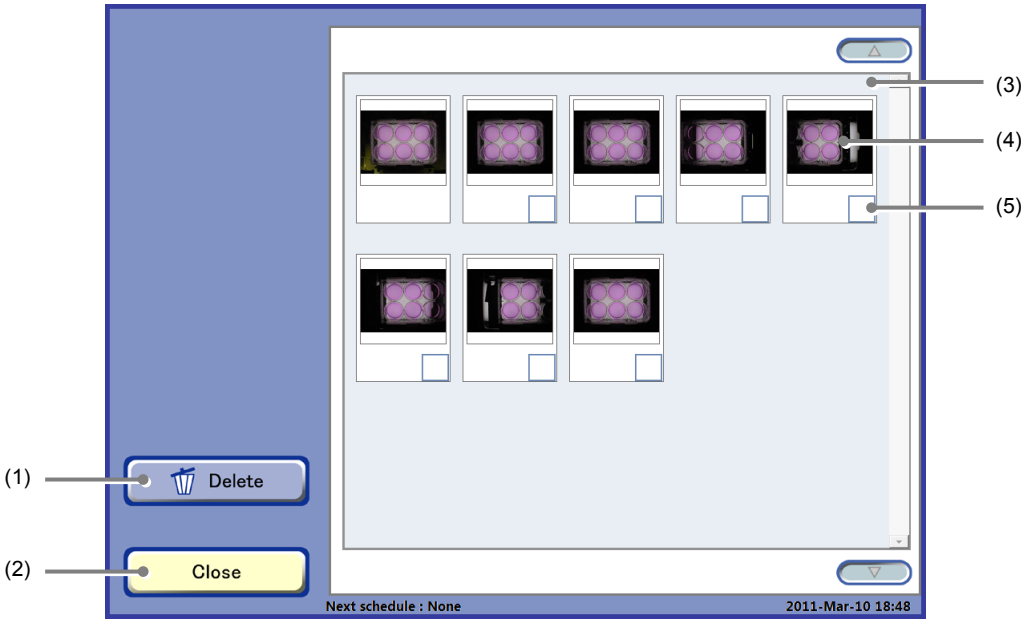
4.5.4.2 Displaying the macro thumbnail images

(1) Press the List button in the Macro live observation window.

The Live observation macro image list window appears.



Macro live observation window



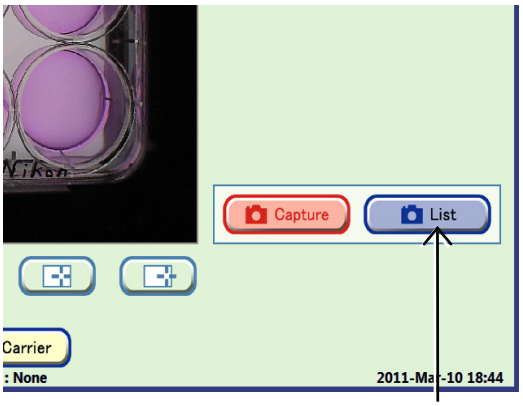
Live observation macro image list window

No.	Name	Function
(1)	Delete button	Press this button to delete the selected image.
(2)	Close button	Press this button to display the Macro live observation window.
(3)	Macro thumbnail images	This area displays images in the captured order.
(4)	Thumbnail image	Press an image to display the Live observation micro image display window.
(5)	Image selection button	Press this button to select an image. When selected, a checked symbol appears in the check box for the selected image.

4.5.4.3 Deleting a macro image

(1) Press the List button in the Macro live observation window.

The Live observation macro image list window appears.



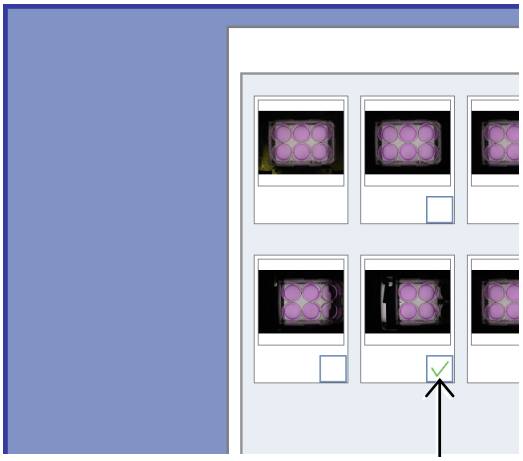
Macro live observation window

(2) From the macro thumbnail images, press the Image selection button below the image to be deleted.

A checked symbol appears in the check box for the image corresponding to the selected Image selection button.

Multiple images can be selected.

> The first displayed image cannot be deleted because it is a macro image automatically captured for the Select sample window.

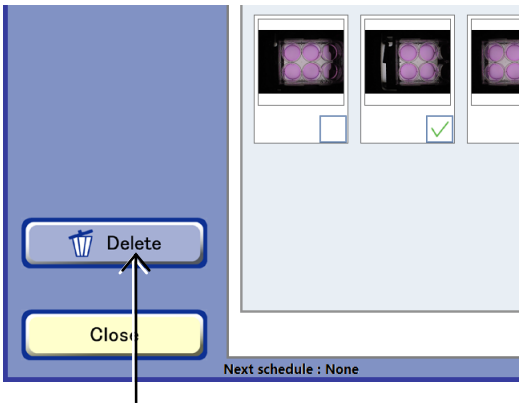


Live observation macro image list window

(3) Press the Delete button.

The image delete confirmation box appears.

Press the OK button to delete the selected macro image from the Live observation macro image list window.



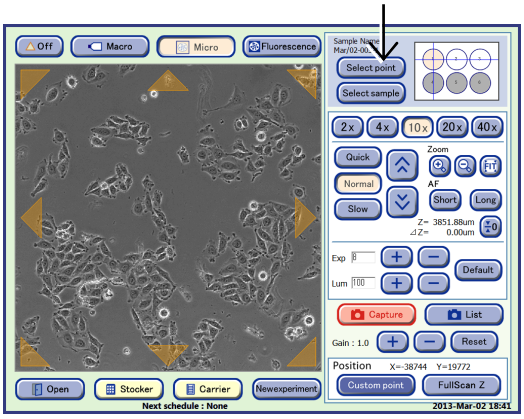
4.5.5 Redisplaying the Registered Observation Point

4.5.5.1 Live observation by redisplaying observation point

The observation point used for the most recent scheduled observation or a registered custom observation point can be redisplayed to check the current status of that observation point.

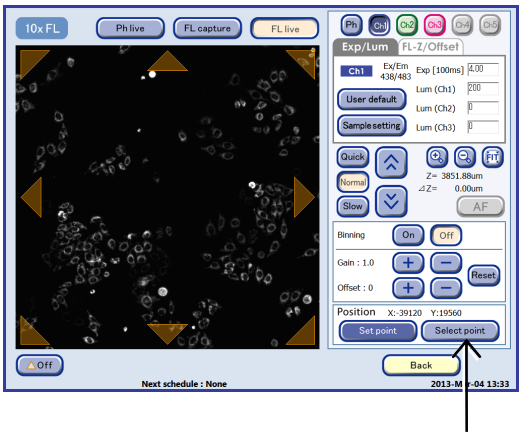
(1) Press the Select point button in the Ph live observation window.

The Select point window appears.



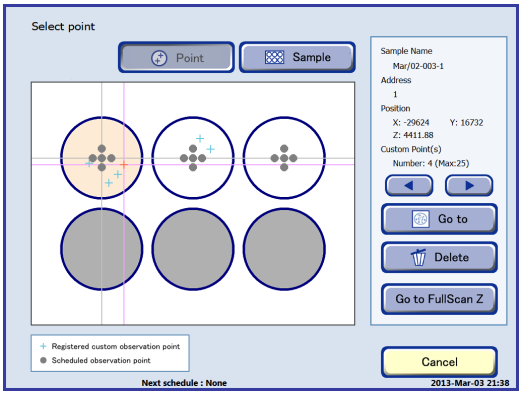
Ph live observation window

Press the Select point button in the Position area of the FL live observation window during a Ph live observation.

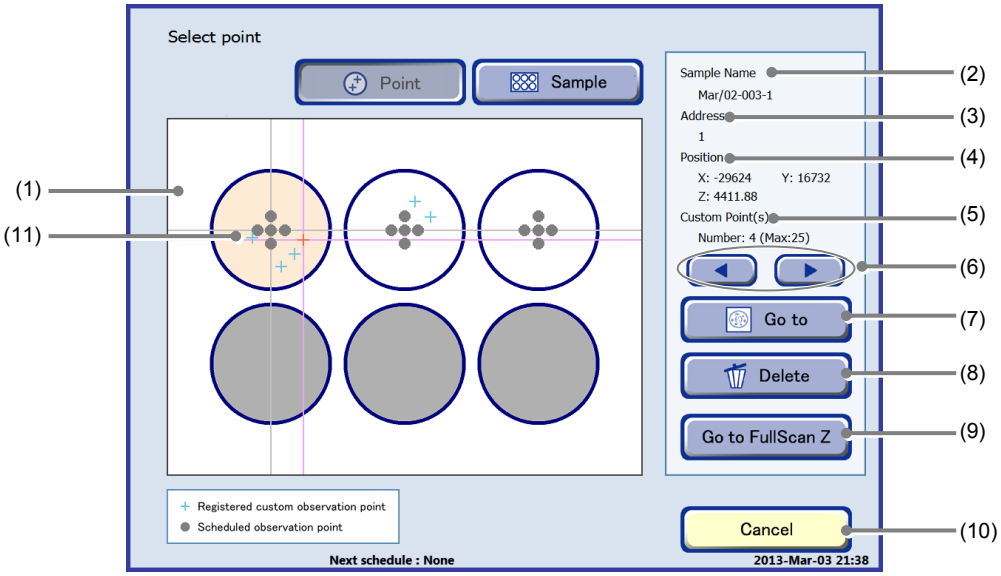


FL live observation window

Observation points registered in the Ph live observation window (section 4.5.1), the FL live observation window (section 4.5.2), the Observation condition setting window (section 4.6.2), or the Full Scan image display window (section 4.7.3.4) are displayed with crosshair marks in the Select point window.



Select point window



Select point window

No.	Name	Function
(1)	Holder map display	Press an observation point on the holder map display to display the image of the position.
(2)	Sample Name	This area displays the sample name.
(3)	Address display	This area displays the address of the sample selected in the holder map.
(4)	Coordinates of the observation point	This area displays the coordinates (X, Y, Z) for the observation point on the selected image, where (0, 0, 0) is the center of the holder.
(5)	Custom observation point count	This area displays the number of observation points registered for the selected sample.
(6)	◄/► button	Press this button to select the observation point in numeric order.
(7)	Go to button	Press this button to display the selected observation point in the Ph live observation window or the FL live observation window.
(8)	Delete button	Press this button to delete selected observation point.
(9)	Go to FullScan Z button	Press this button to display the image at the custom observation point registered with the FullScan Z button in the Ph live observation window or the FL live observation window. (Enabled when FullScan Z is registered in the Ph live observation window)
(10)	Cancel button	Press this button to return to the Ph live observation window or the FL live observation window.
(11)	Observation point	● The observation point used for the most recent scheduled observation. + The registered custom observation point.

(2) Display the observation point image.

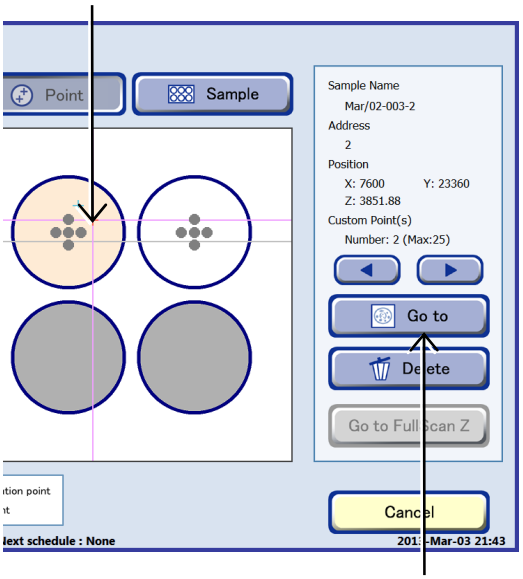
1. Select the observation point.

Select a registered custom observation point by pressing the cross-hair mark.

Select a scheduled observation point by pressing the circle mark.

Alternatively, the observation point can be selected by pressing the ◄/► buttons.

The color of the selected crosshair mark turns red.



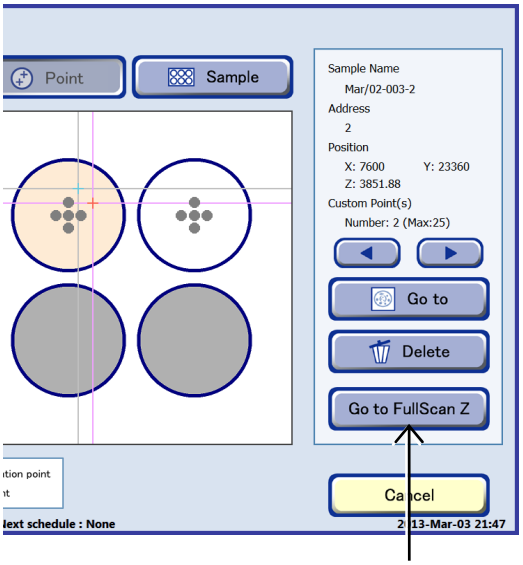
2. Press the Go to button.

The live image of the selected observation point appears in the Ph live observation window or the FL live observation window.

> To delete an observation point, select the point and press the Delete button.

To display the live image of the observation point registered with FullScan Z, first select the sample registered with FullScan Z and then press the Go to FullScan Z button.

The live image at the FullScan Z position appears in the Ph live observation window or the FL live observation window.

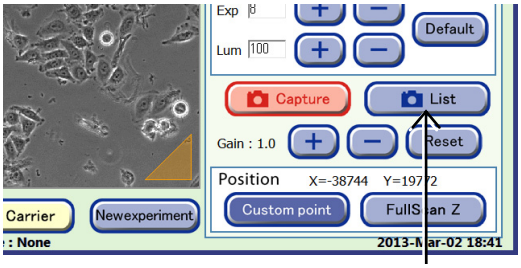


4.5.5.2 Live observation by redisplaying live observation point

Selecting a captured live observation micro thumbnail image enables you to check the current status of the same observation point as the selected image.

(1) Press the Image List button [List] in the Ph live observation window.

The Live observation micro image list window appears.

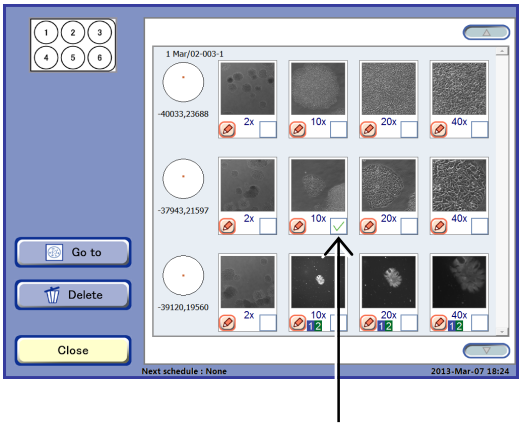


Ph live observation window

(2) Press the image selection button below the image to display the same observation point from the micro thumbnail images.

A checked symbol appears in the check box for the image corresponding to the selected Image selection button.

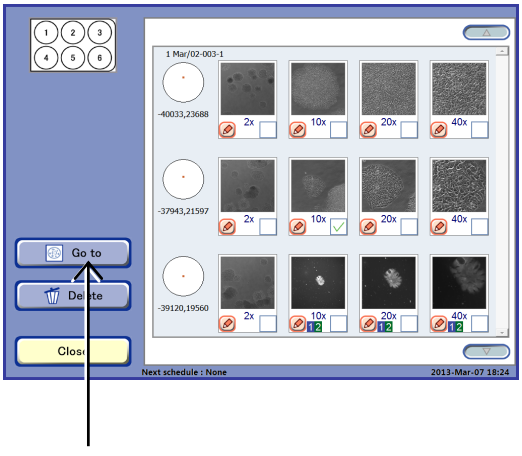
> In this case, select only one image.
> If multiple images are selected, the observation point cannot be retrieved.



Live observation micro image list window

(3) Press the Go to button.

The Ph live observation window appears and the current image at the observation point of the selected image appears.



4.5.6 Setting Scheduled Observation during Live Observation

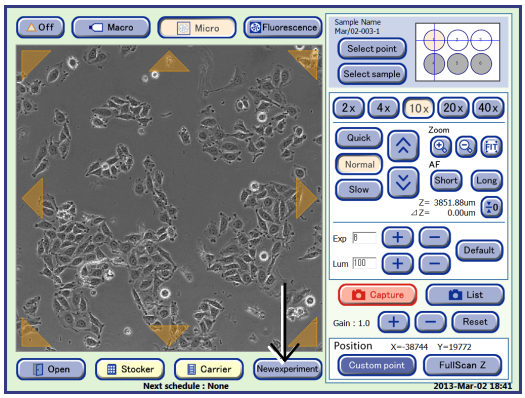
Scheduled observation can be set during live observation.

In order to avoid transferring due to floating cells or to suspend observation until there is no condensation in the container, the sample can be held on stage until the specified scheduled observation start time or scheduled observation can be started immediately without returning the sample to the stocker.

> When holding the sample on the stage, scheduled observation cannot be set if there are other schedules scheduled for times before you wish to start your scheduled observation.

(1) Press the New experiment button in the Ph live observation window.

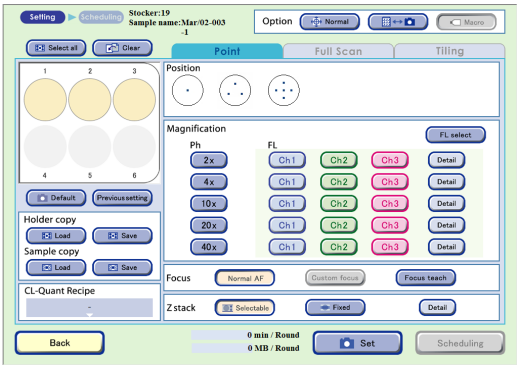
The Observation condition setting window for the sample being observed appears.



Ph live observation window

(2) Set the schedule observation condition in the Observation condition setting window.

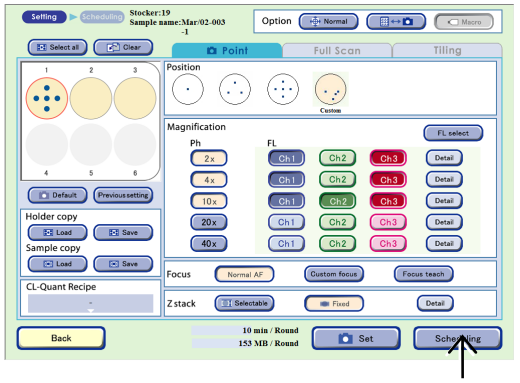
For details on setting scheduled observation, see Section 4.6.2, “Setting the Scheduled Observation Conditions.”



Observation condition setting window

(3) After setting schedule observation conditions, press the Scheduling button.

The Scheduling window appears.

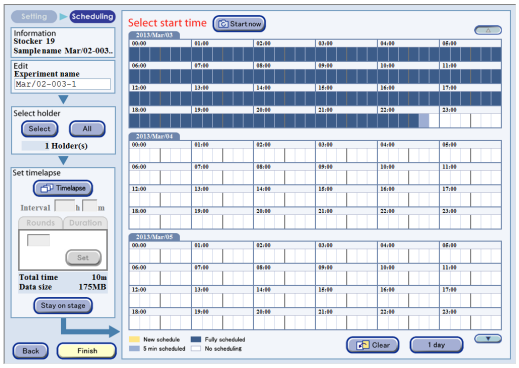


Observation condition setting window

(4) Set the schedule in the Scheduling window.

For details on setting a normal schedule, see Section 4.6.3.1, “Setting the schedule for each sample.”

To perform a scheduled observation with the sample held on the stage without returning it to the stocker, see step (5).



Scheduling window

(5) Perform scheduled observation with the sample held on the stage.

There are four ways to set the execution of scheduled observation with the sample held on stage without returning it to the stocker.

a. When immediately starting scheduled observation (with no timelapse set)

b. When immediately starting scheduled observation (with timelapse set)

c. When holding a sample on the stage until the specified scheduled observation start time (with no timelapse set)

d When holding a sample on the stage until the specified scheduled observation start time (with timelapse set)

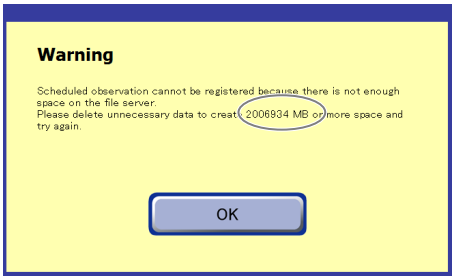
When schedule cannot be set:

If sufficient space to save the observation data cannot be allocated on the file server, an insufficient data space warning dialog box is displayed during scheduled observation setting.

When this dialog is displayed, more space must be allocated by deleting unnecessary observation data. (To delete observation data, it is necessary to log in as an administrator.)

In addition, it is recommended that the data space circled in the figure as shown on the right be written down before starting schedule data deletion. The data space displayed in the dialog is the estimated amount to be deleted.

See Section 7.2 “Deleting the Observation Data” for details.



Insufficient data space warning dialog box

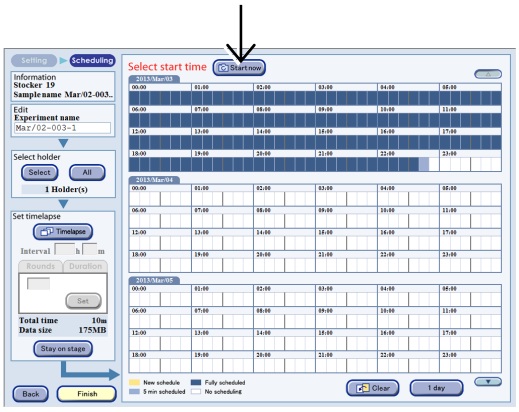
The following describes each case.

a. When immediately starting scheduled observation (with no timelapse set)

a-1. Press the Start now button.

A scheduled observation immediate start confirmation dialog box appears.

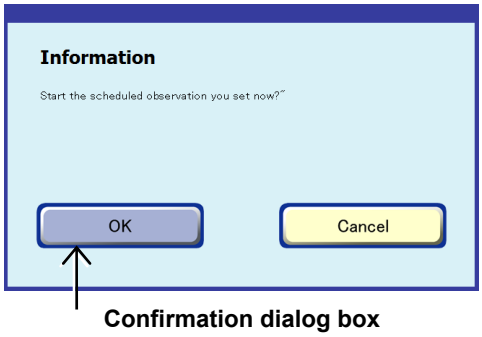
If a scheduled observation is scheduled in the immediate future, the already registered schedule has priority. After the registered scheduled observation completes, the schedule set with the Start now button is executed.



Scheduling window

a-2. Press the OK button to close the dialog box.

The Scheduling window appears and the scheduled observation starts.



Confirmation dialog box

b. When immediately starting scheduled observation (with timelapse set)

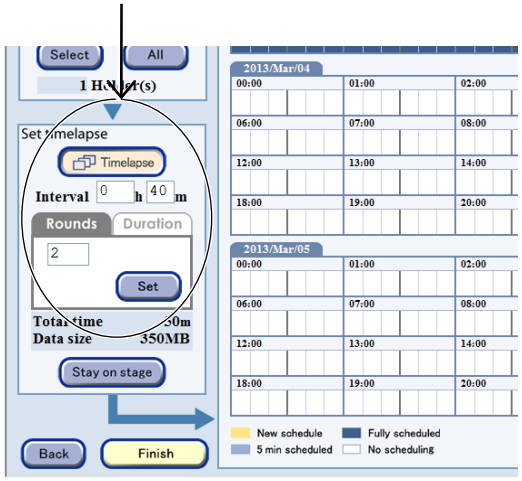
b-1. Press the Timelapse button.

b-2. Press the Interval (the h field or the m field of the Interval) button, and then enter the interval by hours in the h field, and 5 minute increments in the m field in the Keyboard window.

A time shorter than previously required for observation cannot be entered.

b-3. Press the number for Rounds (Rounds tab) or the desired observation period (d, h, m on the Duration tab) to enter in the Keyboard window and press the Set button.

The observation period (Total time) is displayed in 5 minute intervals under the condition for which Timelapse was set.

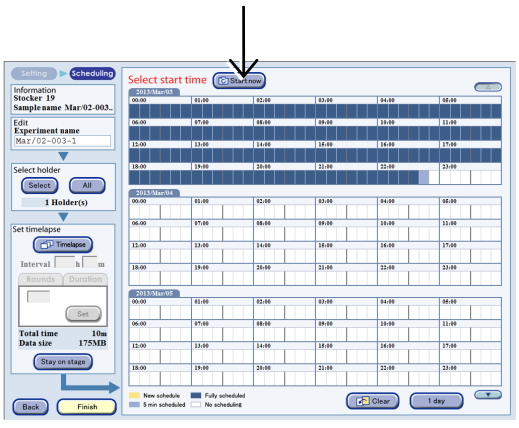


Scheduling window

b-4. Press the Start now button.

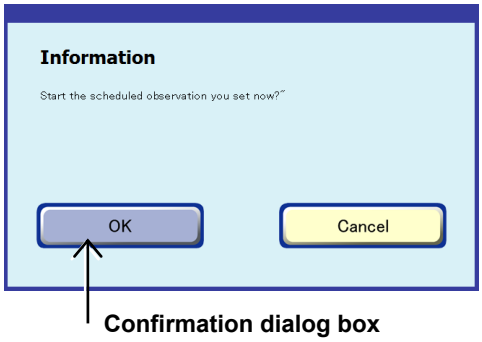
A scheduled observation immediate start confirmation dialog box appears.

> Timelapse cannot be set if the Focus teach button is pressed in the Observation condition setting window.



b-5. Press the OK button to close the dialog box.

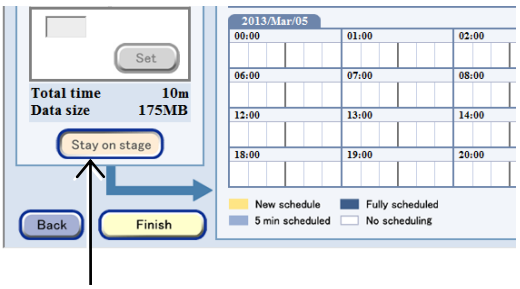
The Scheduling window appears and the scheduled observation starts.



Confirmation dialog box

c. When holding a sample on the stage until the specified scheduled observation start time (with no timelapse set)

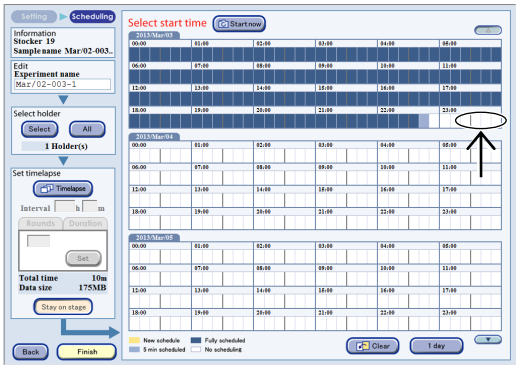
c-1. Press the Stay on stage button.



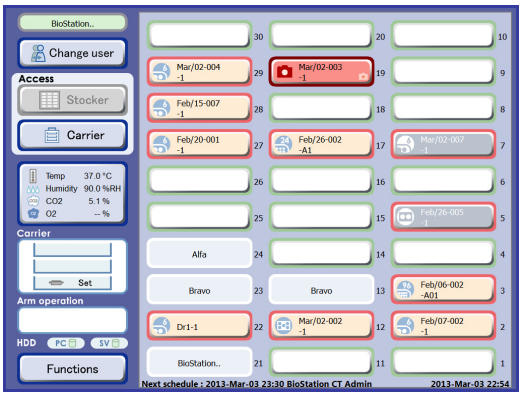
Scheduling window

c-2. Press the desired time zone (white part) to start scheduled observation in the Schedule setting field to set the time for scheduled observation.

The System status screen appears.



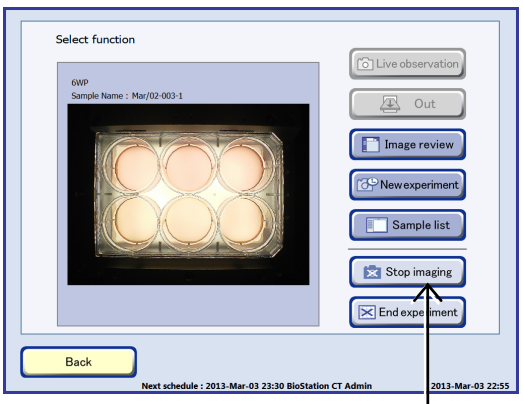
The sample is held on the stage until the set scheduled observation completes.



System status screen

To stop holding the sample on the stage, press the Cancel holding on Stop imaging button in the Select function window or the Image review window.

For details, see “To cancel scheduled observation of sample held on stage” described later.



Select function window

d. When holding a sample on the stage until the specified scheduled observation start time (with timelapse set)

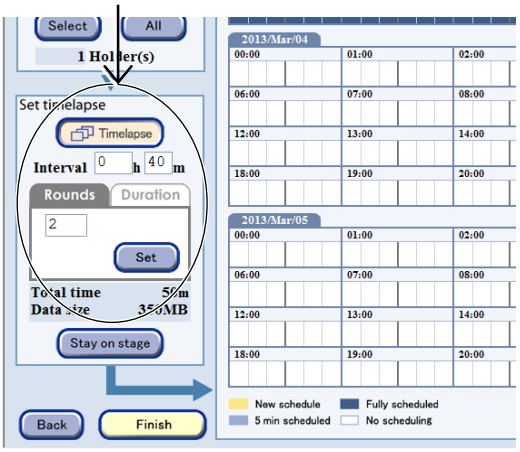
d-1. Press the Timelapse button.

d-2. Press the Interval (the h field or the m field of the Interval) button, and then enter the interval by hours in the h field, and 5 minute increments in the m field in the Keyboard window.

A time shorter than previously required for observation cannot be entered.

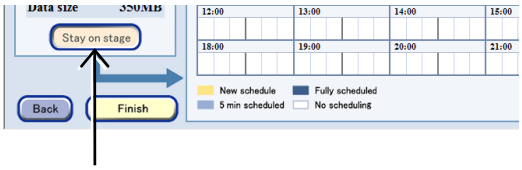
d-3. Press the number for Rounds (Rounds tab) or the desired observation period (d, h, m on the Duration tab) to enter in the Keyboard window and press the Set button.

The observation period (Total time) is displayed in 5 minute intervals under the condition for which Timelapse was set.



Scheduling window

d-4. Press the Stay on stage button.

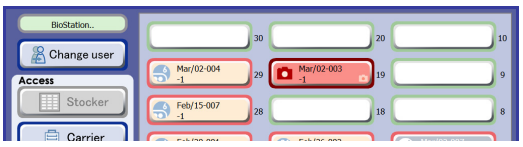


d-5. Press the desired time zone (white part) to start scheduled observation in the Schedule setting field to set the time for scheduled observation.

The System status screen appears.

> Timelapse cannot be set if the Focus teach button is pressed in the Observation condition setting window.

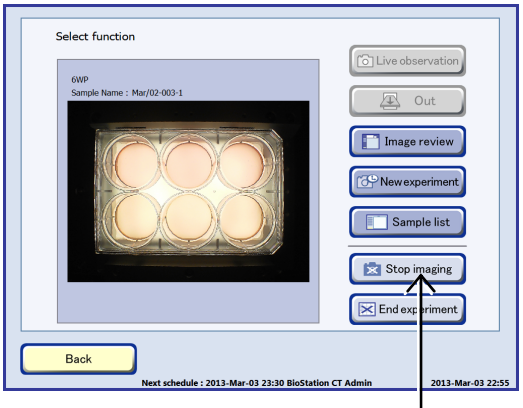
The sample is held on the stage until the set scheduled observation completes.



System status screen

To stop holding the sample on stage, press the Cancel holding on Stop imaging button in the Image review window.

For details, see “To cancel scheduled observation of sample held on stage” described later.

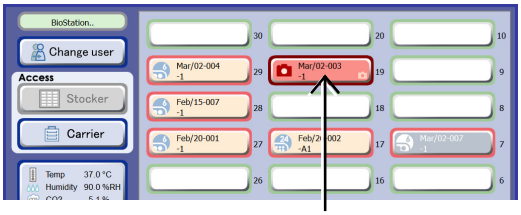


Select function window

To cancel scheduled observation of sample held on stage

1. **Press the Stocker button displayed in red on the System status screen.**

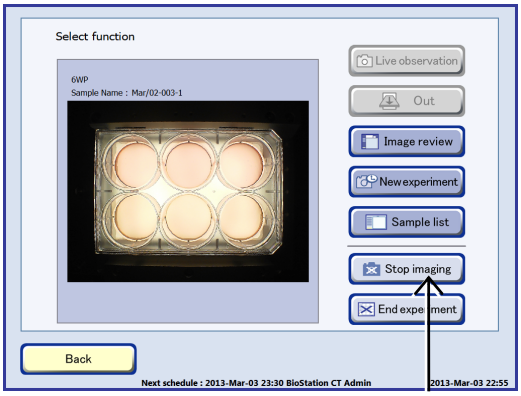
   The Select function window is displayed.



System status screen

2. **Press the Stop imaging button in the Select function window.**

   A cancel holding on stage confirmation dialog box appears.



Select function window

   Alternatively, press the Stop imaging button in the Image review window.

   A cancel holding on stage confirmation dialog box appears.

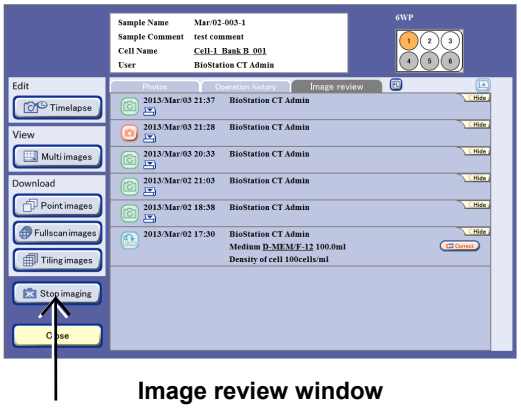
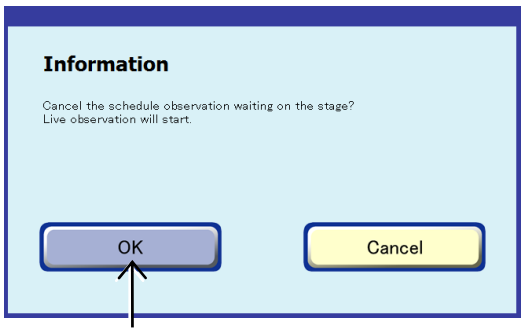


Image review window

3. **Press the OK button to close the dialog box.**

   Holding on stage is canceled and the Ph live observation window appears.



Confirmation dialog box

4.5.7 Ending Live observation

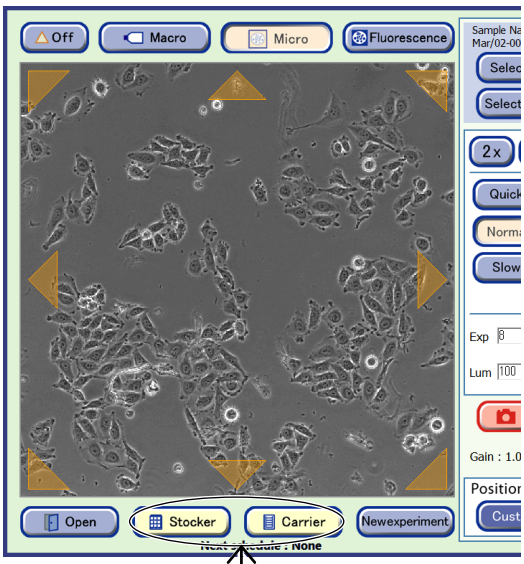
(1) To end live observation, press the Stocker button or the Carrier button in the Ph live observation window.

Press the Stocker button to transport the holder on the stage to the stocker.

Press the Carrier button to transport the holder on the stage to the carrier. For loading procedures, see Section 4.3.3, “Unloading samples.”

When the holder is transported, the Ph live observation window is closed.

> If the Carrier button is disabled, no carrier is set on the carrier slider or all carriers are full.
> To transport the holder to a carrier, set a carrier that has at least one vacant position onto the carrier slider.



Ph live observation window

4.6 Scheduled Observation (Automatic Observation)

This section describes the procedure for automatically observing samples cultured in a stocker in accordance with a preset schedule.

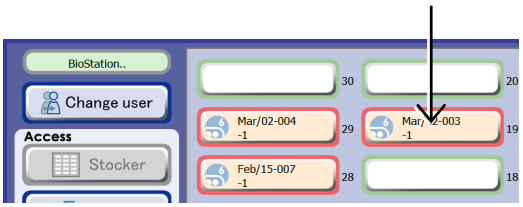
4.6.1 Selecting a Sample

(1) Select the sample to schedule observation.

a. To open the Observation condition setting window from the Select function window

a-1. On the System status screen, press the button for the stocker that contains the sample to be scheduled.

The Select function window appears.



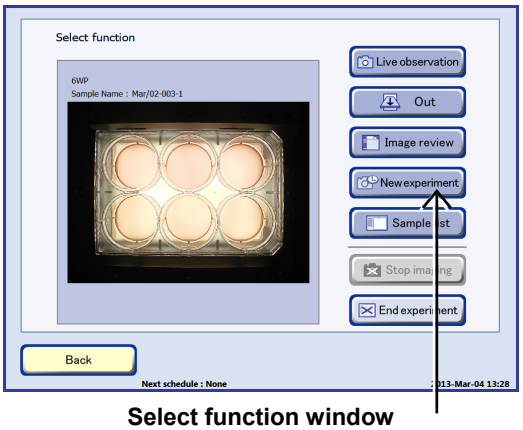
System status screen

a-2. Press the New experiment button.

The Observation condition setting window for the selected sample appears.

If the Observation condition setting window is opened from the Select function window, as samples have been already selected, go to step (3) of section 4.6.2, “Setting Scheduled Observation Conditions.”

To check the observation schedule for the selected sample, go to step (2).

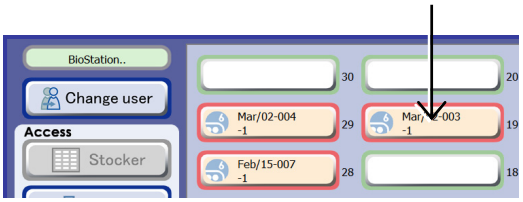


Select function window

b. To open the Schedule confirmation window from the Image review window

b-1. On the System status screen, press the button for the stocker that contains the sample to be scheduled.

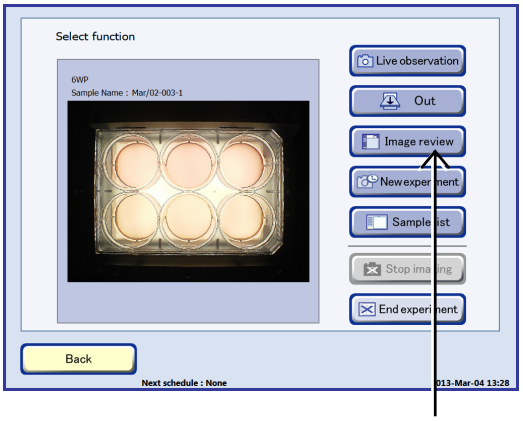
The Select function window appears.



System status screen

b-2. Press the Image review button.

The Image review window for the selected sample appears.



Select function window

b-3. Press the Timelapse button on the left side of the Image review window.

The Schedule confirmation window appears.

If the Schedule confirmation window is opened from the Image review window, the Schedule confirmation window for the sample displayed in the Image review window appears.
Since a sample is already selected, go to step (3) of section 4.6.2, “Setting Scheduled Observation Conditions.”

To check the observation schedule for the selected sample, go to step (2).

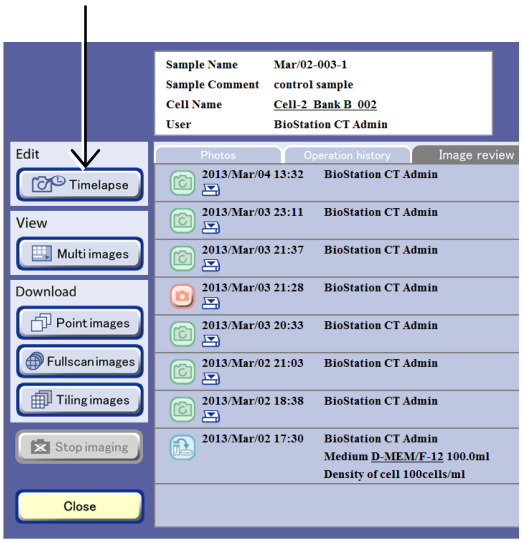
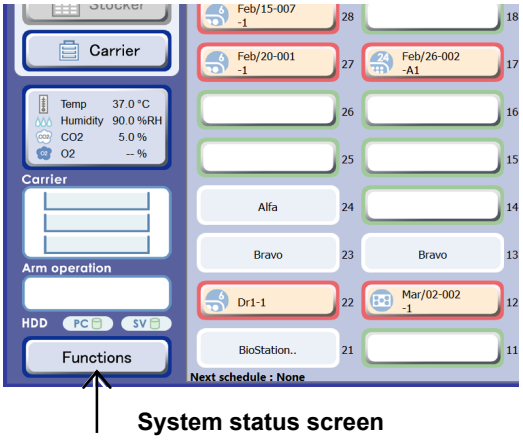


Image review window

c. To open the Schedule confirmation window from the Functions window

c-1. Press the Functions button on the System status screen.

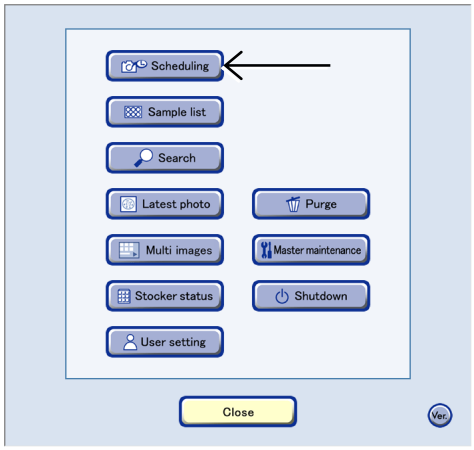
The Functions window appears.



System status screen

c-2. Press the Scheduling button.

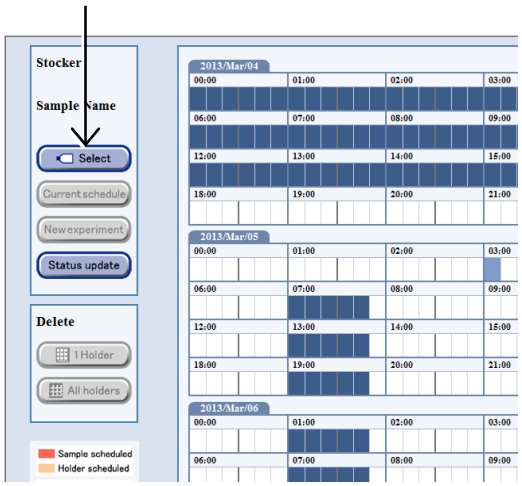
The Schedule confirmation window appears.



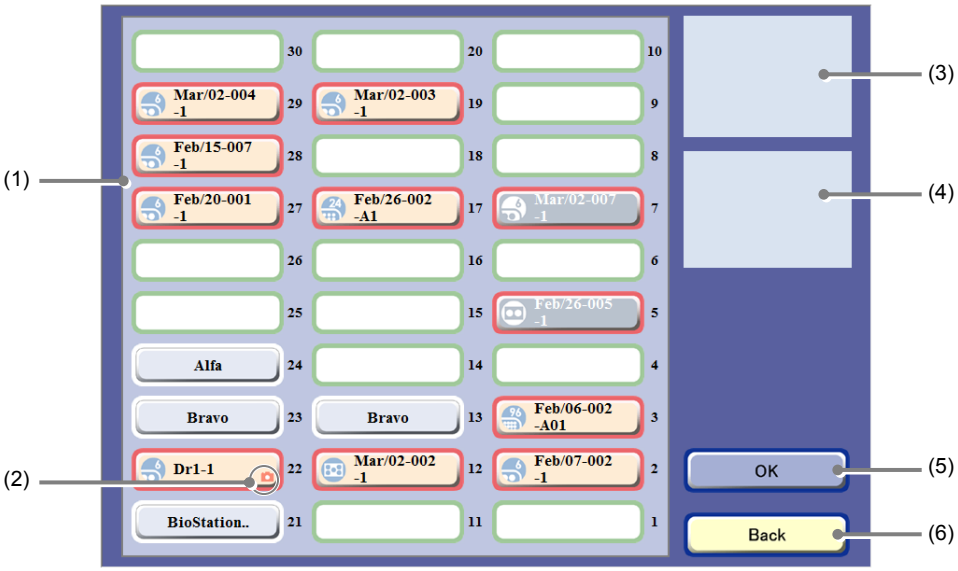
Functions window

c-3. Press the Select button.

The Sample selection window appears.



Schedule confirmation window



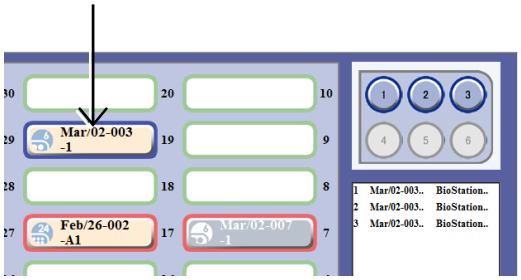
Sample selection window

No.	Name	Function
(1)	Stocker display area	Select a stocker that contains the sample to be scheduled.
(2)	Scheduled observation icon	This icon indicates that a scheduled observation is set for the sample in the stocker.
(3)	Holder map display area	This area displays a holder map in the selected stocker.
(4)	Sample list display area	This area displays a sample list in the selected stocker. When a 96- or 48-well plate is selected, the Sample list is not displayed.
(5)	OK button	Press this button to open the Schedule confirmation window for the selected sample.
(6)	Back button	Press this button to return to the Schedule confirmation window.

c-4. Select the button for the stocker that contains the sample to be scheduled.

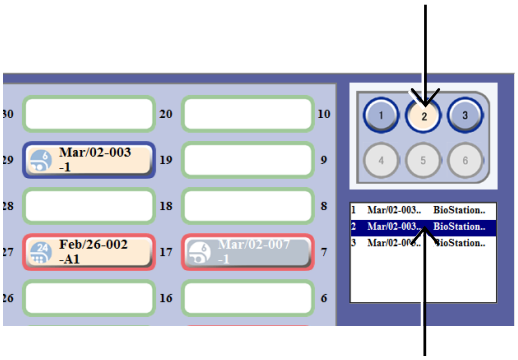
The frame color of the selected stocker button turns blue and the holder map and the Sample list are displayed.

To set the same schedule for all the samples in the holder, go to step c-6 and press the OK button.

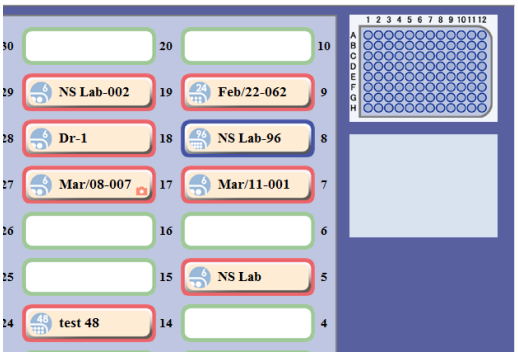


Sample selection window

c-5. To schedule a single sample, select the sample from the holder map or the Sample list.



When a 96- or 48-well plate is selected, the Sample list is not displayed.
Go to step c-6.



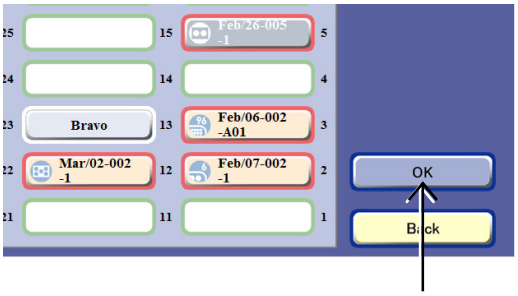
Sample selection window
(When 96-/48-well plate is selected)

c-6. Press the OK button.

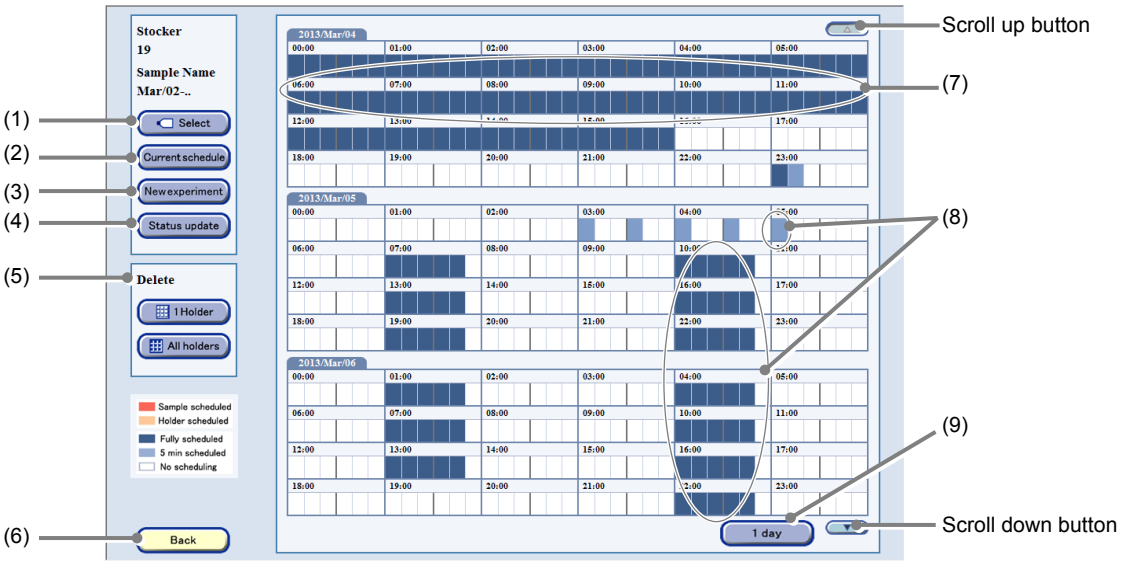
The Sample selection window closes and the Schedule confirmation window appears.

After the sample is selected, the Current Schedule button and the New experiment button are enabled in the Schedule confirmation window.

When a schedule is already set up, and when the sample selected in the Sample selection window is the same as the previous one, the schedule is displayed in red. The schedule for other samples stored in the same holder is displayed in orange.



The Schedule confirmation window shows the schedule for a single day or three days in one screen, with each 10 minute period categorized by color.

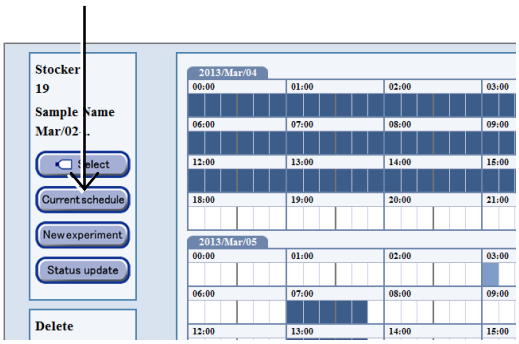


Schedule confirmation window

No.	Name	Function
(1)	Select button	Press this button to display the Sample selection window for scheduled observation.
(2)	Current schedule button	Press this button to display the Current schedule window. One-week observation schedule for the selected sample is displayed in the Current schedule window.
(3)	New experiment button	Press this button to display the Observation condition setting window.
(4)	Status update button	Press this button to display the latest schedule setting.
(5)	Delete area	Delete the observation schedule. **1 Holder button:** Delete all observation schedule settings in the selected stocker at the same time. **All holders button:** Delete all observation schedules for the samples cultured by the same user and in the same vessel in all stockers at the same time.
(6)	Back button	Press this button to close the Schedule confirmation window.
(7)	Elapsed periods	The time frame already elapsed is displayed in blue because it cannot be scheduled.
(8)	Scheduled periods	An already scheduled time frame is displayed in blue in 10 minute units or in light blue if the time for scheduled observation is no more than 5 minutes. A schedule can be set in an already scheduled time frame if any vacant time is available.
(9)	Displayed days switch button	Press this button to switch the number of days (1 or 3) displayed in a single screen.

(2) To check the observation schedule for the selected sample, press the Current schedule button.

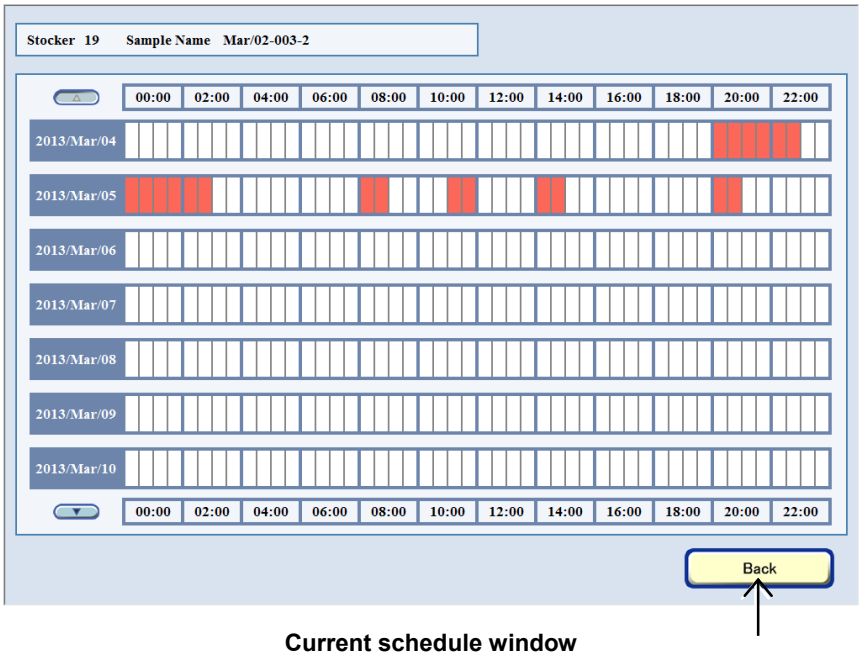
The Current schedule window appears.



Schedule confirmation window

The Current schedule window shows the one-week observation schedule for the selected sample. The period of time in which scheduled observation is set is displayed in red.

1. Press the Back button to return to the Schedule confirmation window after checking the Schedule.



Current schedule window

The procedure for selecting a sample is now complete.

To set scheduled observation conditions, go to section 4.6.2 “Setting the Scheduled Observation Conditions.”

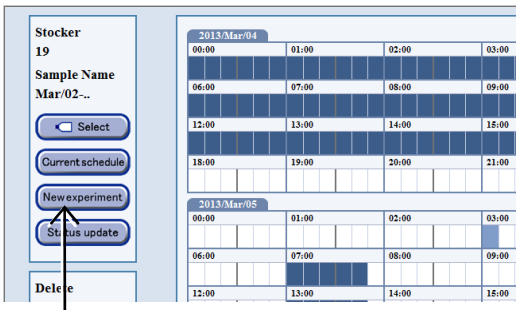
4.6.2 Setting the Scheduled Observation Conditions

(1) Set the observation conditions for schedule observation.

> To copy already registered scheduled observation settings, see Section 4.6.5.1, “Copying standby scheduled observation settings.”

(2) Press the New experiment button.

The Observation condition setting window appears.



Schedule confirmation window

(3) Select an appropriate observation method from the observation method selection tabs.

There are three observation methods: Point observation, Full Scan observation, and Tiling observation.

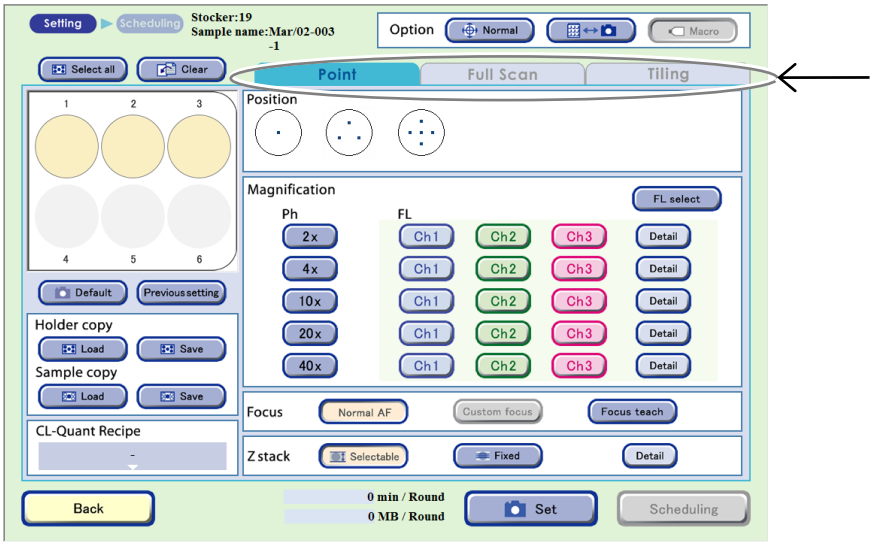
Point: An observation method which specifies the observation position from default or custom.
See Section 4.6.2.1, “Point observation.”

Full Scan: An observation method which captures the entire culture area of the Sample as tiled images.
See Section 4.6.2.2, “Full Scan observation.”

* Full Scan observation is not possible when a 75 cm^2 culture flask or a part of 25 cm^2 culture flask (25CF(ob), 25CF_A(ob), 25CF_A) or Nunc 4-well multi dish is used.

Tiling: An observation method that specifies the area to be observed at high magnification from the entire culture area captured by Full Scan and captures the specified area by tiling.
See Section 4.6.2.3, “Tiling observation.”

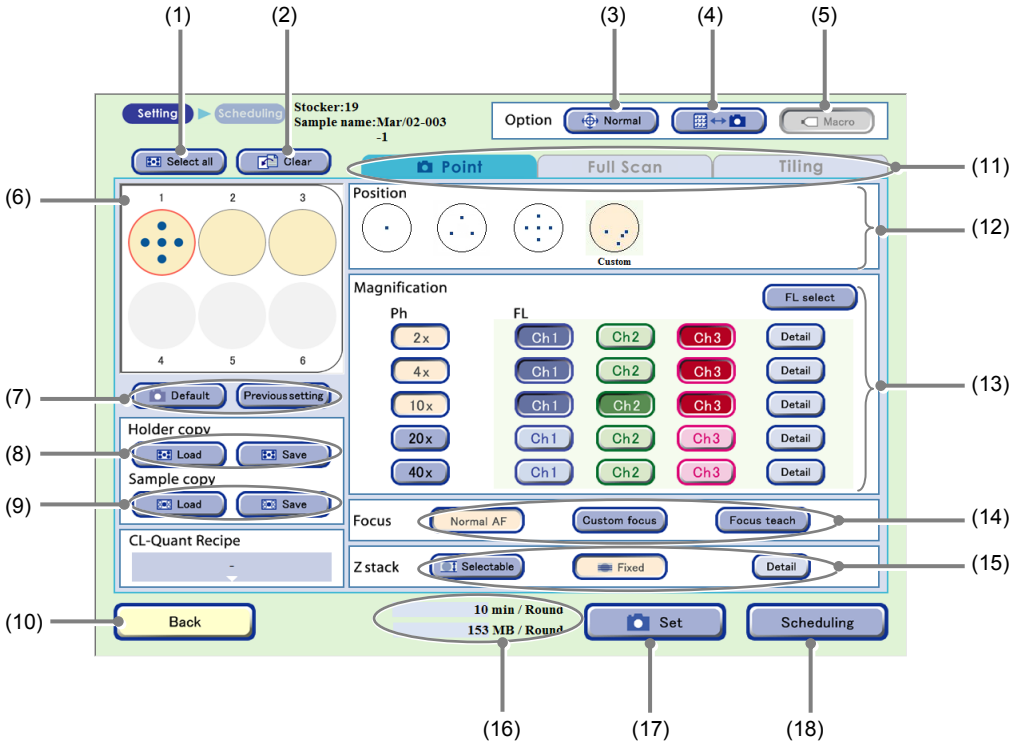
* To specify a desired observation area as a capture area by Tiling observation, a Full Scan image must be captured by Full Scan and the captured area must be registered as a custom observation point in the Full Scan image display window in advance.



Observation condition setting window

4.6.2.1 Point observation

This section describes the setting procedure when Point observation is selected as the observation method.



Observation condition setting window (Point tab selected)

No.	Name	Function
(1)	Select all button	Press this button to select all samples of the holder. The selected sample is marked with a red frame.
(2)	Clear button	Press this button to clear the observation condition settings.
(3)	Stage speed selection button	Select the stage speed. **Normal:** Set the stage speed to normal. **Slow:** Set the stage speed to slow.
(4)	Scheduled observation mode selection	Select the scheduled observation mode. **button: (Normal mode)** The holder is returned to the stocker when each round in scheduled observation is finished. **Keep button: (Stage exclusive mode)** The holder is kept on the stage from the first round to the last round for scheduled observation.
(5)	Macro button	Select whether to capture a macro image during scheduled observation. (Enabled only during stage exclusive mode) **ON (Concave):** Enable macro capture. **OFF (Convex):** Disable macro capture.

No.	Name	Function
(6)	Sample selection area	Select the sample for scheduled observation. (All samples are selected in the Observation condition setting window by default.) The selected sample is marked with a red frame. To cancel the selection, press the selected sample again.
(7)	Settings load buttons	The scheduled observation conditions are loaded. **Default button:** Load the Scheduled observation default setting. (See Chapter 5, “Environmental Settings.”) **Previous setting button:** Load the previous observation settings.
(8)	Holder copy area	Capture conditions of the holder are loaded and saved. **Load button:** Load the saved capturing conditions for the holder into the vessel being displayed. **Save button:** Save the capturing conditions for the vessel being displayed on a holder basis.
(9)	Sample copy area	Capture conditions of each sample are loaded and saved. **Load button:** Load the saved capturing conditions into the selected sample. **Save button:** Save capture conditions of the selected sample.
(10)	Back button	Press this button to return to the previous window without saving the settings.
(11)	Observation method selection tabs	Select the observation method. There are three observation methods: Point observation, Full Scan observation, and Tiling observation. Select the Point tab here.
(12)	Observation position setting area	Select the default observation position or a custom observation point. **Default observation position:** An observation position preregistered on this product by default. **Custom observation point:** An observation position newly registered for a live observation, scheduled observation, or in the Full scan image display window. Two ways for displaying the custom observation point are shown below. ● When the custom observation point is registered for the sample selected from the sample selection area. ● When multiple samples are selected from the sample selection area. (Note that a sample without a registered custom observation point cannot be set even though the Set button is pressed.)

No.	Name	Function
(13)	Magnification/FL channel setting area	Select the observation magnification and FL channel button. **Magnifications button:** Select the magnification to be used to capture a phase contrast image in scheduled observation. **FL channel button:** Press the buttons for the magnifications and channels of the fluorescence images to be captured. Multiple channels can be selected. **Detail button:** The FL image exposure conditions setting window appears. Set the exposure time and the intensity of each excitation light source. (See Section 4.6.2.4, “Setting exposure conditions for fluorescence images.”) **FL select button:** The FL channel selection window appears. When up to 5 types of fluorescence filter cube are mounted, channels (Ch1 to Ch3) to be used when setting scheduled observation conditions can be changed to the added fluorescence filter channels (Ch4, Ch5). (See Section 4.6.2.5, “Changing the fluorescence channel (optional).”)
(14)	Focus Type area	Select the focus type. **Normal AF:** Perform autofocus at the specified observation position (the center of the sample when a 96- or 48-well plate is used). **Custom focus:** Autofocus is not performed. Instead, the Z position of the registered custom observation point is used. (Enabled only when Custom observation point is selected.) **Focus teach button:** Register custom observation points (X, Y, Z) during scheduled observation when Normal AF is pressed.
(15)	Z stack area	Specify the number of Z stack images. **Selectable button:** Enable the Z stack capture function in selectable pitches. Select a capture range and pitch for each magnification. The number of images to be captured is selected automatically. **Fixed button:** Enable the Z stack capture function in fixed pitch. Select the number of images to be captured for each magnification. The pitch and capture area are selected automatically. **Detail button:** Display the setting window for selectable pitches or fixed pitches.
(16)	Time required for observation/data size	The time and data size required for one round of scheduled observation is displayed when the Set button is pressed.
(17)	Set button	Press this button to set the Observation setting for the selected sample.
(18)	Scheduling button	Press this button to display the Scheduling window.

(1) Set the mode for scheduled observation.

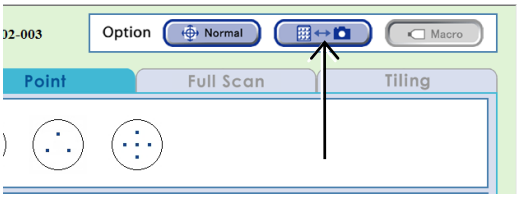
There are two modes available for scheduled observations: The Normal mode to return the holder to the stocker each time 1 Round is finished, and the Stage exclusive mode that keeps the holder on the stage from the first Round to the last Round.

> The procedure for setting scheduled observation varies depending on whether Normal mode or Stage exclusive mode is used. For details, see Section 4.6.3, “Setting Schedules.”

To use the Normal mode for scheduled observation

a. Switch the Scheduled observation mode selection to (Normal mode).

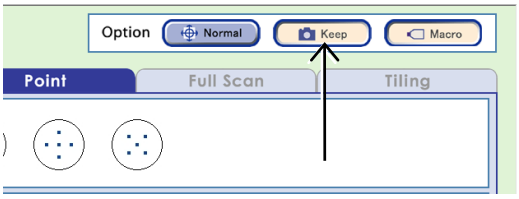
The figure on the right shows the mode button set to the Normal mode.



To use the Stage exclusive mode for scheduled observation

a. Switch the Scheduled observation mode selection to Keep (Stage exclusive mode).

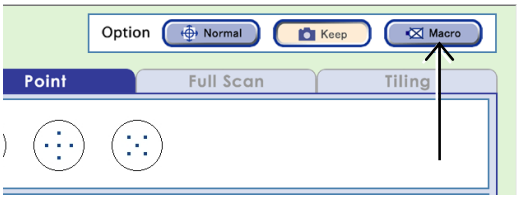
The figure on the right shows the mode button set to the Stage exclusive mode.



b. Switch the Macro button to either enable or disable macro capture.

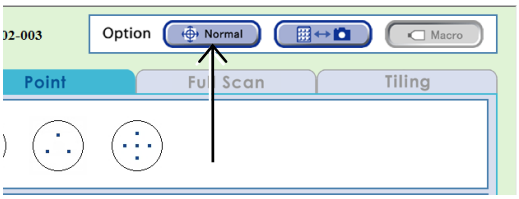
The figure on the right shows the Macro button set to OFF (to disable the macro capture).

The Macro button is only effective for the Stage exclusive mode. Be sure to perform macro capture when using the Normal mode for scheduled observation.



(2) If necessary, use the Stage speed selection button to switch the stage speed when shifting the observation position.

Each time the Stage speed selection button is pressed, the mode is switched between Normal (normal speed) and Slow (slow speed).



(3) Select the sample for scheduled observation.

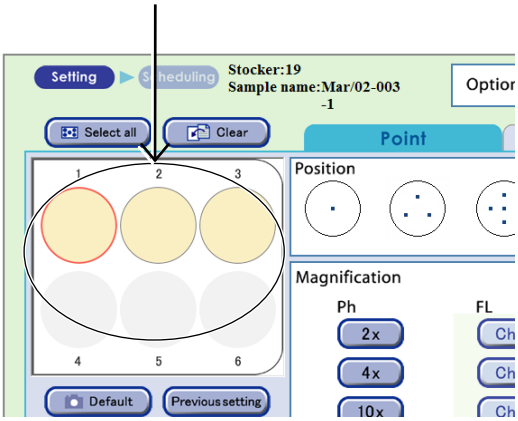
All samples are selected in the Observation condition setting window by default.

When a vessel other than 96-/48-well plate is used

Press the Sample button in the Sample selection area to select the sample for scheduled observation. The selected sample is marked with a red frame.
To cancel the selection, press the selected sample again.

To select or unselect all samples in the holder, press the Select all button.

To use the sample and the Observation setting used in the previous observation, press the Previous setting button.



Observation condition setting window

When using a 96-, 48-well plate

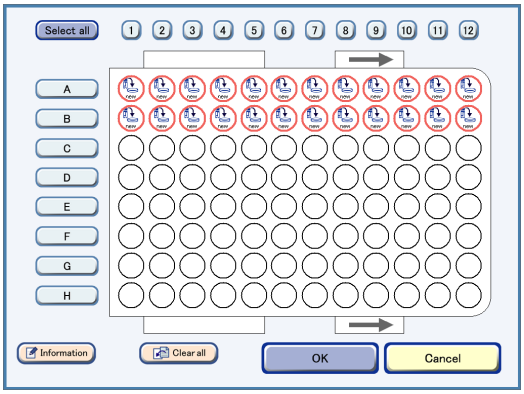
Press the Sample selection area to display the 96-well or 48-well plate sample selection window.

Press the target sample to select it.
The selected sample is marked with a red frame.

Pressing the Select all button selects or unselects all samples.

Pressing one of the vertical alphabet buttons selects all samples in that row. Pressing one of the horizontal number buttons selects all samples in that column. When the selected sample is pressed again, the selection is canceled.

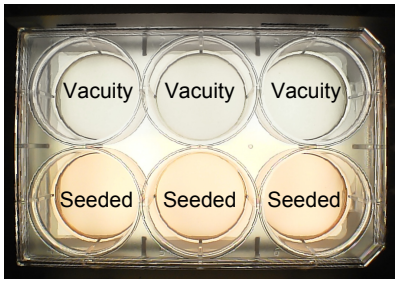
Pressing the OK button applies the selection and reopens the Observation condition setting window.



Well plate sample selection window (96-well)

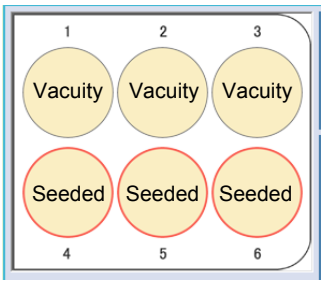
If a well plate is used and the selected samples contain an empty well on which cells are not seeded, autofocus on other wells may not work correctly.

Because images may become out of focus, exclude empty wells before selecting samples and setting the observation conditions.



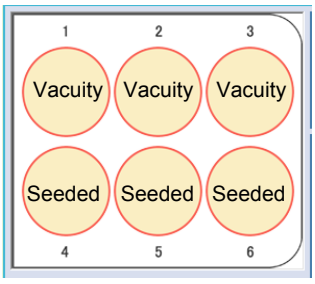
Well plate

Good



Selecting only wells on which cells are seeded

Not good



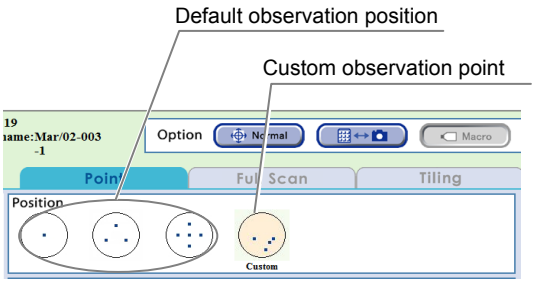
Selecting wells including empty wells

(4) Select the observation position.

Select the default observation position or a custom observation point.

Two ways for displaying the custom observation point are shown below.

- **When the custom observation point is registered for the sample selected from the sample selection area.**
- **When multiple samples are selected from the sample selection area.**
  (Note that a sample without a registered custom observation point cannot be set even though the Set button is pressed in step 9.)



Observation condition setting window

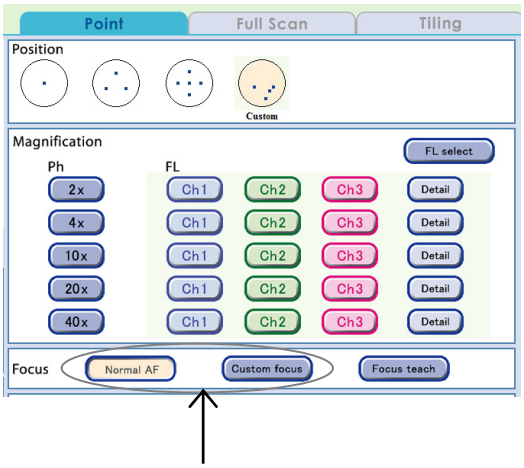
(5) Select the focus type.

Focus type can be selected for samples with a custom observation point registered.

The following describes each case.

a. When a default observation point is selected

Focus type cannot be selected.
Normal AF is selected and autofocus is performed at the specified observation position (the center of the sample when a 96- or 48-well plate is used).



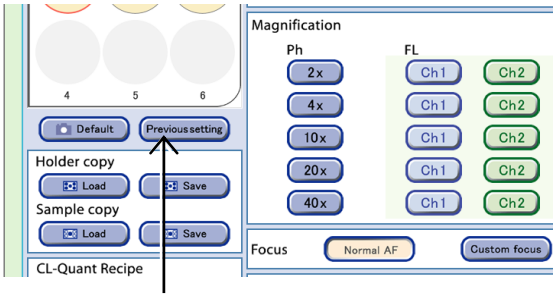
b. When a custom observation point is selected

Either Custom focus or Normal AF can be selected.

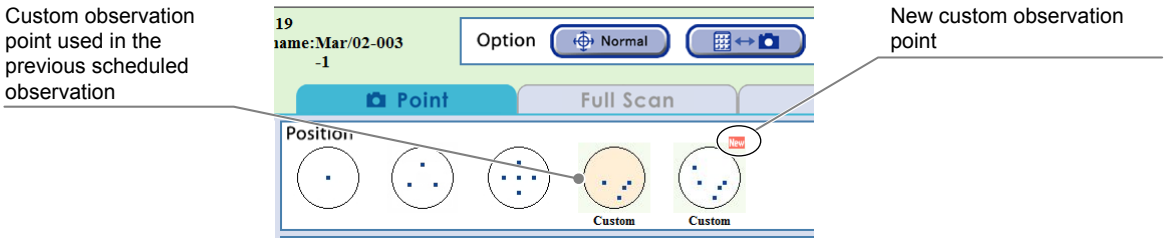
With Custom focus, scheduled observation is performed at the Z position of the registered custom observation point without performing autofocus.

Select Normal AF to perform autofocus at the specified observation position.

When the custom observation point is changed after the previous scheduled observation, the custom observation point (on the left) used in the previous scheduled observation and the new custom observation point (on the right) are both displayed by pressing the Previous setting button in step (3).



To use the new custom observation point changed from the observation position in the previous scheduled observation, select the custom observation point (displayed in NEW) on the right.



If the sample unloaded so the medium can be changed is reloaded, the custom observation point remains without being deleted. When a new custom observation point is registered without the current custom observation point being deleted, the new custom observation point is added to the current custom point. Delete unnecessary custom observation point in the relevant Live observation window.

A custom observation point can be registered as shown below.
(Up to 25 custom observation points can be registered per sample.)

a. Registration during live observation

For information on registering during live observation, see Section 4.5.1.2, “Capturing a Ph live image and registering a custom observation point” or Section 4.5.2.2, “Displaying a FL live image and registering a custom observation point.”

b. Registration during scheduled observation

A custom observation points (X, Y, Z) can be registered using the following procedure. In this case, the Autofocus setting is registered as the custom observation point (Z).

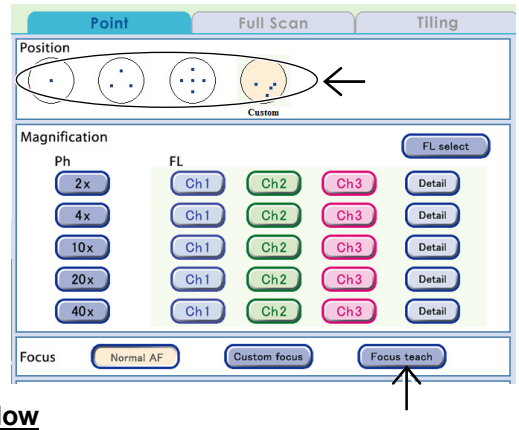
The custom observation point is registered automatically during scheduled observation so that it can be used at the next scheduled observation.

b-1. Select the default observation position or a custom observation point.

If a custom observation point is selected, press the Normal AF button to enable the Focus teach button.

b-2. Press the Focus teach button.

When the scheduled observation is performed by selecting the Focus teach button, observation is allowed only once and cannot be repeated.



c. Registration in the Full Scan image display window

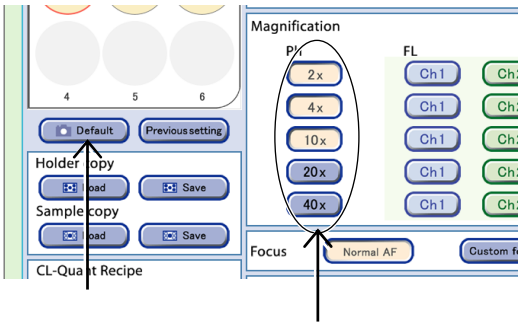
For details on registration, see Page 233.

(6) Select magnifications to be used for scheduled observation.

The figure on the right shows an example that the three magnifications (2x, 4x, and 10x) are selected.

To use the default settings of the magnifications and the observation position, press the Default button.

For details on the default settings, see Chapter 5, “Environmental Settings.”

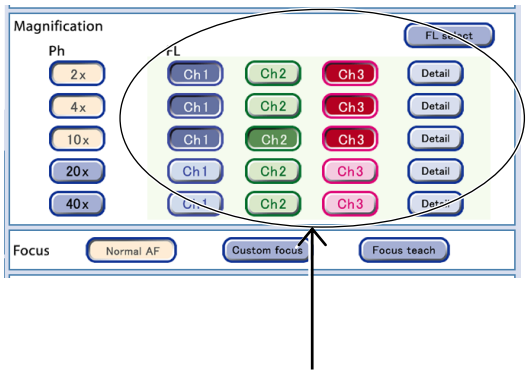


(7) To capture the fluorescence image, select the FL channel button.

To change the exposure conditions for capturing fluorescence images, press the Detail button. For details, see Section 4.6.2.4, “Setting exposure conditions for fluorescence images.”

When up to 5 types of fluorescence filter cube are mounted, channels (Ch1 to Ch3) to be used can be changed to the added fluorescence filter channels (Ch4, Ch5). For details, see Section 4.6.2.5, “Changing the fluorescence channel (optional).”

When the Fluorescence unit is not connected, the FL channel button is disabled.



(8) Specify the number of Z stack images.

If the default setting is acceptable, go to step (9).

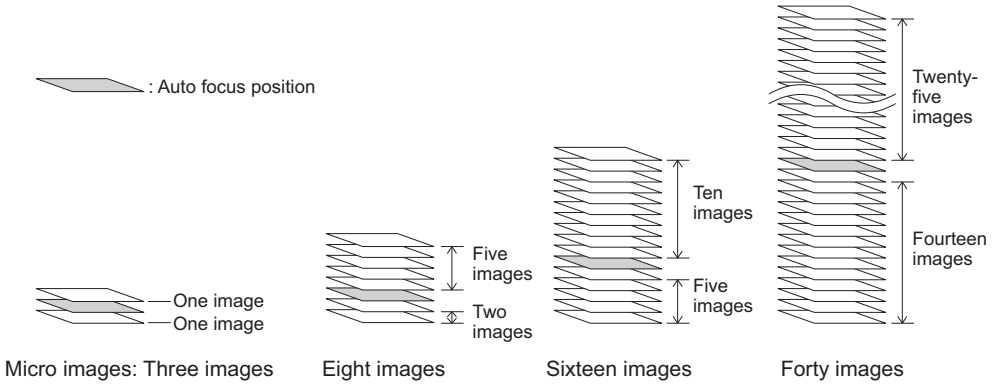
For details on the default settings, see Chapter 5, “Environmental Settings.”

Z stack

Micro images captured in a scheduled observation are included in a set of images shifted in the Z-axis direction around the autofocus position. The image set is called a Z stack image.
For Z stack images, there are two ways to capture images; Fixed pitch for capturing images in accordance with a fixed pitch and selectable pitch for capturing images in accordance with a specified range and pitch.

Fixed pitch images

Images are captured at a predefined range and pitch by selecting the number of images (1, 3, 8, 16, or 40).
The autofocus position in a Z stack image captured with a fixed pitch is defined for each image count by default as shown below. However, if a position near the upper or lower limit of the Z-axis is set for the autofocus position, the image count of the upper part and the lower part will change. (Example: If the lowest position in the Z-axis is set as the autofocus position in fixed pitch capturing of eight images, the lower part image count is zero and the upper part image count is seven.)



Additionally, if a custom observation point is selected and the Custom focus button is pressed to capture an image for in scheduled observation, the AF position described above will be the Z position registered as the custom observation point.

Selectable pitch images

To capture images using a selectable pitch, determine the number of images to be captured by selecting the range in the Z-axis direction (0, 100, 200, 300, or 400 µm) and pitch (5, 10, or 20 µm).
When the selected range is 0 µm, a Z stack is not generated and only one image is captured.
When the selected range is 400 µm and the pitch is 5 µm, the number of capturing images is the maximum (81 images).

* The function called “Micro set” in BioStation CT Ver.3.3 and earlier is now referred to as “Fixed-pitch Z stack.”

Press the Selectable or Fixed button to select the pitch setting.

a. Using Selectable pitch

Selecting a capture range and pitch for each magnification automatically specifies the number of images to be captured.
Note that the magnifications in the Observation condition setting window change in conjunction with the magnifications selected in the Selectable pitch setting window.

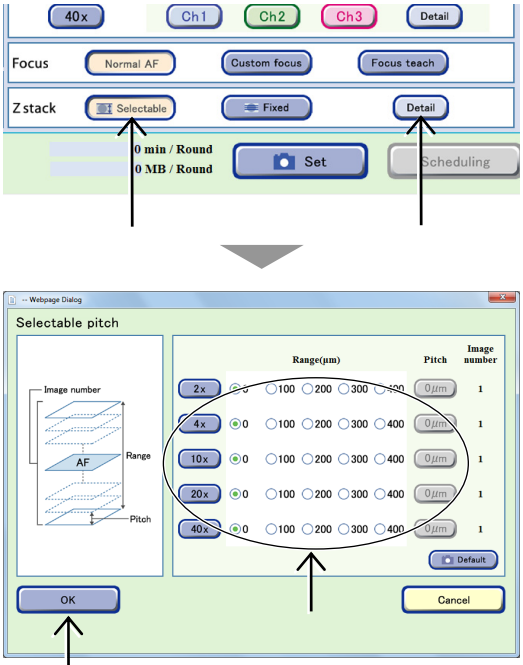
a-1. Press the Selectable button and then press the Detail button.

The Selectable pitch setting window appears.

a-2. Select a Range and Pitch for each magnification.

Depending on the selected range and pitch, the number of images to be captured is determined and displayed on the right side of the window.

a-3. Press the OK button.



Selectable pitch setting window

b. Using Fixed pitch

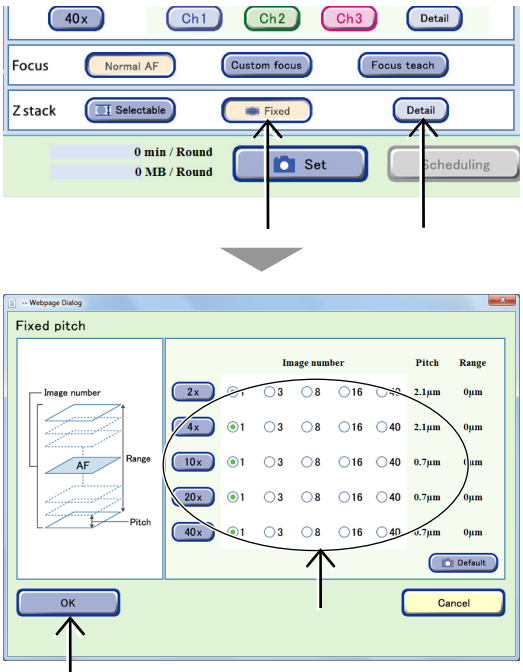
Selecting the number of capturing images for each magnification automatically specifies a corresponding capture range and pitch.
Note that the magnifications in the Observation condition setting window change in conjunction with the magnifications selected in the Fixed pitch setting window.

b-1. Press the Fixed button and then press the Detail button.

The Fixed pitch setting window appears.

b-2. Directly select the number of images to be captured (1, 3, 8, 16, or 40).

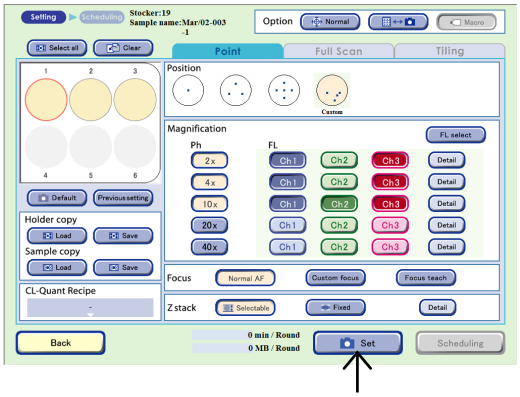
b-3. Press the OK button.



Fixed pitch setting window

(9) To specify the selected observation setting, press the Set button.

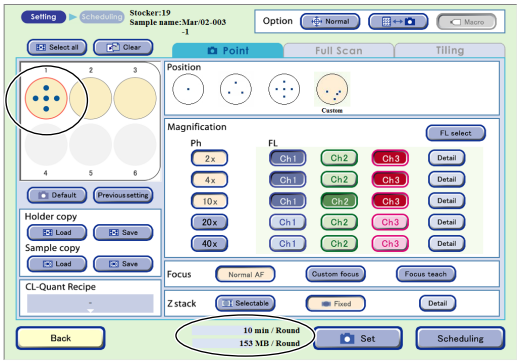
The observation setting is set up for the selected sample.



Samples set for scheduled observations are displayed as shown in the figure on the right.

The time and data size required for one round of scheduled observation is displayed.

When the observation setting is set, the icon appears on the Observation method selection tab.



The procedure for setting the point observation conditions is now complete.

To set schedules, see Section 4.6.3, "Setting Schedules."

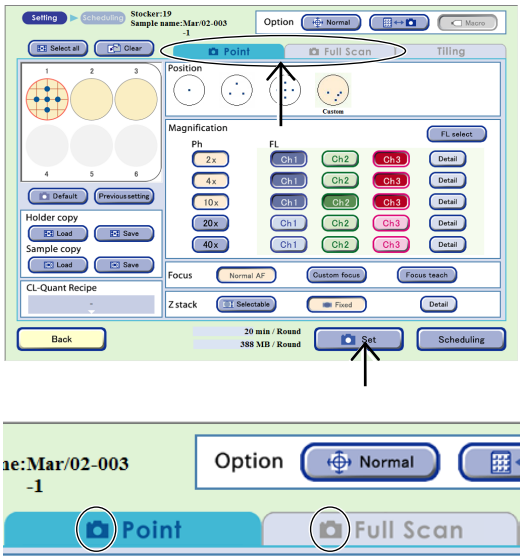
High magnification images of any point and a low magnification image of entire sample can be captured at the same time with one scheduled observation setting by combining Point observation that enables high magnification observation and Full Scan observation which performs low magnification observation. Full Scan observation is not possible when a 75 cm^2 culture flask or a part of 25 cm^2 culture flask (25CF(ob), 25CF_A(ob), 25CF_A) or Nunc 4-well multi dish is used.

Perform the setting in accordance with the following procedure.

1. Set the scheduled observation condition for Point observation and press the Set button.
2. Select the Full Scan observation method selection tab and set the Full Scan scheduled observation condition. (See Section 4.6.2.2.)
3. Press the Set button.

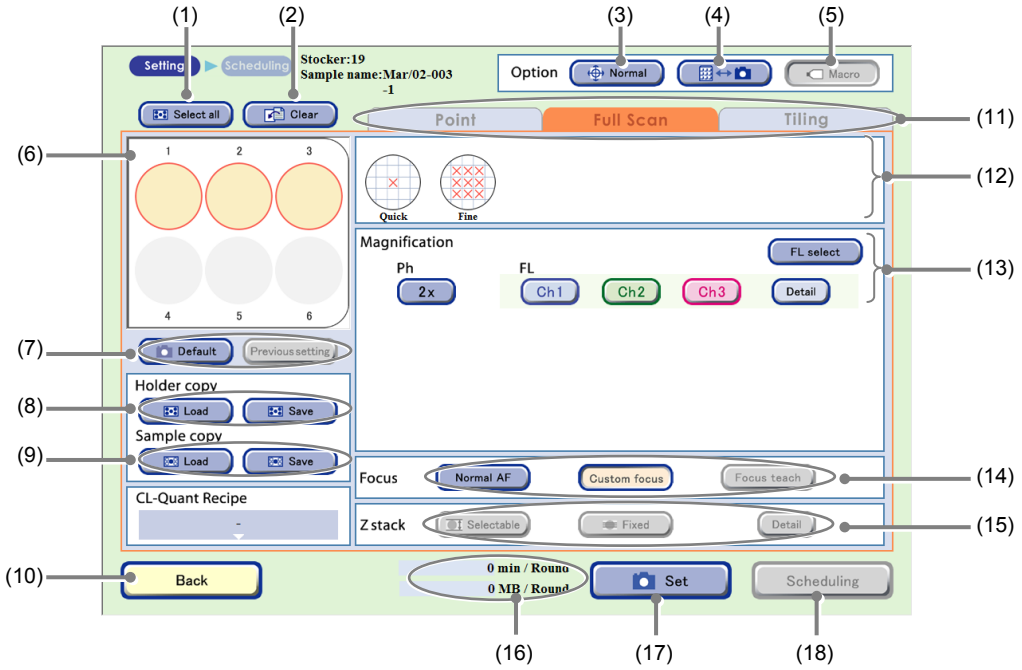
   The observation setting is set to the selected sample and the total time and data size required for a round of both scheduled observations is displayed in the time required for observation area.

   The icon appears on the Observation method selection tab for which the observation setting is set.



4.6.2.2 Full Scan observation

This section describes the setting procedure when Full Scan observation is selected as the observation method.



Observation condition setting window (Full Scan tab selected)

No.	Name	Function
(1)	Select all button	Press this button to select all samples of the holder. The selected sample is marked with a red frame.
(2)	Clear button	Press this button to clear the observation condition settings.
(3)	Stage speed selection button	Select the stage speed. **Normal:** Set the stage speed to normal. **Slow:** Set the stage speed to slow.
(4)	Scheduled observation mode selection	Select the scheduled observation mode. **button: (Normal mode)** The holder is returned to the stocker when each round in scheduled observation is finished. **Keep button: (Stage exclusive mode)** The holder is kept on the stage from the first round to the last round for scheduled observation.
(5)	Macro button	Select whether to capture a macro image during scheduled observation. (Enabled only during stage exclusive mode) **ON (Concave):** Enable macro capture. **OFF (Convex):** Disable macro capture.
(6)	Sample selection area	Select the sample for scheduled observation. (All samples are selected in the Observation condition setting window by default.) The selected sample is marked with a red frame. To cancel the selection, press the selected sample again.

No.	Name	Function
(7)	Settings load buttons	The scheduled observation conditions are loaded. **Default button:** Load the Scheduled observation default setting. (See Chapter 5, “Environmental Settings.”) **Previous setting button:** Load the previous observation settings.
(8)	Holder copy area	Capture conditions of the holder are loaded and saved. **Load button:** Load the saved capturing conditions for the holder into the vessel being displayed. **Save button:** Save the capturing conditions for the vessel being displayed on a holder basis.
(9)	Sample copy area	Capture conditions of each sample are loaded and saved. **Load button:** Load the saved capturing conditions into the selected sample. **Save button:** Save capture conditions of the selected sample.
(10)	Back button	Press this button to return to the previous window without saving the settings.
(11)	Observation method selection tabs	Select the observation method. There are three observation methods: Point observation, Full Scan observation, and Tiling observation. Select the Full Scan tab here.
(12)	AF position setting area	Select the autofocus position. **Quick:** Autofocus is performed at the center of the sample. **Fine:** Autofocus is performed at multiple points. Disabled when a 96-well plate or 48-well plate is used.
(13)	Magnification/FL channel setting area	Select the observation magnification and FL channel button. **Magnification button:** Select the magnification to be used to capture a phase contrast image in scheduled observation. The observation magnification displayed in the Magnification area depends on the combination of the type of culture vessel and Quick or Fine in the AF position setting area. **FL channel button:** Press the buttons for the magnifications and channels of the fluorescence images to be captured. Multiple channels of the same magnification can be selected. **Detail button:** The FL image exposure conditions setting window appears. Set the exposure time and the intensity of each excitation light source. (See Section 4.6.2.4, “Setting exposure conditions for fluorescence images.”) **FL select button:** The FL channel selection window appears. When up to 5 types of fluorescence filter cube are mounted, channels (Ch1 to Ch3) to be used when setting scheduled observation conditions can be changed to the added fluorescence filter channels (Ch4, Ch5). (See Section 4.6.2.5, “Changing the fluorescence channel (optional).”)

No.	Name	Function
(14)	Focus Type area	Select the focus type. **Normal AF:** Autofocus is performed at the center of the sample. **Custom focus:** Autofocus is performed near the custom observation point. (Enabled only for sample with FullScan Z registered with live observation.) **Focus teach button:** (When Full Scan is selected, this button is disabled.)
(15)	Z stack area	When Full Scan is selected, this button is disabled.
(16)	Time required for observation/data size	The time and data size required for one round of scheduled observation is displayed when the Set button is pressed.
(17)	Set button	Press this button to set the Observation setting for the selected sample.
(18)	Scheduling button	Press this button to display the Scheduling window.

* The optional software “CL-Quant” allows analyzing the captured Full Scan images and saving the combined images.

(1) Set the mode for scheduled observation.

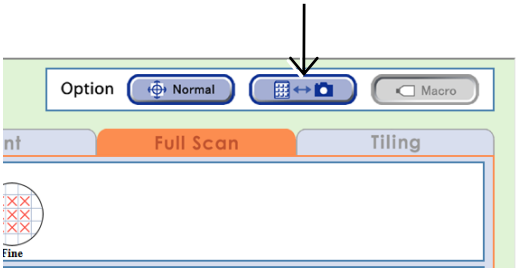
There are two modes available for scheduled observations: The Normal mode to return the holder to the stocker each time 1 Round is finished, and the Stage exclusive mode that keeps the holder on the stage from the first Round to the last Round.

> The procedure for setting scheduled observation varies depending on whether Normal mode or Stage exclusive mode is used. For details, see Section 4.6.3, “Setting Schedules.”

To use the Normal mode for scheduled observation

a. Switch the Scheduled observation mode selection to (Normal mode).

The figure on the right shows the mode button set to the Normal mode.

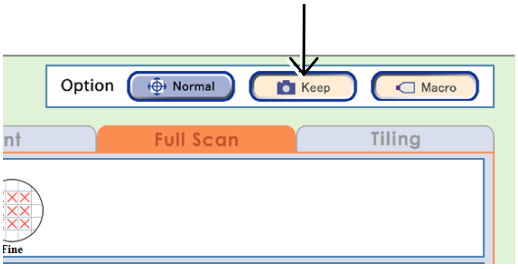


Observation condition setting window

To use the Stage exclusive mode for scheduled observation

a. Switch the Scheduled observation mode selection to Keep (Stage exclusive mode).

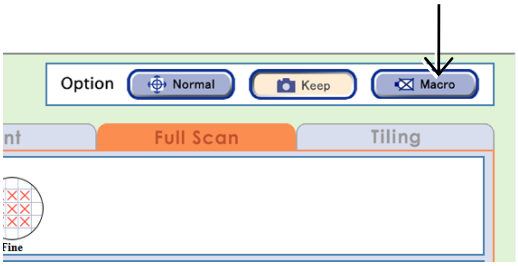
The figure on the right shows the mode button set to the Stage exclusive mode.



b. Switch the Macro button to either enable or disable macro capture.

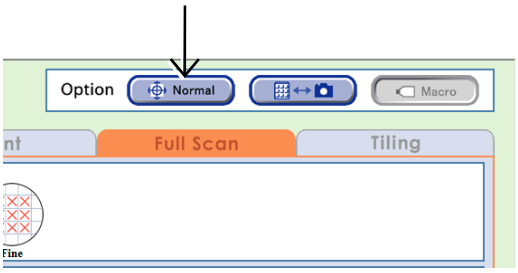
The figure on the right shows the Macro button set to OFF (to disable the macro capture).

The Macro button is effective only in the stage exclusive mode. Be sure to perform the macro capture when using the normal mode for scheduled observation.



(2) If necessary, use the Stage speed selection button to switch the stage speed when shifting the observation position.

Each time the Stage speed selection button is pressed, the mode is switched between Normal (normal speed) and Slow (slow speed).



(3) Select the sample for scheduled observation.

All samples are selected in the Observation condition setting window by default.

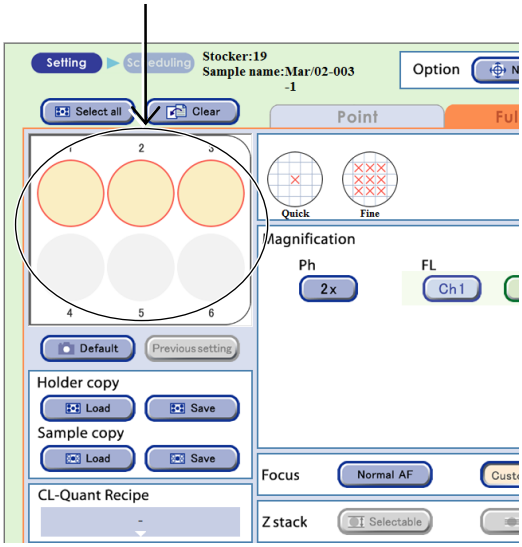
<u>When a vessel other than a 96-, 48-well plate is used</u>

When the Full Scan tab is selected, all samples appear as selected (red frame).

If there is a sample for which observation conditions do not need to be set, press the selected sample once more to deselect it.

To select or unselect all samples in the holder, press the Select all button.

To use the sample and the same observation setting used in the previous observation, press the Previous setting button.



<u>When using a 96-, 48-well plate</u>

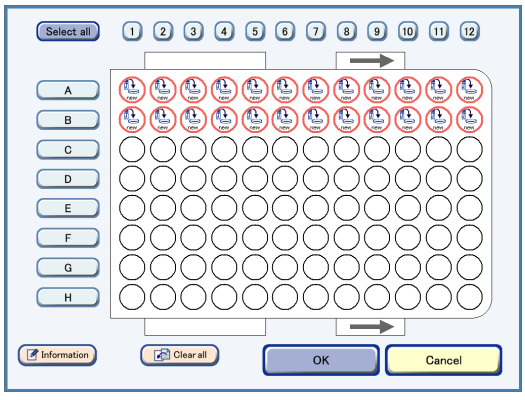
Press the Sample selection area to display the 96-well or 48-well plate sample selection window.

Press the target sample to select it.
The selected sample is marked with a red frame.

Pressing the Select all button selects or unselects all samples.

Pressing one of the vertical alphabet buttons selects all samples in that row. Pressing one of the horizontal number buttons selects all samples in that column. When the selected sample is pressed again, the selection is canceled.

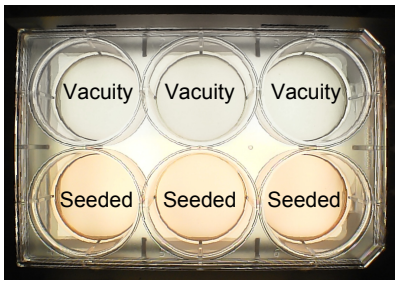
Pressing the OK button reflects the selection and reopens the Observation condition setting window.



Well plate sample selection window (96-well)

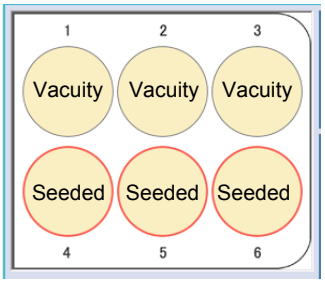
If a well plate is used and the selected samples contain an empty well on which cells are not seeded, autofocus on other wells may not work correctly.

Because images may become out of focus, exclude empty wells before selecting samples and setting the observation conditions.



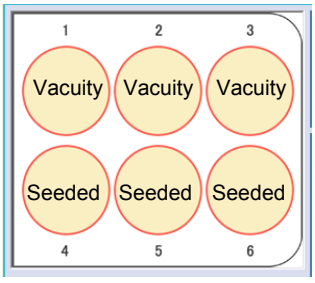
Well plate

Good



Selecting only wells on which cells are seeded

Not good

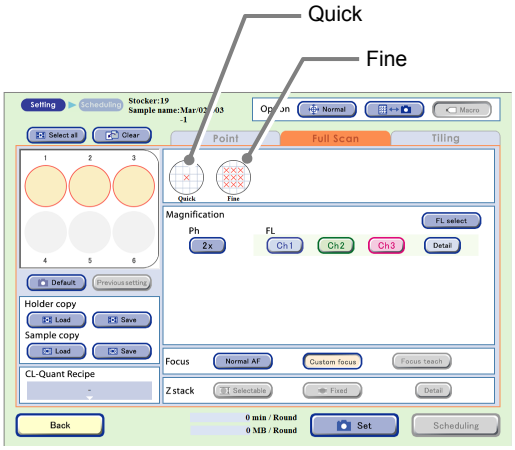


Selecting wells including empty wells

(4) Select the autofocus position.

Select Quick to perform AF at the center of the sample.

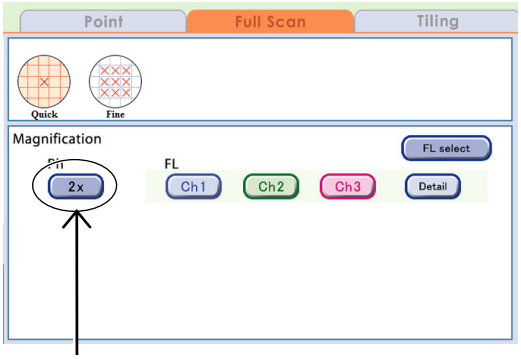
Select Fine to perform AF at more than one position. (This is not available when a 96-well plate or 48-well plate is used.)
Because the AF is performed at more than one position, a high-precision image can be acquired. (However, it takes time to observe one round.)



Observation condition setting window

(5) Select magnifications to be used for scheduled observation.

The observation magnification displayed in the Magnification area depends on the combination of the type of culture vessel and Quick or Fine in the AF position setting area.



The magnifications, the number and exposure time of images captured by Full Scan observation for each culture vessel are as follows: (* Reference value)

Culture vessel	35 mm dish			60 mm dish			
AF position mode	Quick		Fine	Quick	Fine		
Observation magnification	2x	4x	10x	2x	2x	4x	10x
Number of AF position	1	1	9	1	4	4	9
Number of images captured	10x10	20x20	10x10	14x14	7x7	14x14	15x15
Exposure (min) - Ph only	15	30	55	10	15	25	40
Data size - Ph only	396	1567	3521	309	309	1227	3166
Exposure (min) - Ph + FL 1ch (*)	55	75	150	45	45	65	120
Data size - Ph + FL 1ch (*)	786	3130	7036	615	615	2452	6330

Culture vessel	100 mm dish				6-well			
AF position mode	Quick	Fine			Quick	Fine		
Observation magnification	2x	2x	4x	10x	2x	2x	4x	10x
Number of AF position	1	16	16	25	1	4	4	25
Number of images captured	20x20	5x5	10x10	15x15	10x10	5x5	10x10	7x7
Exposure (min) - Ph only	10	20	30	55	15	30	40	115
Data size - Ph only	314	314	1251	4396	475	475	1881	5748
Exposure (min) - Ph + FL 1ch (*)	45	50	65	165	65	80	100	275
Data size - Ph + FL 1ch (*)	626	626	2501	8790	943	943	3756	11490

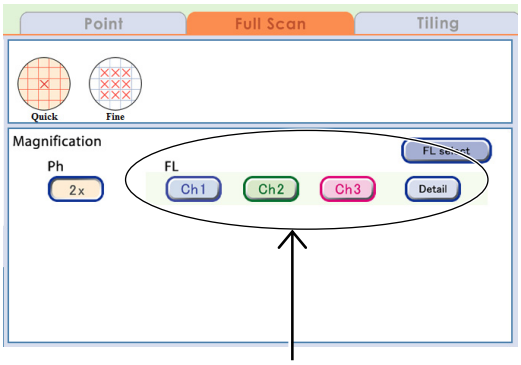
Culture vessel	12-well			24-well		
AF position mode	Quick		Fine	Quick		Fine
Observation magnification	2x	4x	10x	2x	4x	10x
Number of AF position	1	1	4	1	1	1
Number of images captured	6x6	12x12	11x11	4x4	12x12	15x15
Exposure (min) - Ph only	15	30	65	25	30	50
Data size - Ph only	349	1361	4549	322	1222	4240
Exposure (min) - Ph + FL 1ch (*)	55	70	185	55	70	160
Data size - Ph + FL 1ch (*)	686	2711	9086	622	2422	8459

Culture vessel	48-well		96-well	
AF position mode	Quick	Fine	Quick	Fine
Observation magnification	4x	10x	4x	10x
Number of AF position	1	1	1	1
Number of images captured	6x6	10x10	4x4	8x8
Exposure (min) - Ph only	45	55	60	80
Data size - Ph only	1393	3793	1285	4885
Exposure (min) - Ph + FL 1ch (*)	85	155	105	210
Data size - Ph + FL 1ch (*)	2743	7543	2485	9685

Culture vessel	25cm^2 culture flask				75cm^2 culture flask
AF position mode	Quick	Fine			
Observation magnification	2x	2x	4x	10x	
Number of AF position	1	4	4	25	
Number of images captured	14x14	7x7	14x14	12x12	Not applicable
Exposure (min) - Ph only	10	10	15	40	
Data size - Ph only	155	155	614	2814	
Exposure (min) - Ph + FL 1ch (*)	25	25	35	115	
Data size - Ph + FL 1ch (*)	308	308	1226	5626	

The reference values in this table are by culture vessel unit. (by one dish or one well-plate)

(6) To capture the fluorescence image, select the FL channel button.



To change the exposure conditions for capturing fluorescence images, press the Detail button. For details, see Section 4.6.2.4, “Setting exposure conditions for fluorescence images.”

When up to 5 types of fluorescence filter cube are mounted, channels (Ch1 to Ch3) to be used can be changed to the added fluorescence filter channels (Ch4, Ch5). For details, see Section 4.6.2.5, “Changing the fluorescence channel (optional).”

When the optional Fluorescence unit is not connected, the FL channel button is disabled.

(7) Select the focus type.

Focus type can be selected for sample with FullScan Z registered in the Ph live observation window.

The following describes each case.

a. Sample name without registered FullScan Z button

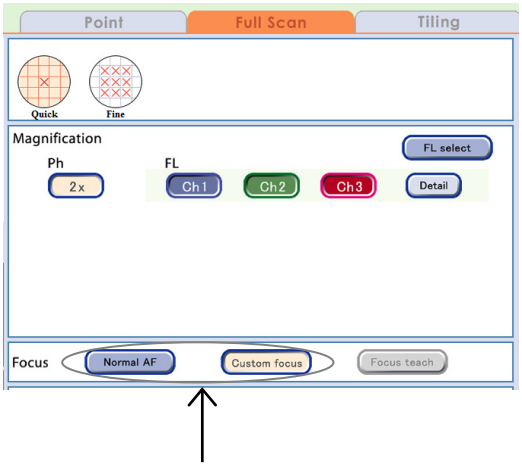
The Focus type cannot be selected.
Normal AF is selected and autofocus is performed at the center of sample.

b. Sample name with registered FullScan Z button

Either Custom focus or Normal AF can be selected.

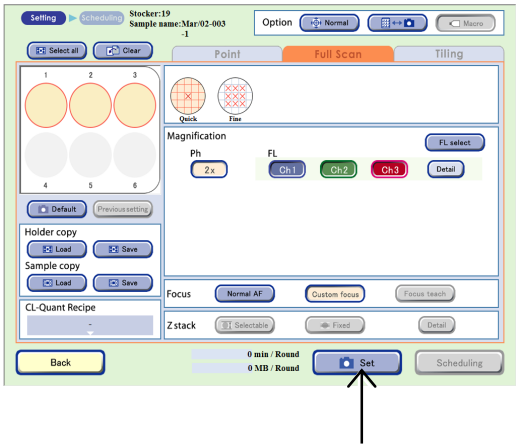
Custom focus performs autofocus near the custom observation point of the registered FullScan Z.

Select Normal AF to perform autofocus at the center of the sample.



(8) To specify the selected observation setting, press the Set button.

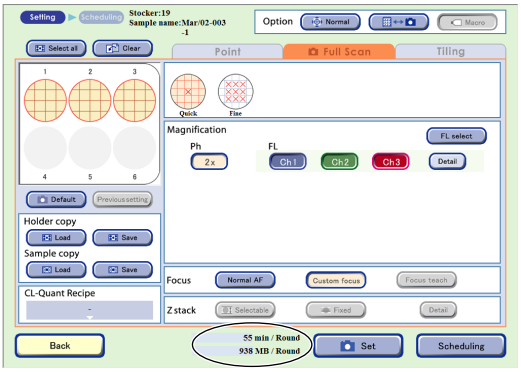
The observation setting is set up for the selected sample.



Samples set for scheduled observations are displayed as shown in the figure on the right.

The time and data size required for one round of scheduled observation is displayed.

When the observation setting is set, the icon appears on the Observation method selection tab.



The procedure for setting the full scan observation conditions is now complete.

To set schedules, see Section 4.6.3, "Setting Schedules."

A low magnification image of entire sample and high magnification images of any point can be captured at the same time with one Scheduled observation setting by combining Full Scan which is low magnification observation and Point observation or Tiling observation that enables high magnification.

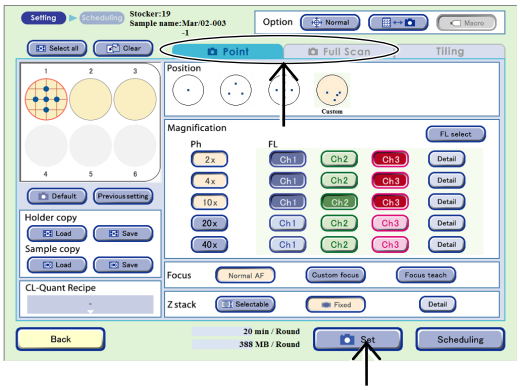
* To specify a desired observation area as a capture area by Tiling observation, a Full Scan image must be captured by Full Scan and the captured area must be registered in the Full Scan image display window in advance.

Perform the setting in accordance with the following procedure.

1. Set the Full Scan observation scheduled observation condition and press the Set button.
2. Switch to Point observation or Tiling observation and set the scheduled observation conditions. (See Section 4.6.2.1 for details on settings on the Point tab and Section 4.6.2.3 for settings on the Tiling tab.)
3. Press the Set button.

   The observation setting is set to the selected sample and the total time and data size required for a round of both scheduled observations is displayed in the time required for observation area.

   The icon appears on the Observation method selection tab for which the observation setting is set.



4.6.2.3 Tiling observation

This section describes the setting procedure when Tiling observation is selected as the observation method.

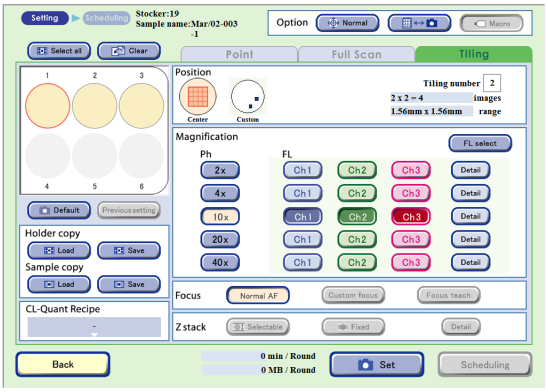
The procedure for Tiling observation depends on the method used to specify the position to be observed.

a. Tiling capture by autofocusing at the center of the entire sample (Center)

Go to step (5).

b. Tiling capture by autofocusing at the center of each specified capture area (Custom)

To perform Tiling observation at a desired observation position (Custom), a Full Scan image must be captured by Full Scan and the captured area must be registered as Tiling observation area in the Full Scan image display window in advance.
The Full Scan image must be captured by Full Scan in advance.
Set after completing image capturing in accordance with the procedures from sections 4.6.2.2, “Full Scan observation” to 4.6.3, “Setting Schedules.”



Observation condition setting window (Tiling tab selected)

(1) Display the Full Scan image display window.

1. The Image review window is displayed.

For the procedure for displaying the Image review window, see Section 4.6.1, “Selecting a Sample.”

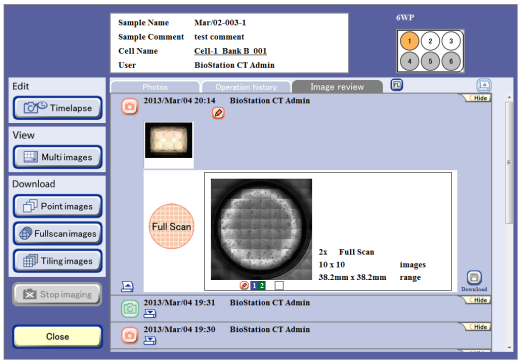
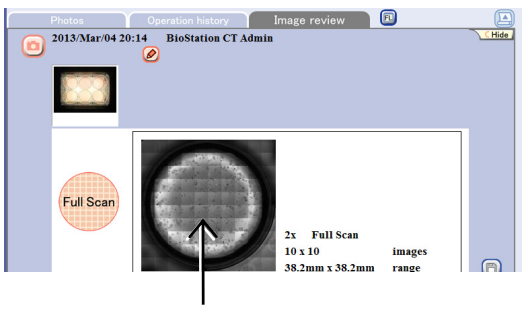


Image review window

2. Press the thumbnail image of the Full Scan image.

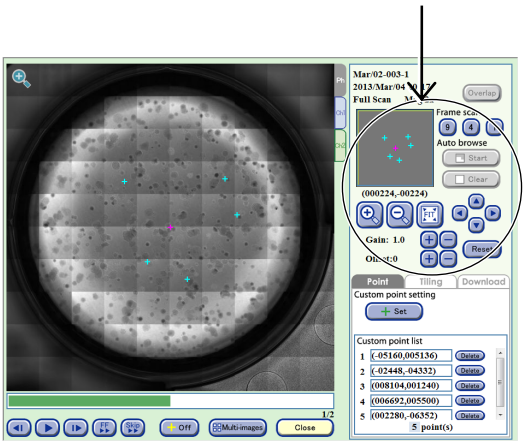
The Full Scan image display window appears.



(2) On the Point tab of the Full Scan image display window, register the center of the area on which tiled observation is to be performed as the custom observation point.

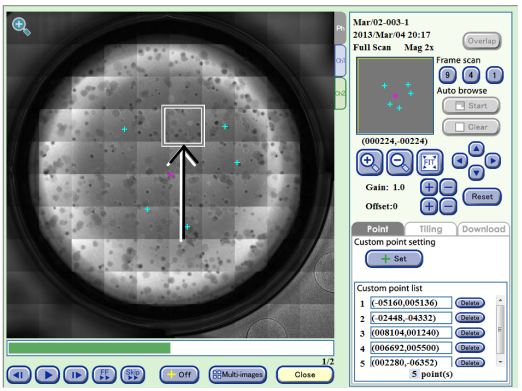
1. In the image display Area select area the center of the area on which tiled observation is to be performed.

Enlarge, reduce, or shift the field of view to move the position to be registered as a custom observation point to the center of view.



Full Scan image display window (Point tab)

Alternatively, press a point in the tiled image displayed in the image display area to enlarge the selected point.

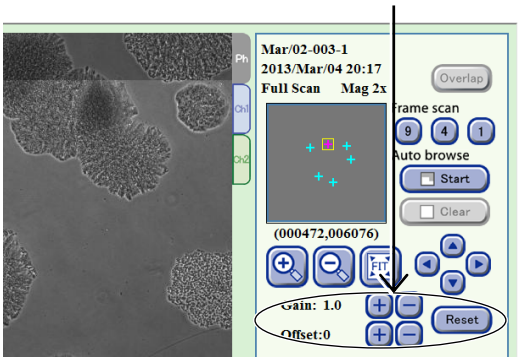


For more information on the operation of the Full Scan image display window, see Section 4.7.3.4, “Viewing the Full Scan image and preparing download.”

Use Gain and Offset to adjust the brightness of a displayed image when searching for an area to observe.

Use the Gain +/- button to adjust the contrast.

Use the Offset +/- button to adjust the brightness.

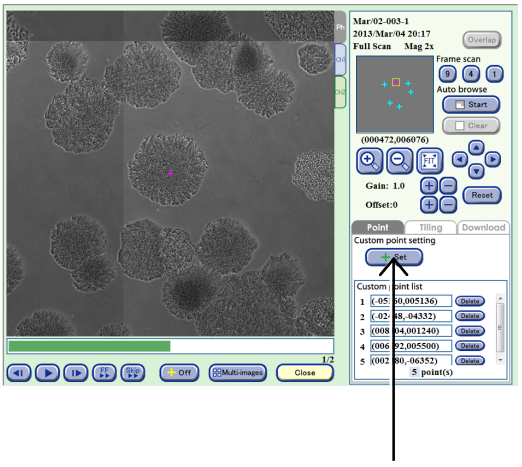


2. Press the Set button.

The XY coordinates of the center of view displayed in the image display area are registered as a custom observation point.

The registered custom observation point is added to the custom point list field and indicated with a light blue pointer (crosshair) in the image display area and observation point display area.

The pointer in the image display area can be shown/hidden using the +Off button.

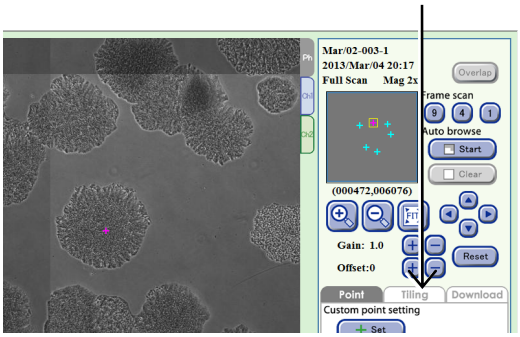


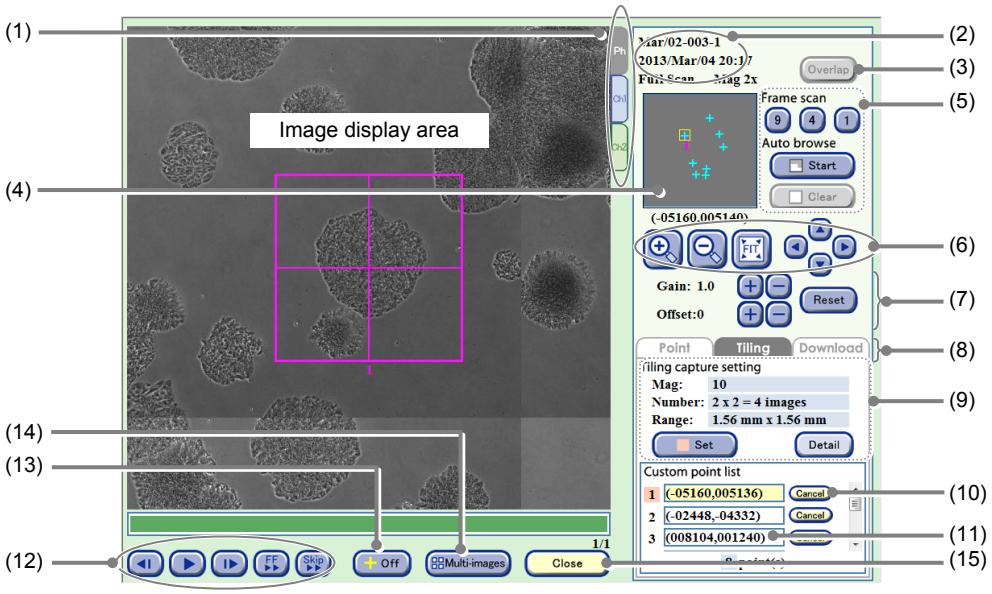
3. To perform tiled observation of more than one area, register the center of each area as a custom observation point.

(3) Register the area on which Tiling observation is to be performed.

1. Press the Tiling tab.

The Tiling tab window appears.





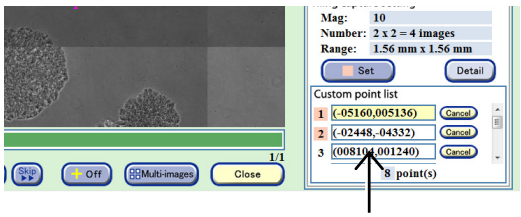
Full Scan image display window (Tiling tab)

No.	Name	Function
(1)	Channel tabs	These tabs are displayed when fluorescence images are captured. Select a tab to switch the image displayed among Ph (a phase contrast image) and Ch1 to 3 (FL channel images).
(2)	Sample information	This area displays the observation point, sample name, and captured date and time.
(3)	Overlap button	Press this button to overlay the fluorescence image and the phase contrast image.
(4)	Observation point display area	This area displays all custom observation points registered in the entire sample area or the position and size of observation field of view displayed in the image display area.
(5)	Scan playback function	Scanning of the entire field of view is played back automatically. **Frame scan:** Play back scan of entire field of view with the selected frame size. **Auto browse:** Play back scan with the displayed magnification from the currently displayed point. Also enables pause, resume, or clear playback of scan.
(6)	Display size change buttons	**+, - buttons:** Enlarge or reduce the image. **FIT button:** Display the image on the full screen. **Field of view 1 frame move button:** Shift the field of view up/down, left/right by one frame.

No.	Name	Function
(7)	Brightness adjustment of a displayed image	Adjust the brightness of a displayed image. **Gain:** Press the +/- button to adjust the contrast by ±0.1. **Offset:** Adjust the brightness. Press the +/- button to adjust the brightness by ±5 for a phase contrast image or by ±10 for a fluorescence image. **Reset button:** Reset the brightness of a displayed image.
(8)	Operation window switch tabs	Press a tab to switch the operation window. **Point tab:** Enable registration of custom observation point. **Tiling tab:** Enable registration of capture area for Tiling observation. (See Section 4.6.2.3, “Tiling observation.”) **Download tab:** Enable download preparation for an Full Scan image. (See Section 4.7.3.4, “Viewing the Full Scan image and preparing download.”)
(9)	Tiling capture area setting area	This area displays the set tiling capture magnification, count, and capture range. **Detail button:** Display the Tiling observation condition change window and allows changing the tiled capture magnification and count. **Set button:** Register the position displayed in the image display area as tiled captured area.
(10)	Cancel button	Press this button to cancel the tiled capture area registered with the Set button.
(11)	Custom point list field	This field displays the registered custom observation point. Select a Custom observation point from the list moves the field of view to that position.
(12)	Timelapse images playback button	**► button:** Play back timelapse images continuously. During playback, this button changes to the pause button and pressing the button pauses playback. **I►/◄I button:** Play one frame forward /one frame backward **FF button:** Fast-forward an image. **Skip button:** Skip the image for the predefined frame during playback.
(13)	+Off button	Press this button to display or hide pointers displayed in the image display area.
(14)	Multi-images button	Press this button to display the Multi images display window. Full Scan observation thumbnail images are registered in the Multi images display window. (See Section 4.9, “Multi Images Display of Captured Images.”)
(15)	Close button	Press this button to close the Full Scan image display window.

2. From the Custom observation point list, select a custom observation point within the area on which tiling capture is to be performed.

The specified custom observation point appears in the image display area.



Full Scan image display window (Tiling tab)

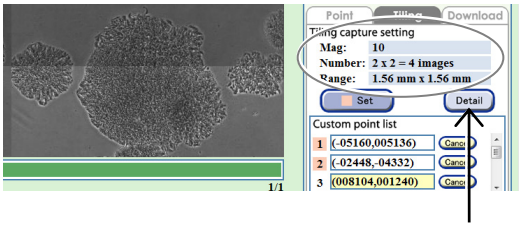
3. Check the tiling observation condition displayed in the image display area.

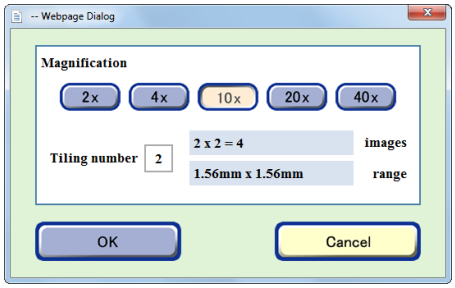
Press the Detail button as necessary to change the tiling capture magnification and tiling count setting.
Press the Detail button to display the Tiling observation condition change window.

Select the Magnification button to change the maginification. (Multiple selections are not allowed.)

To change the tiling count, select a tiling area from the number selection box showing a number from 1 to 20 which is displayed by pressing the Tiling number field. (Selecting 5 captures 25 images in a 5×5 grid.)

Press the OK button to confirm the change.



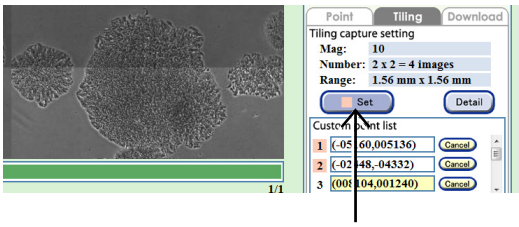


Tiling observation condition change window

> One tiling observation condition can be set for each sample.

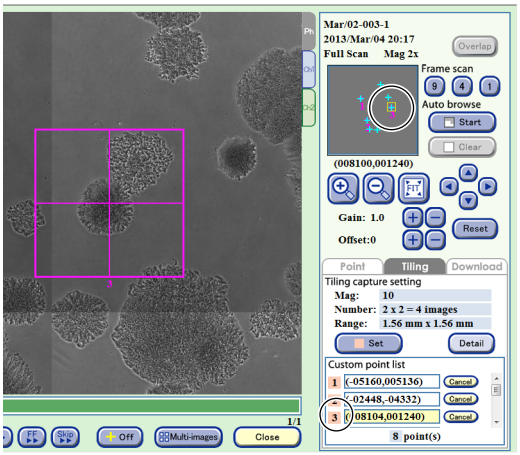
4. Press the Set button.

A Tiling observation area is registered.

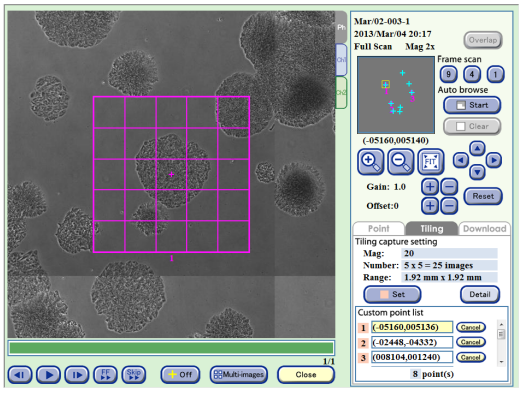


When registered, the number of the corresponding point in the Custom point list field turns pink.

The registered tiling observation area pointer is displayed in the observation point display area in pink and the number of the custom observation point in the Custom point list field is displayed near the pointer.



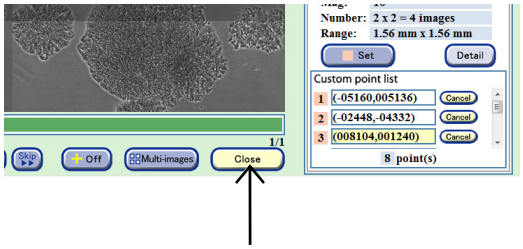
In the window on the right, the tiling capture magnification is 20x, capture count is 25 in 5×5 grid, and the tiling capture area is 1.92 mm × 1.92 mm.



Capture area that is not adjacent to each other can also be captured as tiling image. In that case, repeat steps 2 to 4 for each Tiling observation area to register.

5. **Press the Close button.**

   The Full scan image display window closes and the Image review window appears.



(4) Set the observation conditions for schedule observation.

1. **With the sample for Tiling observation selected, press the Timelapse button on the left side of the Image review window.**

   The Schedule confirmation window appears.

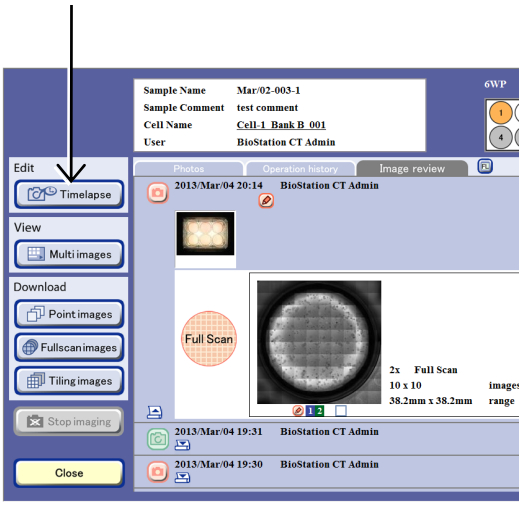
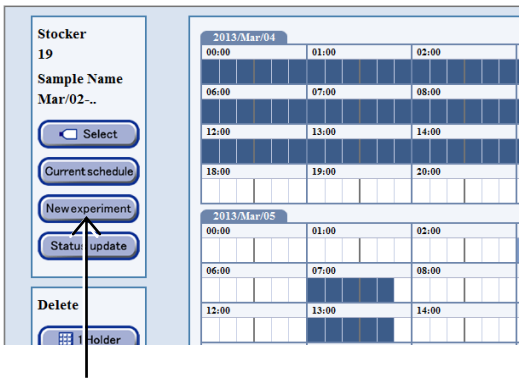


Image review window

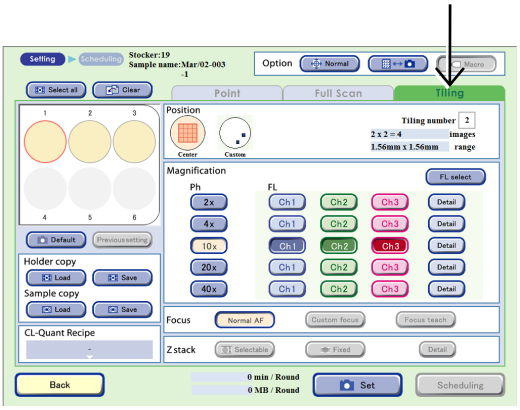
2. **Press the New experiment button.**

   The Observation condition setting window appears.

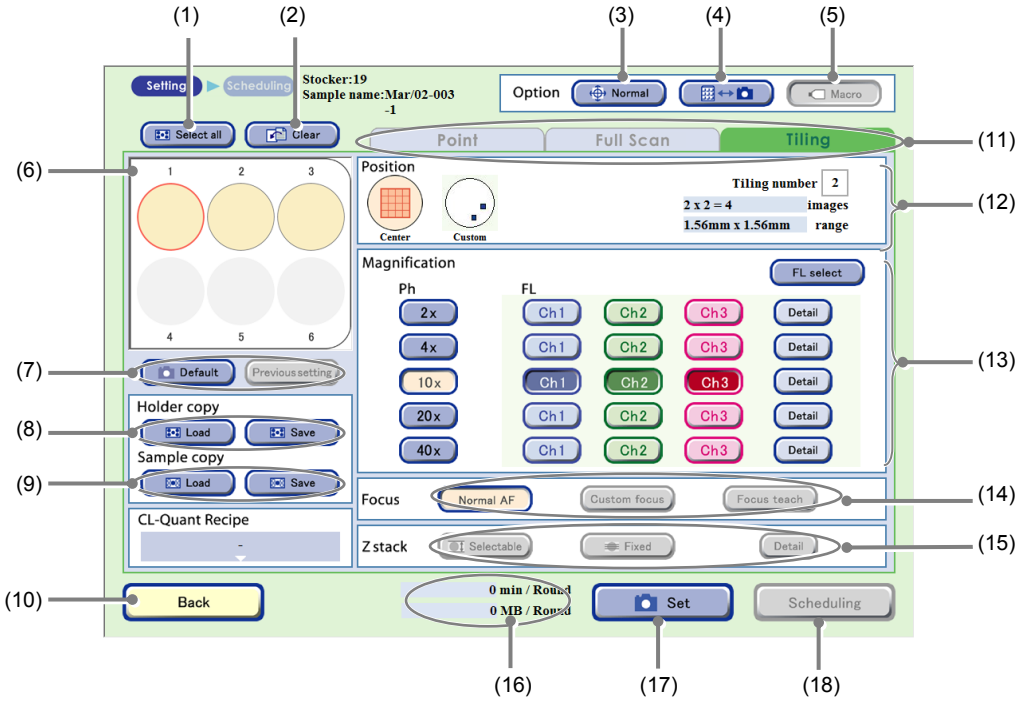


Schedule confirmation window

3. **Select Tiling observation on the Observation method selection tab.**



Observation condition setting window



Observation condition setting window (Tiling tab selected)

No.	Name	Function
(1)	Select all button	Press this button to select all samples of the holder. The selected sample is marked with a red frame.
(2)	Clear button	Press this button to clear the observation condition settings.
(3)	Stage speed selection button	Select the stage speed. **Normal:** Set the stage speed to normal. **Slow:** Set the stage speed to slow.
(4)	Scheduled observation mode selection	Select the scheduled observation mode. **button: (Normal mode)** The holder is returned to the stocker when each round in scheduled observation is finished. **Keep button: (Stage exclusive mode)** The holder is kept on the stage from the first round to the last round for scheduled observation.
(5)	Macro button	Select whether to capture a macro image during scheduled observation. (Enabled only during stage exclusive mode) **ON (Concave):** Enable macro capture. **OFF (Convex):** Disable macro capture.
(6)	Sample selection area	Select the sample for scheduled observation. (All samples are selected in the Observation condition setting window by default.) The selected sample is marked with a red frame. To cancel the selection, press the selected sample again.

No.	Name	Function
(7)	Settings load buttons	The scheduled observation conditions are loaded. **Default button:** Load the Scheduled observation default setting. (See Chapter 5, “Environmental Settings.”) **Previous setting button:** Load the previous observation settings.
(8)	Holder copy area	Capture conditions of the holder are loaded and saved. **Load button:** Load the saved capturing conditions for the holder into the vessel being displayed. **Save button:** Save the capturing conditions for the vessel being displayed on a holder basis.
(9)	Sample copy area	Capture conditions of each sample are loaded and saved. **Load button:** Load the saved capturing conditions into the selected sample. **Save button:** Save capture conditions of the selected sample.
(10)	Back button	Press this button to return to the previous window without saving the settings.
(11)	Observation method selection tabs	Select the observation method. There are three observation methods: Point observation, Full Scan observation, and Tiling observation. Select the Tiling tab here.
(12)	Observation position setting area	Select the default observation position or a custom observation point. Select the default observation position (Center) or the custom observation point (Custom). **Center:** Autofocus is performed at the center of the entire sample and tiling capture is performed. **Custom:** Autofocus and tiling capture is performed at the center of each specified area. **Tiling number:** Specify/display the tiling area.
(13)	Magnification/FL channel setting area	Select the observation magnification and FL channel button. **Magnification button:** Select an observation magnification to be used for scheduled observation. (Multiple observation magnifications cannot be selected for Tiling observation.) **FL channel button:** Press the buttons for the magnifications and channels of the fluorescence images to be captured. Multiple channels of the same magnification can be selected. **Detail button:** The FL image exposure conditions setting window appears. Set the exposure time and the intensity of each excitation light source. (See Section 4.6.2.4, “Setting exposure conditions for fluorescence images.”) **FL select button:** The FL channel selection window appears. When up to 5 types of fluorescence filter cube are mounted, channels (Ch1 to Ch3) to be used when setting scheduled observation conditions can be changed to the added fluorescence filter channels (Ch4, Ch5). (See Section 4.6.2.5, “Changing the fluorescence channel (optional).”)

No.	Name	Function
(14)	Focus Type area	Select the focus type. **Normal AF:** Autofocus is performed at the center of the sample. **Custom focus:** (When Tiling is selected, this button is disabled.) **Focus teach button:** (When Tiling is selected, this button is disabled.)
(15)	Z stack area	When Tiling is selected, this button is disabled.
(16)	Time required for observation/data size	The time and data size required for one round of scheduled observation is displayed when the Set button is pressed.
(17)	Set button	Press this button to set the Observation setting for the selected sample.
(18)	Scheduling button	Press this button to display the Scheduling window.

(5) Set the mode for scheduled observation.

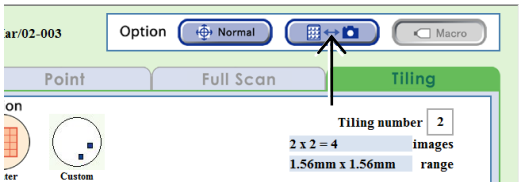
There are two modes available for scheduled observations: The Normal mode to return the holder to the stocker each time 1 Round is finished, and the Stage exclusive mode that keeps the holder on the stage from the first Round to the last Round.

> The procedure for setting scheduled observation varies depending on whether Normal mode or Stage exclusive mode is used. For details, see Section 4.6.3, “Setting Schedules.”

To use the Normal mode for scheduled observation

a. Switch the Scheduled observation mode selection to (Normal mode).

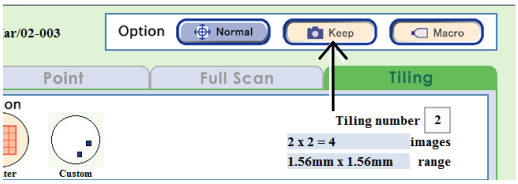
The figure on the right shows the mode button set to the Normal mode.



To use the Stage exclusive mode for scheduled observation

a. Switch the Scheduled observation mode selection to Keep (Stage exclusive mode).

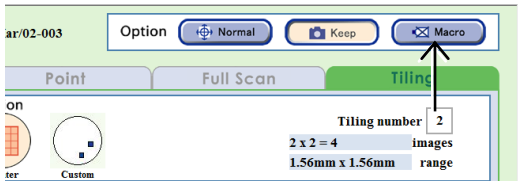
The figure on the right shows the mode button set to the Stage exclusive mode.



b. Switch the Macro button to either enable or disable the macro capture.

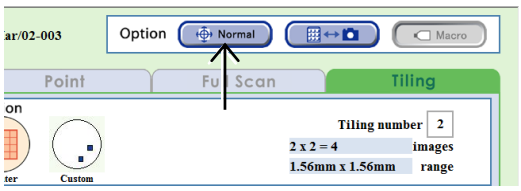
The figure on the right shows the Macro button set to OFF (to disable the macro capture).

The Macro button is only effective for the Stage exclusive mode. Be sure to perform the macro capture when using the Normal mode for scheduled observation.



(6) If necessary, use the Stage speed selection button to switch the stage speed when shifting the observation position.

Each time the Stage speed selection button is pressed, the mode is switched between Normal (normal speed) and Slow (slow speed).



(7) Select the sample for scheduled observation.

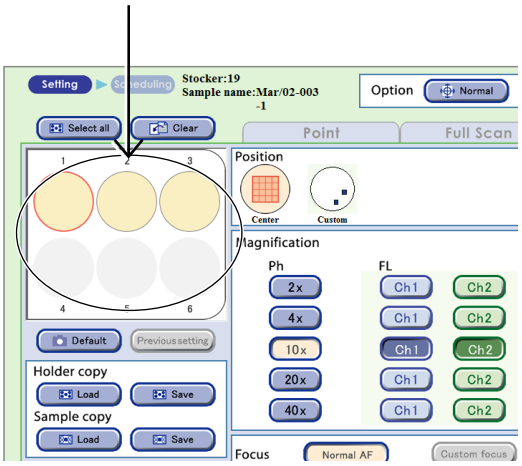
All samples are selected in the Observation condition setting window by default.

When a vessel other than 96-, 48-well plate is used

Press the Sample button in the Sample selection area to select the sample for scheduled observation.
The selected sample is marked with a red frame.
To cancel the sample, press the Sample button again.

To select or unselect all samples in the holder, press the Select all button.

To use the sample and the observation setting that was used in the previous observation, press the Previous setting button.



Observation condition setting window

When using a 96-, 48-well plate

Press the Sample selection area to display the 96-well or 48-well plate sample selection window.

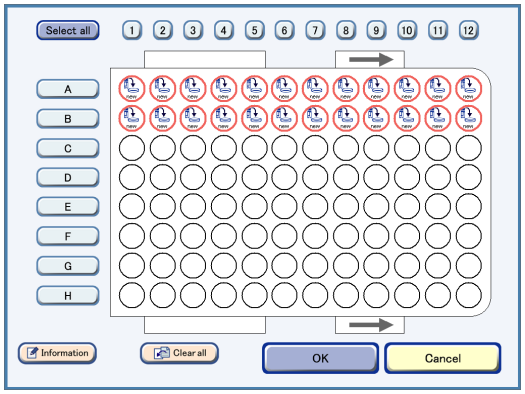
Press the target sample to select it.
The selected sample is marked with a red frame.

Pressing the Select all button selects or unselects all samples.

Pressing one of the vertical alphabet buttons selects all samples in that row. Pressing one of the horizontal number buttons selects all samples in that column.

When the selected sample is pressed again, the selection is canceled.

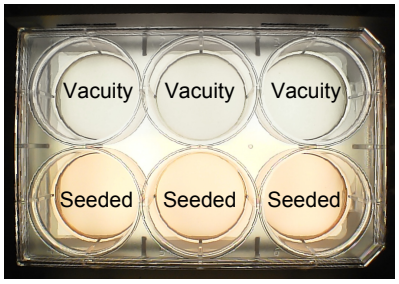
Press the OK button to apply the selection and reopen the Observation condition setting window.



Well plate sample selection window (96-well)

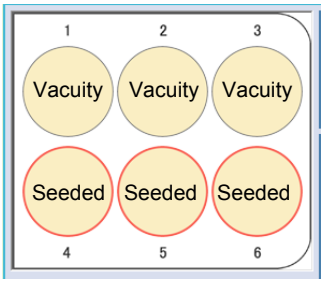
If a well plate is used and the selected samples contain an empty well on which cells are not seeded, autofocus on other wells may not work correctly.

Because images may become out of focus, exclude empty wells before selecting samples and setting the observation conditions.



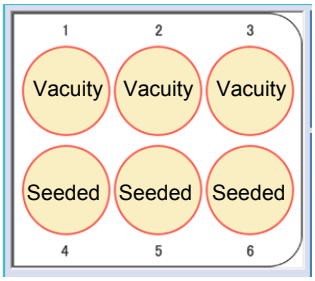
Well plate

Good



Selecting only wells on which cells are seeded

Not good



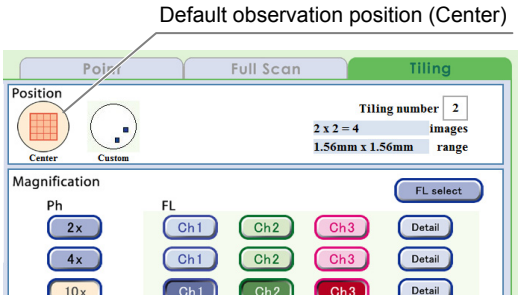
Selecting wells including empty wells

(8) Select the observation position.

Select the default observation position (Center) or the custom observation point (Custom).

a. When Center is selected

Autofocus is performed at the center of the entire sample and tiling capture is performed.



Observation condition setting window

a-1. Select a magnification to be used for scheduled observation.

Multiple magnifications cannot be selected for Tiling observation.

The figure on the right shows an example with 10x selected.

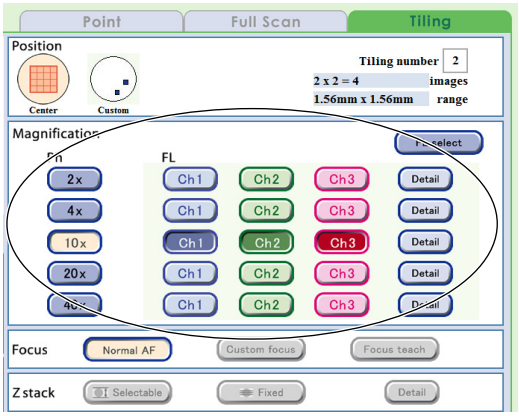
To use the default settings of the magnification and the observation position, press the Default button.

a-2. To capture the fluorescence image, press the FL channel button for the selected observation magnification.

To change the exposure conditions for capturing fluorescence images, press the Detail button. For details, see Section 4.6.2.4, “Setting exposure conditions for fluorescence images.”

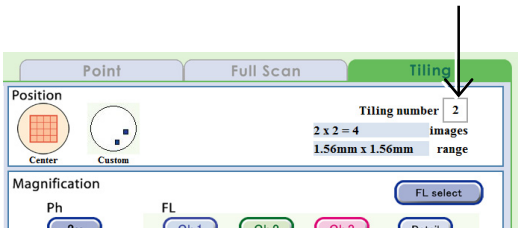
When up to 5 types of fluorescence filter cube are mounted, channels (Ch1 to Ch3) to be used can be changed to the added fluorescence filter channels (Ch4, Ch5). For details, see Section 4.6.2.5, “Changing the fluorescence channel (optional).”

When the optional Fluorescence unit is not connected, the Channel button is disabled.



a-3. When using Center, set the tiling area in the Tiling number field.

Select a tiling area from the number selection box showing a number from 1 to 20 which is displayed by pressing the Tiling number field. (Selecting 5 captures 25 images in a 5×5 grid.)

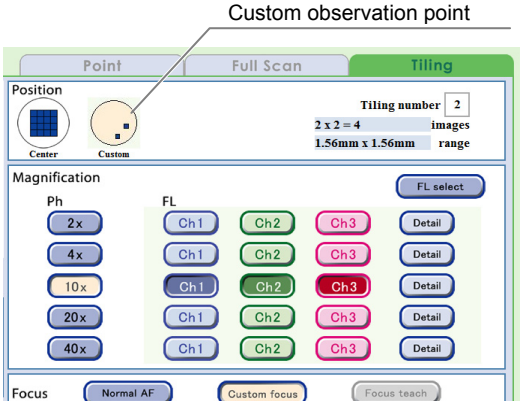


b. When Custom is selected

Autofocus is performed on each tiling observation area registered in the Full Scan image display window and tiling capture is performed.

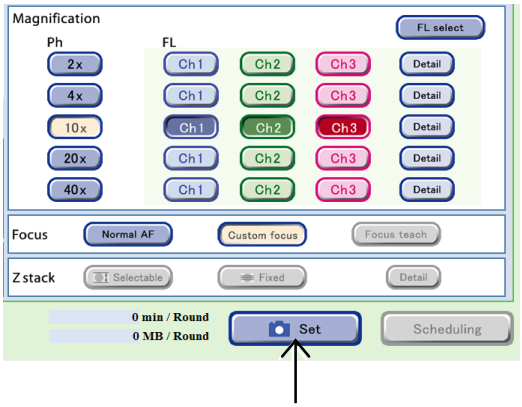
When using Custom, the area to be captured must be registered as a Tiling observation area in advance. (See steps (1) to (3).)

When Custom is selected, the magnification and FL channel cannot be changed in the Observation condition setting window. The magnification when the tiling observation area is set in the Full Scan image display window is used.



(9) To specify the selected observation setting, press the Set button.

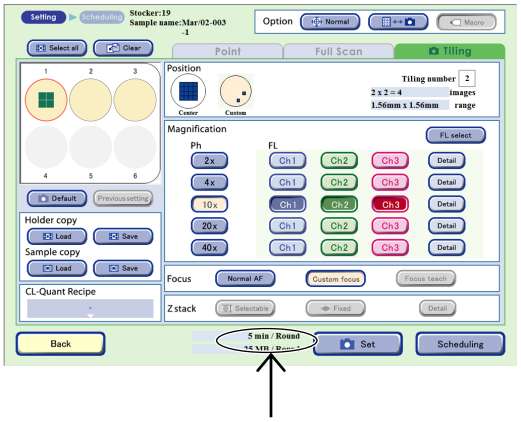
The observation setting is set up for the selected sample.



Samples set for scheduled observations are displayed as shown in the figure on the right.

The time and data size required for one round of scheduled observation is displayed.

When the observation setting is set, the icon appears on the Observation method selection tab.



The procedure for setting the observation conditions is now complete.

To set schedules next, see Section 4.6.3, "Setting Schedules."

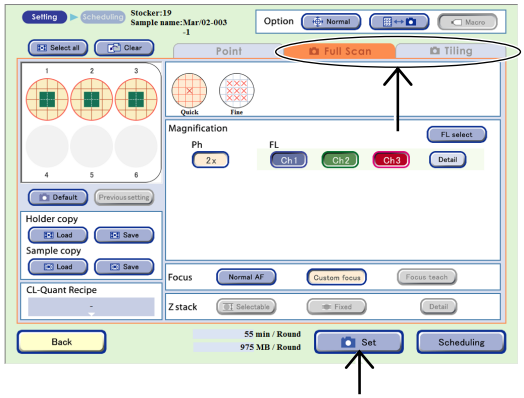
A high magnification image in the specified area and a low magnification image in the entire area can be captured at the same time with one scheduled observation setting by combining Tiling observation that enables high magnification observation and Full Scan observation which performs low magnification observation.

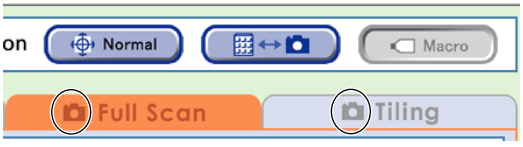
Perform the setting in accordance with the following procedure.

1. Set the scheduled observation condition for Tiling observation and press the Set button.
2. Select the Full Scan observation method selection tab and set the Full Scan observation scheduled observation condition. (See Section 4.6.2.2.)
3. Press the Set button.

   The observation setting is set to the selected sample and the total time and data size required for a round of both scheduled observations is displayed in the time required for observation area.

   The icon appears on the Observation method selection tab for which the observation setting is set.



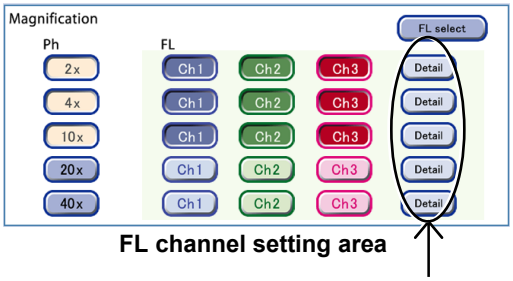


4.6.2.4 Setting exposure conditions for fluorescence images

This section describes how to set the exposure time and brightness of each excitation light source when a fluorescence image is captured.

(1) In the FL channel setting area in the Observation condition setting window, press the Detail button.

Press the Detail button in the FL channel settings area. The FL image exposure conditions setting window appears.



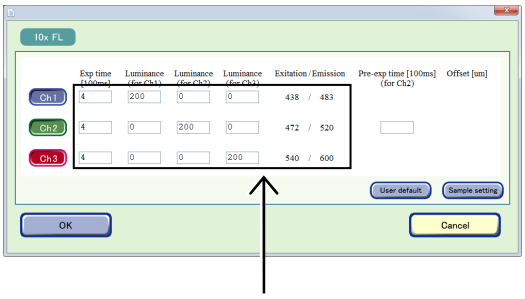
FL channel setting area

(2) Set the exposure time and the intensity of each excitation light source.

Press the entry field to set the exposure conditions (exposure time and intensity of each excitation light source) for each channel.

The unit of the exposure time is [100ms], but up to the 2nd decimal digit (i.e. unit: 1 ms) can be entered.
The maximum value of the intensity of excitation light source is 240.

When multiple samples are selected, no value is displayed for each entry field.
The registered exposure conditions for a sample are used as the default exposure conditions.

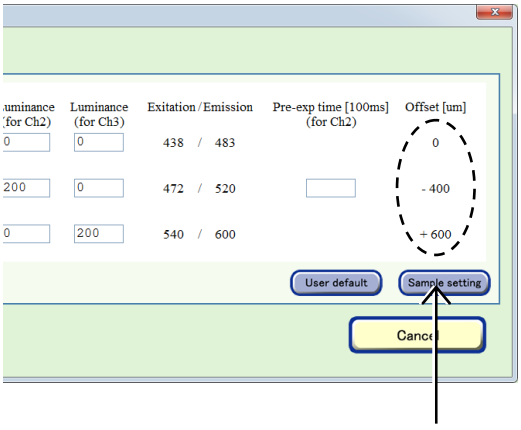


FL image exposure conditions setting window

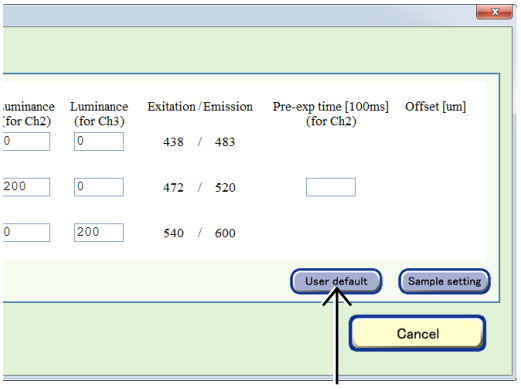
When the Sample setting button is pressed, the registered exposure conditions are applied. Exposure conditions are registered in live observation. For details on registration, see Section 4.5.2.2, “Displaying a FL live image and registering a custom observation point.”

When the exposure conditions for one of the selected multiple samples have been registered, the values are applied by pressing the Sample setting button.

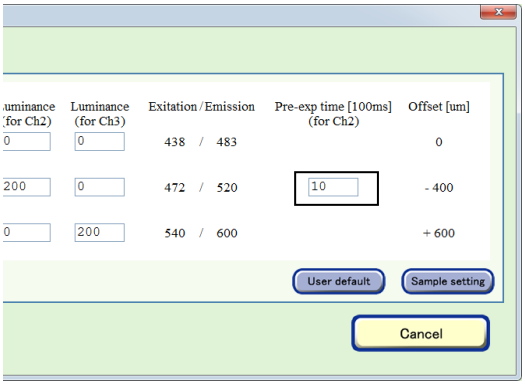
In addition, when Z position correction from a phase contrast image for each channel is registered on the FL-Z/Offset tab in the FL live observation window, the registered values are loaded to the Offset [um] area of each channel.



When the User default button is pressed, the default values specified with the environmental settings are applied. For details on setting the default values of the exposure conditions for each user, see Chapter 5, “Environmental Settings.”



(3) If a sample has a strong autofluorescence signal at Ch2 (GFP), set a fluorescence pre-exposure.



Some samples including cells and medium have an autofluorescence which becomes apparent especially when they are observed near the GFP fluorescence wavelength. The autofluorescence is likely to be subject to photobleaching faster than the fluorescent molecules to be observed. Fluorescence illumination immediately before image acquisition can minimize the influence of the autofluorescence on the captured image.

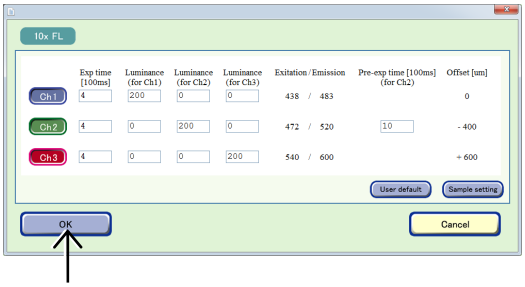
Press the Pre-exposure field to enter the exposure time in the Keyboard window.

The unit of the exposure time is [100ms], but up to the 2nd decimal digit (i.e. unit: 1 ms) can be entered.

> Set the exposure time of fluorescence pre-exposure longer than the actual exposure time of capturing.
>
> Note that fluorescence is subject to photobleaching and becomes less visible by setting a too long fluorescence pre-exposure time.

(4) Press the OK button.

The Observation condition setting window appears again.



> When a channel is selected for fluorescence observation, the observation magnification button is automatically selected. When fluorescence observation is selected, phase contrast images are always captured as a set. When the selected observation magnification button is cancelled, any selected channel for fluorescence observation is also cancelled.

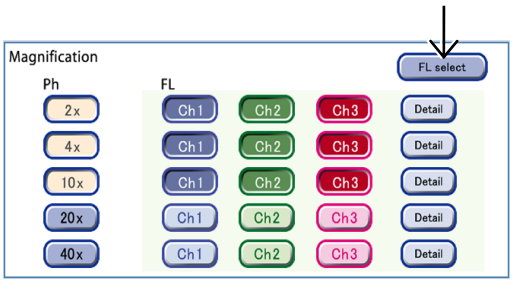
4.6.2.5 Changing the fluorescence channel (optional)

When up to 5 types of fluorescence filter cube are mounted, channels (Ch1 to Ch3) to be used when setting scheduled observation conditions can be changed to the added fluorescence filter channels (Ch4, Ch5).

Follow the procedure below to change the fluorescence channel to be used.

(1) Press the FL select button in the FL channel setting area of the Observation condition setting window.

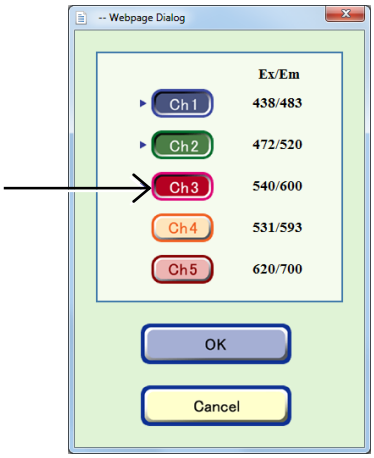
The FL channel selection window appears.



FL channel setting area

(2) Select the button for the fluorescence channel not to be used.

The channel is deselected and the surface becomes convexed.



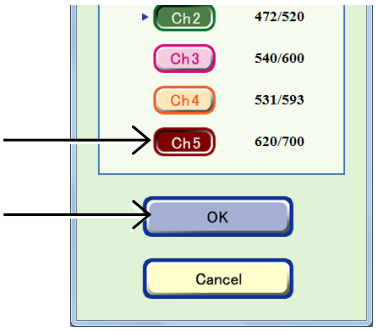
FL channel selection window

(3) Select the desired fluorescence channel button.

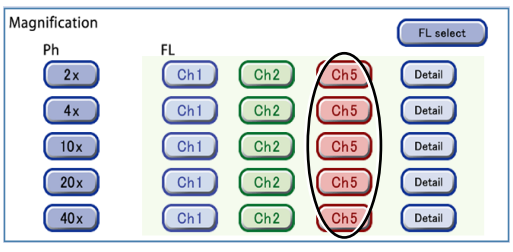
The channel is selected and the surface becomes concaved.

(4) Press the OK button.

The Observation condition setting window appears again.



The selected channel is applied to the FL channel setting area.



FL channel setting area

4.6.3 Setting Schedules

4.6.3.1 Setting schedules for each sample

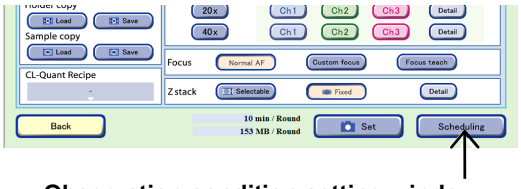
This section describes the procedure for setting a schedule for each sample after setting scheduled observation conditions in Section 4.6.2, “Setting Scheduled Observation Conditions.”

(1) Open the Scheduling window.

Press the Scheduling button in the Observation condition setting window.

The Scheduling window appears.

The schedule can be set by operating the Scheduling window as shown below.



Observation condition setting window

Scheduling window
(For the Normal mode without the Timelapse setting)

* The function called “Interval setting” in BioStation CT Ver.3.3 and earlier is now referred to as “Timelapse setting.” The button name has been changed to the Timelapse button.

No.	Name	Function
(1)	Information area	This area displays the stocker number of the stocker that contains the selected sample and sample name.
(2)	Edit area	Enter the experiment name for schedule observation.
(3)	Select holder area	Set schedules of samples in multiple holders with one schedule setting. **Select button:** Display the Holder selection window which allows selection of multiple samples for scheduled observation. **All button:** Enable batch setting for scheduled observation for samples with same type of vessel and same user. (See Section 4.6.3.2.)

No.	Name	Function
(4)	Set timelapse area	Timelapse is set here. **Timelapse button:** Select to set a time-lapse. When the Stage exclusive mode is used, this button is always selected. **Interval area:** Set the observation interval (time from the start of the first Round until the start of the next Round). Press the h or m Interval field, and enter an interval by hours in the h field and by minutes in 5-minute increments in the m field in the Keyboard window. **Rounds tab:** Set the repetition of the observation. Press the entry field to enter the number in the Keyboard window, then press the Set button. **Duration tab:** Set the desired observation period. Press the d, h and m entry fields to enter the duration in the Keyboard window, then press the Set button. **Total time:** Display the total observation period of this scheduled observation experiment in increments of 5 minutes under the conditions for which Timelapse was set. **Data size:** Display the total data size of this scheduled observation experiment under the conditions for which Timelapse was set. **Stay on stage button:** Hold the sample under live observation on the stage without returning it to the stocker, until scheduled observation starts. (Enabled only during live observation) For details, see “b. When the Timelapse setting is assigned in the Normal mode” or “c. When the stage exclusive mode is assigned” in subsequent pages.
(5)	Back button	Press this button to return to the Observation condition setting window.
(6)	Finish button	Press this button to finish the setting for schedules.
(7)	Start now button	Press this button to start scheduled observation immediately.
(8)	Schedule setting field	Periods displayed in white have no schedule. Press a period for a scheduled observation to set the schedule.
(9)	Clear button	Press this button to clear the registered period displayed in yellow.
(10)	Displayed days switch button	Press this button to switch the number of days (1 or 3) displayed in a single screen.

(2) Set the schedule.

The procedure for setting the scheduled setting differs in the following cases:

a. When the Timelapse setting is not assigned in the Normal mode

b. When the Timelapse setting is assigned in the Normal mode

c. When the Stage exclusive mode is assigned

d. When immediately starting scheduled observation (with no timelapse set)

e. When immediately starting scheduled observation (with timelapse set)

Scheduled observation can be started immediately for a sample undergoing live observation, or a sample can be held on the stage until the specified scheduled observation start time. For details, see Section 4.5.6, “Setting Scheduled Observation During Live Observation.”

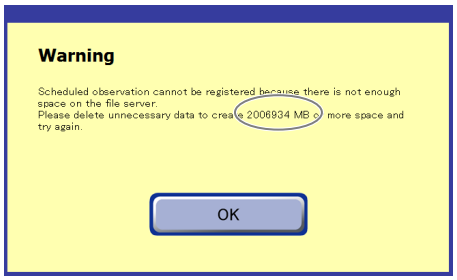
When schedule cannot be set:

If sufficient space to save the observation data cannot be allocated on the file server, an insufficient data space warning dialog box is displayed during scheduled observation setting.

When this dialog is displayed, more space must be allocated by deleting unnecessary observation data. (To delete the observation data, it is necessary to log in as an administrator.)

In addition, it is recommended that the data space circled in the figure as shown on the right be written down before starting schedule data deletion. The data space displayed in the dialog is the estimated amount to be deleted.

See Section 7.2, “Deleting the Observation Data” for details.



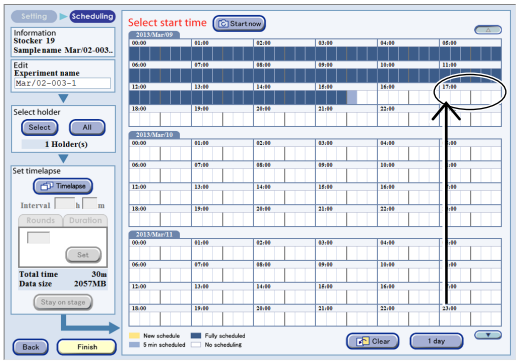
Insufficient data space warning dialog box

The following describes each case.

a. When the Timelapse setting is not assigned in the Normal mode

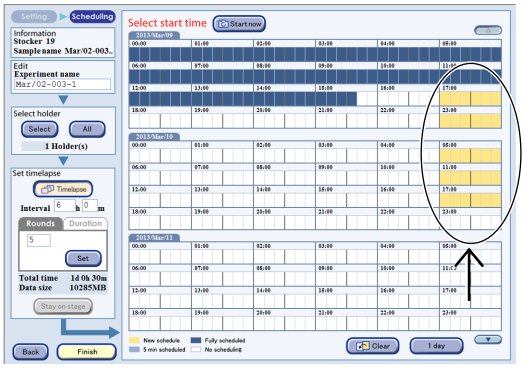
a-1. Press a period displayed in white on the Schedule setting area to set the start time of the observation.

Multiple schedules can be set.
The scheduled period is displayed in units of 10 minutes and colored in yellow.



The figure on the right shows an example of multiple scheduled observations set at 6 hours intervals from 17:00.
To set 55 minutes for the scheduled observation time, press the period of 17:00. The one hour period from 17:00 is displayed in yellow.

To cancel the schedule setting, press the period to be canceled again or press the Clear button (to clear all registrations).



In the example in the above figure, the period from 17:00 can be pressed even if a 5 minute schedule is already registered between 17:00 and 17:10.
In this case, the set schedule starts at 17:05. In other words, if a schedule is registered already, a new schedule may not start at the exact time (17:00).
All schedule settings can be verified in the Observation condition setting window.

Go to step (3).

b. When the Timelapse setting is assigned in the Normal mode

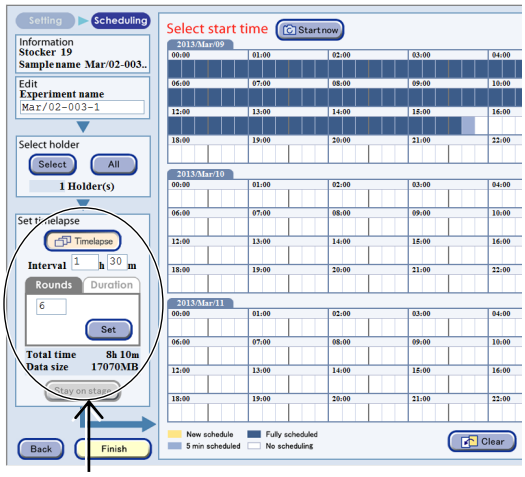
b-1. Press the Timelapse button.

b-2. Press the Interval field (the h field and the m field of the Interval field), and then enter the interval by hours in the h field, and 5 minute increments in the m field in the Keyboard window.

A time shorter than the observation time required per one round cannot be entered.

b-3. Enter a number for the number for rounds (Rounds tab) or the desired observation period (d, h, m on the Duration tab) using the Keyboard window and then press the Set button.

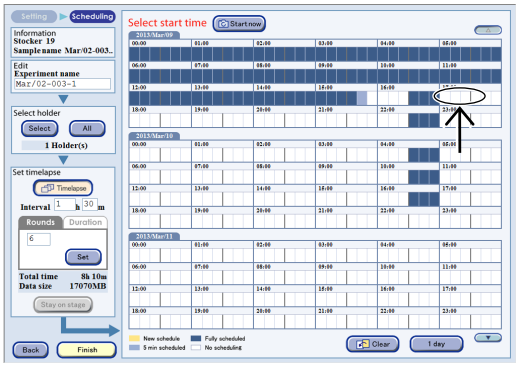
The observation period (Total time) is displayed in 5 minute intervals under the condition for which Timelapse was set.



The Scheduling window (When the Timelapse setting is assigned in the Normal mode)

b-4. To set the time for scheduled observation, in the Schedule setting field press the time zone (white part) in which you desire scheduled observation to start.

Multiple schedules can be set.
The scheduled period is displayed in units of 10 minutes and colored in yellow.



> The start time of the timelapse cannot be set within the period during which scheduled observation has already been scheduled. However, whether to skip or delay the schedule can be selected if the ongoing Round falls at the same time.

The figure on the right shows an example of Timelapse setting in which scheduled observation is set to a Round (40 min), Interval (1 h 30 m) and Rounds (6) from 17:00.

Note that a new schedule cannot be registered if the observation time for an already-scheduled observation overlaps even for one interval. In the example in the figure on the right, the time 17:30 cannot be selected since a scheduled observation has already been registered for 22:30. Note that the entire schedule is invalid for registration of a scheduled observation in this case.

To cancel the schedule setting, press the period to be canceled again or press the Clear button (to clear all registrations).

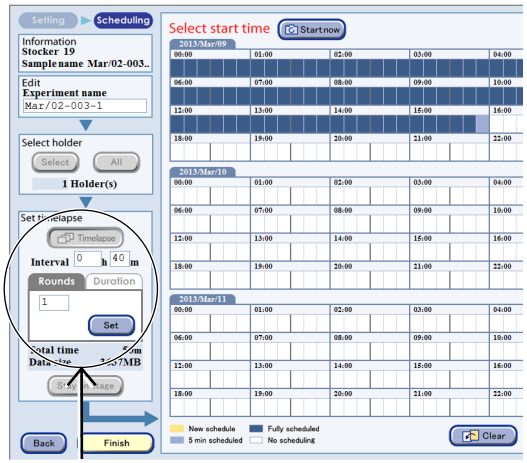
> Timelapse cannot be set when the Focus teach button is pressed in the Observation condition setting window.

Go to step (3).

c. For Stage exclusive mode observation

c-1. To change the observation interval, press the h or m Interval field.

In the keyboard window, enter the interval in hours in the h field and in 1 minute increments in the m field.
A time shorter than the observation time required per one round cannot be entered.



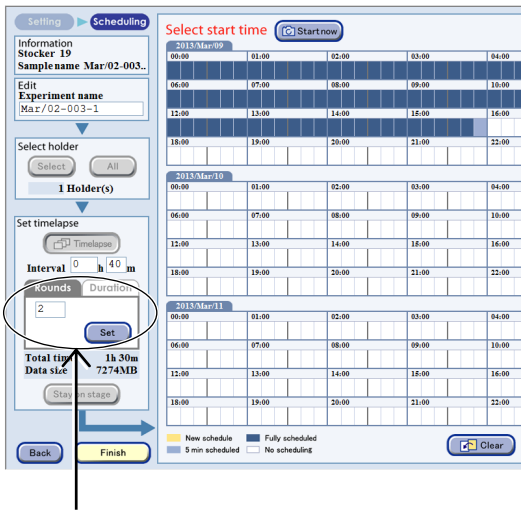
Scheduling window
(For Stage exclusive mode)

c-2. To change the number of rounds, press the Rounds tab, and to change the desired observation period, press d, h and m on the Duration tab.

The keyboard window appears.

Enter a value in the Keyboard window, and then press the Set button.

The observation period (Total time) is recalculated, and then the sum of the 5 minute intervals is displayed.
When the observation period is recalculated, the load time is not included in the observation period per Round in the Stage exclusive mode. The load time is then added.

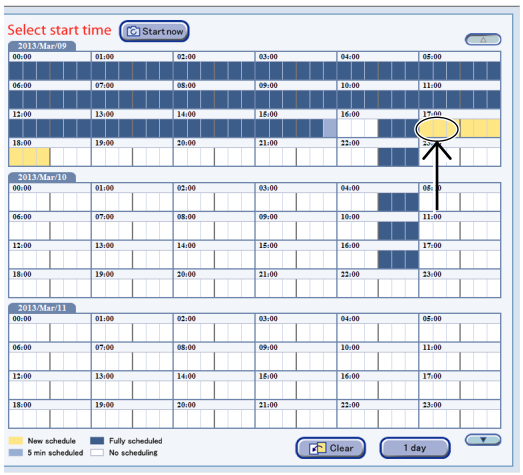


c-3. To set the time for scheduled observation, in the Schedule setting field press the time zone (white part) in which you desire scheduled observation to start.

Multiple schedules can be set. The scheduled period is displayed in units of 10 minutes and colored in yellow.

The figure on the right shows an example of Timelapse settings with a 40min/round, Interval of 40m, 2 Rounds and 17:00 as the start time of the scheduled observation.

To cancel the schedule setting, press the period to be canceled again or press the Clear button (to clear all registrations).



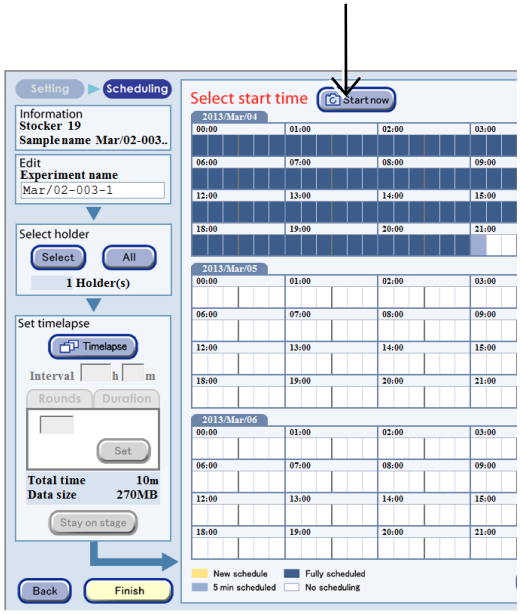
Go to step (3).

d. When immediately starting scheduled observation (with no timelapse set)

d-1. Press the Start now button.

A scheduled observation immediate start confirmation dialog box appears.

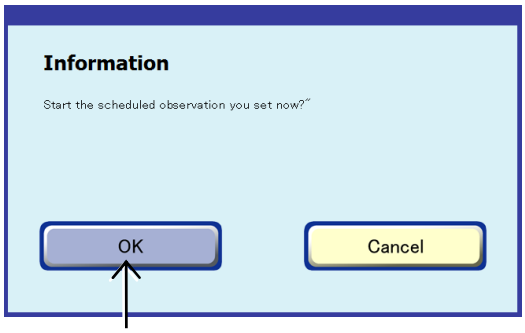
> If a scheduled observation is scheduled in the immediate future, an already registered schedule has priority. After the registered scheduled observation completes, the schedule set with the Start now button is executed.



Scheduling window

d-2. Press the OK button to close the dialog box.

The Scheduling window appears and the scheduled observation starts.



Confirmation dialog box

Go to step (3).

e. When immediately starting scheduled observation (with timelapse set)

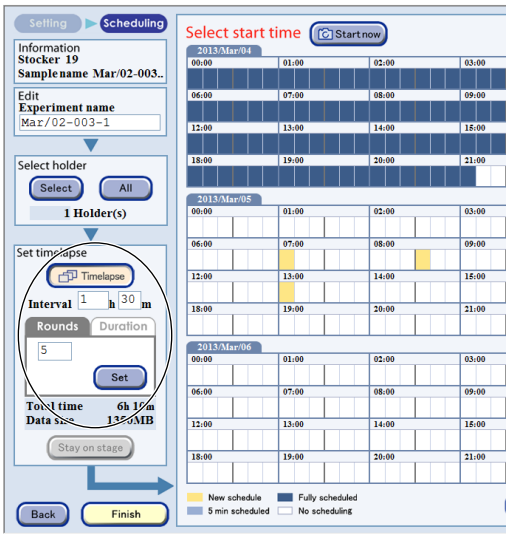
e-1. Press the Timelapse button.

e-2. Press the Interval field (the h field and the m field of the Interval field), and then enter the interval by hours in the h field, and 5 minute increments in the m field in the Keyboard window.

A time shorter than the observation time required per one round cannot be entered.

e-3. Enter a number for the number for rounds (Rounds tab) or the desired observation period (d, h, m on the Duration tab) using the Keyboard window and then press the Set button.

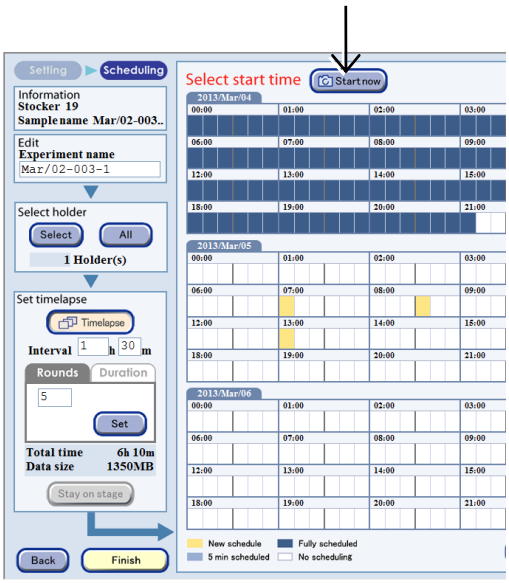
The observation period (Total time) is displayed in 5 minute intervals under the condition for which Timelapse was set.



Scheduling window

e-4. Press the Start now button.

A scheduled observation immediate start confirmation dialog box appears.

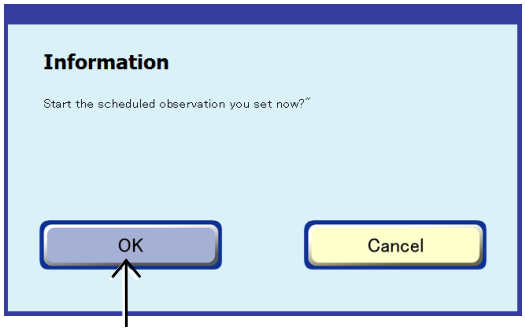


> Timelapse cannot be set if the Focus teach button is pressed in the Observation condition setting window.

> The start time of the timelapse cannot be set within the period during which scheduled observation has already been scheduled. However, whether to skip or delay the schedule can be selected if the time of the ongoing Round falls at the same time.

e -5. Press the OK button to close the dialog box.

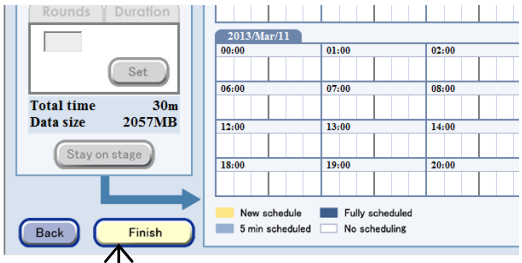
The Scheduling window appears and the scheduled observation starts.



Confirmation dialog box

(3) Press the Finish button.

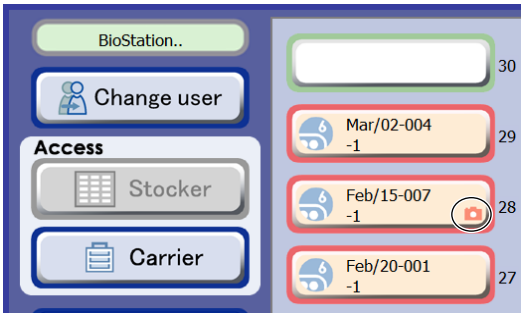
The Scheduling window closes.



Scheduling window

> The registered schedule setting is deleted if a sample is unloaded with “No return” selected as the purpose of unloading.
>
> If [Medium change] is selected as the purpose of unloading, the registered schedule setting is preserved even though a sample is unloaded.

If scheduled observation is scheduled, a camera symbol appears on the corresponding stocker button on the System status screen.



System status screen

The procedure for setting scheduled observation is now complete.

4.6.3.2 Batch setting schedules to multiple samples

This section describes the procedure for setting schedules of samples in multiple holders with one schedule setting.

When culturing and observing a large number of samples, scheduled observation can be set efficiently by batch scheduling.

Schedules can be set in batches either on all holders for the same type of vessel within a stocker or on selected holders.

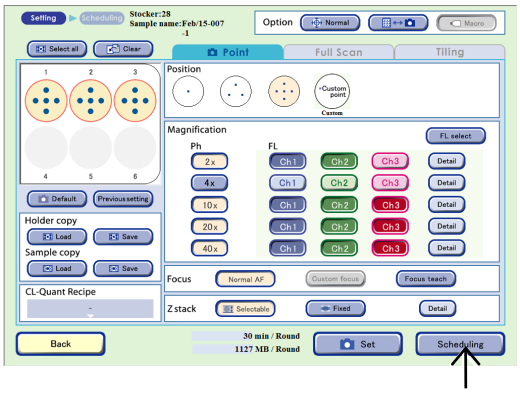
Batch setting for all vessels of the same type within a stocker

(1) Set scheduled observation conditions in the Observation condition setting window.

For details, see Section 4.6.2, “Setting the Schedule Observation Conditions.”

(2) Press the Scheduling button.

The Scheduling window appears.

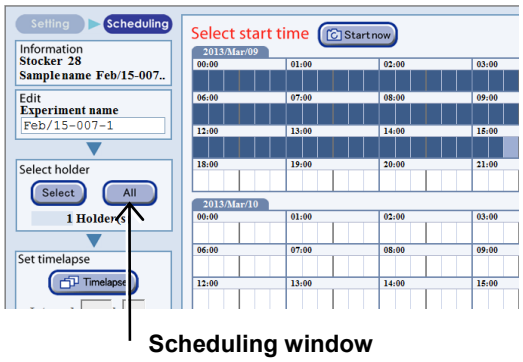


Observation condition setting window

(3) Press the All button.

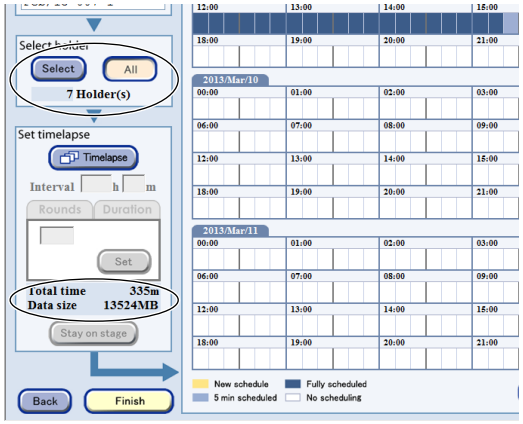
Press this before setting the time table.

When the All button is pressed, the same schedule is applied to all vessels of the same type within the stocker.



Scheduling window

For the observation setting in the example on the right, if there are seven holders that contain the same type vessels for the same user, a 30 min/Round schedule is applied to the seven holders and the total required observation time is displayed as 335 min (30 min × 7).

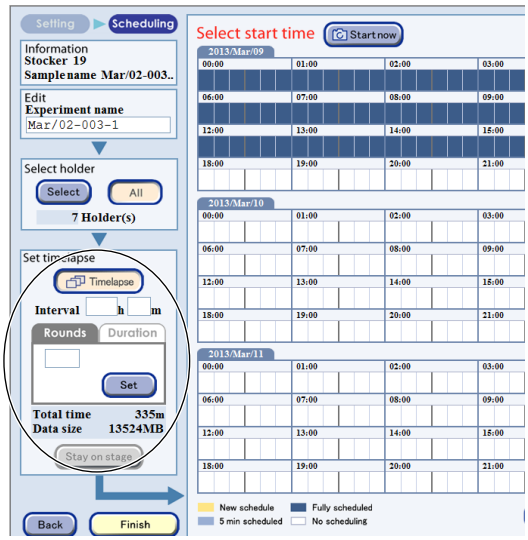


(4) Set the time table.

Go to step (5) if timelapse is not set.

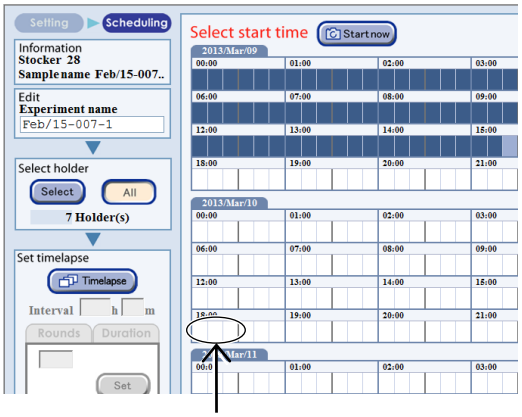
To configure timelapse settings, press the Timelapse button and then specify the observation interval (Interval area), the number of repetitions (Rounds tab), and the desired observation period (Duration tab).

For details, see Page 168, “b. When the Timelapse setting is assigned in the Normal mode.”

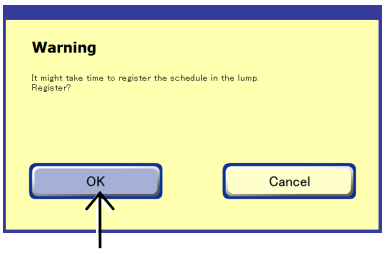


(5) To set the time for scheduled observation, in the Schedule setting field press the time zone (white part) in which you desire scheduled observation to start.

A confirmation dialog box appears.



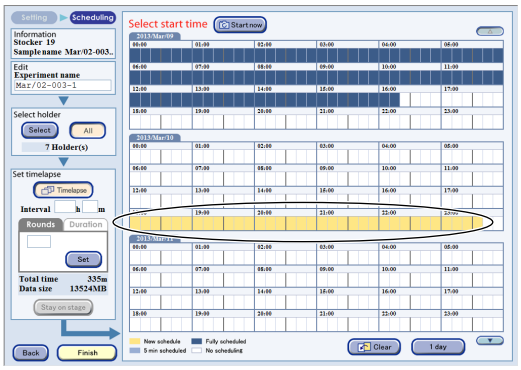
(6) Press the OK button.



Confirmation dialog box

A registered schedule is shown in yellow.

Select other periods for registration if necessary.



Scheduling window

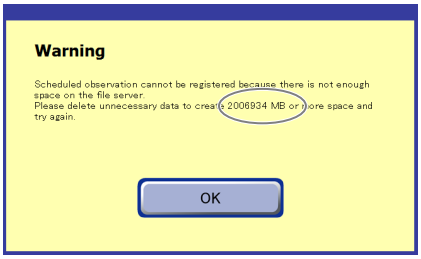
When schedule cannot be set:

If sufficient space to save the observation data cannot be allocated on the file server, an insufficient data space warning dialog box is displayed during scheduled observation setting.

When this dialog is displayed, more space must be allocated by deleting unnecessary observation data. (To delete the observation data, it is necessary to log in as an administrator.)

In addition, it is recommended that the data space circled in the figure as shown on the right be written down before starting schedule data deletion. The data space displayed in the dialog is the estimated amount to be deleted.

See Section 7.2, “Deleting the Observation Data” for details.

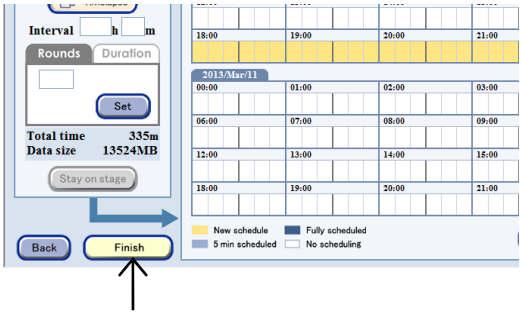


Insufficient data space warning dialog box

(7) After registration, press the Finish button.

The Scheduling window closes.

Batch set scheduled observations can be confirmed in the Schedule confirmation window.
For details, see Section 4.6.3.3, “Checking batch set scheduled observations.”

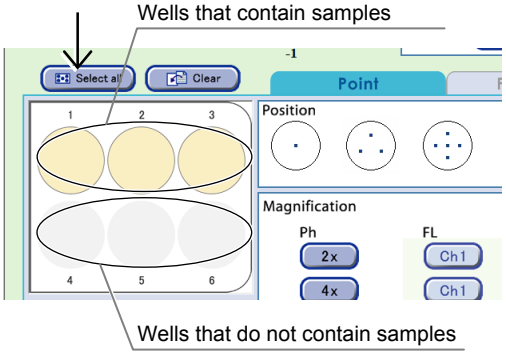


The registered schedule setting is deleted if a sample is unloaded with “No return” selected as the purpose of unloading.

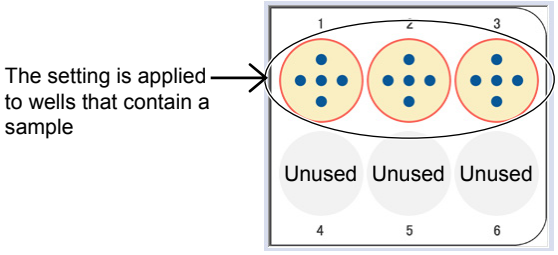
If [Medium change] is selected as the purpose of unloading, the registered schedule setting is preserved even though a sample is unloaded.

When the scheduled observation condition is set for a holder that has an unused well or unused part that does not contain samples, the setting is not applied to the unused well or unused part even if all samples are selected with the Select all button.

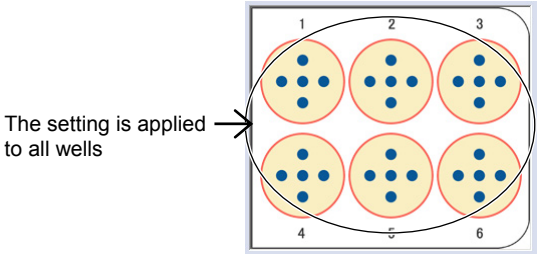
However, if scheduled observation is set in a batch for multiple holders based on that holder, the setting is applied to all samples even if there are holders without an unused well or unused part.



Observation condition setting window



Holder with 3 wells out of 6 used



Holder with all 6 wells used

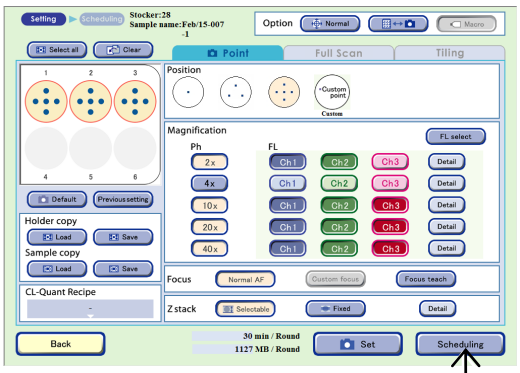
Batch setting to selected holders

(1) Set scheduled observation conditions in the Observation condition setting window.

For details, see Section 4.6.2, “Setting the Schedule Observation Conditions.”

(2) Press the Scheduling button.

The Scheduling window appears.

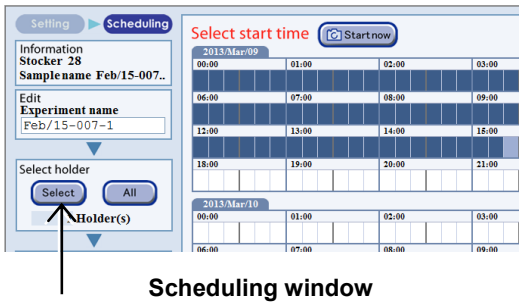


Observation condition setting window

(3) Press the Select button.

The Holder selection window appears.

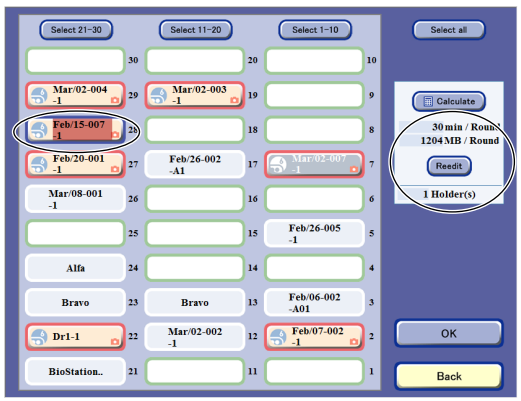
Press the Select button before setting the time table.



Scheduling window

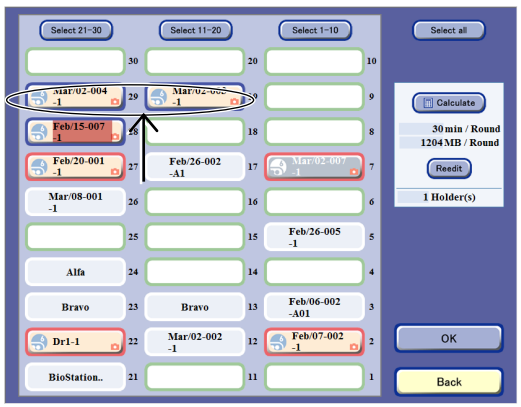
Selected holders are displayed with a blue frame.
Selectable holders are displayed with a red frame.

The time required for observation and the number of observation setting holders can be set on the right side of the window.



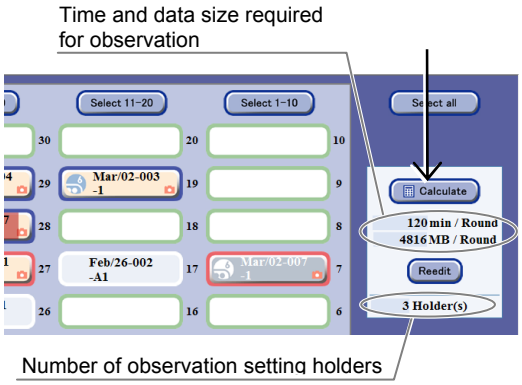
Holder selection window

(4) Select a stocker to apply the scheduled observation.



(5) Press the Calculate button.

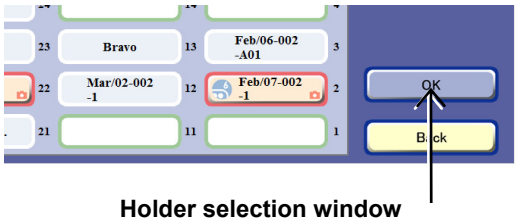
The total time and total data size required for the observation and the number of observation setting holders for the selected holders will be updated.



Holder selection window

(6) Press the OK button.

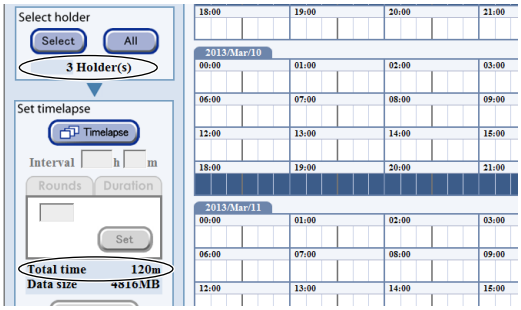
The Scheduling window appears.



Holder selection window

(7) The observation setting is applied to the selected holder.

For the observation setting in the example on the right, three holders are selected, and a 30 min/Round schedule is applied to those three holders, so the total required observation time is displayed as 120 min (30 min × 3).



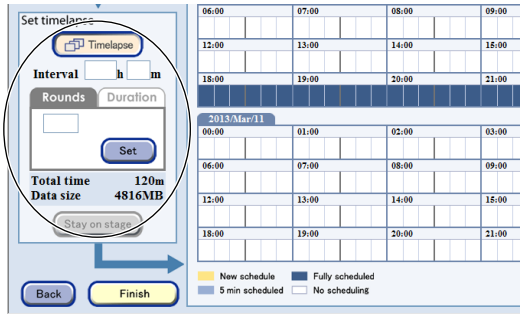
Scheduling window

(8) Set the time table.

Go to step (9) if timelapse is not set.

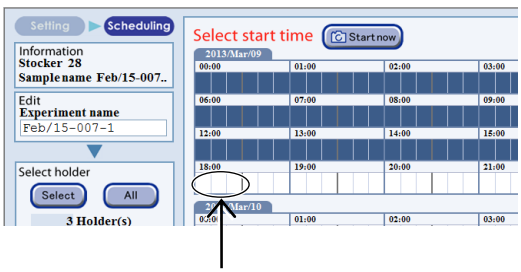
To configure Timelapse settings, press the Timelapse button and then specify the observation interval (Interval area), the number of repetitions (Rounds tab), and the desired observation period (Duration tab).

For details, see Page 168, “b. When the Timelapse setting is assigned in the Normal mode.”

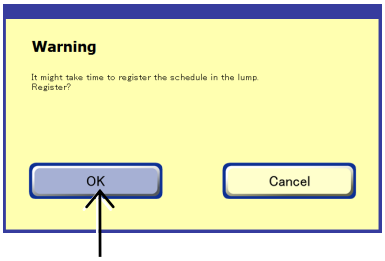


(9) To set the time for scheduled observation, in the Schedule setting field press the time zone (white part) in which you desire scheduled observation to start.

A confirmation dialog box appears.



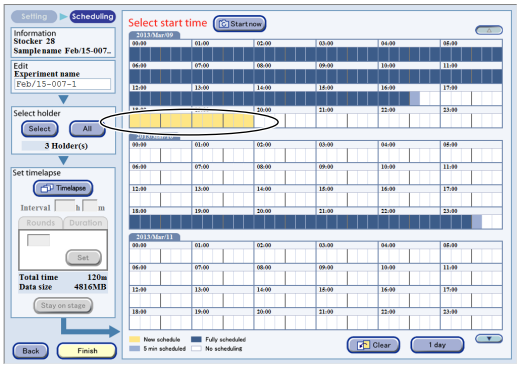
(10) Press the OK button.



Confirmation dialog box

A registered schedule is shown in yellow.

Select other periods for registration if necessary.



Scheduling window

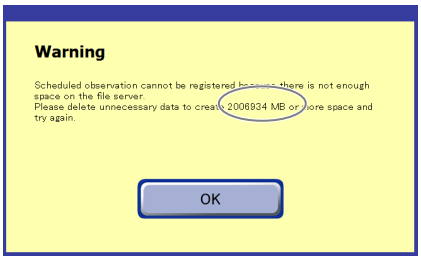
When schedule cannot be set:

If sufficient space to save the observation data cannot be allocated on the file server, an insufficient data space warning dialog box is displayed during scheduled observation setting.

When this dialog is displayed, more space must be allocated by deleting unnecessary observation data. (To delete the observation data, it is necessary to log in as an administrator.)

In addition, it is recommended that the data space circled in the figure as shown on the right be written down before starting schedule data deletion. The data space displayed in the dialog is the estimated amount to be deleted.

See Section 7.2, “Deleting the Observation Data” for details.

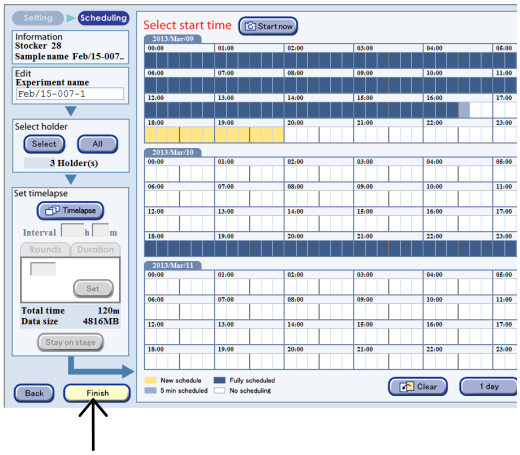


Insufficient data space warning dialog box

(11) After registration, press the Finish button.

The Scheduling window closes.

Batch-scheduled scheduled observations can be confirmed in the Schedule confirmation window. For details, see Section 4.6.3.3, “Checking batch set scheduled observations.”



The registered schedule setting is deleted if a sample is unloaded with “No return” selected as the purpose of unloading.

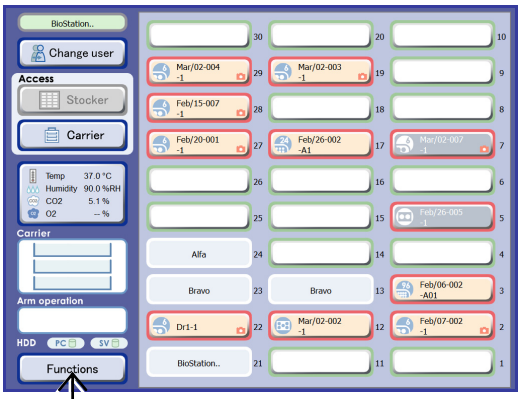
If [Medium change] is selected as the purpose of unloading, the registered schedule setting is preserved even though a sample is unloaded.

4.6.3.3 Checking batch set scheduled observations

Check batch set scheduled observations by holder or sample.

(1) Press the Functions button on the System status screen.

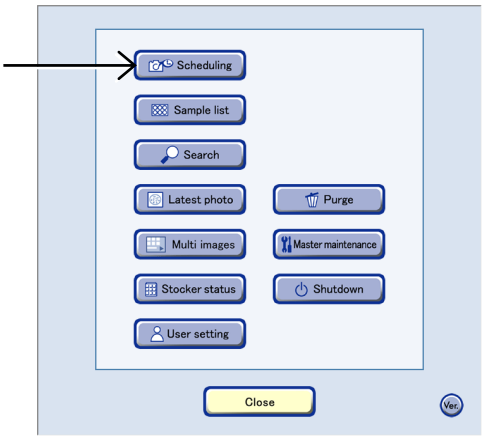
The Functions window appears.



System status screen

(2) Press the Scheduling button.

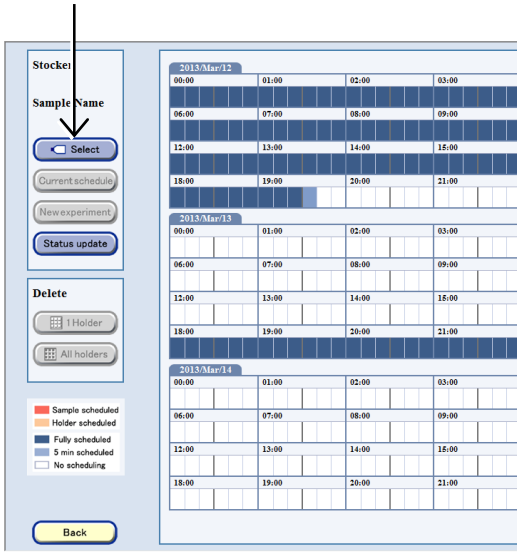
The Schedule confirmation window appears.



Functions window

(3) Press the Select button.

The Sample selection window appears.

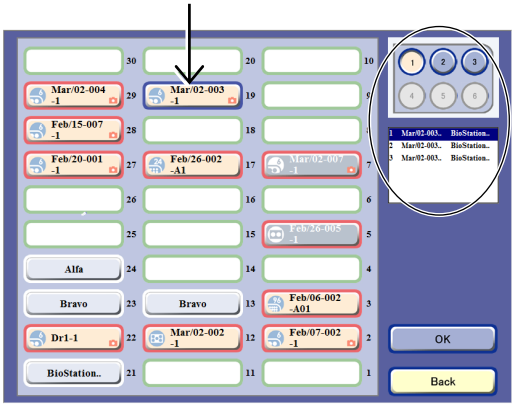


Schedule confirmation window

(4) Press the button of the stocker that contains samples for which scheduled observations are to be confirmed.

Selected holders are displayed with a blue frame. Selectable holders are displayed with a red frame.

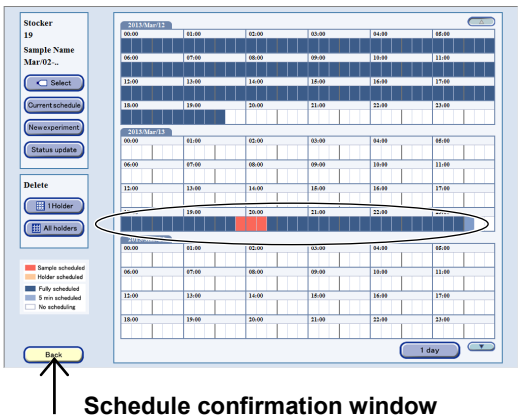
To confirm a schedule for one sample, select the sample on the Holder map or in the Sample list.



Sample selection window

In the Schedule confirmation window, scheduled observations that are scheduled for the selected holder are displayed in red (when an individual sample is selected in the Sample selection window) or in orange (when samples are selected as a holder in the Sample selection window).

The scheduled observations set for the holders of the same type vessels in the stocker selected in the Sample selection window are shown in blue.



Schedule confirmation window

(5) Press the Back button.

The Schedule confirmation window closes.

4.6.4 Saving and Loading Observation Conditions

There are two ways to save capture conditions for scheduled observations; saving the observation conditions of the executed schedule afterward and saving when waiting for execution. The saved capture conditions can be loaded when setting a new scheduled observation.

4.6.4.1 Saving capture conditions for the executed scheduled observation

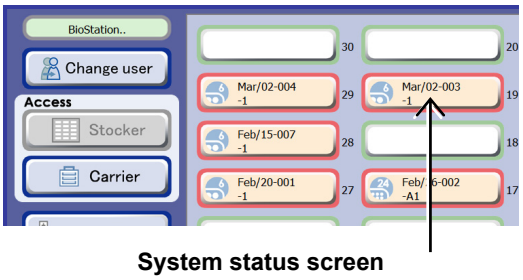
Capture conditions (observation points, observation magnification, number of images of Z stacking, fluorescence, etc.) for the executed scheduled observation can be saved for either a holder or a sample. The X and Y values or X, Y, and Z values are saved for an observation point, whereas the values for the channel, brightness and exposure time are saved for fluorescence conditions.

Also, the capture conditions are saved by holder or by sample. Capture conditions for an entire holder can be saved by selecting a desired holder. Capture conditions for an individual sample can be saved by selecting a desired sample.

(1) Display the Image review window.

1. **On the System status screen, press the holder for which the observation conditions are to be saved.**

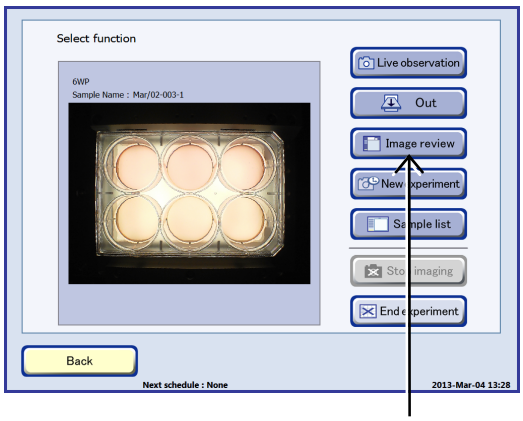
   The Select function window is displayed.



System status screen

2. **Press the Image review button in the Select function window.**

   The Image review window of the selected sample appears.



Select function window

(2) Select the observation history in which the capture conditions are to be saved.

In the Image review window, press the icon of the observation history for which capture conditions are to be saved. The Capture conditions confirmation window appears.

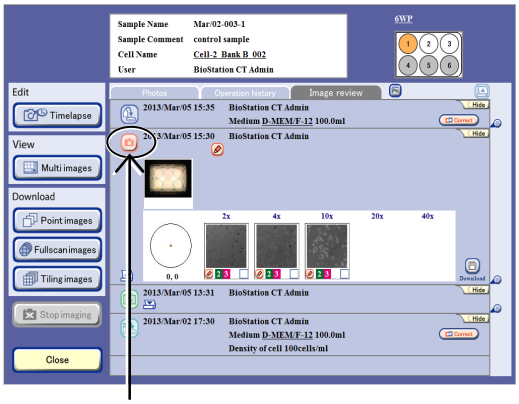


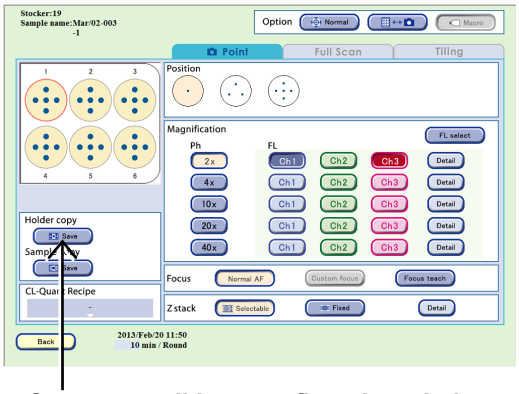
Image review window

(3) Save the capture conditions.

a. Saving capture conditions for each holder

a-1. Press the Save button in the Holder copy area.

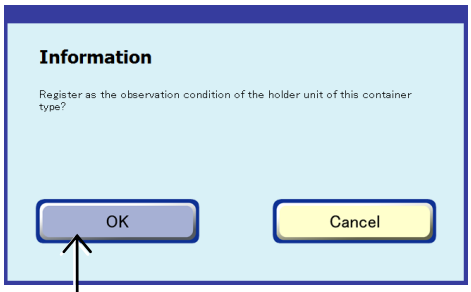
A confirmation dialog box appears.



Capture conditions confirmation window

a-2. Press the OK button to close the dialog box.

Capture conditions are saved for each holder.



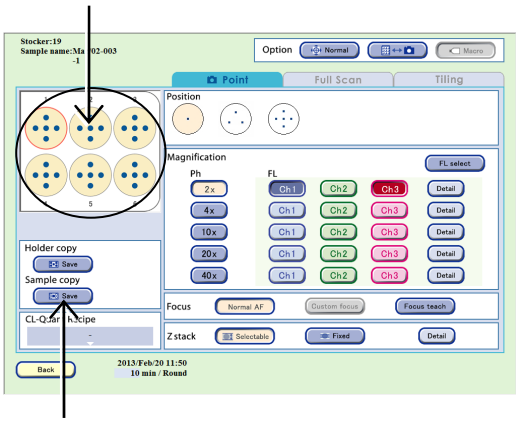
Confirmation dialog box

b. Saving capture conditions for each sample

b-1. Select a sample.

Select the sample for which capture conditions are to be saved by pressing the sample selection area in the Capture conditions confirmation window.

When a 96-/48-well plate is used, press the sample selection area to open the respective 96-, 48-well plate sample selection window and then select a sample in the window.



Capture conditions confirmation window

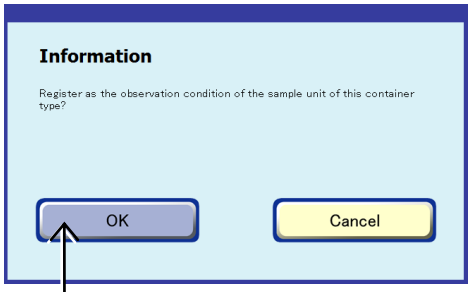
b-2. Save the capture conditions.

Press the Save button in the Sample copy area.

A confirmation dialog box appears.

b-3. Press the OK button to close the dialog box.

The capture conditions are saved for each sample.



Confirmation dialog box

4.6.4.2 Saving capture conditions for standby scheduled observations

Capture conditions (observation points, observation magnification, number of images of Z stacking, fluorescence, etc.) for a standby scheduled observation can be saved for either a holder or a sample.

The X and Y values or X, Y, and Z values are saved for an observation point, and values for the channel, brightness and exposure time are saved for fluorescence conditions.

Also, the capture conditions are saved by holder or by sample. Capture conditions for an entire holder can be saved by selecting the desired holder. Capture conditions for an individual sample can be saved by selecting a desired sample.

(1) Display the Schedule confirmation window.

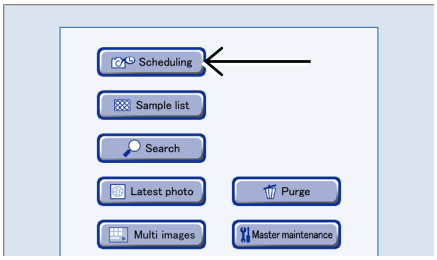
1. Press the Functions button on the System status screen.

The Functions window appears.

System status screen

2. Press the Scheduling button.

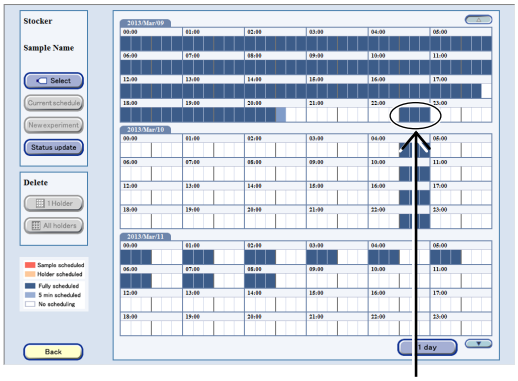
The Schedule confirmation window appears.



Functions window

(2) Select the period of the standby schedule.

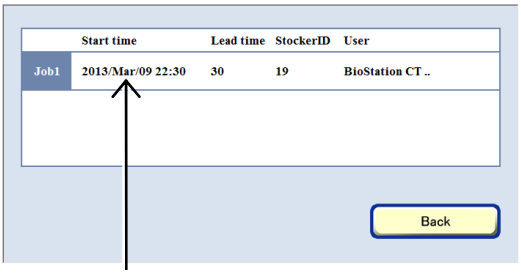
Press the period of the standby schedule in the Schedule confirmation window. The Scheduled observation selection window appears.



Schedule confirmation window

(3) Select the desired observation schedule for which capture conditions are to be saved.

In the Scheduled observation selection window, press the desired observation schedule for which capture conditions are to be saved. The Observation condition setting window appears.



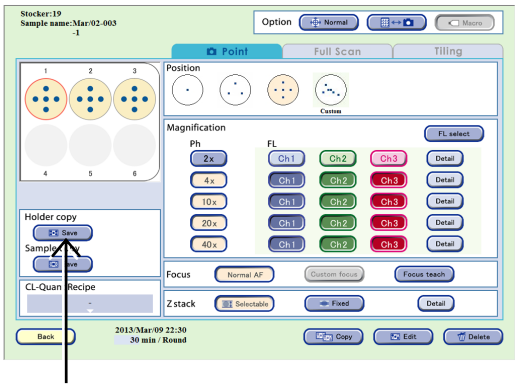
Scheduled observation selection window

(4) Save the capture conditions.

a. Saving capture conditions for each holder

a-1. Press the Save button in the Holder copy area.

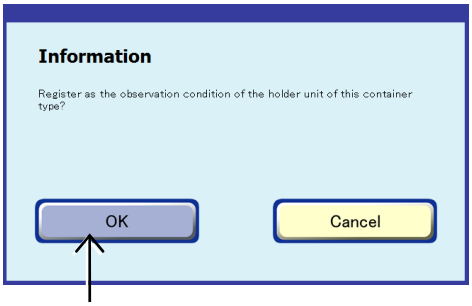
A confirmation dialog box appears.



Capture conditions confirmation window

a-2. Press the OK button to close the dialog box.

Capture conditions are saved for each holder.



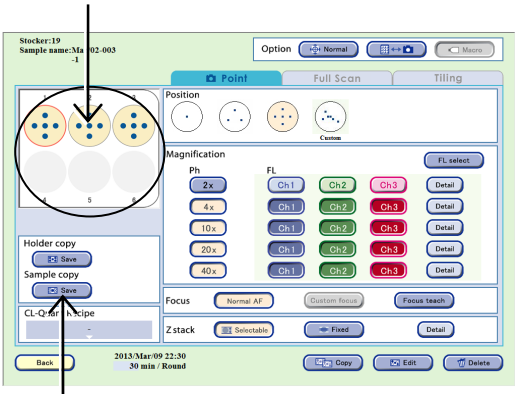
Confirmation dialog box

b. Saving capture conditions for each sample

b-1. Select a sample.

Press the sample selection area on the Capture conditions confirmation window to select a desired sample for which capture conditions are to be saved.

When a 96-/48-well plate is used, press the sample selection area to open the respective 96-, 48-well plate sample selection window and select a sample in the window.



Capture conditions confirmation window

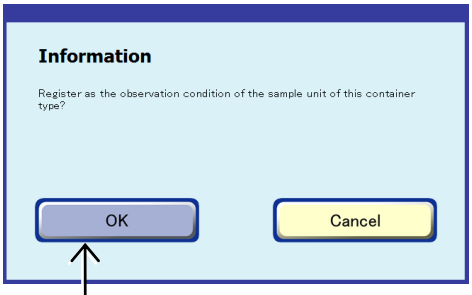
b-2. Save the capture conditions.

Press the Save button in the Sample copy area.

A confirmation dialog box appears.

b-3. Press the OK button to close the dialog box.

The capture conditions are saved for each sample.



Confirmation dialog box

4.6.4.3 Loading saved capture conditions

When setting scheduled observations, the saved capture conditions can be loaded if the culture vessels are the same type.

Capture conditions can be loaded by loading either conditions saved by holder unit or by sample unit.

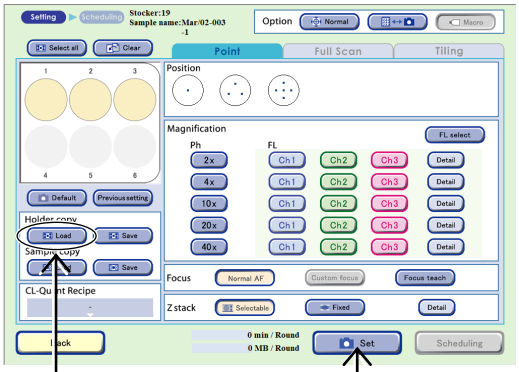
a. Loading capture conditions for each holder

a-1. Press the Load button in the Holder copy area.

Capture conditions saved for a holder are loaded.

a-2. Press the Set button.

Loaded capture conditions are set by holder.



Observation condition setting window

b. Loading capture conditions for each sample

b-1. Select the sample.

In the Sample selection area of the Observation condition setting window, select the sample for which capture conditions are to be set.

When a 96-/48-well plate is used, press the sample selection area to open the respective 96-, 48-well plate sample selection window and select a sample in the window.

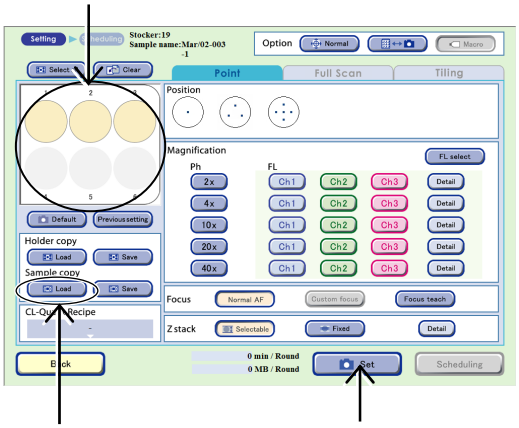
b-2. Press the Load button in the Sample copy area.

Capture conditions saved for a sample are loaded.

When multiple samples are selected as loading destination, the saved capture conditions are loaded for all the selected samples.

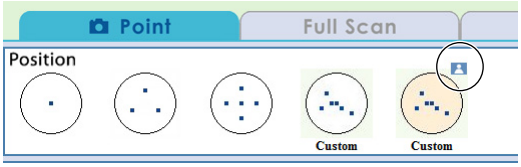
b-3. Press the Set button.

Loaded capture conditions are set by sample.



Observation condition setting window

When capture conditions with custom observation point are loaded, a custom observation point icon will be displayed in the observation position setting area.



4.6.5 Copying, Editing, and Deleting Observation Settings

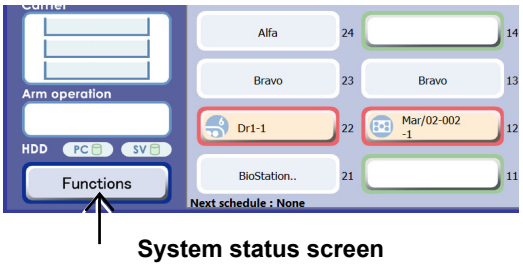
4.6.5.1 Copying standby scheduled observation settings

This section describes the procedure for adding a Round by copying the standby scheduled observation settings.

(1) Display the Schedule confirmation window.

1. Press the Functions button on the System status screen.

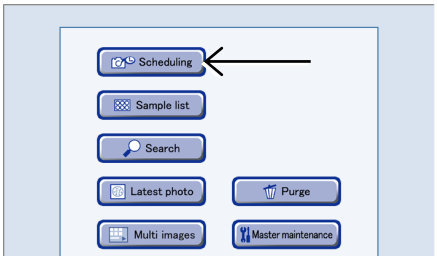
The Functions window appears.



System status screen

2. Press the Scheduling button.

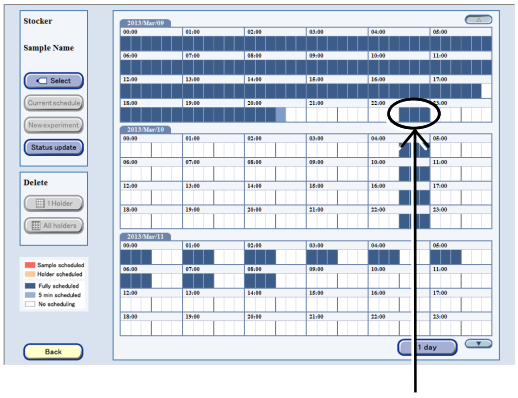
The Schedule confirmation window appears.



Functions window

(2) In the Schedule confirmation window, press the period of the schedule to be copied.

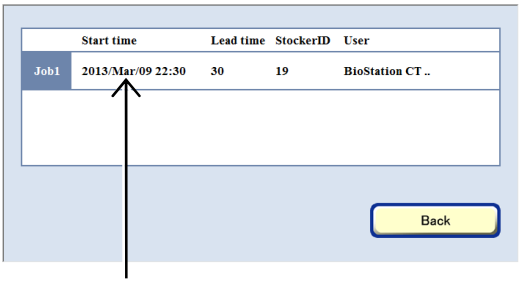
When a scheduled period is pressed, the Scheduled observation selection window appears.



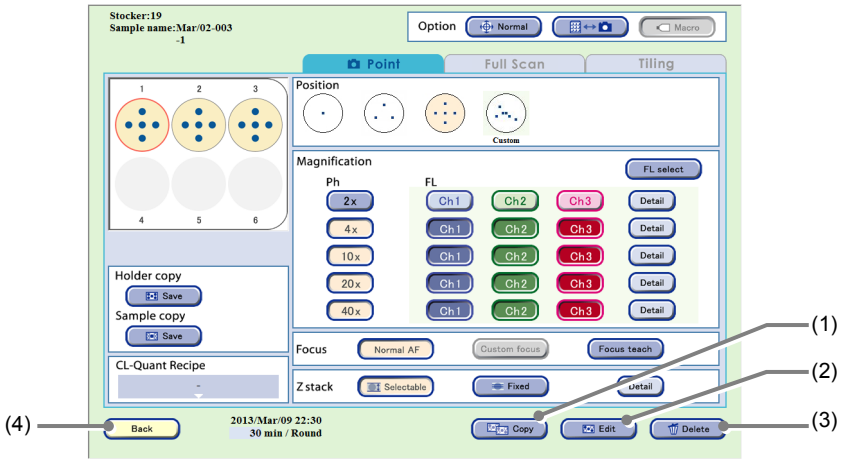
Schedule confirmation window

(3) Select the original scheduled observation in the Scheduled observation selection window.

The Capture conditions confirmation window appears.



Scheduled observation selection window

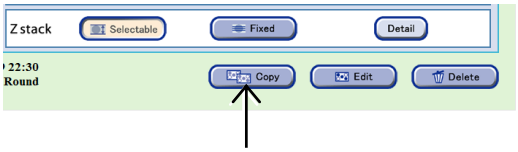


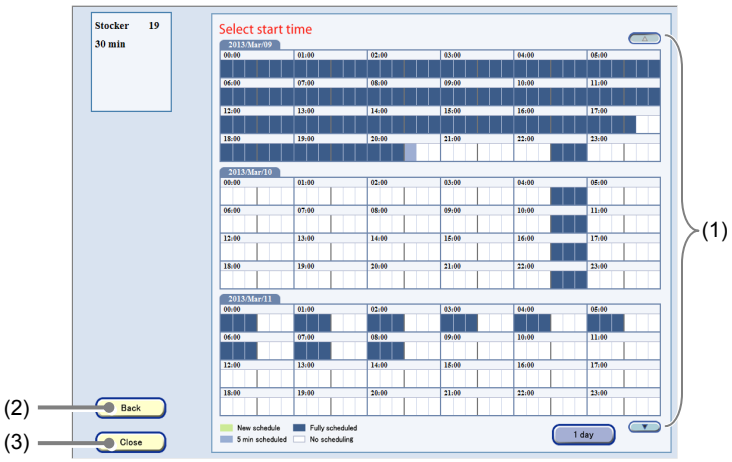
Capture conditions confirmation window

No.	Name	Function
(1)	Copy button	Press this button to copy the selected schedule.
(2)	Edit button	Press this button to display the observation modification window.
(3)	Delete button	Press this button to delete the selected schedule.
(4)	Back button	Press this button to close the Capture conditions confirmation window.

(3) Press the Copy button.

The Observation condition copy destination window in which the copy destination of the schedule can be specified appears.





Observation condition copy destination window

No.	Name	Function
(1)	Schedule setting field	Periods displayed in white have no schedule. Press the period for a scheduled observation to set a schedule.
(2)	Back button	Press this button to return to the Capture conditions confirmation window.
(3)	Close button	Press this button to close the Observation condition copy destination window.

(4) Press the copy destination time period of the schedule.

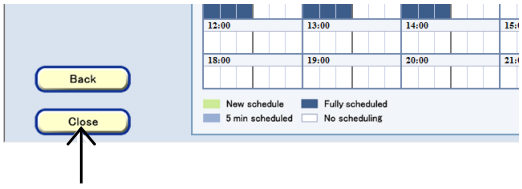
The selected period is displayed in green.

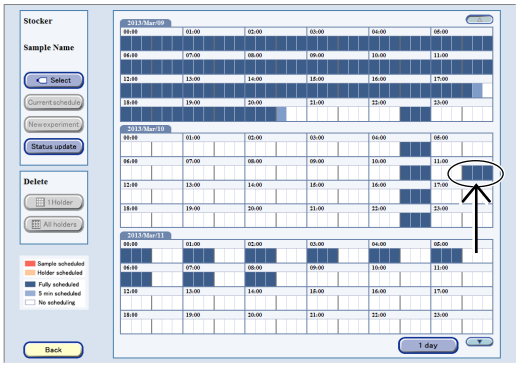
To cancel the setting, press the period again.

(5) Press the Close button.

The Observation condition copy destination window closes.

The Schedule confirmation window shows schedules of observations in red.





Schedule confirmation window

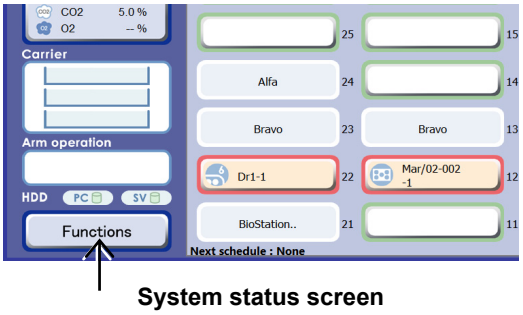
4.6.5.2 Editing standby observation conditions settings

This section describes the procedure for editing the standby scheduled observation settings.

(1) Display the Schedule confirmation window.

1. Press the Functions button on the System status screen.

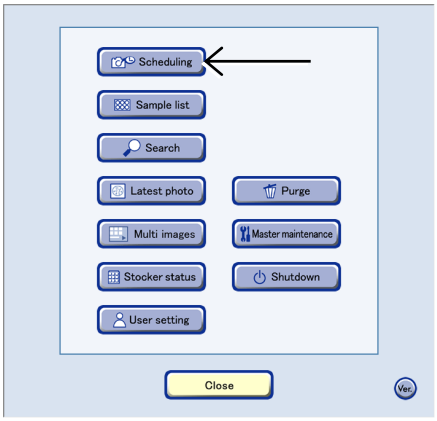
The Functions window appears.



System status screen

2. Press the Scheduling button.

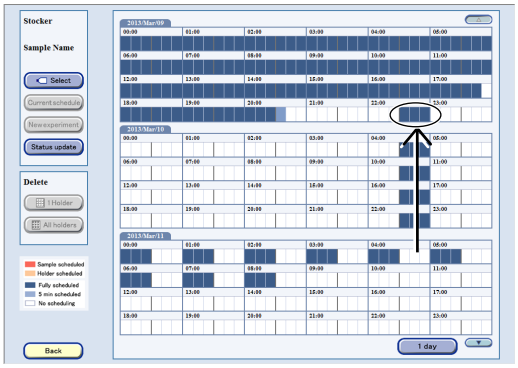
The Schedule confirmation window appears.



Functions window

(2) In the Schedule confirmation window, press the period with the schedule settings to be edited.

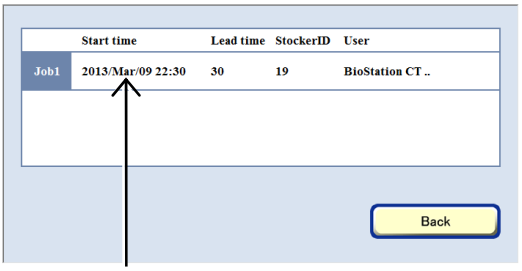
When a scheduled period is pressed, the Scheduled observation selection window appears.



Schedule confirmation window

(3) Select the observation schedule in the Scheduled observation selection window.

The Capture conditions confirmation window appears.



Scheduled observation selection window

(4) Press the Edit button.

The Scheduled Observation condition modification window appears.

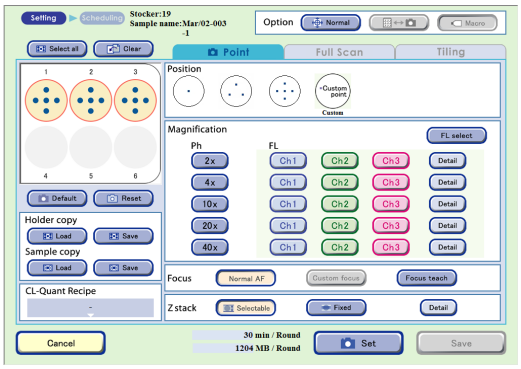
> If scheduled observation is in operation or if the Stage exclusive mode is set, schedule settings cannot be edited.

Capture conditions confirmation window

(5) Modify the observation settings.

For information on how to edit scheduled observation settings, see Section 4.6.2, “Setting the Scheduled Observation Conditions.”

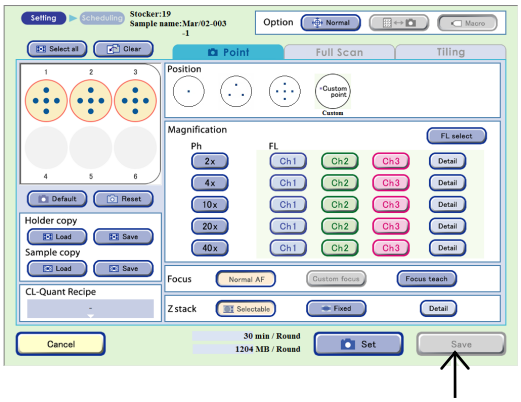
> If the modified schedule for observation conditions setting becomes longer and exceeds the free period, it cannot be registered. Change the observation conditions setting.



Observation condition modification window

(6) Press the Save button to finish the edit.

The modified schedule is saved, and the Schedule confirmation window appears again.



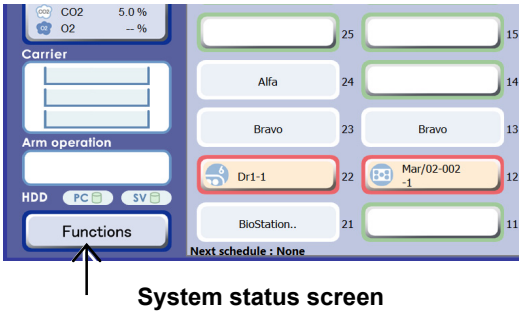
4.6.5.3 Deleting standby scheduled observation settings

This section describes the procedure for deleting the standby scheduled observation settings.

(1) Display the Schedule confirmation window.

1. Press the Functions button on the System status screen.

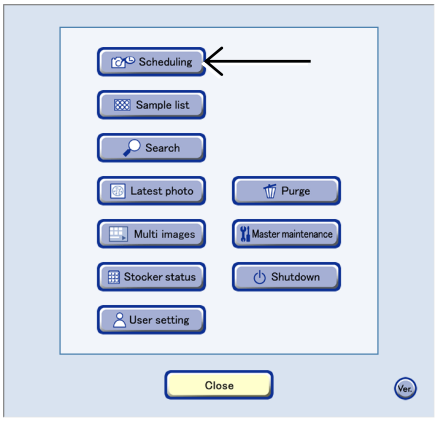
The Functions window appears.



System status screen

2. Press the Scheduling button.

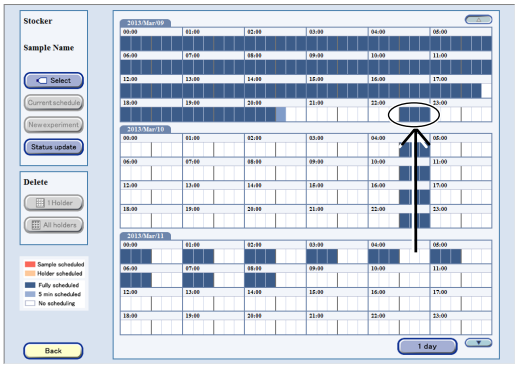
The Schedule confirmation window appears.



Functions window

(2) In the Schedule confirmation window, press the scheduled period to be deleted.

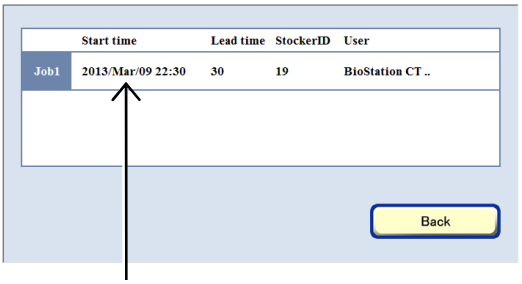
When a scheduled period is pressed, the Scheduled observation selection window appears.



Schedule confirmation window

(3) In the Scheduled observation selection window, select the scheduled observation setting to be deleted.

The Capture conditions confirmation window appears.



Scheduled observation selection window

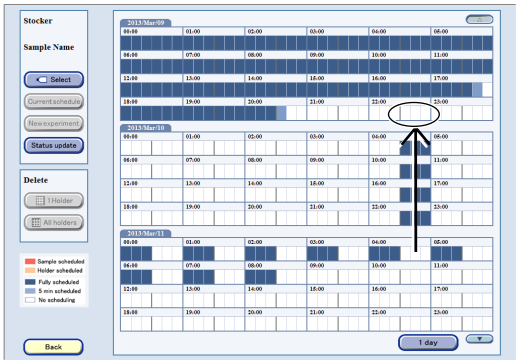
(4) Press the Delete button.

If scheduled observation is in operation, schedule settings cannot be deleted.

Capture conditions confirmation window

A schedule delete confirmation dialog box appears.

Press the OK button to delete the selected schedule Setting. The Schedule confirmation window appears.



Schedule confirmation window

See the next section for details on the procedure for batch deleting the schedule settings of multiple samples.

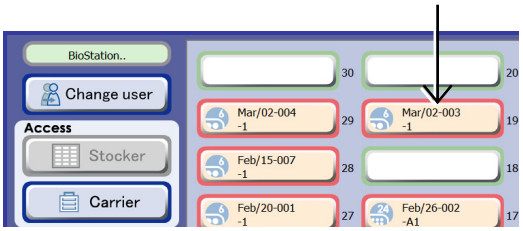
4.6.5.4 Batch deleting standby scheduled observation settings (applied to a selected holder)

This section describes the procedure for batch deleting the standby scheduled observation settings.
Batch deleting of scheduled observation settings is applied to all scheduled observations that are set for the samples in the selected holder.

When the stocker button is used to select a holder

(1) Press the button of the stocker that contains the holder whose schedule is to be deleted.

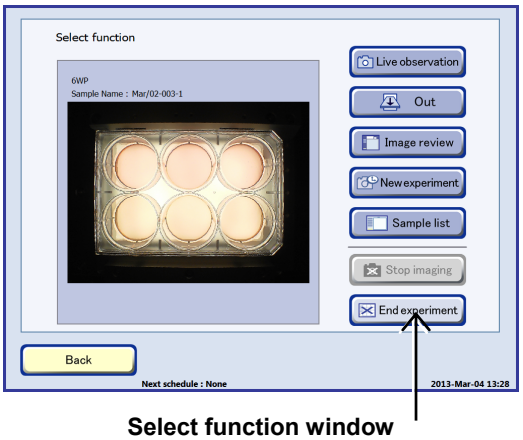
The Select function window appears.



System status screen

(2) Press the End experiment button.

All observation schedule settings in the selected stocker are deleted at the same time.



Select function window

When a holder is selected in the Schedule confirmation window

(1) Open the Schedule confirmation window.

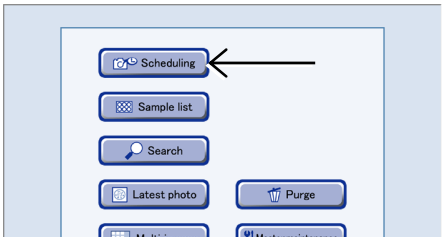
1. Press the Functions button on the System status screen.

The Functions window appears.

System status screen

2. Press the Scheduling button.

The Schedule confirmation window appears.

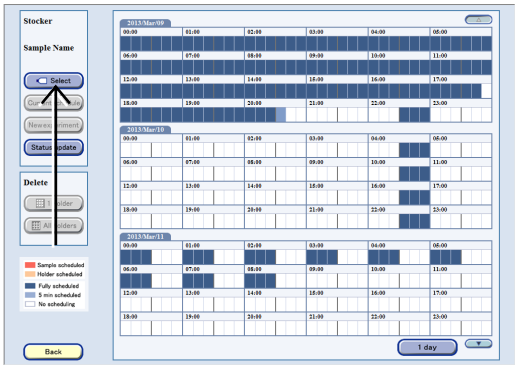


Functions window

(2) Select the samples for which the schedules are to be deleted.

1. Press the Select button.

The Sample selection window appears.



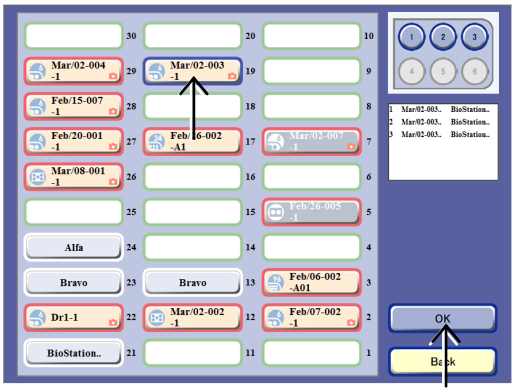
Schedule confirmation window

2. Press the button for the stocker that contains the samples whose schedule is to be deleted.

The frame color of the selected stocker button turns blue.

3. Press the OK button.

The Sample selection window is closed and the Schedule confirmation window appears.

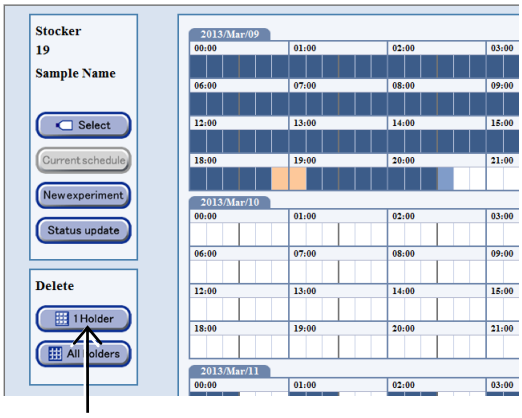


Sample selection window

(3) Delete the observation schedule.

1. Press the 1 Holder button in the Delete area.

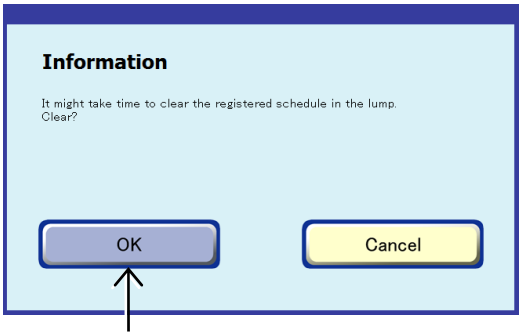
A schedule batch delete confirmation dialog box appears.



Schedule confirmation window

2. Press the OK button to close the dialog box.

All observation schedule settings in the selected stocker are deleted at the same time.



Confirmation dialog box

4.6.5.5 Batch deleting standby scheduled observation settings (applied to all culture vessels of the same type)

This section describes the procedure for batch deleting the standby scheduled observation settings. Batch deleting of scheduled observation settings is applied to all samples cultured by the same user in the same type of vessel in all stockers.

(1) Open the Schedule confirmation window.

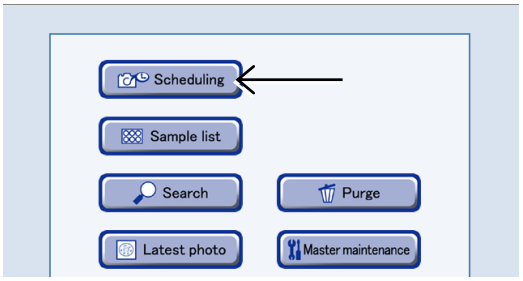
1. Press the Functions button on the System status screen.

The Functions window appears.

System status screen

2. Press the Scheduling button.

The Schedule confirmation window appears.

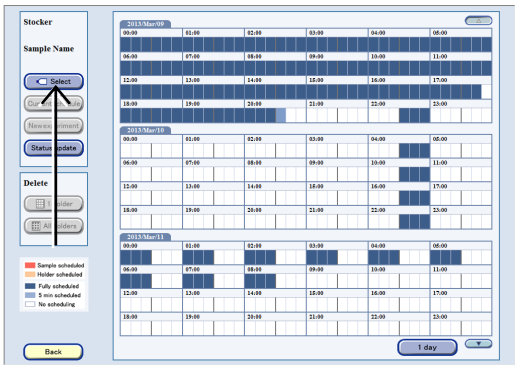


Functions window

(2) Select the samples for which the schedules are to be deleted.

1. Press the Select button.

The Sample selection window appears.



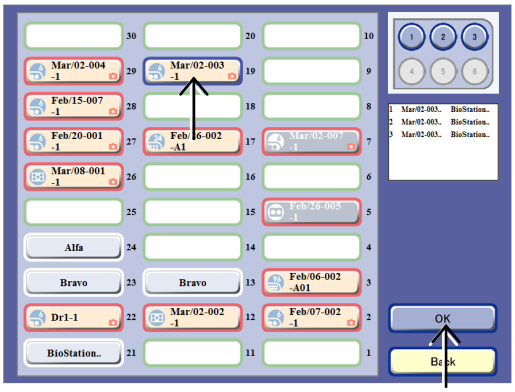
Schedule confirmation window

2. Press the button for the stocker that contains the samples whose schedule is to be deleted.

The frame color of the selected stocker button turns blue.

3. Press the OK button.

The Sample selection window is closed and the Schedule confirmation window appears.

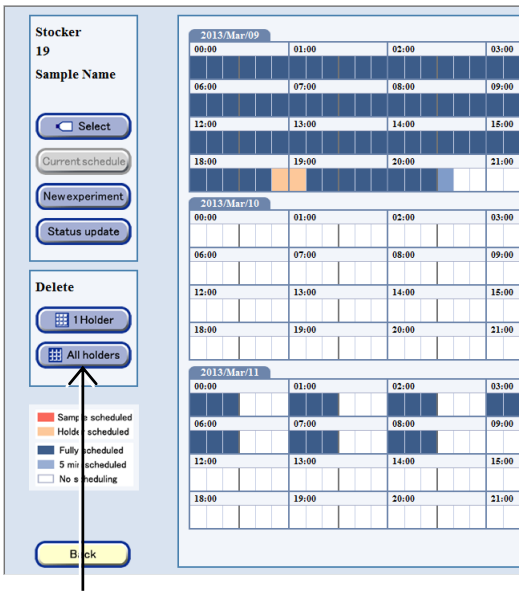


Sample selection window

(3) Delete the observation schedule.

1. Press the All holders button in the Delete area.

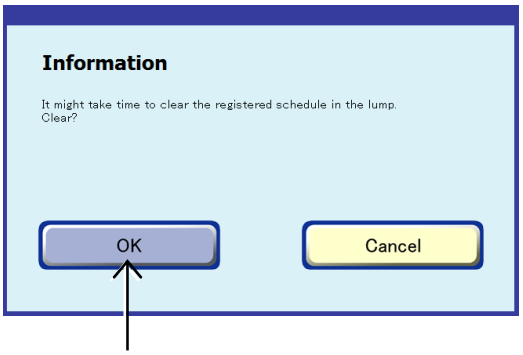
A schedule batch delete confirmation dialog box appears.



Schedule confirmation window

2. Press the OK button to close the dialog box.

All observation schedules for the samples cultured by the same user and in the same vessel in all stockers are batch deleted.



Confirmation dialog box

4.7 Displaying and Editing the Observation Data

4.7.1 Displaying a List of the Observation Data

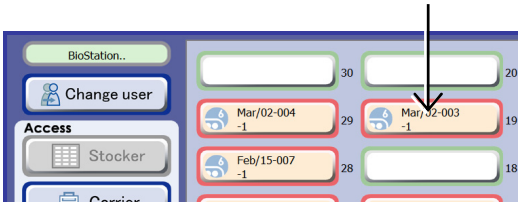
This section describes the procedure for checking the observation data of each sample.

(1) Display the Image review window.

a. To select a stocker where a sample is being cultured

a-1. On the System status screen, press the button of the stocker where the sample is stored.

The Select function window appears.



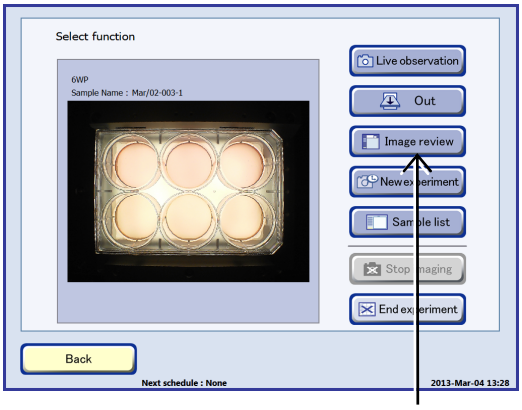
System status screen

a-2. Press the Image review button.

The Image review window for the selected sample appears.

An observation data list of samples stored in the selected holder is displayed in the Image review window.

For details on the Image review window, see Section 4.7.2, “Image Review Window.”

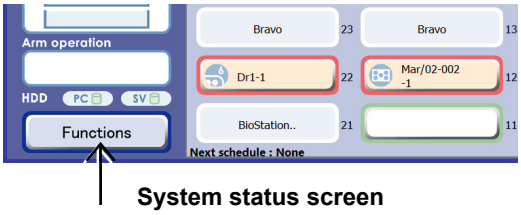


Select function window

b. To select sample(s) in the sample list

b-1. Press the Functions button on the System status screen.

The Functions window appears.



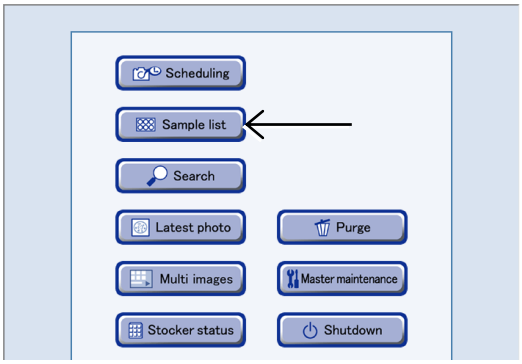
System status screen

b-2. Press the Sample list button in the Functions window.

The Search result sample list appears.

All samples cultured by the User are displayed in this Sample list window.

> To display the list of the already observed culture samples in the Sample list window, use the search function.
> For details on the search function, see Section 4.10, “Searching for the Observation Data.”



Functions window

b-3. In the sample list in the Sample list window, press the sample name of the sample to be displayed.

The Image review window for the selected sample appears.
For details on the Image review window, see Section 4.7.2, “Image review window.”

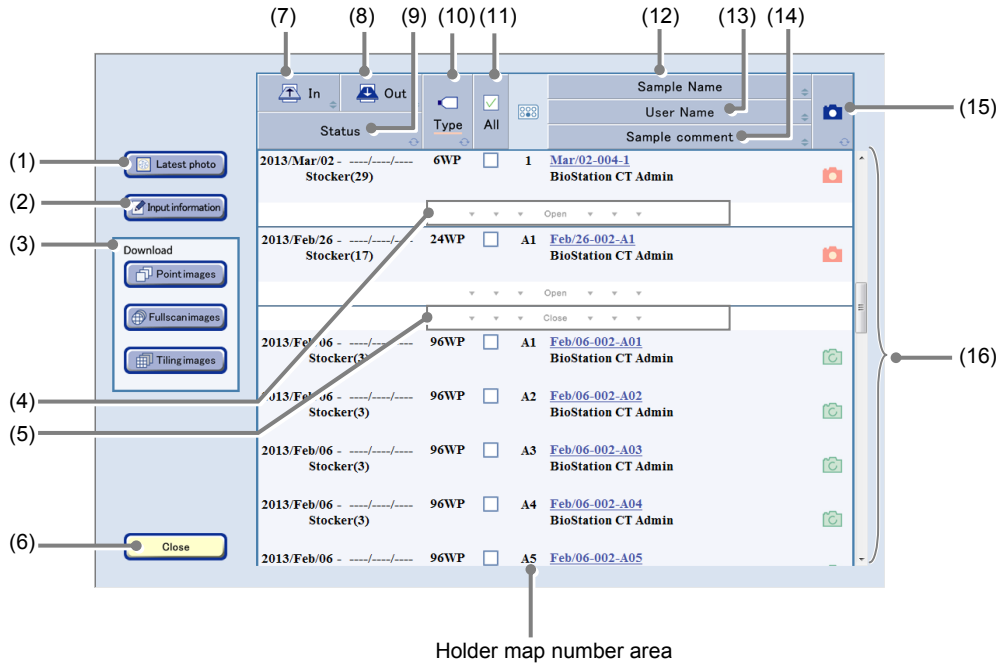
In the Sample list window, samples are listed in accordance with their stocker numbers and grouped by holder.
The display order in the sample list can be changed by the sort function at the top of the Sample list window.
Press the Open area to display all sample names in the holder. Press the Close area to display only the first sample.

> For details on vessel name abbreviations used in the Sample list window, see the list on Page 57, “Abbreviation and description for vessels.”

> If a name of the sample whose observation data is deleted is pressed, a dialog box appears indicating that the history cannot be found on the file server. If observation data has been stored in an external PC using Ver. 3.7 or earlier, it can be uploaded and displayed again. For details on the procedure for uploading observation data, see the “BioStation CT Ver. 3.8 Instructions (PC Operations).”

mark	Switch the display order of the sort result between ascending order and descending order.
mark	Display the sort result from top to bottom cyclically so that the first sample comes after the last sample.

* The most recently pressed sort button is underlined in pink.



Sample list window

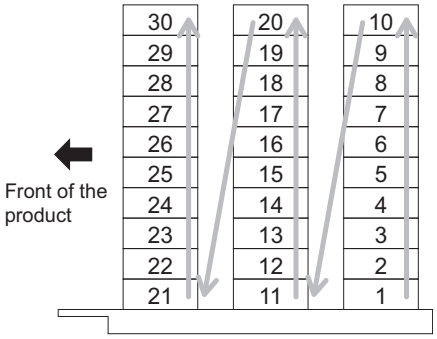
No.	Name	Function
(1)	Latest photo button	Press this button to display the Latest photo list window. (See Section 4.7.6.)
(2)	Input information button	Press this button to display the Basic information batch input window for selected multiple samples. (See Section 4.7.5.)

No.	Name	Function
(3)	Download preparation area	Prepare for downloading. (See Section 4.11.) **Point images button:** Perform download preparation of the Point images of selected multiple samples. **Fullscan images button:** Perform download preparation of the Full Scan images of selected multiple samples. **Tiling images button:** Perform download preparation of the Tiling images of selected multiple samples.
(4)	Open area	Press the area to display all sample names in the holder.
(5)	Close area	Press the area to display only the first sample in the holder.
(6)	Close button	Press this button to close the Sample list window.
(7)	In sort button	Press this button to sort the list in order of loaded date.
(8)	Out sort button	Press this button to sort the list in order of unloaded date.
(9)	Status sort button	Press this button to sort the list in order of status.
(10)	Type sort button	Press this button to sort the list by the type of culture vessels.
(11)	All check button	Press this button to select all displayed samples or cancel the selection.
(12)	Sample Name sort button	Press this button to sort the list in order of sample name.
(13)	User Name sort button	Press this button to sort the list in order of user name.
(14)	Sample comment sort button	Press this button to sort the list in order of sample comment.
(15)	Observation status sort button	Press this button to sort the list in order of observation status.  (Pink) : Scheduled observation images are included  (Green) : Live observation images are included (No icon) : No observation images
(16)	Sample list	The user name and the sample name of the sample are listed.

Stocker number

The three-row and ten-column stocker is numbered from 1 to 30 as shown in the figure on the right side.

The rear stocker is numbered from 1 to 10, the center stocker is numbered from 11 to 20, and the front stocker is numbered from 21 to 30.



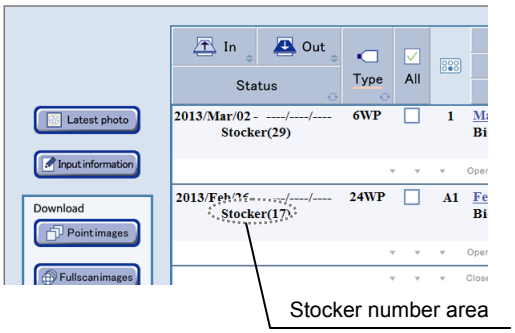
Stocker number

These stocker numbers are displayed in the stocker number area in the window.

When a sample is being unloaded (Medium change), “OUT” is displayed in the Stocker number area.

In the Sample list window displayed using the Sample list button, samples that have been cultured (No return) are not displayed.

Samples that have been cultured (No return) are displayed on the Search result sample list window that is displayed using the Search button.

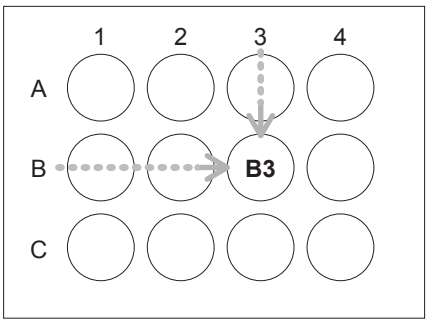


Holder map number

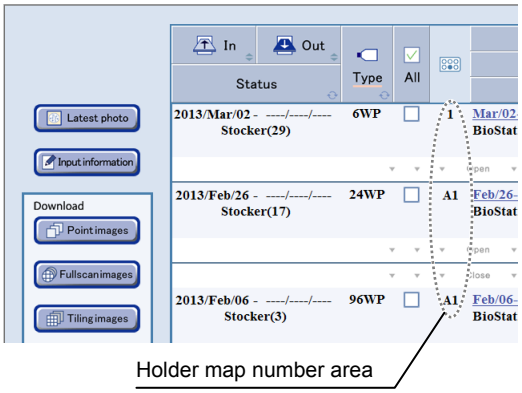
A sample position in a 12-well plate, 24-well plate, 48-well plate or 96-well plate is displayed using a letter of alphabet and a number.

The figure on the right side is an example of a 12-well plate. The columns are named from up to down in alphabetical order and the rows are named from left to right in numerical order. For example, the sample in the third row and the second column is named “B3.”

A sample position in a 6-well plate, flask, or dish is displayed with a number.



Holder map number



4.7.2 Image review window

The observation history and the operation history are displayed along a time-axis in the Image review window. The Display switch tab can switch between the observation history and the operation history.

Photos tab

Press this tab to display the observation history only.

Operation history tab

Press this tab to display the operation history only.

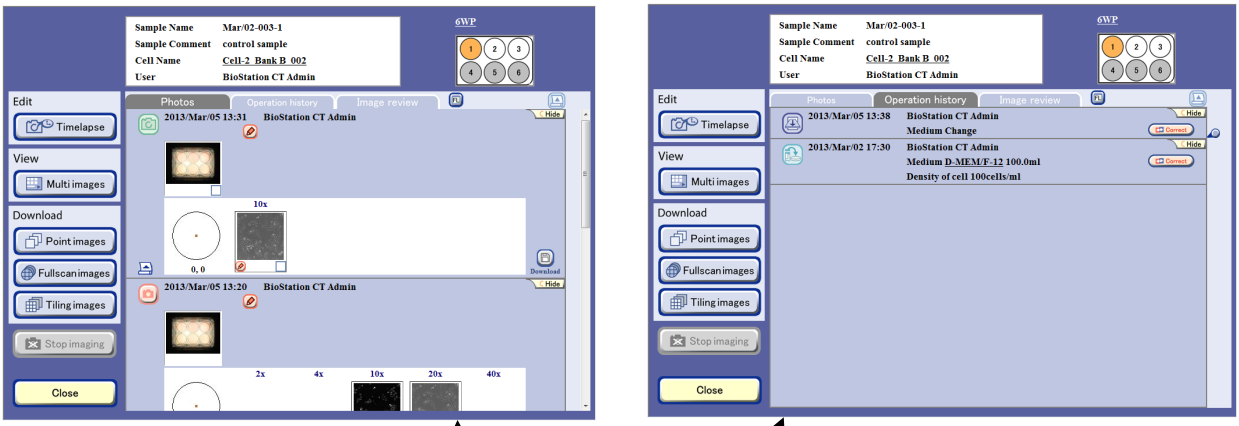


Photo display in the Image review window

Operation display in the Image review window

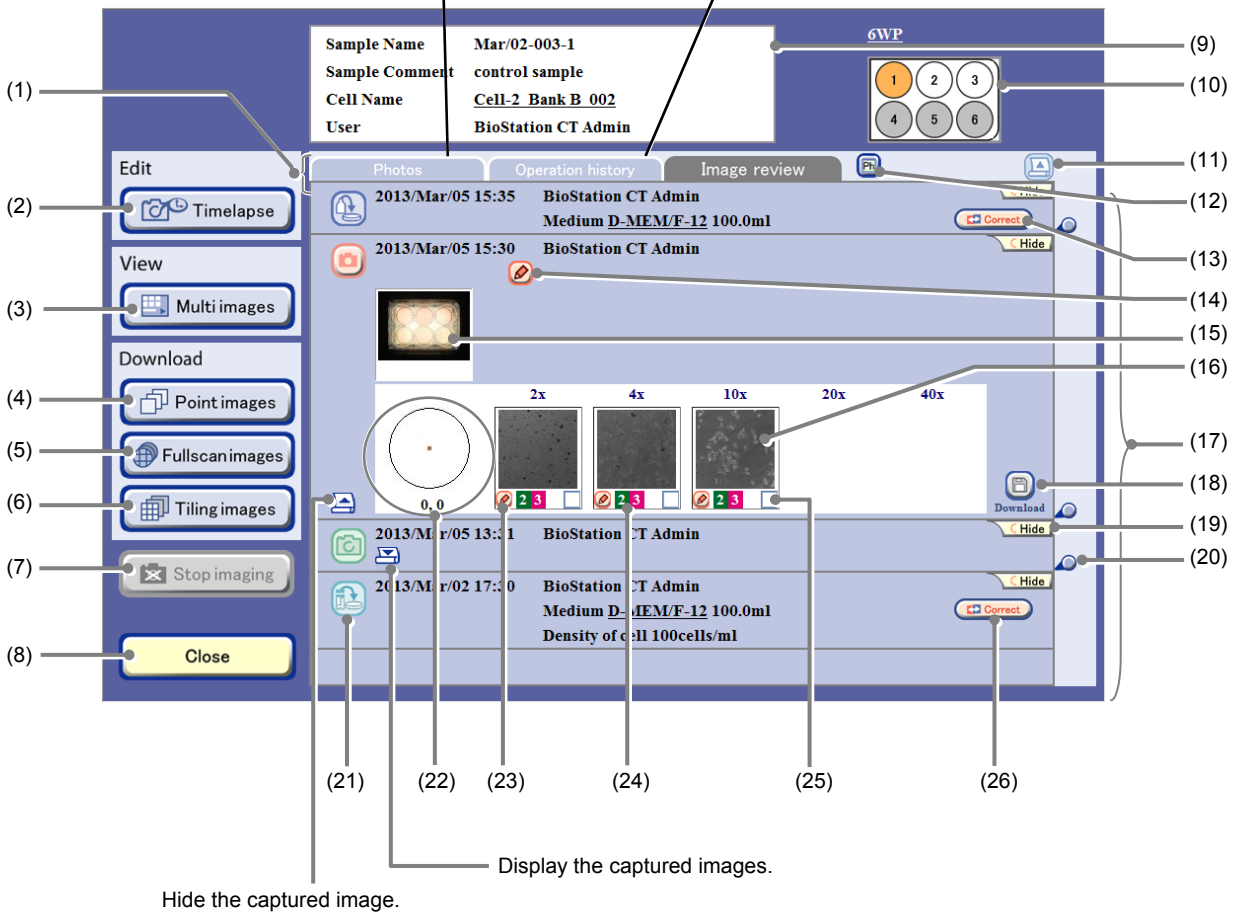


Image review window

No.	Name	Function
(1)	Display switch tab	**Photos tab:** Display the observation history only. **Operation history tab:** Display the operation history only. **Image review tab:** Display both the observation history and the operation history.
(2)	Timelapse button	Press this button to display the Schedule confirmation window.
(3)	Multi images button	Press this button to display the Multi images display window with selected multiple scheduled observation images next to each other. (See Section 4.9, “Multi Images Display of Captured Image.”)
(4)	Point images button	Press this button to perform the download preparation of all Timelapse images captured by the Point observation.
(5)	Fullscan images button	Press this button to perform the download preparation of all Timelapse images captured by the Full Scan observation.
(6)	Tiling images button	Press this button to perform the download preparation of all Timelapse images captured by the Tiling observation.
(7)	Stop imaging button	Press this button to cancel the execution of the scheduled observation in stage exclusive mode or when some sample is waiting on the stage before performing scheduled observation, stop the wait state of the sample and cancel the scheduled observation. (Enabled only while executing)
(8)	Close button	Press this button to display the previous window.
(9)	Basic information display area	This area displays the Basic information edit window. Edit the basic information of a sample.
(10)	Holder map	This area displays the position of the sample of the displayed history. Samples can be selected also by pressing the Holder map button. The selected sample is shown in orange.
(11)	 Expand button	Press this button to hide all captured images.
(12)	Thumbnail display switch button	Press this button to switch between phase contrast images and fluorescence images for thumbnails when fluorescence images are included.  : Display the phase contrast thumbnail images.  : Display the fluorescence thumbnail images.
(13)	 Load information edit button	Press this button to display the Load information edit window. (See Section 4.7.4.3.)
(14)	Observation history comment button	Press this button to display the Observation history comment edit window. (See Section 4.7.4.4.)
(15)	Thumbnail images (Macro image)	This area displays the macro thumbnail images. Press the macro thumbnail image to display the Macro image display window.
(16)	Thumbnail images (Micro image)	This area displays the micro (Ph and/or FL) thumbnail images. Press the micro thumbnail image to display each image display window.

No.	Name	Function
(17)	Observation history display area	This area displays the history in observation/operation date order. In Live observation, an observation history is added each time a sample is observed. When the Scheduled observation mode is set to the Normal mode, a new observation history is created when each Round is finished. When the Stage exclusive mode is set for scheduled observation, a new observation history is created when 1 Round observation is finished.
(18)	 Download preparation button	Press this button to display the Image download setting window.
(19)	 Hide button	Press this button to hide the observation data.
(20)	 Redisplay button	Press this button to redisplay the hidden observation data.
(21)	Observation status icon	This icon displays the observation status. (See Section 4.7.3)
(22)	Observation positions and Coordinate of the observation point	This area displays the observation positions in the culture vessel. The coordinate (x,y) for the observation point on the selected image is displayed, where (0,0) is the center of the sample.
(23)	Micro image comment button	Press this button to display the Micro image comment edit window. (See Section 4.7.4.5.)
(24)	FL channel number	Display the FL channel number with which the fluorescence image was captured. Multi is displayed when images were captured using 4 or more channels.
(25)	Download image selection button	Press this button to select the image for download. A checked symbol appears in the check box for the image.
(26)	 Medium change information edit button	Press this button to display the Medium change information edit window. (See Section 4.7.4.2.)

Information data in the Image review window cannot be updated automatically.
To update the information data, return to the previous window, and then open the Image review window again.

4.7.3 Displaying Images of the Culture Sample and History Information

This section describes the procedure for displaying the captured images of culture sample and their history information in the Image review window. The following buttons and links are used to switch the Image review window to another window.

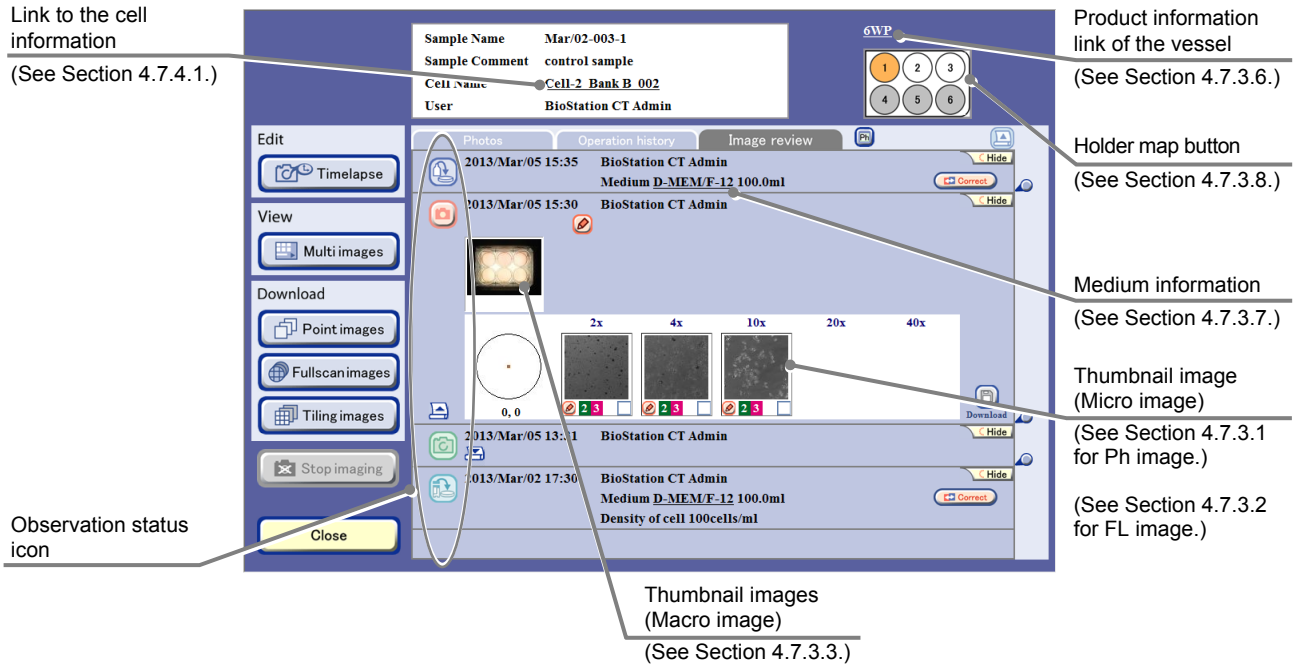


Image review window

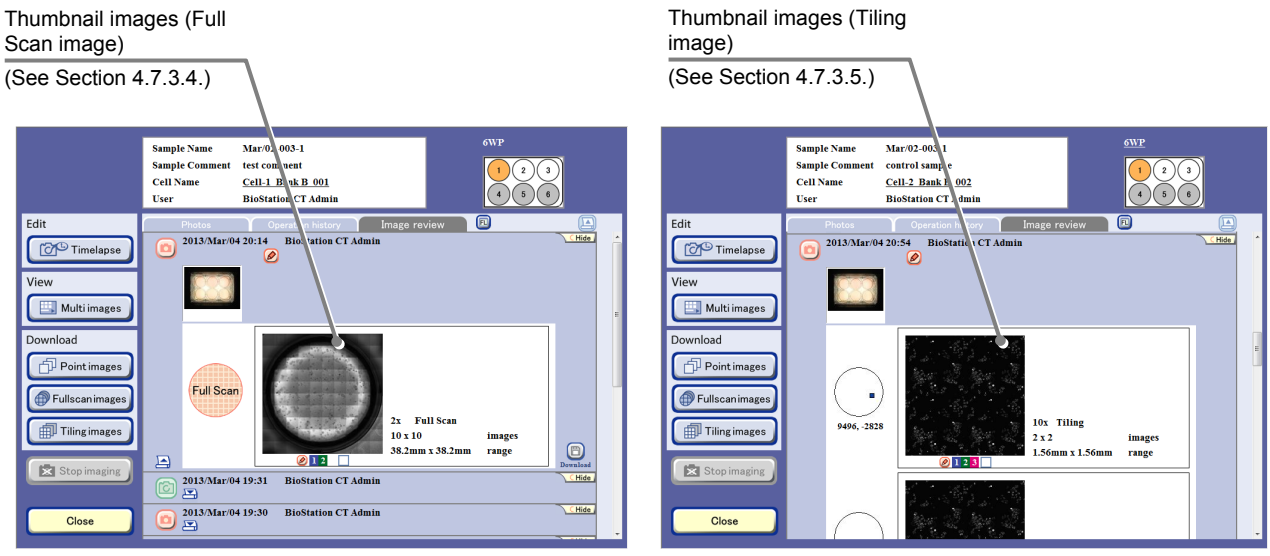


Image review window (Full Scan image)

Image review window (Tiling image)

The observation status icon on the left side of each observation data indicates the history as follows:

Icon/Button	Color	Detail
	Blue	This history icon indicates that culturing has started.
Normal mode 	Red	These observation history buttons indicate that scheduled observation has been performed. Macro images and micro images (Ph images and FL images) at each observation position are displayed in order of magnification. Press either of these buttons to check capture conditions. If one of the following error messages is displayed next to the icon, schedule observation has been skipped. The details are as follows: Error (Loader unit) : An error occurred in the loading unit. Error (Observation unit) : An error occurred in the observation unit. Error (Macro camera) : An error occurred in the macro camera. Error (Micro camera) : An error occurred in the micro camera. Error (PC disk full) : Insufficient disk space on the control PC
Stage exclusive mode 	Orange	
	Green	This icon indicates that a live observation has been performed. Captured macro images, micro images (Ph image and FL image) for each observation position are displayed in order of capturing.
Normal mode 	Red	This button indicates that a scheduled observation was skipped. Press this button to check capture conditions. One of the following messages will be shown. System stop : The microscope was stopped. Live observation (with a user name) : The user skipped a scheduled observation during a live observation. Delay (without a user name) : The user unlocked the access gate during a live observation. Delay (without a user name) : An operation such as loading or unloading a sample caused the schedule to be canceled.
Stage exclusive mode 	Orange	
	Blue	This icon indicates that the medium has been changed.
	Violet	This icon indicates that the sample was unloaded to check or unloaded with other sample in the same holder.
	Blue	This icon indicates that samples are being unloaded for a medium change or the culture has been finished.

4.7.3.1 Viewing the micro image (Ph image) and preparing download

(1) Press the Expand button for the desired history data to display images in the Image review window.

The history area expands to display the captured thumbnail images.

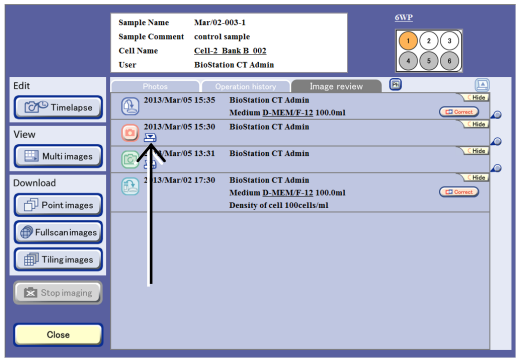


Image review window

(2) Display the Ph image.

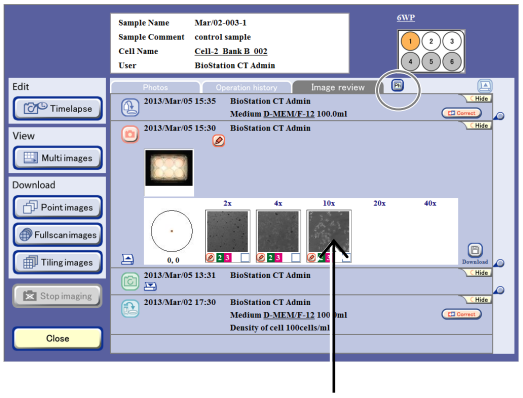
1. Change the Thumbnail display switch button to [Ph].

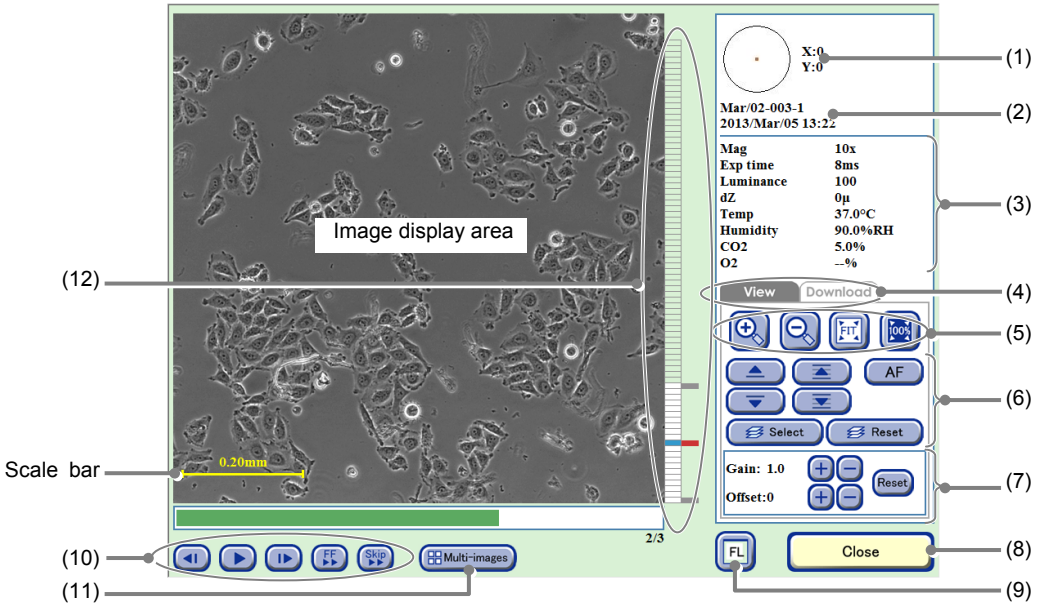
2. Press a thumbnail image of the micro image.

The Point image display window (Ph) appears.

The figure below shows an example of capturing an image count of 21 for a Z stack image in a scheduled observation.

For details on Z stack settings, see Chapter 5, “Environmental Settings” and Section 4.6.2, “Setting the Scheduled Observation Conditions.”





Point image display window (Ph)

No.	Name	Function
(1)	Coordinate of the observation point	This area displays the coordinate (x,y) for the observation point where (0,0) is the center of the sample.
(2)	Sample information	This area displays the observation point, sample name, and captured date and time.

No.	Name	Function
(3)	Image capture conditions and environmental information	This area displays the Image capture conditions and the temperature, humidity, and CO_2 concentration and O_2 concentration (optional) in the CO_2 incubator.
(4)	Operation window switch tabs	Press a tab to switch the operation window. The Download tab includes the functions shown below. **Z stack button:** Create a download file for the micro image selected with the Select button. **Timelapse button:** Create a download file for images captured in all rounds at the same observation point (Z position) as that of the image displayed. **All Z & T button:** Create a download file for all Z stack images captured in all rounds.
(5)	Display size change buttons	**+, - buttons:** Enlarge or reduce the image. **FIT button:** Display the image on the full screen. **100% button:** Display the image with the actual size.
(6)	Z-axis direction display switch	**▲/▼ buttons:** Display the captured images changing the image one by one in the Z-axis direction. **⏫/⏬ buttons:** Continuously play back images in the Z-axis direction. Press the button again to pause the playback. **AF button:** Display the image captured at the autofocus position. **Select button:** Select the Z image to be downloaded. **Reset button:** Reset an image to be downloaded.
(7)	Brightness adjustment of a displayed image	Adjust the brightness of a displayed image. **Gain:** Press the +/- button to adjust the contrast by ±0.1. **Offset:** Press the +/- button to adjust the brightness by ±5. **Reset button:** Reset the brightness of a displayed image.
(8)	Close button	Press this button to close the Point image display window (Ph).
(9)	FL button	Press this button to switch to Point image display window (FL). (See Section 4.7.3.2.)
(10)	Timelapse images playback button	**► button:** Play back timelapse images continuously. During playback, this button changes to the pause button and pressing the button pauses playback. **I►/◄I button:** Play one frame forward /one frame backward **FF button:** Fast-forward an image. **Skip button:** Skip the image for the predefined frame during playback.

No.	Name	Function
(11)	Multi-images button	Press this button to display the Multi images display window. Selected multiple scheduled observation images can be displayed and played back side by side. (See Section 4.9, “Multi Images Display of Captured Images.”)
(12)	Z position display area	Press (click) somewhere in the Z position display area to display the image at the selected position. In the Z position display area, the AF position is indicated in red, and gray marks are displayed at the positions located in increments of ten images from the AF position.

Z stacks

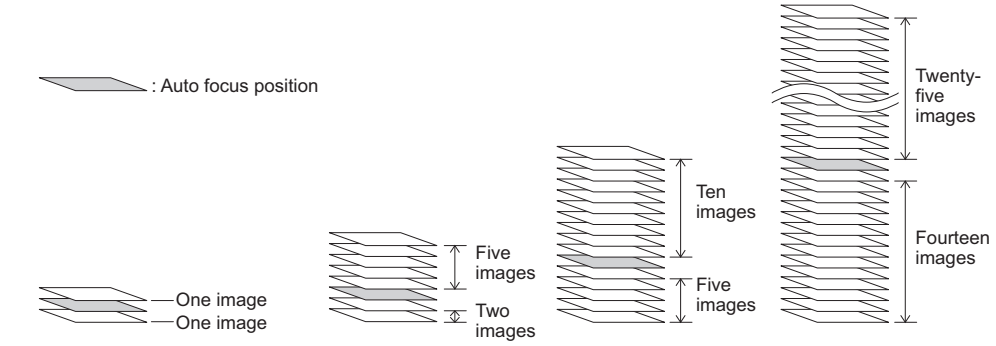
Micro images captured in a scheduled observation are a set of images taken at slightly different positions along the Z-axis centered on the autofocus position. The image set is called a Z stack image.

For Z stack images, there are two ways to capture images; by using a fixed pitch to capture images at a fixed pitch and by using a selectable pitch to capture images at a specified range and pitch.

Fixed pitch images

Images are captured at a predefined range and pitch by selecting the number of images (1, 3, 8, 16, or 40).

The autofocus position in a Z stack image captured using a fixed pitch is defined for each image count by default as shown below. However, if a position near the upper or lower limit of the Z-axis is set as the autofocus position, the image count of the upper part and the lower part will change. (Example: If the lowest position in the Z-axis is set as the autofocus position in a fixed pitch capturing of eight images, the lower part image count is zero and the upper part image count is seven.)



Additionally, if a custom observation point is selected and the custom focus button is pressed to capture an image in scheduled observation, the AF position described above will be the Z position registered as the custom observation point.

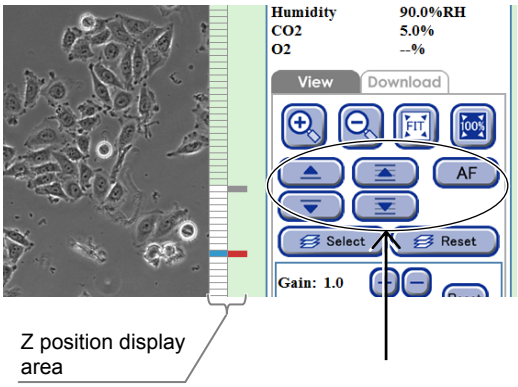
Selectable pitch images

To capture images with using a selectable pitch, determine the number of images to be captured by selecting the range in the Z-axis direction (0, 100, 200, 300, or 400 µm) and pitch (5, 10, or 20 µm).

When the selected range is 0 µm, a Z stack is not generated and only one image is captured.

When the selected range is 400 µm and the pitch is 5 µm, the number of capturing images is the maximum (81 images).

3. **To display the micro images captured at the positions shifted by small increments in the Z-axis direction, press the ▲/▼/⏫/⏬ buttons.**



Press the ▲/▼ buttons to display the captured images changing the image one by one in the Z-axis direction.

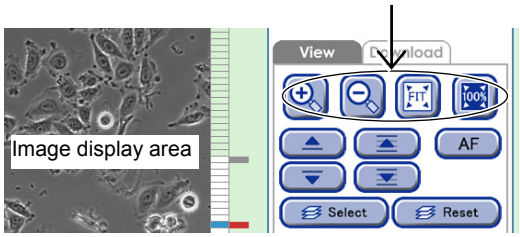
Press the ⏫/⏬ buttons to continuously play back images in the Z-axis direction. Press the button again to pause the playback.

Press (click) somewhere in the Z position display area to display the image at the selected position.

Press the AF button to display the image captured at the autofocus position.

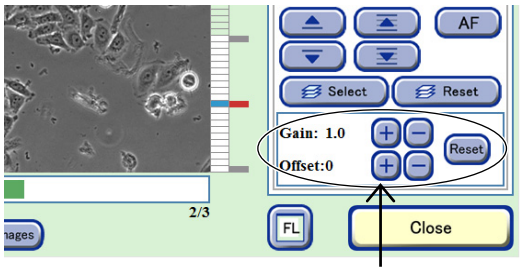
In the Z position display area, the AF position is indicated in red, and gray marks are displayed at the positions located in increments of ten images from the AF position.

4. **Change the display size of the image.**



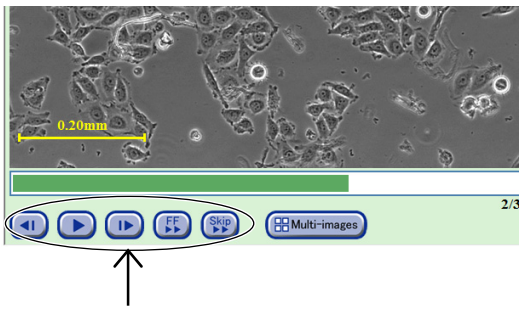
Press +/-, FIT or the 100% button to change the display size of the image.

5. **Adjust the contrast and brightness of an image.**



Use the Gain and Offset +/- buttons to adjust the contrast and brightness of a displayed image.

6. **To play back the displayed timelapse images, press the ► button, and to proceed to the next image or to return to the previous image, press the I► button or the ◄I button.**



To fast-forward the image, press the FF button.

Each time the FF button is pressed, the playback speed is switched. (The speed is switched in the order of 1-step → 2-step → Normal.)

To skip the playback with a frame specified, press the Skip button.

A dialog box to specify the frame to skip is displayed.

When no image is to be downloaded, go to step (6) and close the window.

(3) Select the image to be downloaded.

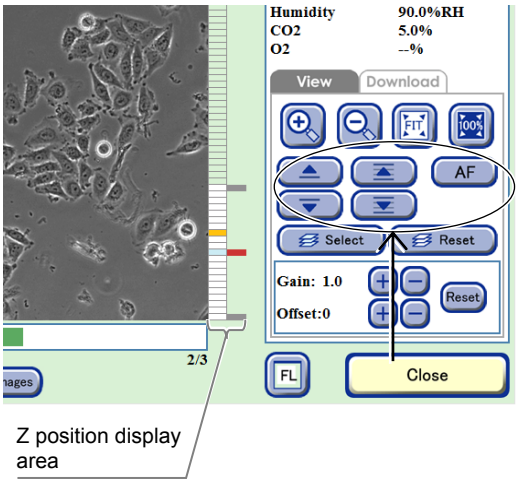
a. To put all selected images into one file

a-1. Press the ▲/▼/▲▲/▼▼ buttons to select the images to be downloaded.

Press (click) somewhere in the Z position display area to display the image at the selected position.

The Z position of the displayed Z stack image is marked in orange (or blue when the Select button is pressed) on the Z position display area.

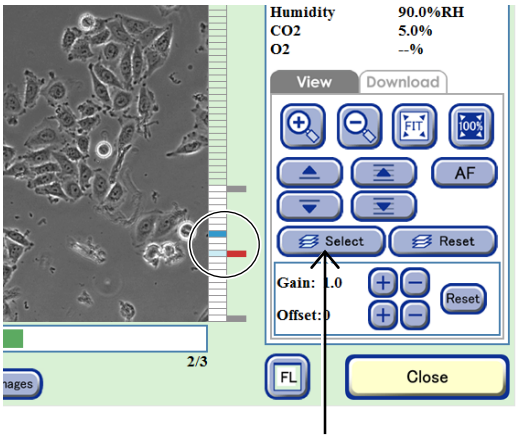
The window example on the right side displays the third image above the AF position.



a-2. Press the Select button.

The Z position of the selected image is displayed in blue.

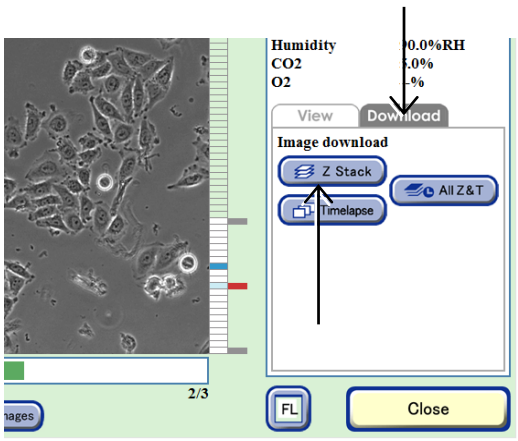
To cancel the selection, press the Reset button.



a-3. Select the Download tab and press the Z Stack button in Image download area.

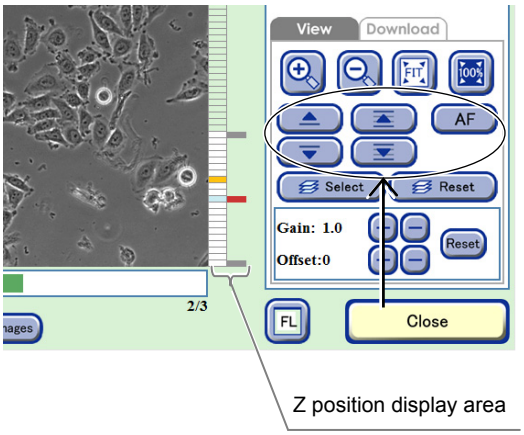
The Image download setting window appears.

Go to step (4).



b. To select and arrange all images for the observation position of the displayed image (Z) in chronological order

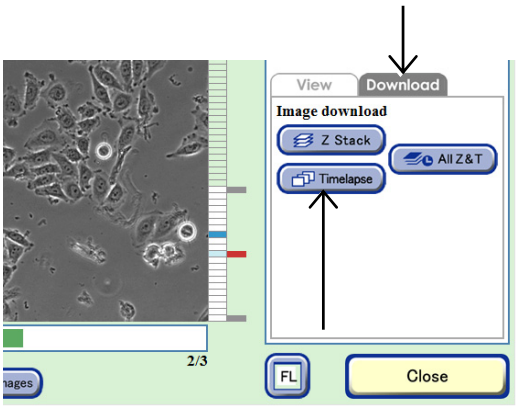
b-1. Press the ▲/▼/▲̄/▼̄ buttons to select the images to be downloaded.



b-2. Select the Download tab and press the Timelapse button in Image download area.

The Image download setting window appears.

Go to step (4).



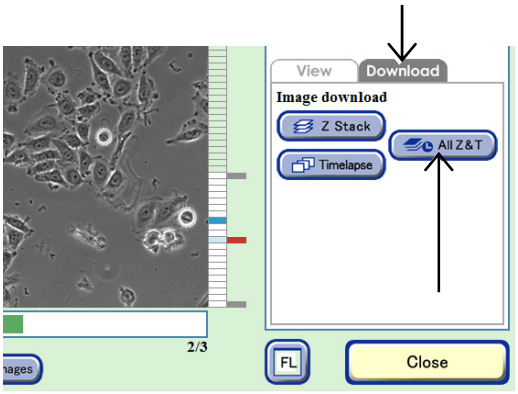
The images downloaded using the Timelapse button are based on the AF position of the Z stack images captured in each round. For example, when the Timelapse button is pressed while an image at a focus position two up from the AF position is displayed in the Image display area, all images at the focus position two up from the AF position will be downloaded for all rounds.
However, download preparation cannot be performed for a round without images at that focus position.

c. To select and arrange all Z stack images captured in all rounds in chronological order

c-1. Select the Download tab and press the All Z & T button in Image download area.

The Image download setting window appears.

Go to step (4).



(4) Set the format conditions of the images to be downloaded.

The allowable conditions depend on the type of image to be downloaded.

Set the format conditions of the images to be downloaded, and then press the OK button.
After download preparation is completed, the download preparation complete dialog box appears.

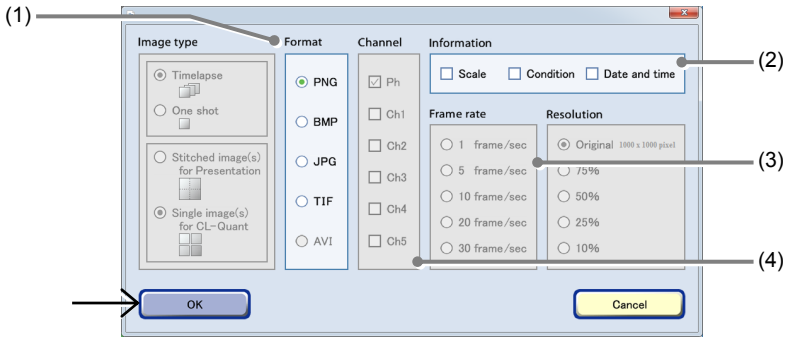


Image download settings window

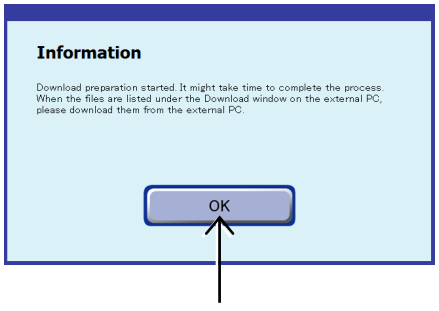
No.	Name	Function
(1)	Format area	Select the format of the images to be downloaded. AVI is enabled only when the Timelapse button in the Image download area is used for download.
(2)	Information area	Select the image information to be added. Three types of image information can be added: Scale, Condition and Date and time. In addition, when AVI is selected for the format, Scale cannot be selected.
(3)	Frame rate area	Select the duration of playback per image. Enabled only when the AVI format is set.
(4)	Channel area	Select the channels to be downloaded. Enabled only when an image captured also by fluorescence capturing is selected and the All Z & T button in the Image download area is used for download.

(5) Press the OK button.

Download preparation for the micro images is completed.

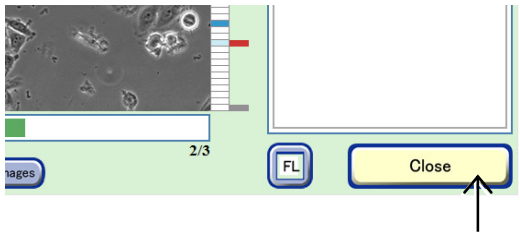
The Point image display window (Ph) appears.

> For details on the procedure for downloading the prepared file data to an external PC, see the "BioStation CT Ver. 3.8 Instructions (PC Operations)."



Download preparation complete dialog box

(6) Press the Close button to close the Point image display window (Ph).



Point image display window (Ph)

Caution

The scale bar displayed in the Point image display window (Ph) is not included in the original image.
An image data file prepared for download includes the original image only.

The image size is calculated as shown below. The dynamic range of the CCD camera is 8 mm × 8 mm (1000 × 1000 pixels), and when the image is captured at 2x, the above size becomes 8/2 mm × 8/2 mm (4 mm × 4 mm) (1 pixel=4 µm).

The image size of each observation magnification is calculated based on the above information.

- **2x of the image size becomes 4 mm × 4 mm (1 pixel=4 µm)**
- **4x of the image size becomes 2 mm × 2 mm (1 pixel=2 µm)**
- **10x of the size becomes 0.8 mm × 0.8 mm (1 pixel=0.8 µm)**
- **20x of the image size becomes 0.4 mm × 0.4 mm (1 pixel=0.4 µm)**
- **40x of the image size becomes 0.2 mm × 0.2 mm (1 pixel=0.2 µm)**

4.7.3.2 Viewing the micro image (FL image) and preparing download

(1) Press the Expand button for the desired history data to display images in the Image review window.

The history area expands to display the captured thumbnail images.

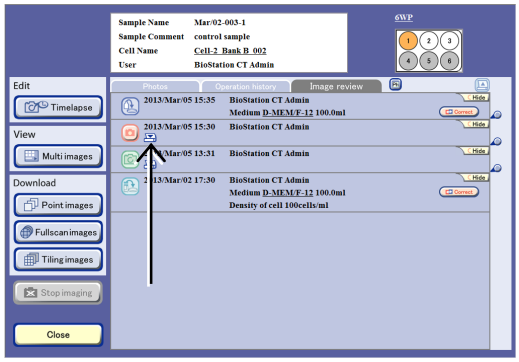


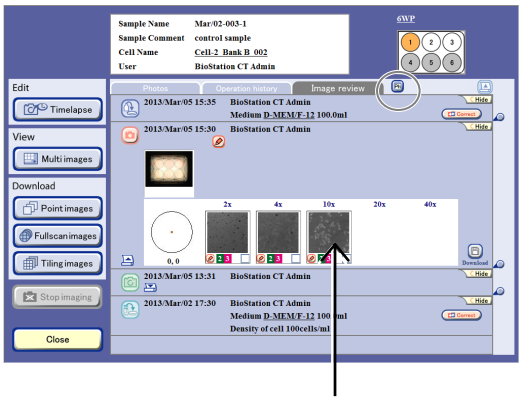
Image review window

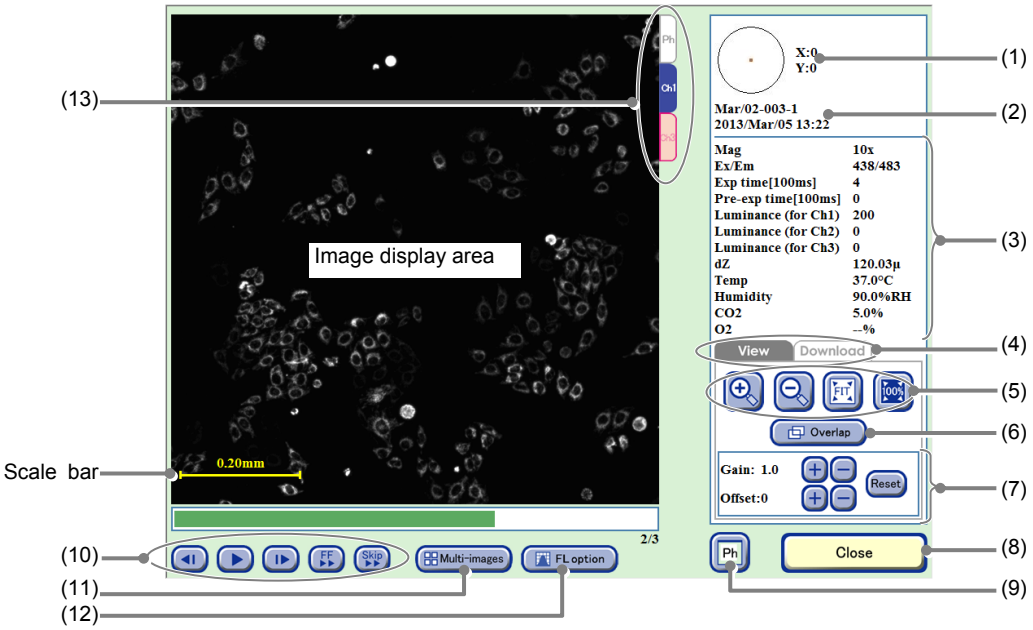
(2) Display the FL image.

1. Change the Thumbnail display switch button to [FL].

2. Press a thumbnail image of the micro image.

The Point image display window (FL) appears.





Point image display window (FL)

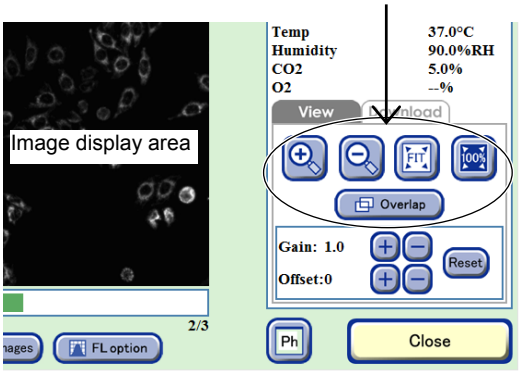
No.	Name	Function
(1)	Coordinate of the observation point	This area displays the coordinate (x,y) for the observation point where (0,0) is the center of the sample.

No.	Name	Function
(2)	Sample information	This area displays the observation point, sample name, and captured date and time.
(3)	Image capture conditions and environmental information	This area displays the Image capture conditions and the temperature, humidity, and CO_2 concentration and O_2 concentration (optional) in the CO_2 incubator.
(4)	Operation window switch tabs	Press a tab to switch the operation window. The Download tab includes the functions shown below. **Timelapse button:** Create a download file for images captured in all rounds at the same observation point as that of the image displayed.
(5)	Display size change buttons	**+, - buttons:** Enlarge or reduce the image. **FIT button:** Display the image on the full screen. **100% button:** Display the image with the actual size.
(6)	Overlap button	Press this button to overlay the fluorescence image and the phase contrast image.
(7)	Brightness adjustment of a displayed image	Adjust the brightness of a displayed image. **Gain:** Press the +/- button to adjust the contrast by ±0.1. **Offset:** Press the +/- button to adjust the brightness by ±10. **Reset button:** Reset the brightness of a displayed image.
(8)	Close button	Press this button to close the Point image display window (FL).
(9)	FL button	Press this button to switch to Point image display window (Ph). (See Section 4.7.3.1.)
(10)	Timelapse images playback button	**► button:** Play back timelapse images continuously. During playback, this button changes to the pause button and pressing the button pauses playback. **I►/◄I button:** Play one frame forward /one frame backward **FF button:** Fast-forward an image. **Skip button:** Skip the image for the predefined frame during playback.
(11)	Multi-images button	Press this button to display the Multi images display window. Selected multiple scheduled observation images can be displayed and played back side by side. (See Section 4.9, “Multi Images Display of Captured Images.”)
(12)	FL option button	Press this button to display analysis results showing a histogram of the intensity value for the images captured in chronological order. This button is displayed only when the image captured in scheduled observation appears. (See Section 4.8, “Analysis of Fluorescence Intensity.”)
(13)	Display switch tabs	Press a tab to switch the channel to check an image.

3. Change the display size of the image.

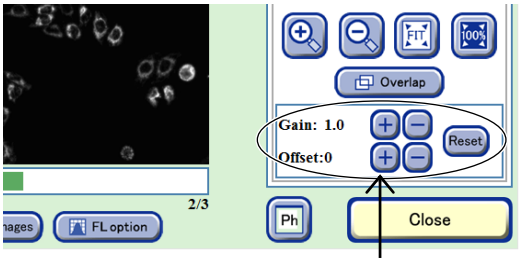
Press +/-, FIT or the 100% button to change the display size of the image.

Overlay the phase contrast image with the fluorescence image by pressing the Overlap button.



4. Adjust the contrast and brightness of an image.

Use the Gain and Offset +/- buttons to adjust the contrast and brightness of a displayed image.



5. To play back the displayed timelapse images, press the ► button, and to proceed to the next image or to return to the previous image, press the I► button or the ◄I button.

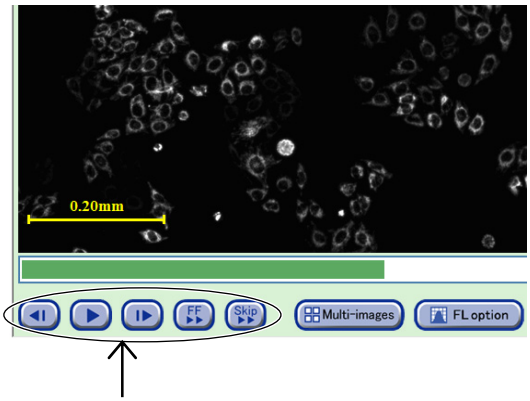
To fast-forward the image, press the FF button.

Each time the FF button is pressed, the playback speed is switched. (The speed is switched in the order of 1-step → 2-step → Normal.)

To skip the playback with a frame specified, press the Skip button.

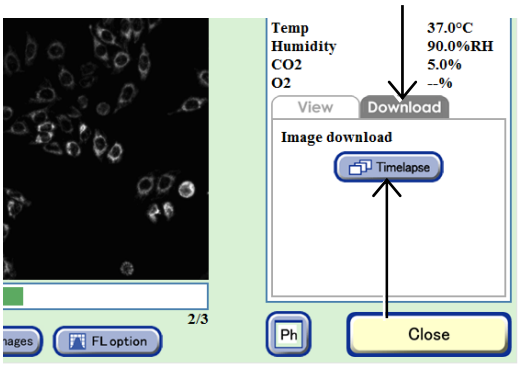
A dialog box to specify the frame to skip is displayed.

When no image is to be downloaded, go to step (6) and close the window.



(3) Select the Download tab and press the Timelapse button in Image download area.

The Image download setting window appears.



(4) Set the format conditions of the images to be downloaded.

Set the format conditions of the images to be downloaded, and then press the OK button.
After download preparation is completed, the download preparation complete dialog box appears.

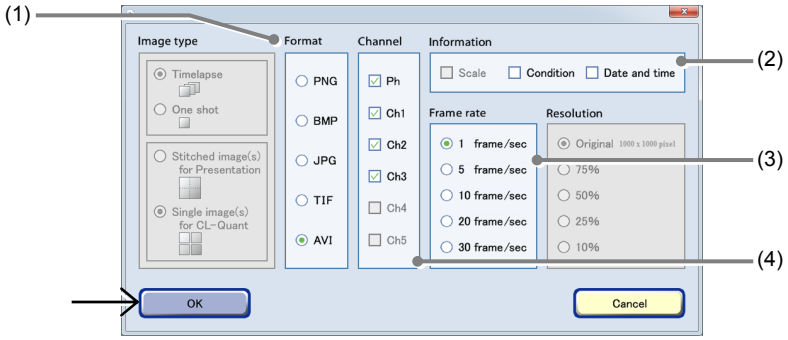


Image download settings window

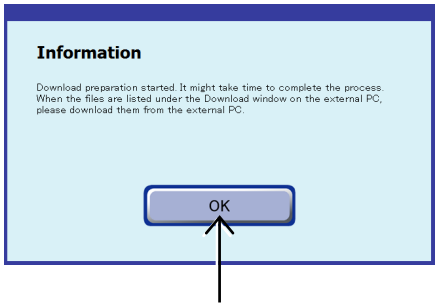
No.	Name	Function
(1)	Format area	Select the format of the images to be downloaded.
(2)	Information area	Select the image information to be added. Three types of image information can be added: Scale, Condition and Date and time. In addition, when AVI is selected for the format, Scale cannot be selected.
(3)	Frame rate area	Select the duration of playback per image. Enabled only when the AVI format is set.
(4)	Channel area	Select the channels to be downloaded.

(5) Press the OK button.

Download preparation for the micro images is completed.

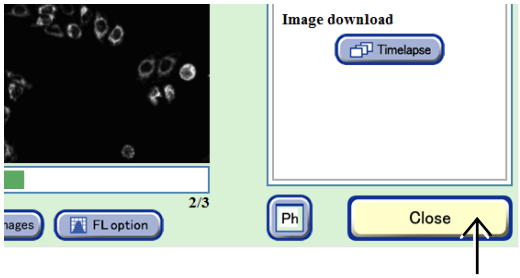
The Point image display window (FL) appears.

> For details on the procedure for downloading the prepared file data to an external PC, see the "BioStation CT Ver. 3.8 Instructions (PC Operations)."



Download preparation complete dialog box

(6) Press the Close button to close the Point image display window (FL).



Point image display window (FL)

Caution

The scale bar displayed in the Point image display window (FL) is not included in the original image.
An image data file prepared for download includes the original image only.

The image size is calculated as shown below. The dynamic range of the CCD camera is 8 mm × 8 mm (1000 × 1000 pixels), and when the image is captured at 2x, the above size becomes 8/2 mm × 8/2 mm (4 mm × 4 mm) (1 pixel=4 µm).

The image size of each observation magnification is calculated based on the above information.

- **2x of the image size becomes 4 mm × 4 mm (1 pixel=4 µm)**
- **4x of the image size becomes 2 mm × 2 mm (1 pixel=2 µm)**
- **10x of the size becomes 0.8 mm × 0.8 mm (1 pixel=0.8 µm)**
- **20x of the image size becomes 0.4 mm × 0.4 mm (1 pixel=0.4 µm)**
- **40x of the image size becomes 0.2 mm × 0.2 mm (1 pixel=0.2 µm)**

Caution

When creating AVI files with the image download function, if the number of frames is too large, the AVI file is fragmented into multiple files because the number of frames in one AVI file is limited.

The maximum number of frames is determined by the number of pixels in the image to be downloaded.

The number of pixels for one side of the image	The maximum number of frames
1000 pixels or less	1000 frames
1001 pixels or more 2000 pixels or less	250 frames
2001 pixels or more 3000 pixels or less	110 frames
3001 pixels or more 4000 pixels or less	60 frames

* **In the Full Scan image display window (Download tab), if the number of vertical pixels is different from the number of horizontal pixels on a download area that has been specified with Area select area, the limitation is applied to the larger one.**

* **For standard images (single images), since the number of pixels is 1000 × 1000 pixels, the maximum number of frames is 1000 frames.**

4.7.3.3 Viewing the macro image and preparing download

(1) Press the Expand button for the desired history data to display images in the Image review window.

The history area expands to display the captured thumbnail images.

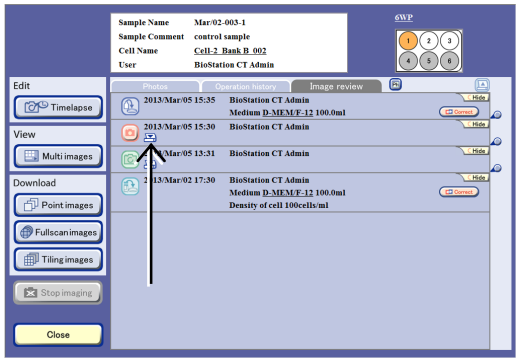
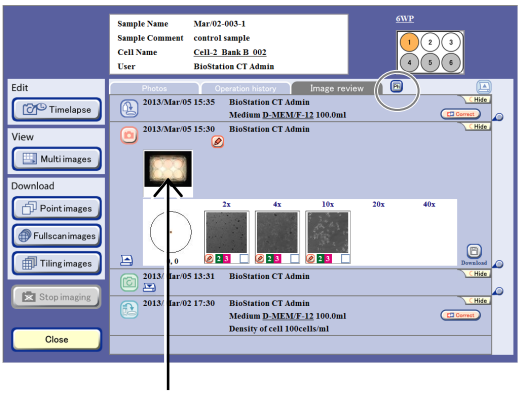


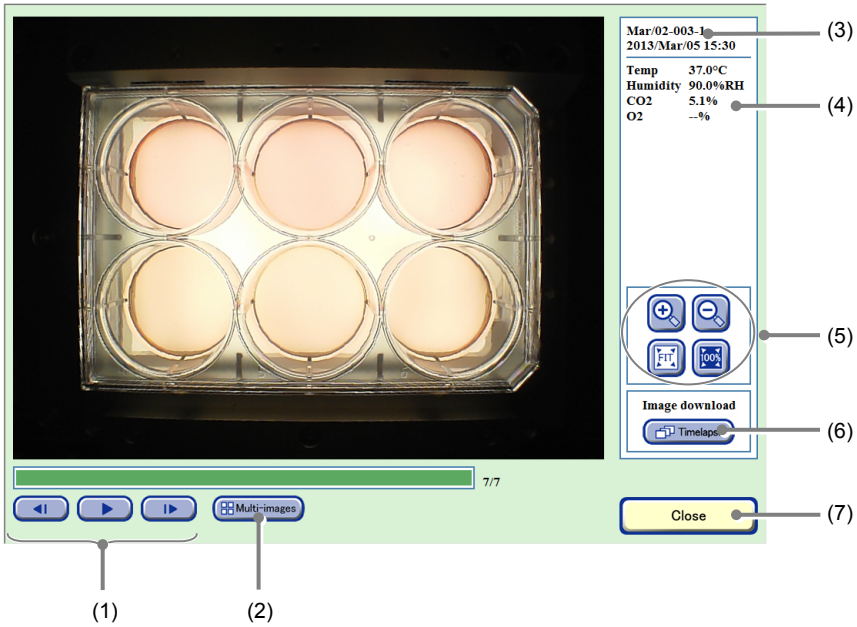
Image review window

(2) Display the macro image.

1. Press a thumbnail image of the macro image.

The Macro image display window appears.



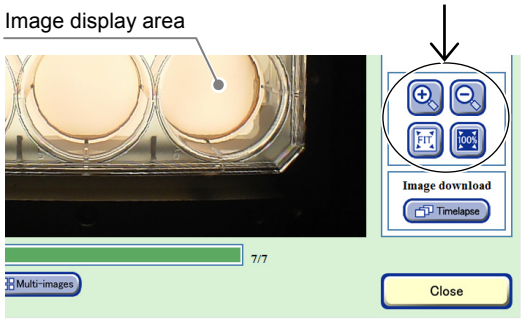


Macro image display window

No.	Name	Function
(1)	Timelapse images playback button	► button: Play back timelapse images continuously. During playback, this button changes to the pause button and pressing the button pauses playback. I►/◄I button: Play one frame forward /one frame backward
(2)	Multi-images button	Press this button to display the Multi images display window. Selected multiple scheduled observation images can be displayed and played back side by side. (See Section 4.9, “Multi Images Display of Captured Images.”)
(3)	Sample information	This area displays the observation point, sample name, and captured date and time.
(4)	Image capture conditions and environmental information	This area displays the Image capture conditions and the temperature, humidity, and CO_2 concentration and O_2 concentration (optional) in the CO_2 incubator.
(5)	Display size change buttons	**+, - buttons:** Enlarge or reduce the image. **FIT button:** Display the image on the full screen. **100% button:** Display the image with the actual size.
(6)	Timelapse button	Press this button to create a download file for images captured in all rounds at the same observation point as that of the image displayed.
(7)	Close button	Press this button to close the Macro image display window.

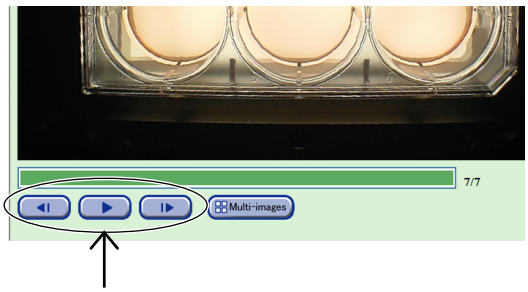
2. Change the display size of the image.

Press the +/-, FIT, or the 100% button to change the display size of the image.



3. To play back the displayed image, press the ► button, and to proceed to the next image or to return to the previous image, press the I► button or the ◄I button.

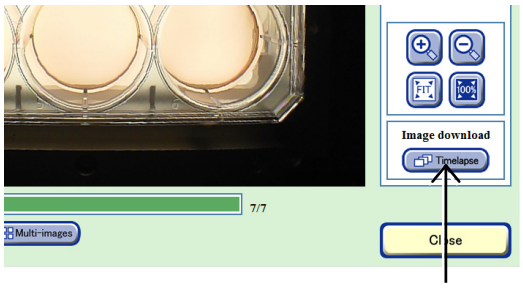
When no image is to be downloaded, go to step (4) and close the window.



(3) To create a download file in which all macro images of the samples displayed on the screen are arranged in chronological order

1. Press the Timelapse button.

The Image download setting window appears.



2. Set the format conditions of the images to be downloaded.

Set the format conditions of the images to be downloaded, and then press the OK button.
After download preparation is completed, the download preparation complete dialog box appears.

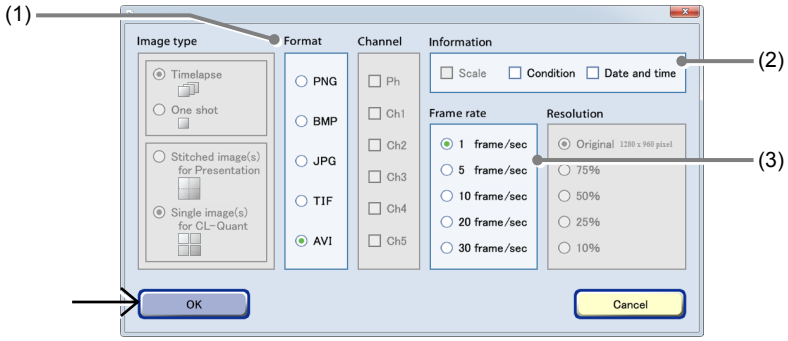


Image download settings window

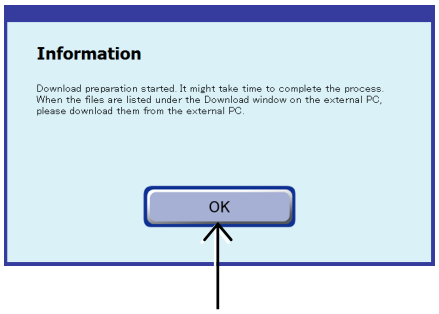
No.	Name	Function
(1)	Format area	Select the format of the images to be downloaded.
(2)	Information area	Select the image information to be added. Two types of image information can be added: Condition and Date and time.
(3)	Frame rate area	Select the duration of playback per image. Enabled only when the AVI format is set.

3. Press the OK button.

Download preparation for the macro images is completed.

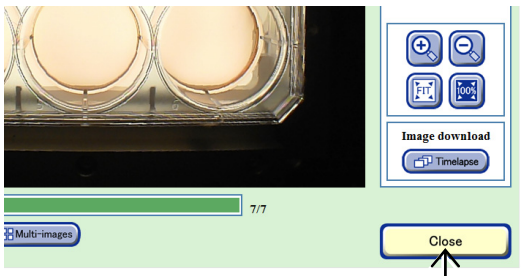
The Macro image display window appears.

> For details on the procedure for downloading the created download file data to an external PC, see the “BioStation CT Ver. 3.8 Instructions (PC Operations).”



Download preparation complete dialog box

(4) Press the Close button to close the Macro image display window.



Macro image display window

4.7.3.4 Viewing the Full Scan image and preparing download

This section describes the procedure for displaying the Full Scan images captured by scheduled Full Scan observation and also the procedure for preparing those images for download using the Full Scan image display window.

Various operations can be performed in the Full Scan image display window such as enlarging, reducing and scrolling the displayed image, playing back images in chronological order, and also preparing images of a specified area for download.

(1) Press the Expand button for the desired history data to display images in the Image review window.

The history area expands to display the captured thumbnail images.

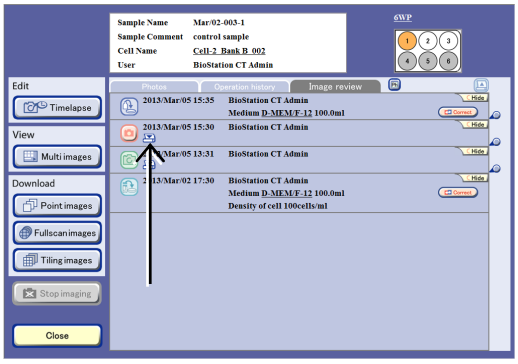
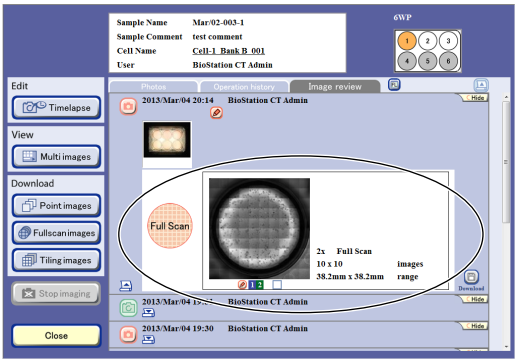


Image review window

If images captured by Full Scan observation are included, “Full Scan” is displayed in the observation position information in the culture vessel and tiled thumbnail images are displayed.

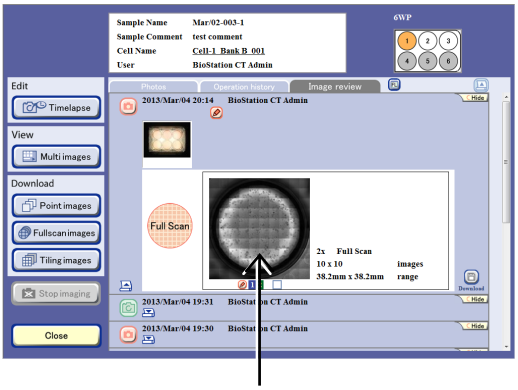
In addition, the observation magnification, number of images, and observation range are displayed on the side of the thumbnail images.

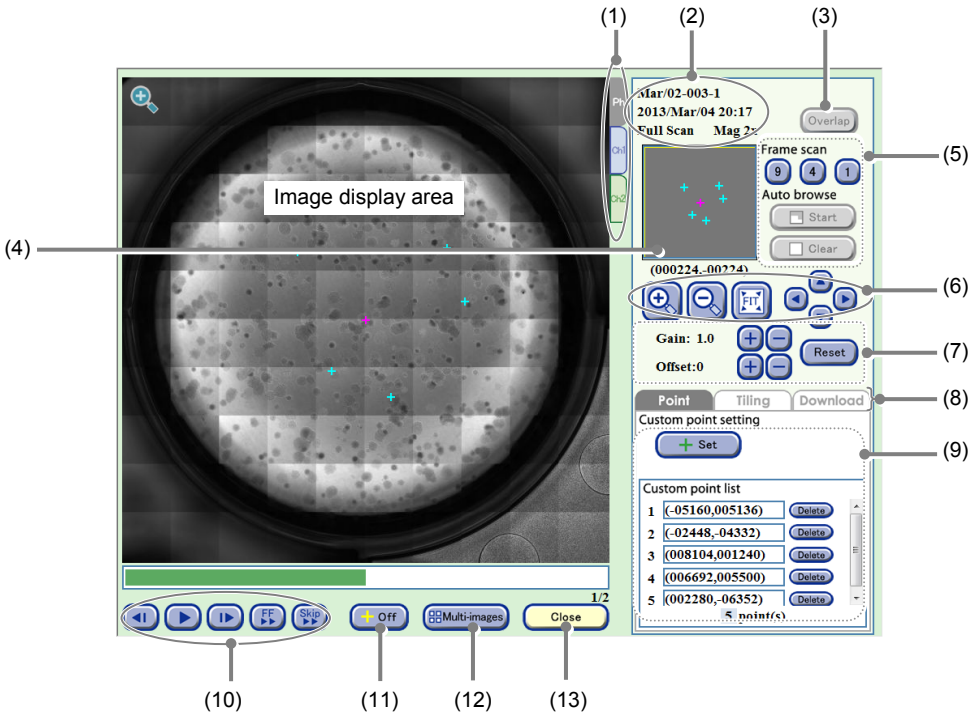


(2) Display the Full Scan image.

1. Press a thumbnail image of the Full Scan images.

The Full Scan image display window appears.





Full Scan image display window (Point tab)

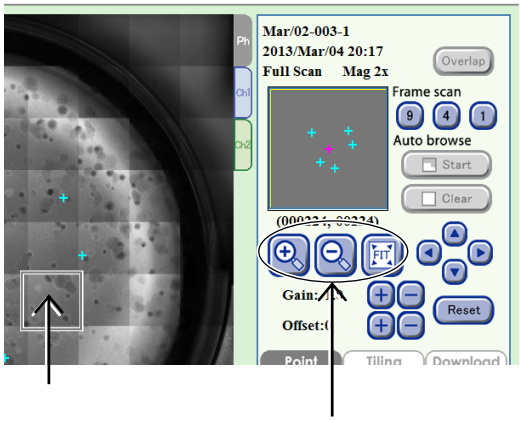
No.	Name	Function
(1)	Channel tabs	These tabs are displayed when fluorescence images are captured. Select a tab to switch the image displayed among Ph (a phase contrast image) and Ch1 to 3 (FL channel images).
(2)	Sample information	This area displays the observation point, sample name, and captured date and time.
(3)	Overlap button	Press this button to overlay the fluorescence image and the phase contrast image.
(4)	Observation point display area	This area displays all custom observation points registered in the entire sample area or the position and size of observation field of view displayed in the image display area.
(5)	Scan playback function	Press this button to play back scan of the entire field of view automatically. **Frame scan:** Plays back scan of entire field of view with the selected frame size. **Auto browse:** Plays back scan with the displayed magnification from the currently displayed point. Also enables pause, resume, or clear playback of scan.
(6)	Display size change buttons	**+, - buttons:** Enlarge or reduce the image. **FIT button:** Display the image on the full screen. **Field of view 1 frame move button:** Shift the field of view up/down, left/right by one frame.

No.	Name	Function
(7)	Brightness adjustment of a displayed image	Adjust the brightness of a displayed image. **Gain:** Press the +/- button to adjust the contrast by ±0.1. **Offset:** Press the +/- button to adjust the brightness by ±5 when the Ph channel tab is displayed. Press the +/- button to adjust the brightness by ±10 when the FL channel tab is displayed. **Reset button:** Reset the brightness of a displayed image.
(8)	Operation window switch tabs	Press a tab to switch the operation window. **Point tab:** Enable registration of custom observation point. **Tiling tab:** Enable registration of capture area for Tiling observation. (See Section 4.6.2.3, “Tiling observation.”) **Download tab:** Enable download preparation for a Full Scan image.
(9)	Custom point setting area	**+Set button:** Register the X-Y coordinate of the center of the field of view displayed in the image display area as the custom observation point. (Up to 25 positions) **Custom point list:** Display the registered custom observation point. Select a custom observation point from the list moves the field of view to that position. **Delete buttons:** Delete the registered custom observation point.
(10)	Timelapse images playback button	► button: Play back timelapse images continuously. During playback, this button changes to the pause button and pressing the button pauses playback. I►/◄I button: Play one frame forward /one frame backward **FF button:** Fast-forward an image. **Skip button:** Skip the image for the predefined frame during playback.
(11)	+Off button	Press this button to display or hide pointers displayed in the image display area.
(12)	Multi-images button	Press this button to display the Multi images display window. Full Scan observation thumbnail images are registered in the Multi images display window. (See Section 4.9, “Multi Images Display of Captured Images.”)
(13)	Close button	Press this button to close the Full Scan image display window.

2. Change the display size of the image.

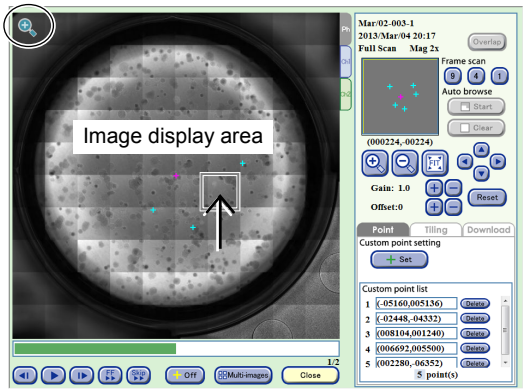
Press the + or - button, or the FIT button to change the display size of the image.

When the entire image is displayed, press a point on the tiled image displayed in the image display area to enlarge the image at the selected point.

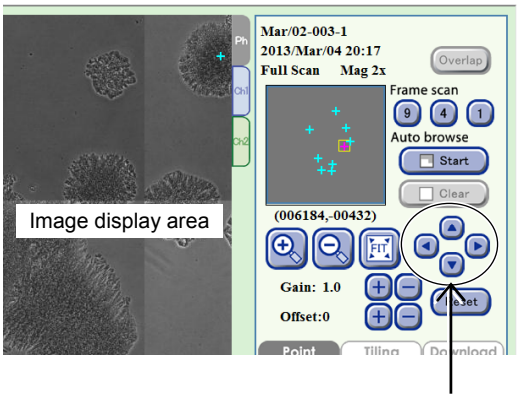


When the magnifying glass icon is displayed in the image display area, select a point in the image display area to enlarge the image at the selected point.

When the magnifying glass icon is not displayed, press a point in the image display area to move the center of the field of view to the pressed position.

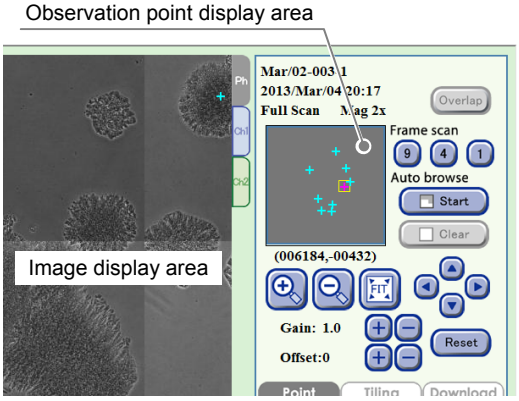


Press the Field of view 1 frame move button to shift the field of view upward, downward, leftward or rightward by the same amount as the size of the current field of view displayed in the image display area.



In the observation point display area, the observation field of view displayed in the image display area is displayed with a yellow rectangle, which indicates the position and size of the observation range relative to the entire image.

The registered custom observation points are indicated with light blue pointers.



3. The entire field of view can be played back automatically.

There are two types of scan playback: Frame scan and Auto browse.

Frame scan:

Scan playback is performed for the entire field of view at the selected field size.

There are three sizes for the field of view: [9] (3×3 images), [4] (2×2 images), and [1] (1 image). Select the size of the field of view. A scan playback start confirmation dialog box appears.

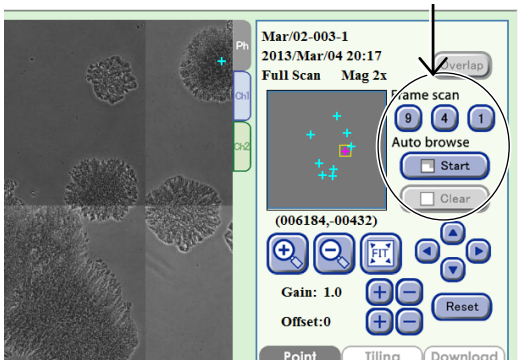
Press the OK button to start the scan playback from the upper left of the image to the right.

Auto browse:

Scan playback is performed using the magnification of the currently displayed point. Scan playback can be paused, resumed or cleared using the buttons.

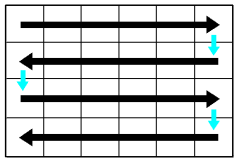
Press the Start button in the Auto browse field. A scan playback start confirmation dialog box appears.

Press the OK button to start the scan playback from the currently displayed position to the right.



The scan playback performs a reciprocating scan to play back.

The first line at the starting point is scanned from left to right (forward), and the second line is scanned from right to left (return).



An area already displayed by scan playback turns green in the observation point display area.

During scan playback, both Frame scan and Auto browse can be performed using the buttons in the Auto browse field.

When scan playback starts, the Start button changes to the Stop button.

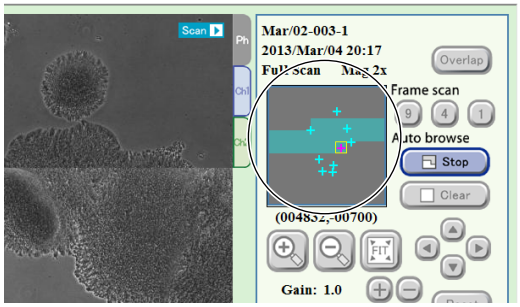
Press the Stop button to pause scan playback. The button name changes to Continue.

Press the Continue button to resume scan playback.
The button name changes to Stop.

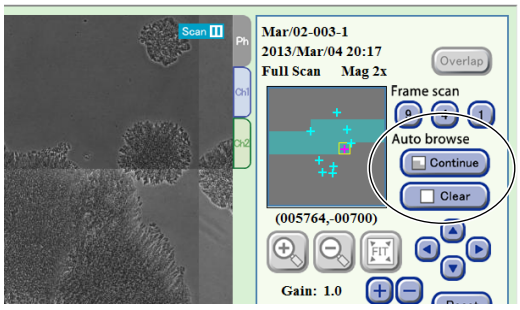
Press the Clear button to restore the original color of the green area in the observation point display area that indicates the area played back by scan playback.

Press the Start button to display the scan playback start confirmation dialog box. Press the OK button to start scan playback from the current position. The button name changes to Stop.

Alternatively, when an enlarged image is displayed, press the Start button in the Auto browse field to start scan playback from the current position at the current size of the field of view.

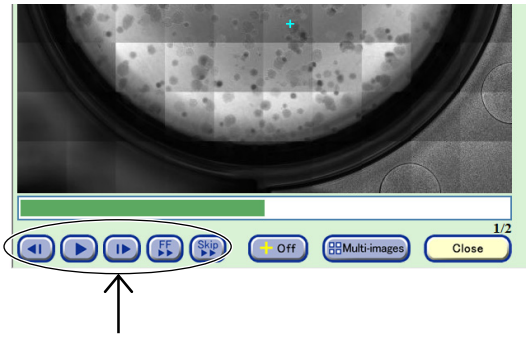


During scan playback



While scan playback is paused

4. **To play back the displayed image, press the ► button, and to proceed to the next image or to return to the previous image, press the I► button or the ◄I button.**



To fast-forward the image, press the FF button.

Each time the FF button is pressed, the playback speed is switched. (The speed is switched in the order of 1-step → 2-step → Normal.)

To skip the playback with a frame specified, press the Skip button.

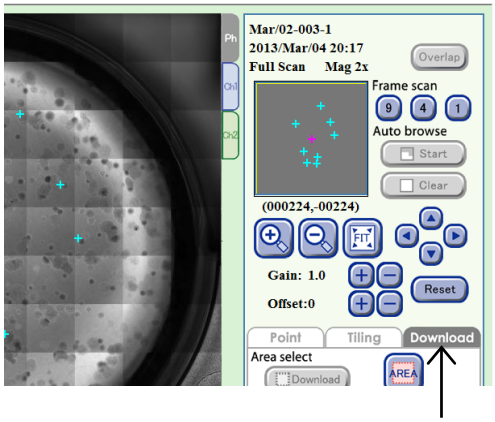
A dialog box to specify the frame to skip is displayed.

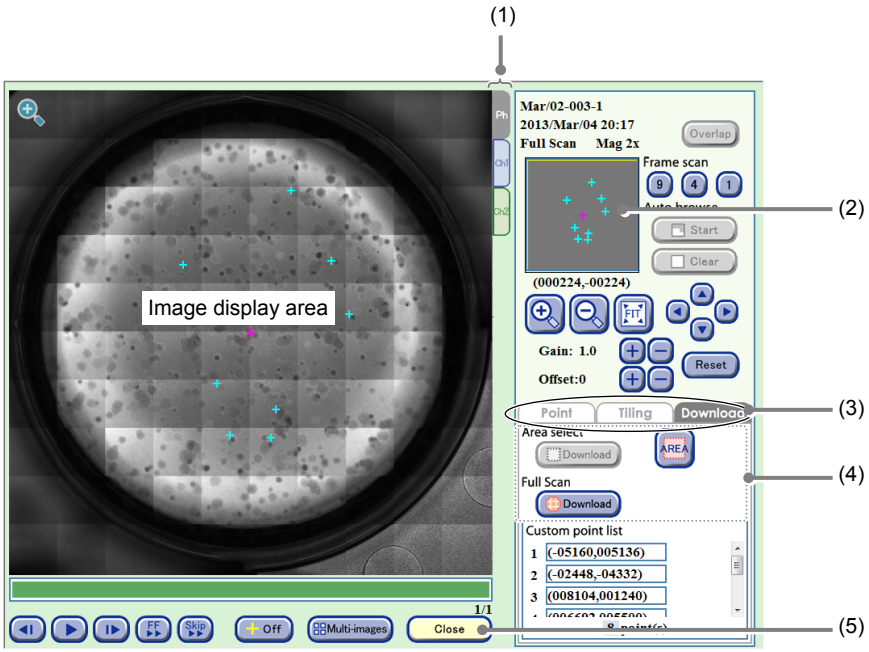
When no image is to be downloaded, go to step (5) and close the window.

(3) Open the Download preparation setting area.

1. **Select the Download tab.**

The Download tab window appears.





Full Scan image display window (Download tab)

No.	Name	Function
(1)	Channel tabs	These tabs are displayed when fluorescence images are captured. Select a tab to switch the image displayed among Ph (a phase contrast image) and Ch1 to 3 (FL channel images).
(2)	Observation point display area	This area displays all custom observation points registered in the entire sample area or the position and size of observation field of view displayed in the image display area.
(3)	Operation window switch tabs	Press a tab to switch the operation window. **Point tab:** Enable registration of custom observation point. **Tiling tab:** Enable registration of capture area for Tiling observation. (See Section 4.6.2.3, “Tiling observation.”) **Download tab:** Enable download preparation for a Full Scan image.
(4)	Download area	**Area select area:** Download the image of the specified area. **Full scan area:** Perform download preparation for a Full Scan image.
(5)	Close button	Press this button to close the Full Scan image display window.

(4) Specify the area to be downloaded.

The setting procedure differs depending on the area to be downloaded.

a. **Download preparation of the Full Scan image**

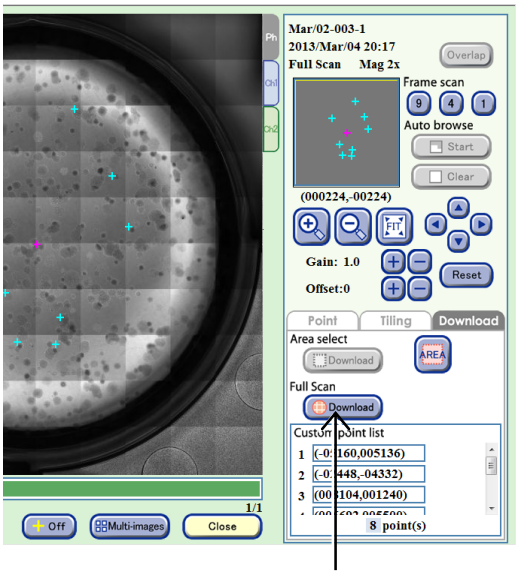
b. **Download preparation of a specified area**

The following describes each case.

a. Download preparation of Full Scan images

a-1. Press the Download button in the Full Scan area.

The Image download setting window appears.



a-2. Set the format conditions of the images to be downloaded.

The allowable conditions depend on the type of image to be downloaded.
The format conditions set here are applied to all images to be downloaded.

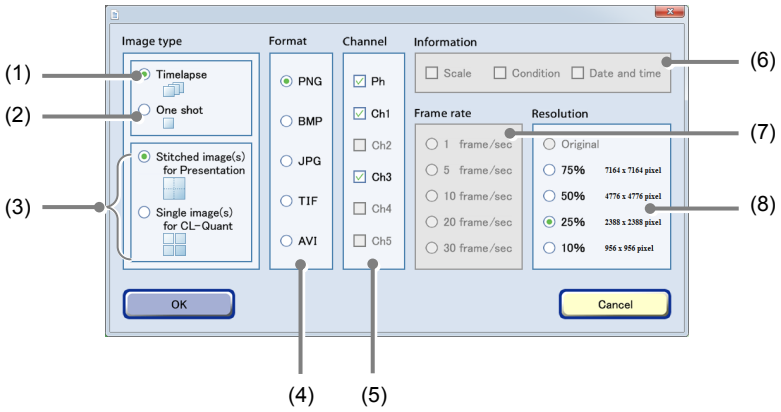


Image download settings window

No.	Name	Function
(1)	Timelapse	A download file is created in which all images taken of the same area as the displayed image are arranged in chronological order.
(2)	One shot	Download preparation of only the currently displayed image is performed.
(3)	Image stitching area	Enabled when download of Full Scan or Tiling is selected. **Stitched image(s) for Presentation** A large image is created by stitching all capture areas. **Single image(s) for CL-Quant** An independent single image for each capture area is created.
(4)	Format area	Select the format of the images to be downloaded.
(5)	Channel area	Select the channels to be downloaded. Enabled only when an image captured also by fluorescence capturing is selected.
(6)	Information area	Select the image information to be added. Three types of image information can be added: Scale, Condition and Date and time. Enabled only when [Original] (no compression) is selected in the Resolution area. Also, when AVI is selected for the format, Scale cannot be selected.
(7)	Frame rate area	Select the duration of playback per image. Enabled only when the AVI format is set.
(8)	Resolution area	Set the compression rate (resolution) of the images. Select [Original] (no compression), [75%], [50%], [25%], or [10%]. The resolution of each compression rate is displayed on the right side.

a-3. After the settings are completed, press the OK button.

After download preparation is completed, the download preparation complete dialog box appears.

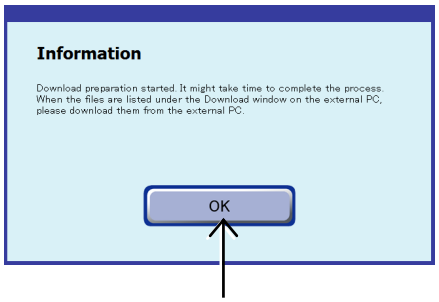
a-4. Press the OK button.

Image download preparation is completed.

The Full Scan image display window appears.

Go to step (5).

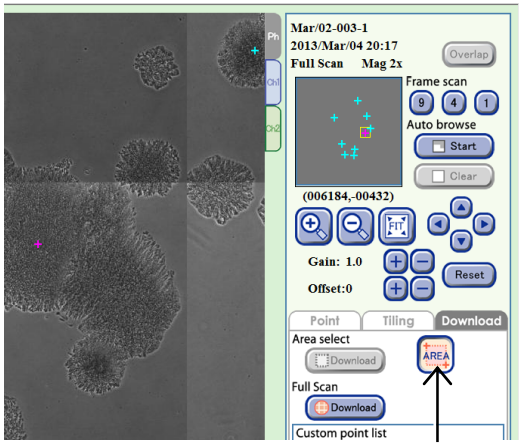
> For details on the procedure for downloading the prepared file data to an external PC, see the “BioStation CT Ver. 3.8 Instructions (PC Operations).”



Download preparation complete dialog box

b. Download preparation of the specified area

b-1. Press the AREA button in the Area select area.

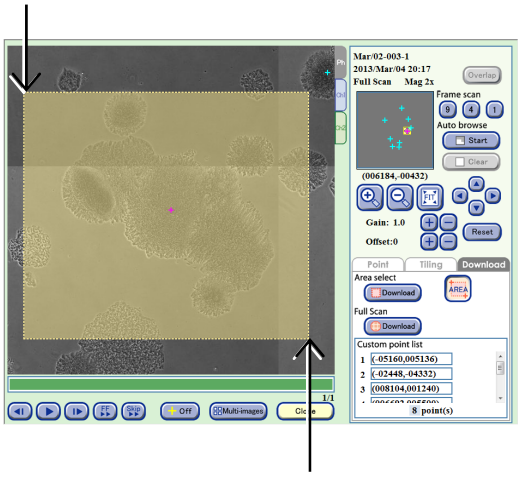


Full Scan image display window (Download tab)

b-2. In the image display area, specify the area to be downloaded by pressing the starting point and ending point of a rectangle.

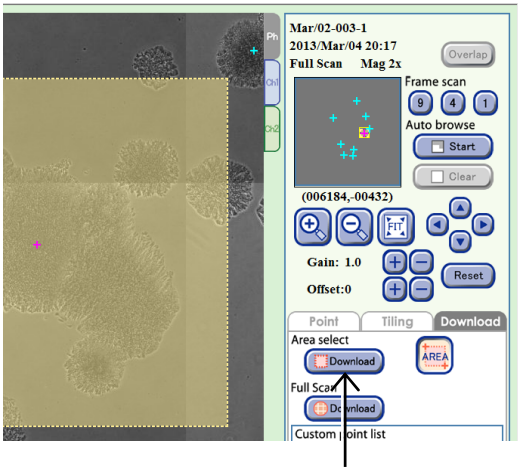
After the rectangle area is specified, the selected area is displayed in yellow in the image display area.

Press the image display area once again to cancel the selected area and display the starting point of the new rectangle area.



b-3. Press the Download button in the Area select area.

The Image download setting window appears.



b-4. Set the format conditions of the images to be downloaded.

The allowable conditions depend on the type of image to be downloaded.
The format conditions set here are applied to all images to be downloaded.

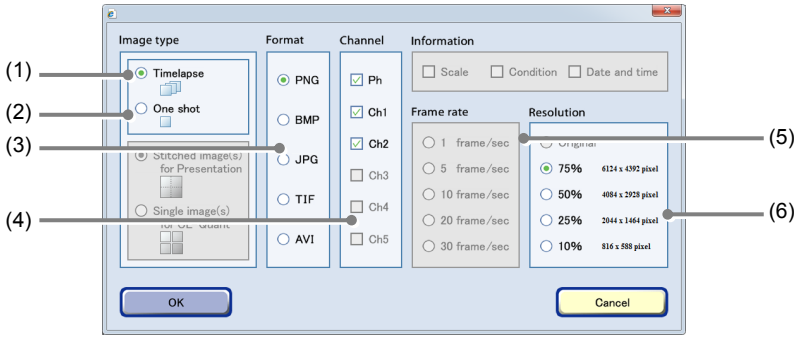
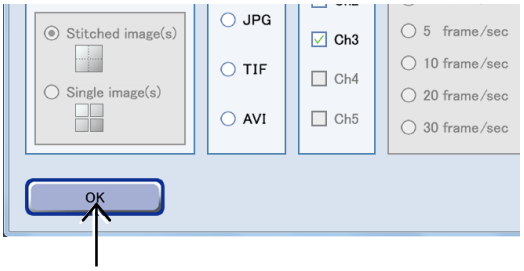


Image download settings window

No.	Name	Function
(1)	Timelapse	A download file is created in which all images taken of the same area as the displayed image are arranged in chronological order.
(2)	One shot	Download preparation of only the currently displayed image is performed.
(3)	Format area	Select the format of the images to be downloaded.
(4)	Channel area	Select the channels to be downloaded. Enabled only when an image captured also by fluorescence capturing is selected.
(5)	Frame rate area	Select the duration of playback per image. Enabled only when the AVI format is set.
(6)	Resolution area	Set the compression rate (resolution) of the images for the specified area. Select [Original] (no compression), [75%], [50%], [25%], or [10%]. The resolution of each compression rate is displayed on the right side.

b-5. After the settings are completed, press the OK button.

After download preparation is completed, the download preparation complete dialog box appears.

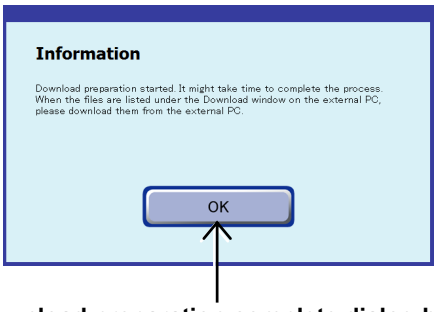


b-6. Press the OK button.

Image download preparation is completed.

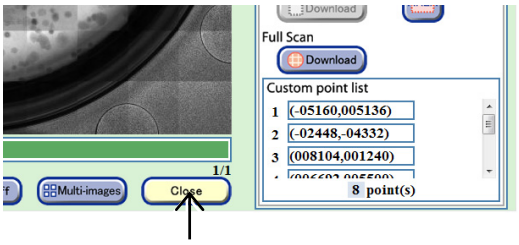
The Full Scan image display window appears.

> For details on the procedure for downloading the prepared file data to an external PC, see the “BioStation CT Ver. 3.8 Instructions (PC Operations).”



Download preparation complete dialog box

(5) Press the Close button to close the Full Scan image display window.



Full Scan image display window

Registering the custom observation point

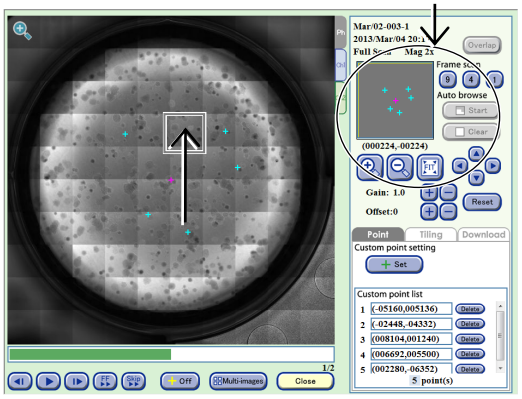
Custom observation points can be registered or deleted in the Full Scan image display window (Point tab).

a. To register custom observation points

a-1. Select the observation position.

Enlarge, reduce, or shift the field of view to move the position to be registered as a custom observation point to the center of view.

Alternatively, press a point in the tiled image displayed in the image display area to enlarge the selected point.



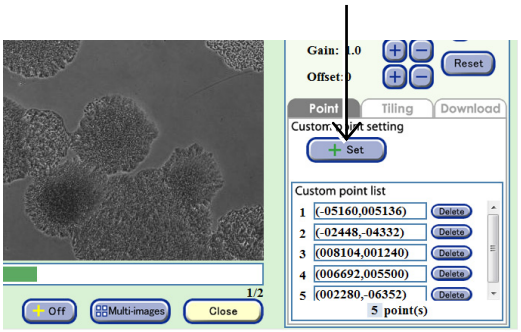
Full Scan image display window (Point tab)

a-2. Press the Set button.

The XY coordinates of the center of view displayed in the image display area are registered as a custom observation point.

The registered custom observation point is added to the custom point list field and indicated with a light blue pointer (crosshair) in the image display area and observation point display area.

The pointer in the image display area can be shown/hidden using the +Off button.



b. To delete custom observation points

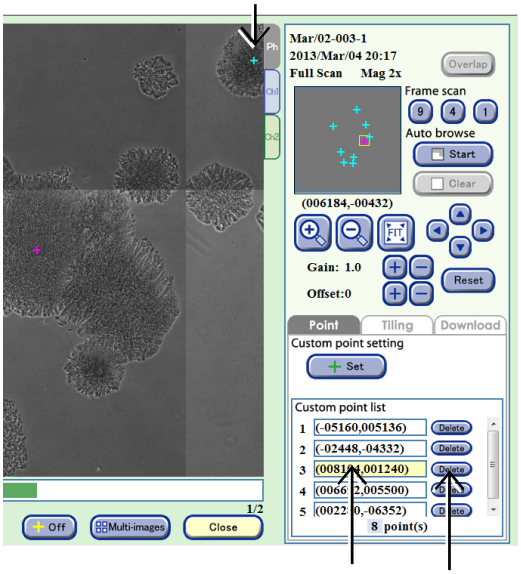
b-1. Select the custom observation point to be deleted.

Select the observation position to be deleted from the custom point list field.

Alternatively, press the light blue pointer displayed in the image display area to select the position.

b-2. Press the Delete button next to the selected custom observation point.

The custom observation point is deleted.



4.7.3.5 Viewing the Tiling image and preparing download

This section describes the procedure for displaying the tiled images captured by scheduled Tiling observation and the procedure for preparing those images to be downloaded using the Tiling image display window.

(1) Press the Expand button for the desired history data to display images in the Image review window.

The history area expands to display the captured thumbnail images.

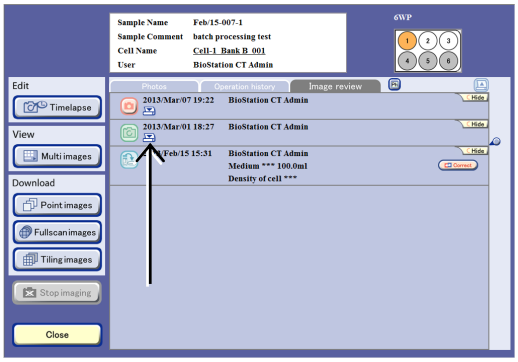
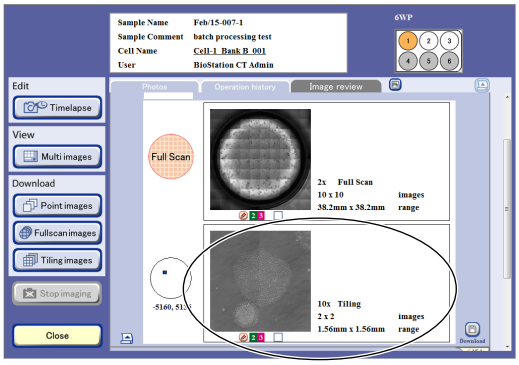


Image review window

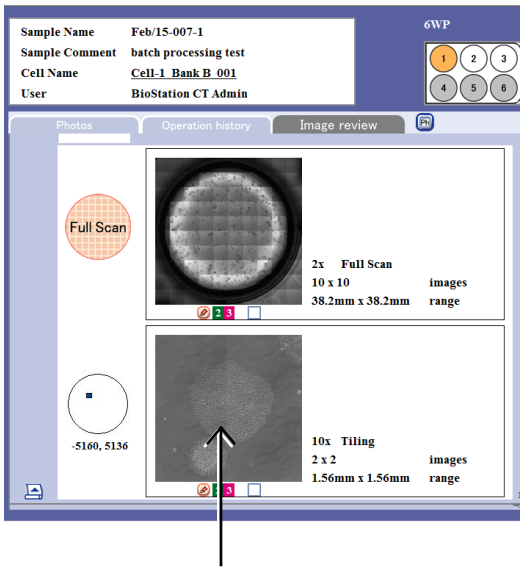
The observation magnification, number of images, and observation range are displayed on the side of the thumbnail images.

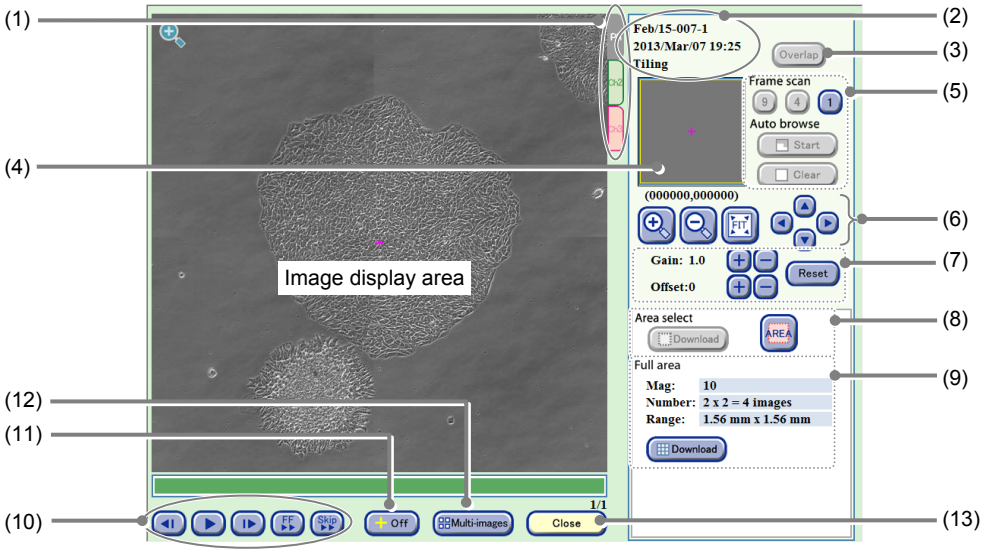


(2) Display the Tiling image.

1. Press a thumbnail image of the Tiling image.

The Tiling image display window appears.





Tiling image display window

No.	Name	Function
(1)	Channel tabs	These tabs are displayed when fluorescence images are captured. Select a tab to switch the image displayed among Ph (a phase contrast image) and Ch1 to 3 (FL channel images).
(2)	Sample information	This area displays the observation point, sample name, and captured date and time.
(3)	Overlap button	Press this button to overlay the fluorescence image and the phase contrast image.
(4)	Observation point display area	This area displays the position and size of the observation field of view displayed in the image display area.
(5)	Scan playback function	Scanning of the entire field of view is played back automatically. **Frame scan:** Play back scan of entire field of view with the selected frame size. **Auto browse:** Play back scan with the displayed magnification from the currently displayed point. Also enables pause, resume, or clear playback of scan.
(6)	Display size change buttons	**+, - buttons:** Enlarge or reduce the image. **FIT button:** Display the image on the full screen. **Field of view 1 frame move buttons:** Shift the field of view up/down, left/right by one frame.
(7)	Brightness adjustment of a displayed image	Adjust the brightness of a displayed image. **Gain:** Press the +/- button to adjust the contrast by ±0.1. **Offset:** Press the +/- button to adjust the brightness by ±5 when the Ph channel tab is displayed. Press the +/- button to adjust the brightness by ±10 when the FL channel tab is displayed. **Reset button:** Reset the brightness of a displayed image.
(8)	Area select	Download the image of the specified area. **Download button:** Prepare to download an image of the specified area on a Tiling image. **AREA button:** Set the area to download by specifying the start point and end point on a Tiling image.

No.	Name	Function
(9)	Full area	Download an entire tiling image. **Tiling information:** Display the observation magnification, observation area, etc. for Tiling observation. **Download button:** Perform download preparation of the entire tiling image.
(10)	Timelapse images playback button	**► button:** Play back timelapse images continuously. During playback, this button changes to the pause button and pressing the button pauses playback. **I►/◄I button:** Play one frame forward /one frame backward **FF button:** Fast-forward an image. **Skip button:** Skip the image for the predefined frame during playback.
(11)	+Off button	Press this button to display or hide pointers displayed in the image display area.
(12)	Multi-images button	Press this button to display the Multi images display window. Register the thumbnail images of Tiling observation on the Multi images display window. (See Section 4.9, “Multi Images Display of Captured Images.”)
(13)	Close button	Press this button to close the Tiling image display window.

2. Change the display size of the image.

Press the +/- or FIT button to change the display size of the image.

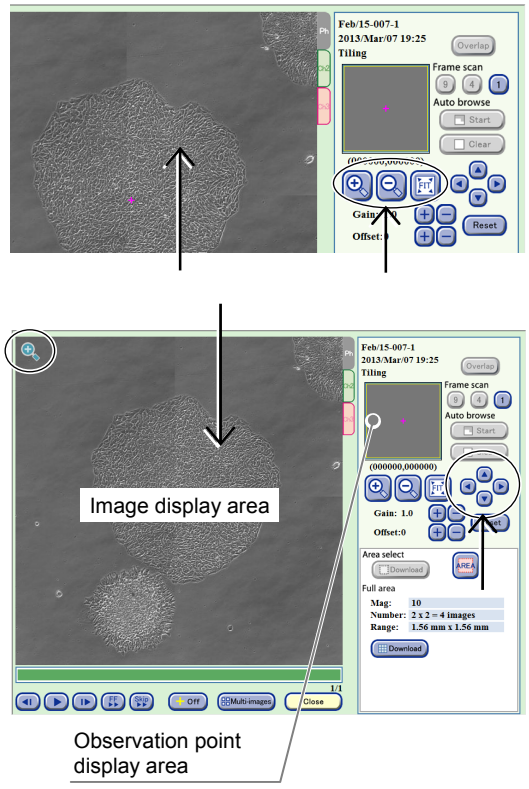
When the entire tiled image is displayed, press a point on the tiled image displayed in the image display area to enlarge the image at the selected point.

When the magnifying glass icon is displayed in the image display area, select a point in the image display area to enlarge the image at the selected point.

When the magnifying glass icon is not displayed, press a point in the image display area to move the center of the field of view to the pressed position.

Press the Field of view 1 frame move button to shift the field of view upward, downward, leftward or rightward by the same amount as the size of the current field of view displayed in the image display area.

In the observation point display area, the observation field of view displayed in the image display area is displayed with a yellow rectangle, which indicates the position and size of the observation range relative to the entire image.



3. The entire field of view can be played back automatically.

There are two types of scan playback: Frame scan and Auto browse.

Frame scan:

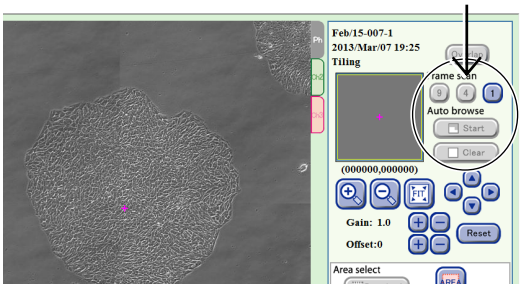
Scan playback is performed for the entire field of view at the selected field size.

There are three sizes for the field of view: [9] (3×3 images), [4] (2×2 images), and [1] (1 image). Press the button for the size of the field of view. A scan playback start confirmation dialog box appears.

Press the OK button to start the scan playback from the upper left of the image to the right.

Auto browse:

Scan play back is performed using the display magnification of the currently displayed point. Scan playback can be paused, resumed or cleared by operating the buttons.

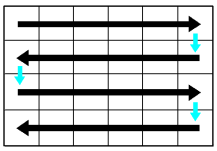


Press the Start button in the Auto browse field. A scan playback start confirmation dialog box appears.

Press the OK button to start the scan playback from the currently displayed position to the right.

The scan playback performs a reciprocating scan to play back.

The first line at the starting point is scanned from left to right (forward), and the second line is scanned from right to left (return).



An area already displayed by scan playback turns green in the observation point display area.

During scan playback, both Frame scan and Auto browse can be performed using the buttons in the Auto browse field.

When scan playback starts, the Start button changes to the Stop button.

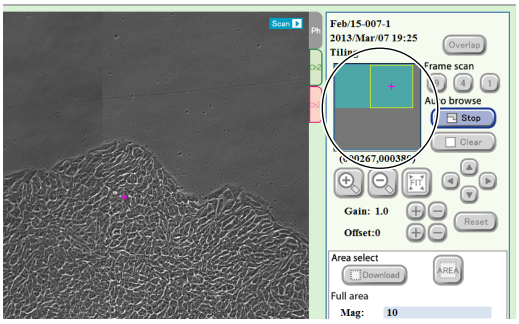
Press the Stop button to pause scan playback. The button name changes to Continue.

Press the Continue button to resume scan playback.
The button name changes to Stop.

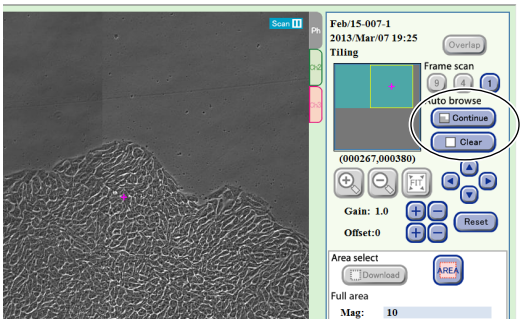
Press the Clear button to restore the original color of the green area in the observation point display area that indicates the area played back by scan playback.

Press the Start button to display the scan playback start confirmation dialog box. Press the OK button to start scan playback from the current position.
The button name changes to Stop.

Alternatively, when an enlarged image is displayed, press the Start button in the Auto browse field to start scan playback from the current position at the current size of the field of view.

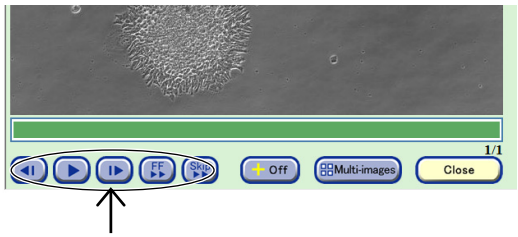


During scan playback



While scan playback is paused

4. To play back the displayed image, press the ► button, and to proceed to the next image or to return to the previous image, press the I► button or the ◄I button.



To fast-forward the image, press the FF button.

Each time the FF button is pressed, the playback speed is switched. (The speed is switched in the order of 1-step → 2-step → Normal.)

To skip the playback with a frame specified, press the Skip button.

A dialog box to specify a frame to skip is displayed.

When no image is to be downloaded, go to step (4) and close the window.

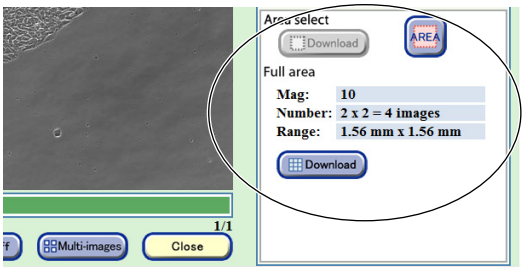
(3) Set the download preparation.

Specify the area to be downloaded.

The setting procedure differs depending on the area to be downloaded.

a. Download preparation of entire tiling image.

b. Download preparation of an image in the area specified in a Tiling image.

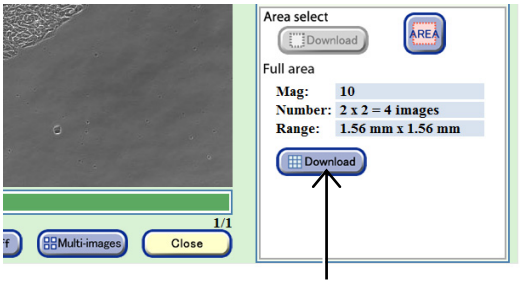


The following describes each case.

a. Download preparation of entire tiling image

a-1. Press the Download button in the Full area.

The Image download setting window appears.



a-2. Set the format conditions of the images to be downloaded.

The allowable conditions depend on the type of image to be downloaded.
The format conditions set here are applied to all images to be downloaded.

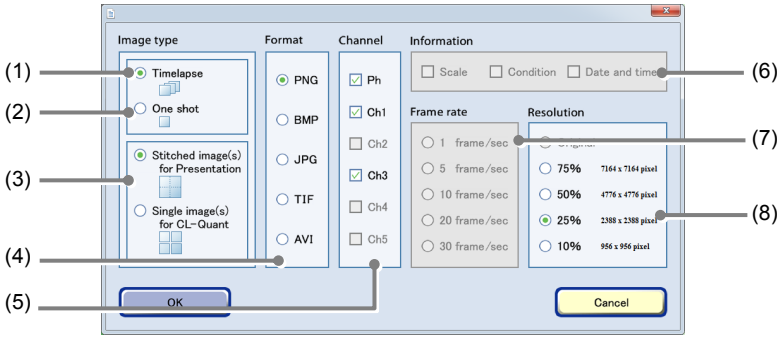
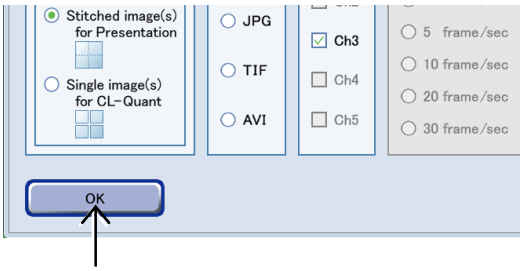


Image download settings window

No.	Name	Function
(1)	Timelapse	A download file is created in which all images taken of the same area as the displayed image are arranged in chronological order.
(2)	One shot	Download preparation of only the currently displayed image is performed.
(3)	Image stitching area	Enabled when download of Full Scan or Tiling is selected. **Stitched image(s) for Presentation** A large image is created by stitching all capture areas. **Single image(s) for CL-Quant** An independent single image for each capture area is created.
(4)	Format area	Select the format of the images to be downloaded.
(5)	Channel area	Select the channels to be downloaded. Enabled only when an image captured also by fluorescence capturing is selected.
(6)	Information area	Select the image information to be added. Three types of image information can be added: Scale, Condition and Date and time. Enabled only when [Original] (no compression) is selected in the Resolution area. Also, when AVI is selected for the format, Scale cannot be selected.
(7)	Frame rate area	Select the duration of playback per image. Enabled only when the AVI format is set.
(8)	Resolution area	Set the compression rate (resolution) of the images. Select [Original] (no compression), [75%], [50%], [25%], or [10%]. The resolution of each compression rate is displayed on the right side.

a-3. After the settings are completed, press the OK button.

After download preparation is completed, the download preparation complete dialog box appears.



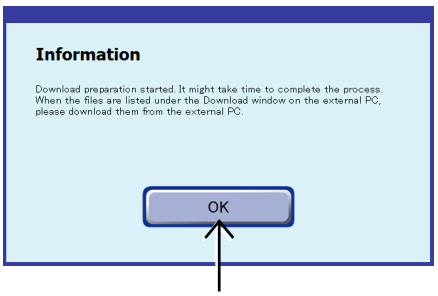
a-4. Press the OK button.

Image download preparation is completed.

The Tiling image display window appears.

Go to step (4).

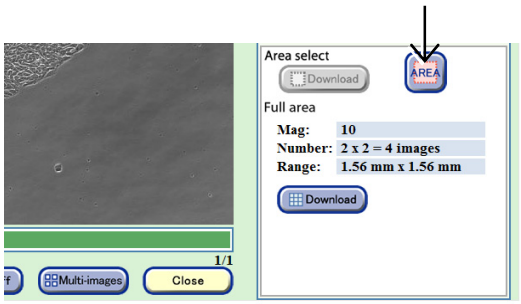
For details on the procedure for downloading the prepared file data to an external PC, see the “BioStation CT Ver. 3.8 Instructions (PC Operations).”



Download preparation complete dialog box

b. Download preparation of an image in the area specified in a Tiling image

b-1. Press the AREA button in the Area select area.

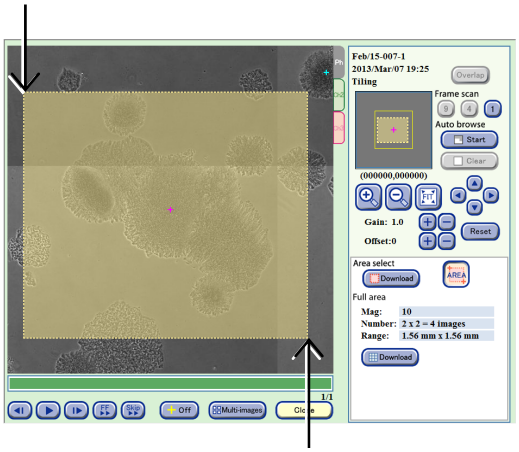


Tiling image display window

b-2. In the image display area, specify the area to be downloaded by pressing the starting point and ending point of a rectangle.

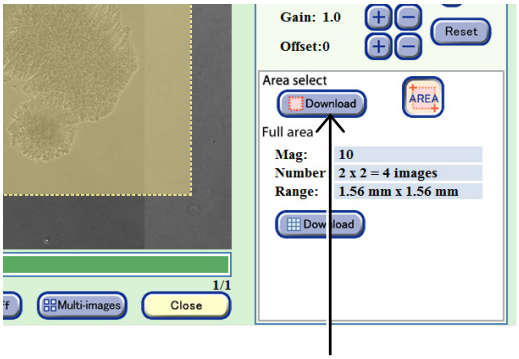
After the rectangle area is specified, the selected area is displayed in yellow in the image display area.

Press the image display area once again to cancel the selected area and display the starting point of the new rectangle area.



b-3. Press the Download button in the Area select area.

The Image download setting window appears.



b-4. Set the format conditions of the images to be downloaded.

The allowable conditions depend on the type of image to be downloaded.
The format conditions set here are applied to all images to be downloaded.

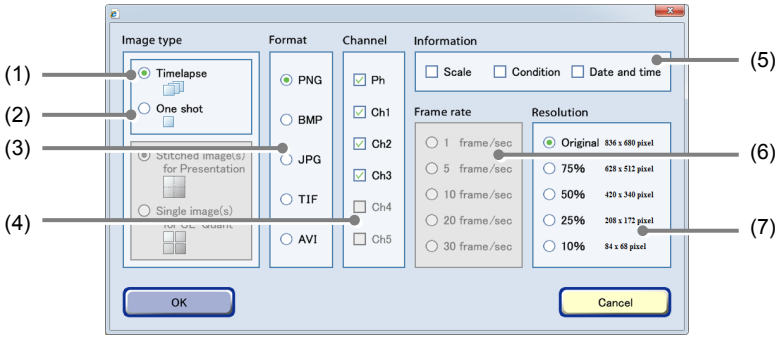
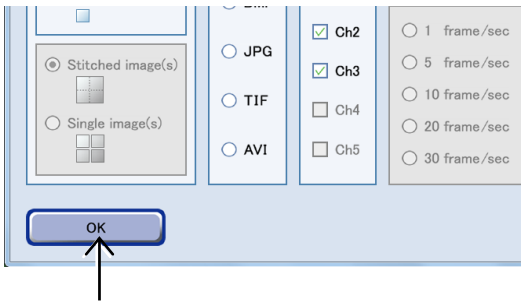


Image download settings window

No.	Name	Function
(1)	Timelapse	A download file is created in which all images taken of the same area as the displayed image are arranged in chronological order.
(2)	One shot	Download preparation of only the currently displayed image is performed.
(3)	Format area	Select the format of the images to be downloaded.
(4)	Channel area	Select the channels to be downloaded. Enabled only when an image captured also by fluorescence capturing is selected.
(5)	Information area	Select the image information to be added. Three types of image information can be added: Scale, Condition and Date and time. Enabled only when [Original] (no compression) is selected in the Resolution area. Also, when AVI is selected for the format, Scale cannot be selected.
(6)	Frame rate area	Select the duration of playback per image. Enabled only when the AVI format is set.
(7)	Resolution area	Set the compression rate (resolution) of the images for the specified area. Select [Original] (no compression), [75%], [50%], [25%], or [10%]. The resolution of each compression rate is displayed on the right side.

b-5. After the settings are completed, press the OK button.

After download preparation is completed, the download preparation complete dialog box appears.

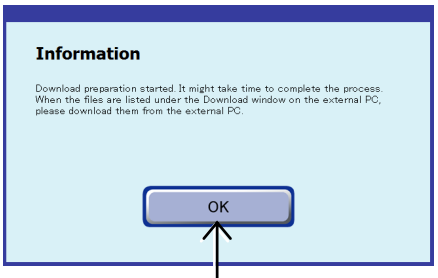


b-6. Press the OK button.

Image download preparation is completed.

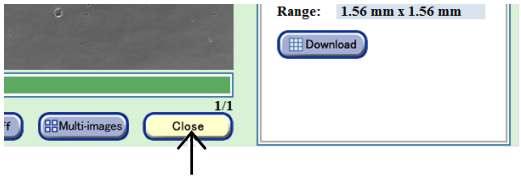
The Tiling image display window appears.

> For details on the procedure for downloading the prepared file data to an external PC, see the “BioStation CT Ver. 3.8 Instructions (PC Operations).”



Download preparation complete dialog box

(4) Press the Close button to close the Tiling image display window.



Tiling image display window

4.7.3.6 Displaying vessel product information

(1) Press the vessel name in the Image review window to display the vessel product information.

The Vessel information window appears.

When a culture vessel name is underlined, its product information is linked and can be opened. When a culture vessel name is not underlined, no information can be opened.

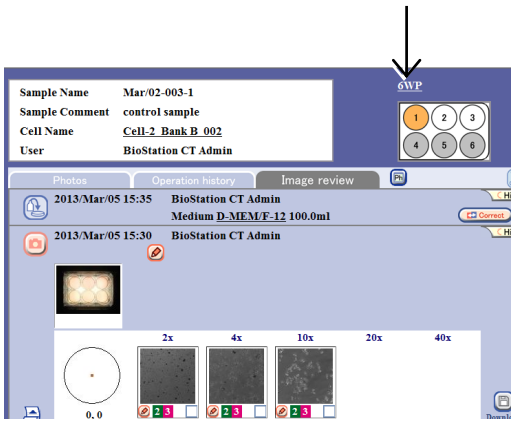
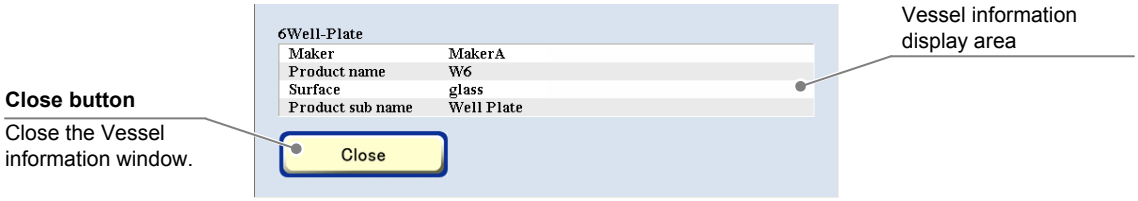
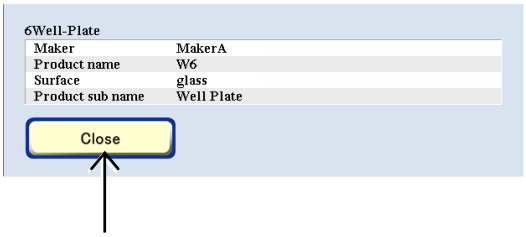


Image review window



Vessel information window

(2) After checking the vessel information, press the Close button to close the window.



4.7.3.7 Displaying medium information

(1) Press the medium name in the Image review window.

The Medium information window appears.

> When a medium name is underlined, its culture information is linked and can be opened.
> When a medium name is not underlined, information cannot be opened.

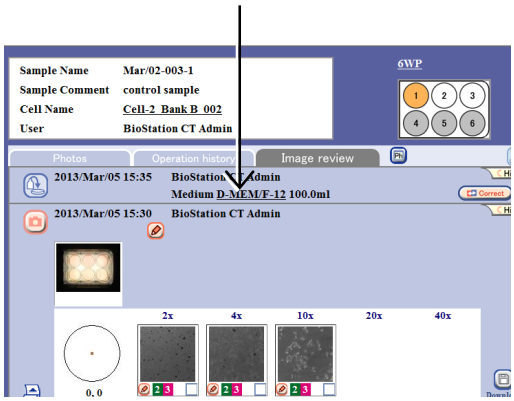
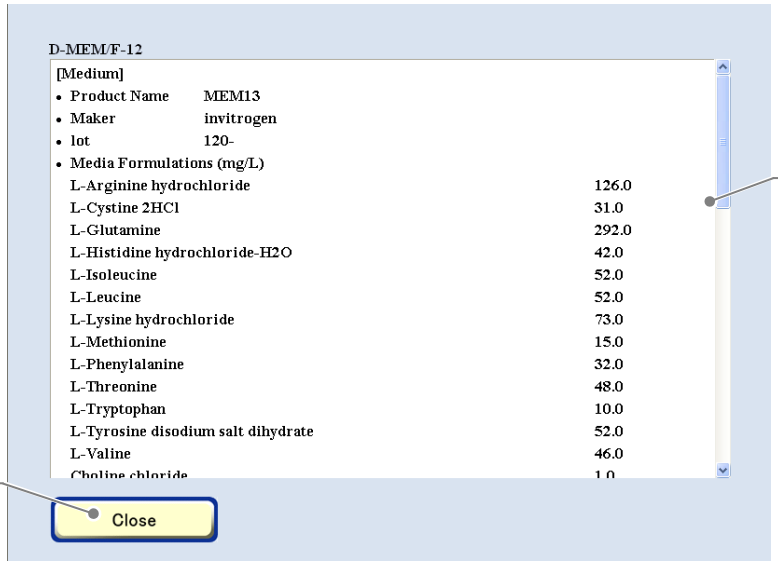
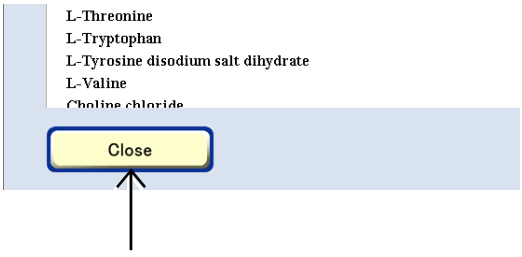


Image review window



Medium information window

(2) After checking the medium information, press the Close button to close the window.



4.7.3.8 Switching to the display of other sample

(1) The observation data of other sample in the same holder can be displayed.

a. When a vessel other than a 96- or 48-well plate is used

a-1. Press the Holder map in the Image review window to select the desired sample.

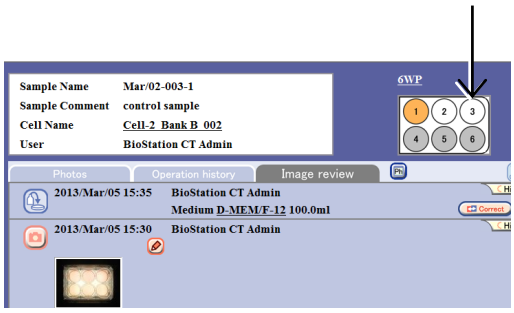


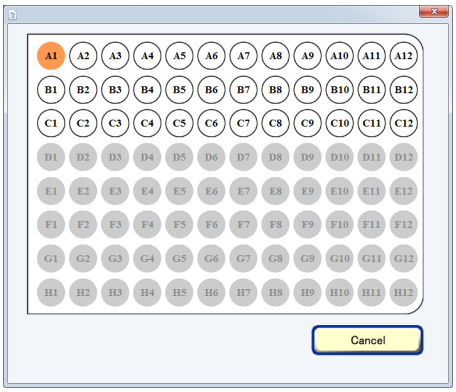
Image review window

b. When a 96- or 48-well plate is used

b-1. Press the Holder map in the Image review window. The 96-well or 48-well plate sample selection window appears.

b-2. Select the desired sample in the well plate sample selection window.

Image review window



Well plate sample selection window (96-well)

(2) The Image review window for the selected sample appears.

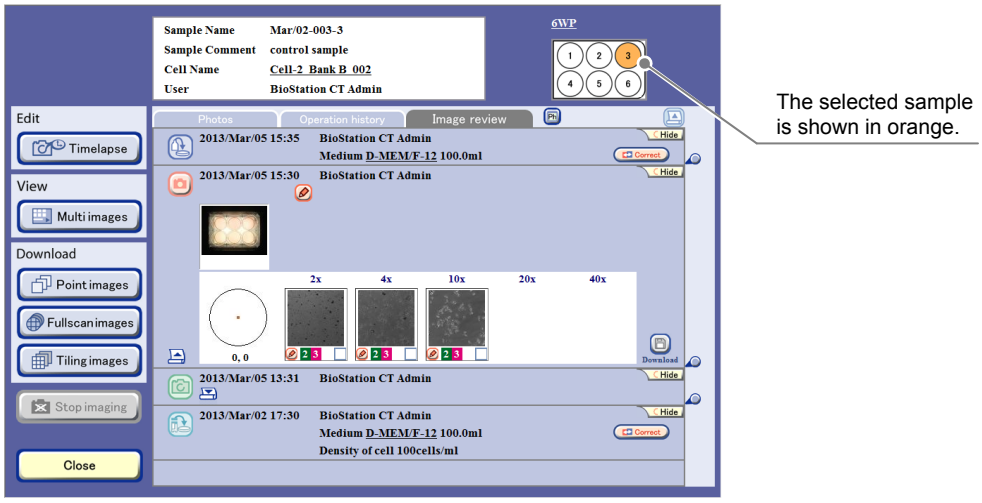


Image review window

4.7.4 Editing the Observation History

This section describes the procedure for editing the history information in the Image review window.
The following buttons are used to switch the Image review window to each edit window.

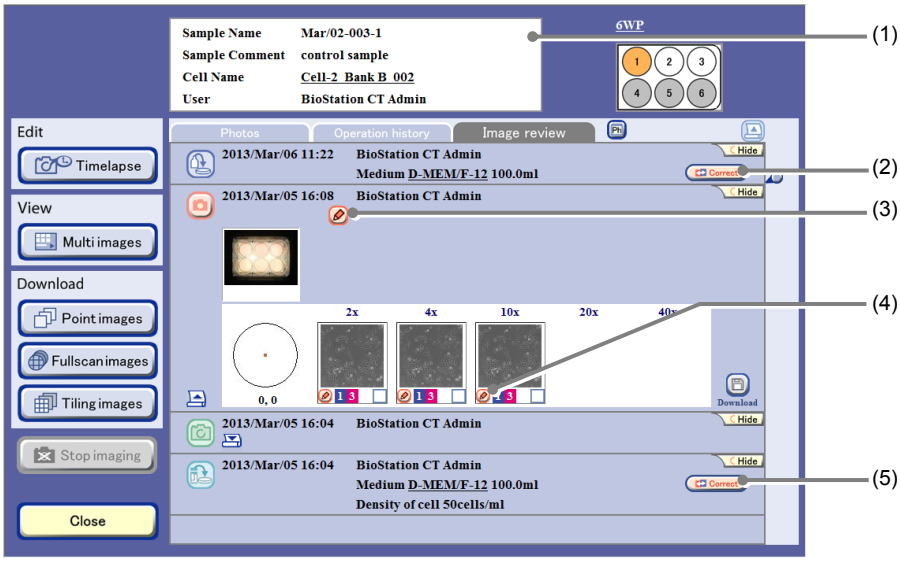


Image review window

No.	Name	Function
(1)	Basic information display area	This area displays the Basic information edit window. (See Section 4.7.4.1.)
(2)	Correct Medium change information edit button	Press this button to display the Medium change information edit window. (See Section 4.7.4.2.)
(3)	Observation history comment button	Press this button to display the Observation history comment edit window. (See Section 4.7.4.4.)
(4)	Micro image comment button	Press this button to display the Micro image comment edit window. (See Section 4.7.4.5.)
(5)	Correct Load information edit button	Press this button to display the Load information edit window. (See Section 4.7.4.3.)

4.7.4.1 Editing the basic information

(1) Press the basic information display area in the Image review window.

The Basic information edit window appears.

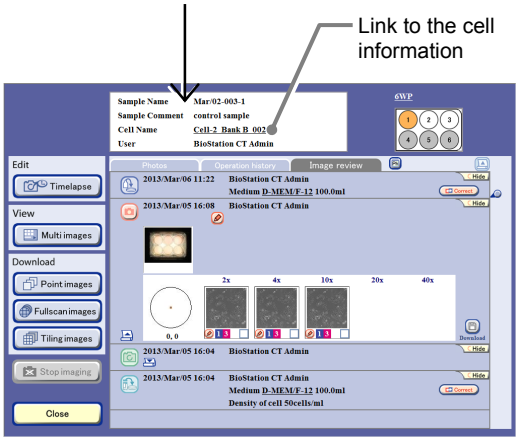
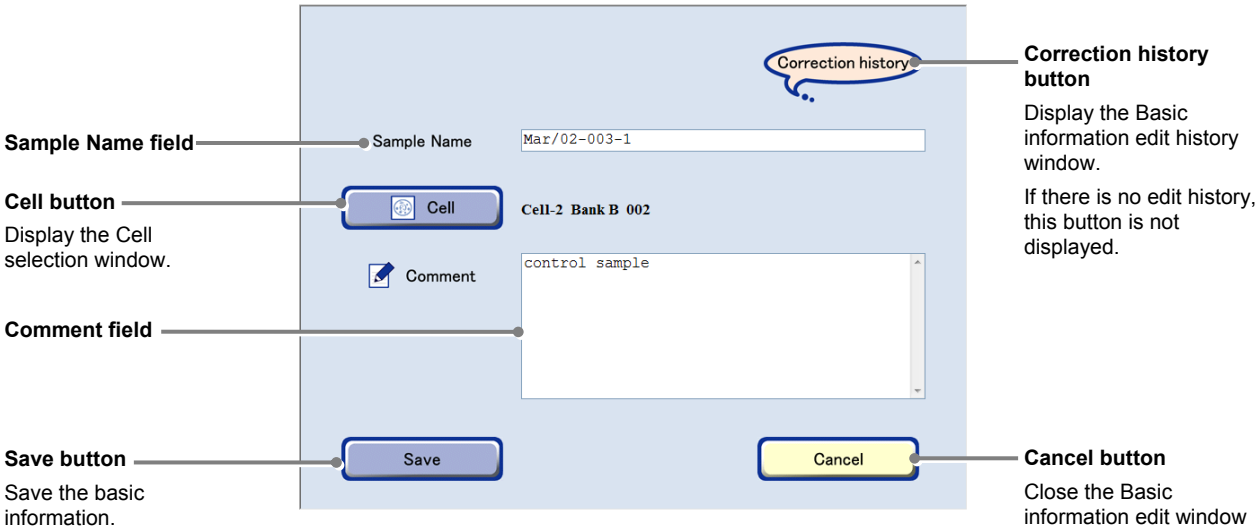


Image review window



Correction history button

Display the Basic information edit history window.

If there is no edit history, this button is not displayed.

Sample Name field

Cell button

Display the Cell selection window.

Comment field

Save button

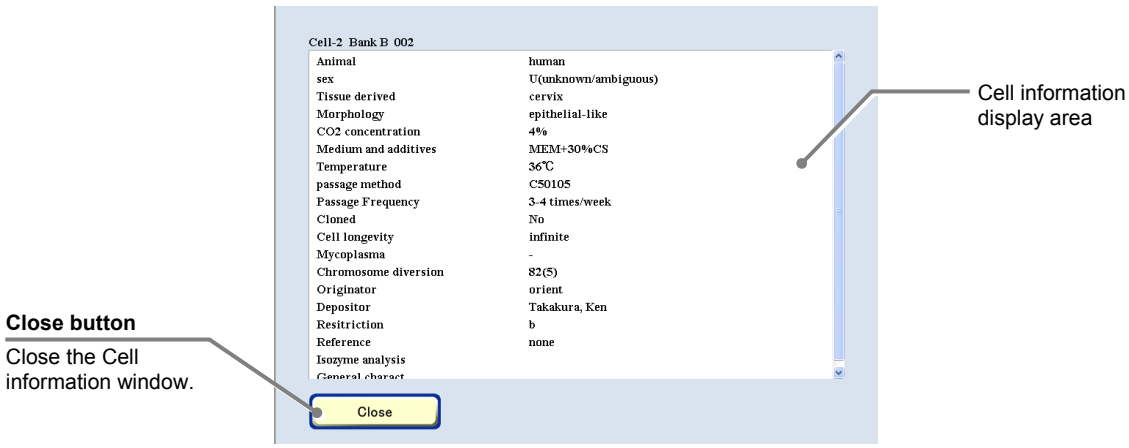
Save the basic information.

Cancel button

Close the Basic information edit window without saving.

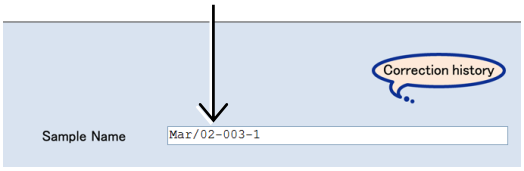
Basic information edit window

When a cell name is underlined in the Basic information display area (cell information link) and the name is pressed, the Cell information window appears. To open the Basic information edit window, press the Basic information display area somewhere other than the cell information link.



Cell information window

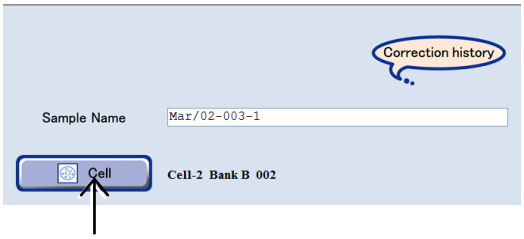
(2) To edit the sample name, press the Sample Name field and enter a name in the Keyboard window.

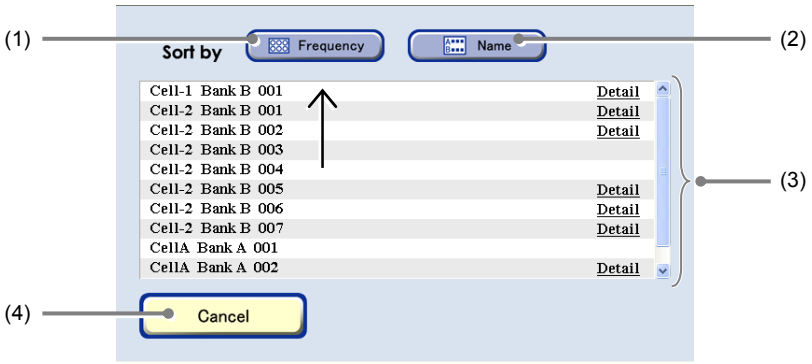


Basic information edit window

(3) To change the cell information, press the Cell button and select the cell to be changed in the Cell selection window.

Press the cell name in the Cell selection window. The cell is selected and the Cell selection window is closed.

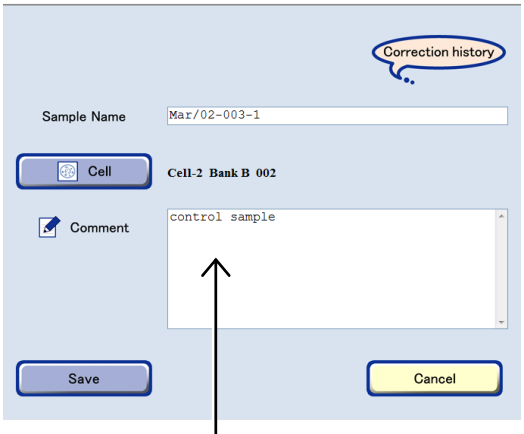




Cell selection window

No.	Name	Function
(1)	Frequency button	Press this button to sort the list in descending order of frequency of the usage.
(2)	Name button	Press this button to sort the cell names in alphabetical order.
(3)	Cell list	Press “Detail” on the right side of the list to display the Cell information window.
(4)	Cancel button	Press this button to close the window without selecting the cell.

(4) To edit a comment, press the Comment field and enter it in the Keyboard window.

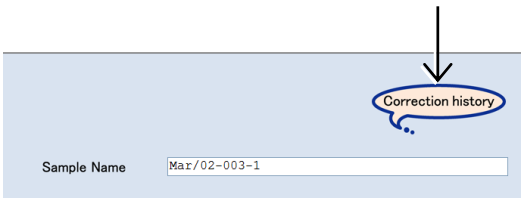


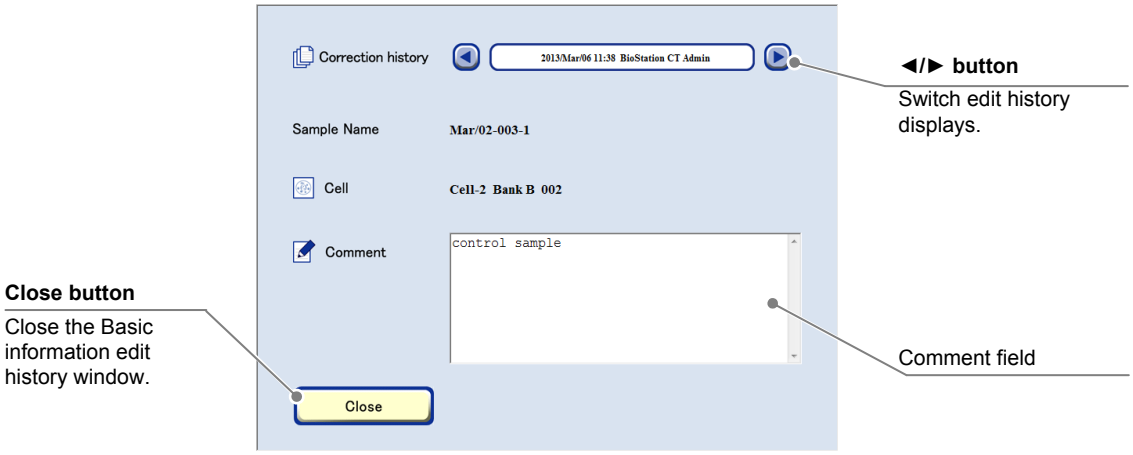
Basic information edit window

(5) Displaying the basic information edit history.

1. Press the Correction history button.

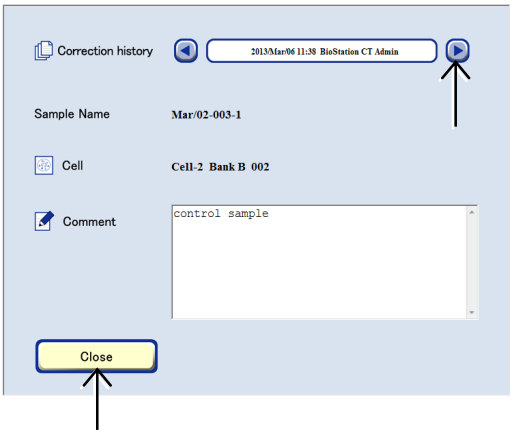
The Basic information edit history window appears.





Basic information edit history window

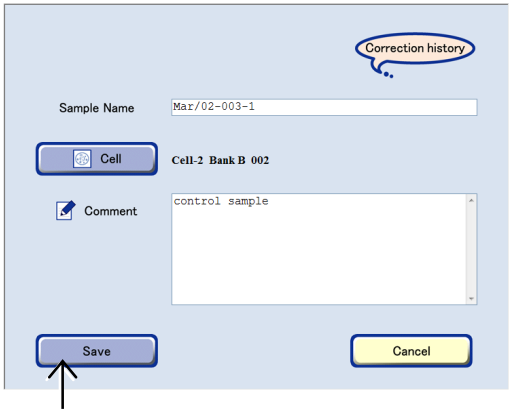
2. Press the ◄/► button to change the history.



3. To close the Basic information edit history window, press the Close button.

(6) Press the Save button in the Basic information edit window.

The edited basic information is saved and the Basic information edit window is closed.



Basic information edit window

4.7.4.2 Editing the medium change information

This section describes the procedure for editing the medium change information in the Image review window.

(1) Press the Correct button in the medium change history area of the Image review window.

The Medium change information edit window appears.

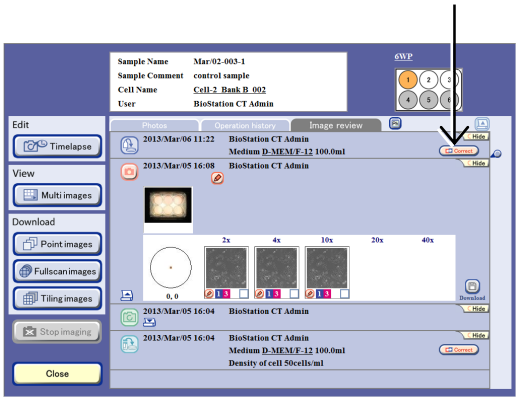
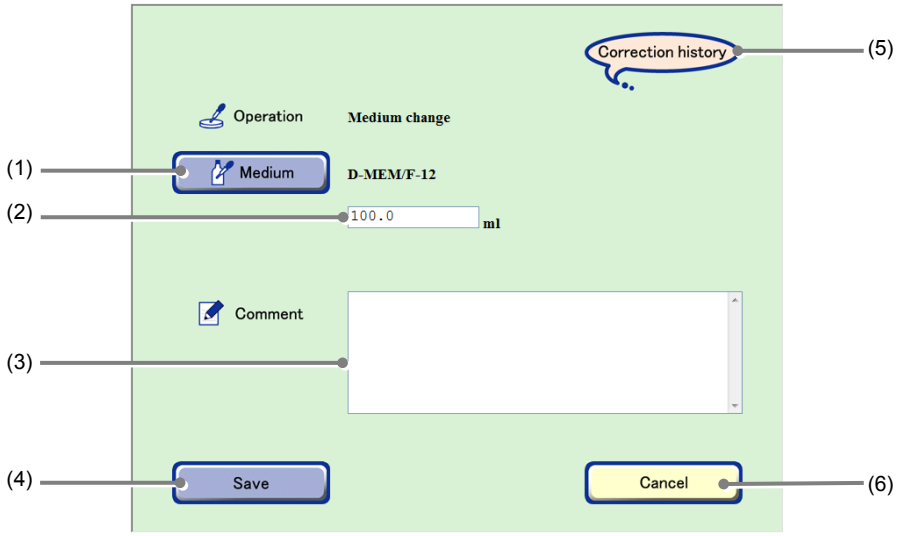


Image review window

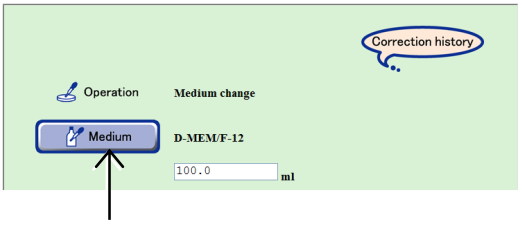


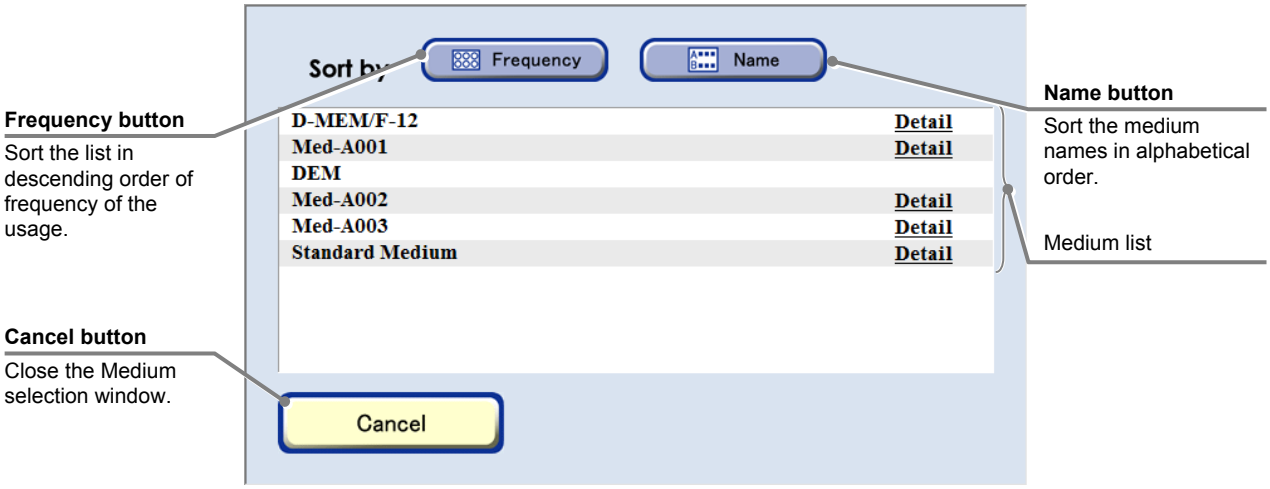
Medium change information edit window

No.	Name	Function
(1)	Medium button	Press this button to display the Medium selection window.
(2)	Medium quantity field	To change the medium quantity, press the Medium quantity field and change the quantity in the Keyboard window.
(3)	Comment field	To change the comment, press the Comment field and change the comment in the Keyboard window.
(4)	Save button	Press this button to save the sample operation information.
(5)	Correction history button	Press this button to display the Medium change information edit history window. If there is no edit history, this button is not displayed.
(6)	Cancel button	Press this button to close the Medium change information edit window without saving.

(2) To change the medium name, press the Medium button.

The Medium selection window appears.

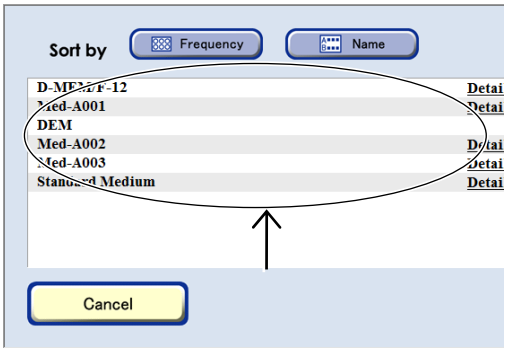




Medium selection window

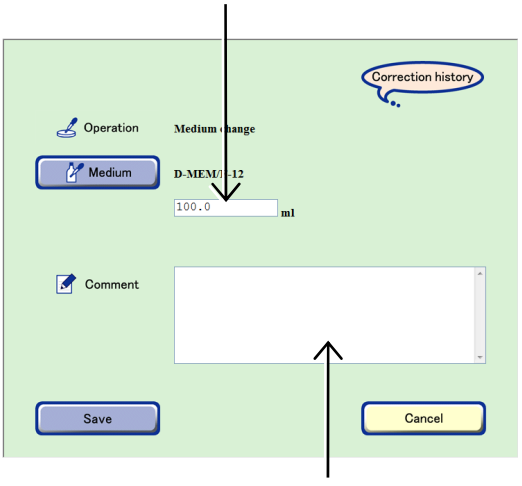
(3) Select the medium name to be changed in the Medium list by pressing it.

The medium name to be changed is selected and the Medium change information edit window appears.



(4) To change the medium quantity, press the Medium quantity field and change the quantity in the Keyboard window.

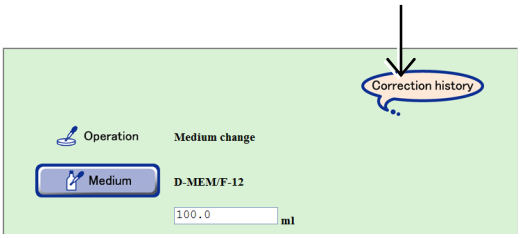
(5) To change the comment, press the Comment field and change the comment in the Keyboard window.



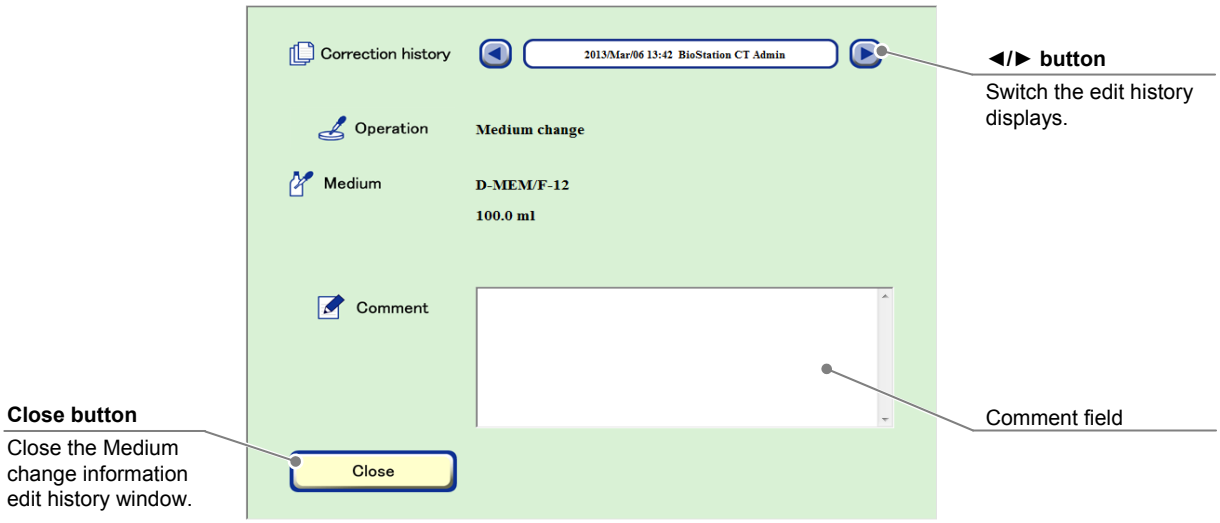
Medium change information edit window

(6) To display the Medium change information edit history window, press the Correction history button.

The Medium change information edit history window appears.



Medium change information edit window

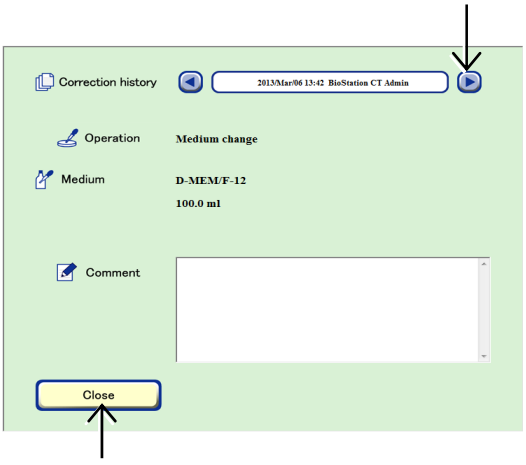


Medium change information edit history window

1. Press the ◄/► button to change the history.

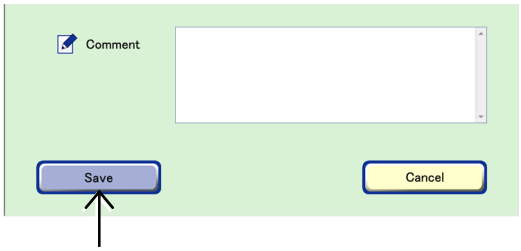
2. To close the Medium change information edit history window, press the Close button.

The Medium change information edit history window is closed.



(7) Press the Save button in the Medium change information edit window.

The edit content is saved and the Medium change information edit window is closed.



Medium change information edit window

4.7.4.3 Editing the load information

This section describes the procedure for editing the load information in the Image review window.

(1) Press the Correct button in the load history area of the Image review window.

The Load information edit window appears.

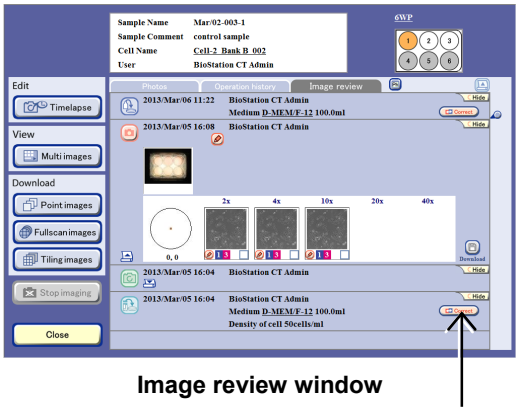
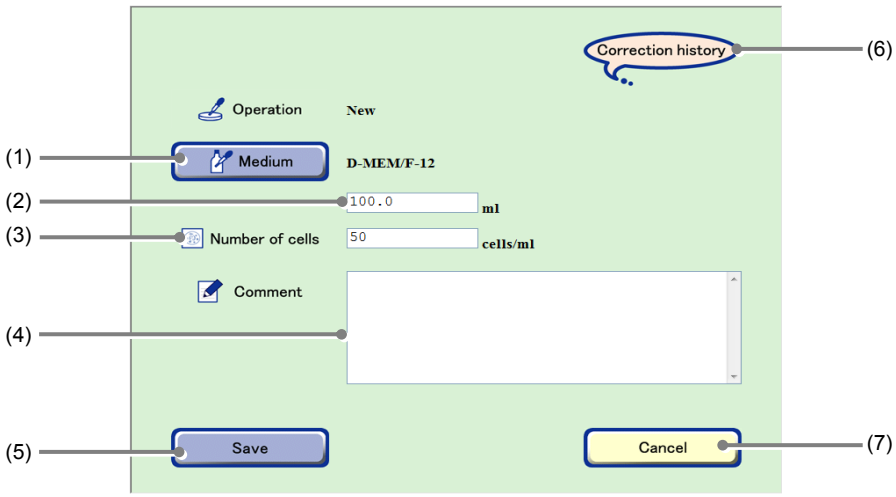


Image review window

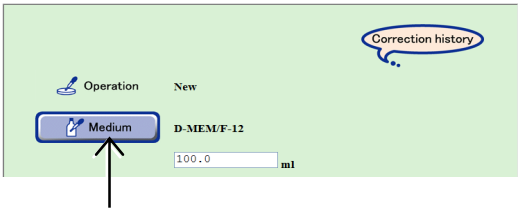


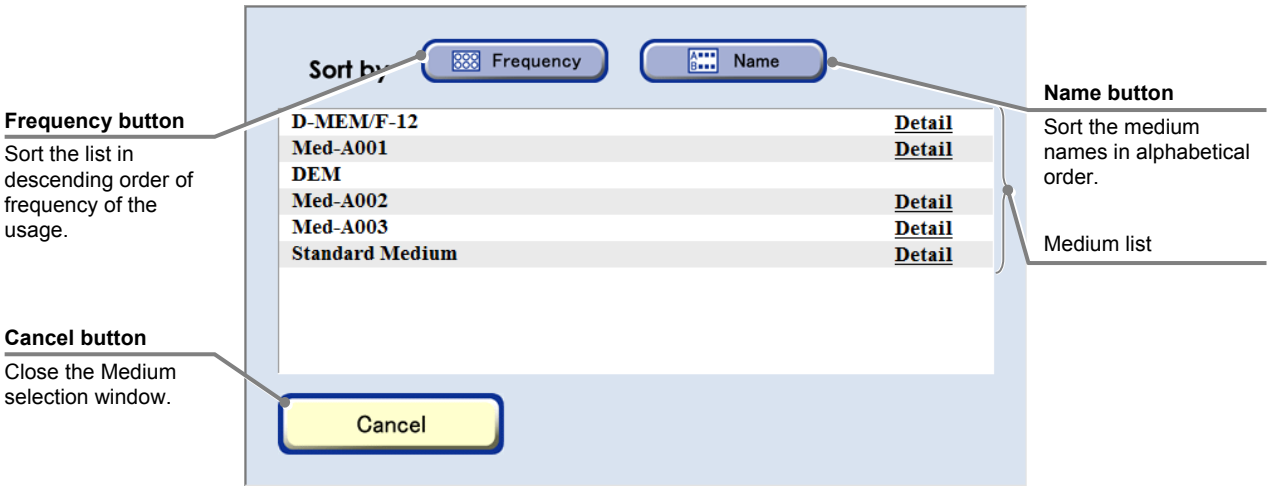
Load information edit window

No.	Name	Function
(1)	Medium button	Press this button to display the Medium selection window.
(2)	Medium quantity field	To change the medium quantity, press the Medium quantity field and change the quantity in the Keyboard window.
(3)	Number of cells field	To change the cell density, press the Number of cells field and modify the number in the Keyboard window.
(4)	Comment field	To change the comment, press the Comment field and change the comment in the Keyboard window.
(5)	Save button	Press this button to save the edited load information.
(6)	Correction history button	Press this button to display the Load information edit history window. If there is no edit history, this button is not displayed.
(7)	Cancel button	Press this button to close the Load information edit window without saving.

(2) To change the medium name, press the Medium button.

The Medium selection window appears.

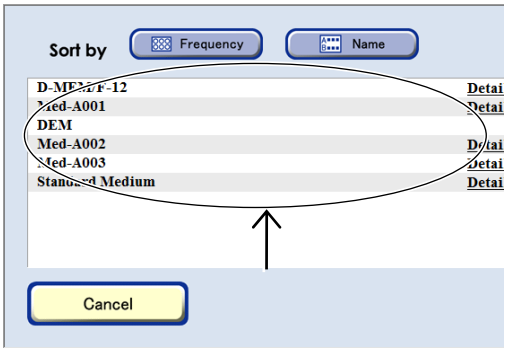




Medium selection window

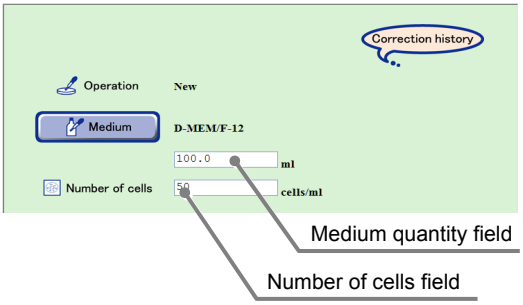
(3) Select the medium name to be changed in the Medium list by pressing it.

The medium name to be changed is selected and the Load information edit window appears.



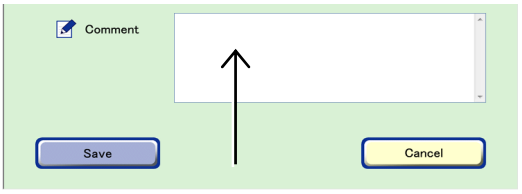
(4) To change the medium quantity, press the Medium quantity field and change the quantity in the Keyboard window.

(5) To change the cell density, press the Number of cells field and modify the number in the Keyboard window.



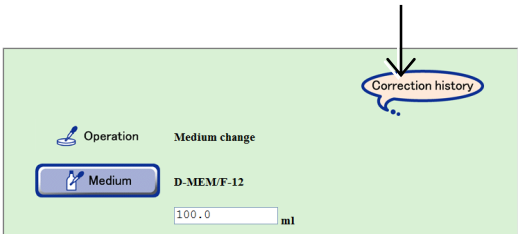
Load information edit window

(6) To change the comment, press the Comment field and change the comment in the Keyboard window.

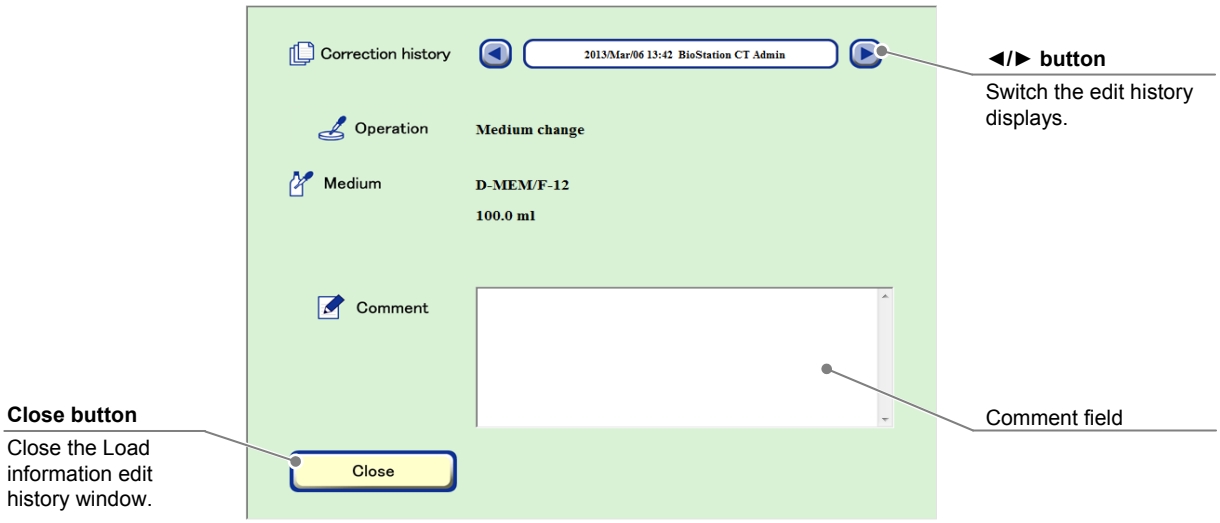


(7) To display the Load information edit history window, press the Correction history button.

The Load information edit history window appears.

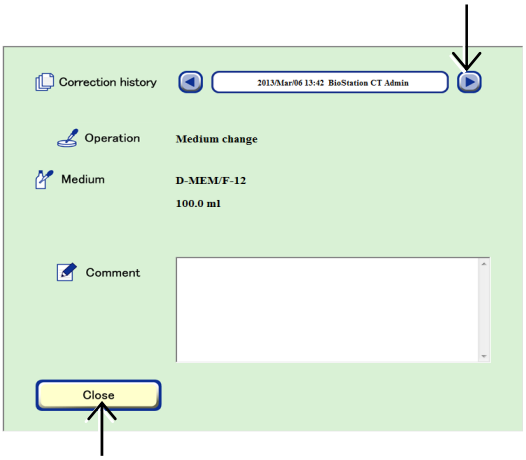


Load information edit window



Load information edit history window

1. Press the ◄/► button to change the history.

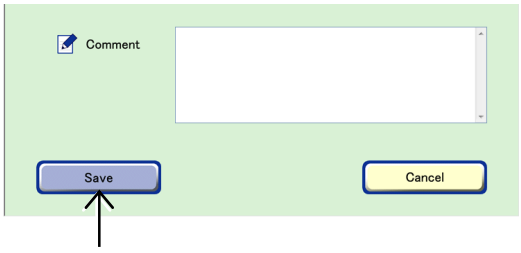


2. To close the Load information edit history window, press the Close button.

The Load information edit history window is closed.

(8) Press the Save button in the Load information edit window.

The edit content is saved and the Load information edit window is closed.



Load information edit window

4.7.4.4 Editing the comment of the observation history

This section describes the procedure for entering or changing the observation history comment.

(1) Press the Observation history comment edit button in the Observation field of the Image review window.

The Observation history comment edit window appears.

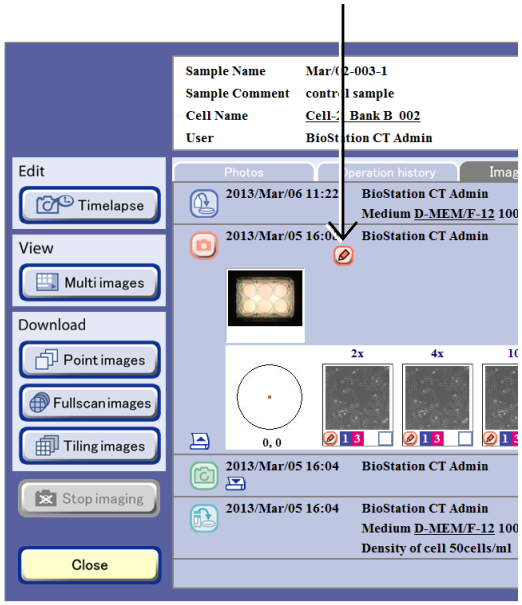
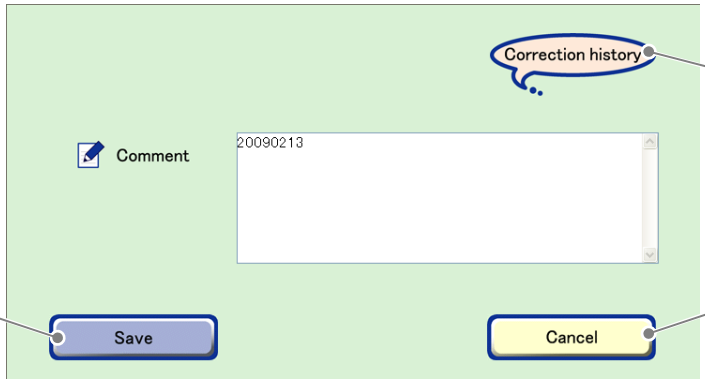


Image review window



Correction history button

Display the Observation history comment edit history window. If there is no edit history, this button is not displayed.

Save button

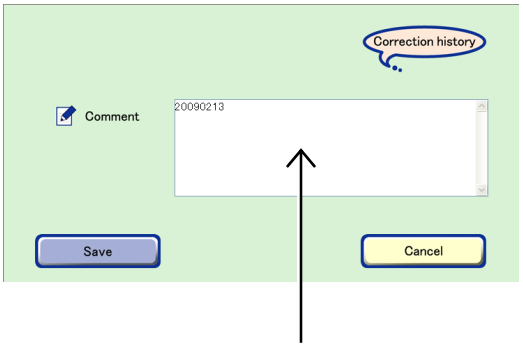
Save the observation history comment.

Cancel button

Close the Observation history comment edit window without saving the comment.

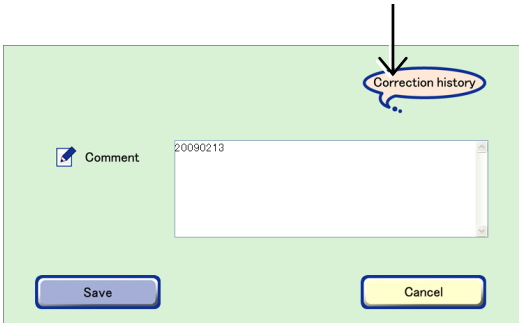
Observation history comment edit window

(2) Press the Comment field and enter a comment in the Keyboard window.

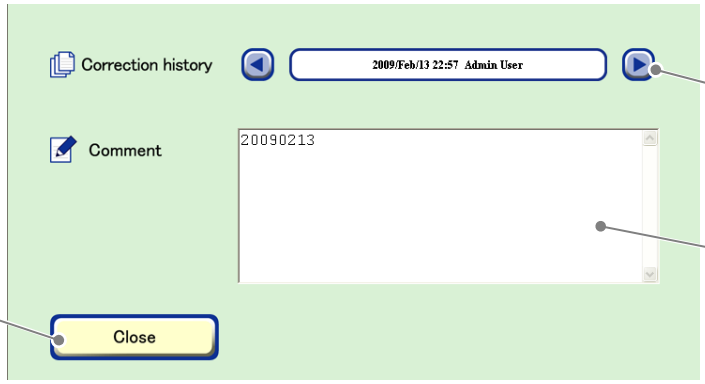


(3) To display the comment edit history of the observation history, press the Correction history button.

The Observation history comment edit history window appears.



Observation history comment edit window



◄/► button
Switch edit history displays.

Comment field

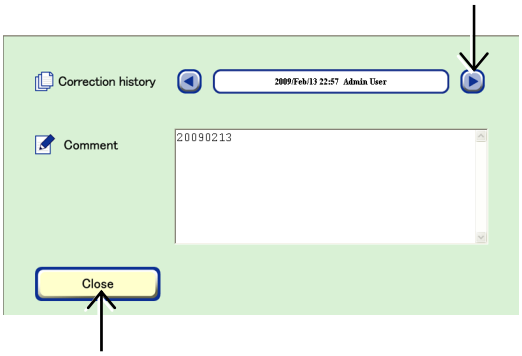
Close button
Close the Observation history comment edit history window.

Observation history comment edit history window

1. Press the ◄/► button to change the history.

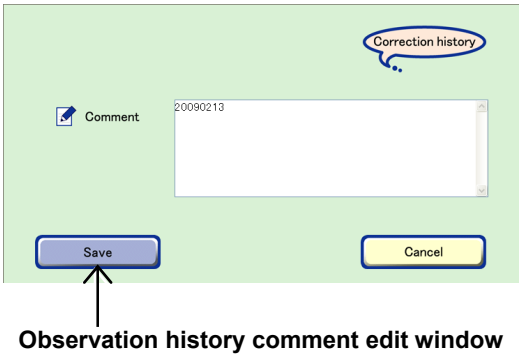
2. To close the Observation history comment edit history, press the Close button.

The Observation history comment edit history window is closed.



(4) Press the Save button in the Observation history comment edit window.

The observation history comment is saved and the Observation history comment edit window is closed.



Observation history comment edit window

4.7.4.5 Editing the comment for the micro image

This section describes the procedure for entering or changing the comment for each microscopic image of cultured sample.

(1) Press the Microscopic image comment edit button below the image in the Image review window.

The Micro image comment edit window appears.

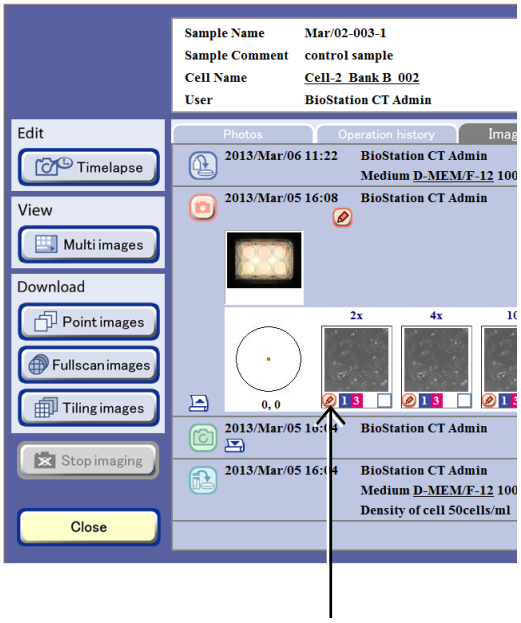
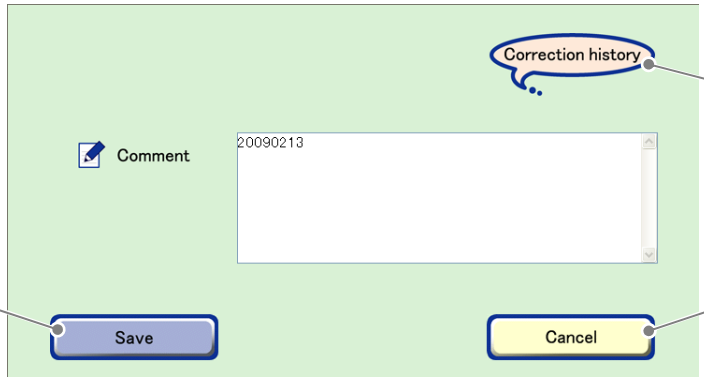


Image review window



Correction history button

Display the Micro image comment edit history window. If there is no edit history, this button is not displayed.

Save button

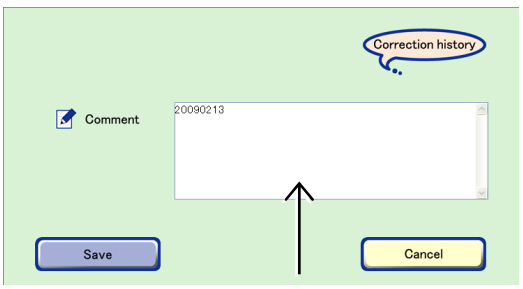
Save the comment for the micro image.

Cancel button

Close the Micro image comment edit window without saving the comment.

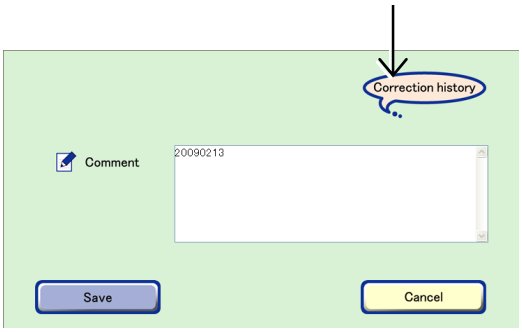
Micro image comment edit window

(2) To change the comment, press the Comment field and change the comment in the Keyboard window.

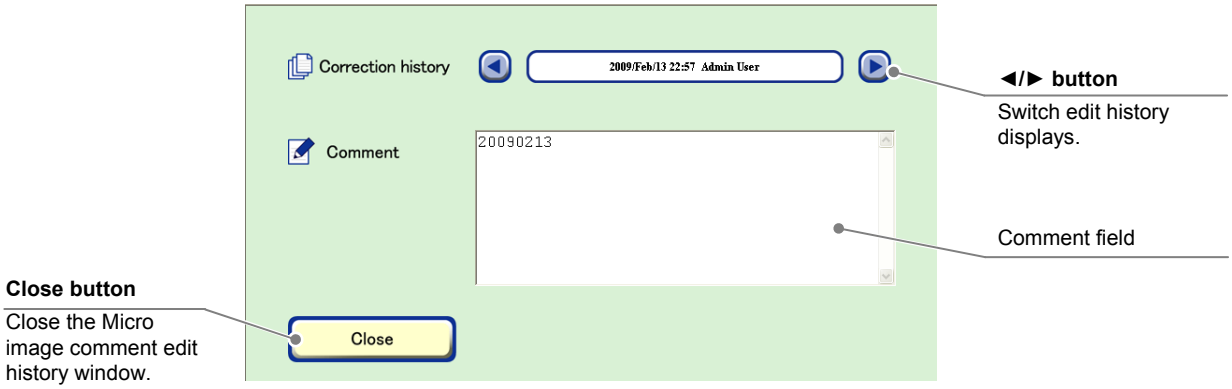


(3) To display the comment edit history of the micro image, press the Correction history button.

The Micro image comment edit history window appears.



Micro image comment edit window

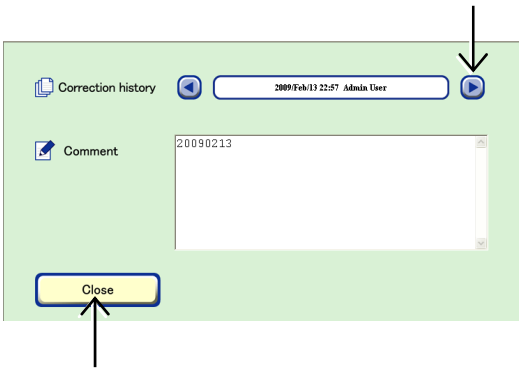


Micro image comment edit history window

1. **Press the ◄/► button to switch the history.**

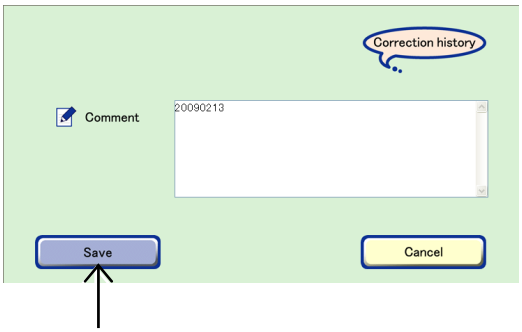
2. **To close the Micro image comment edit history, press the Close button.**

   The Micro image comment edit history window is closed.



(4) Press the Save button in the Micro image comment edit window.

The micro image comment is saved and the Micro image comment edit window is closed.



Micro image comment edit window

4.7.5 Entering the Basic Information in Batch Processing

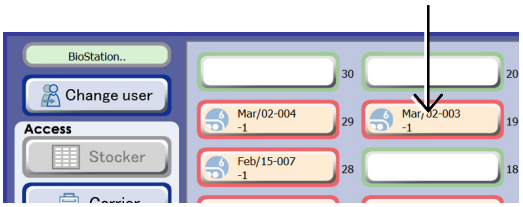
This section describes the procedure for entering the basic information of multiple samples in batch processing.

(1) Display the Sample list window.

a. To select the stocker where the sample is being cultured

a-1. To enter the basic information for a sample, press the button on the System status screen for the stocker where the sample is stored.

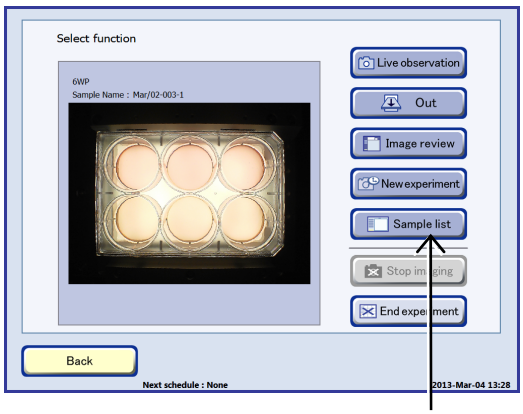
The Select function window appears.



System status screen

a-2. Press the Sample list button.

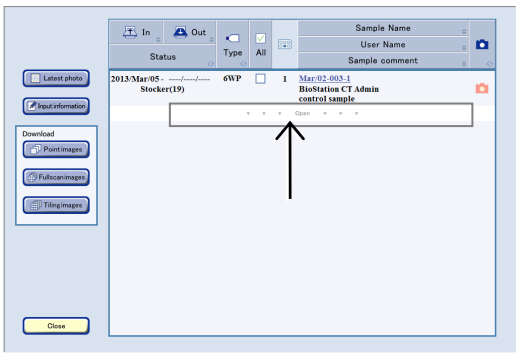
The first sample stored in the selected holder is displayed in the Sample list window.



Select function window

a-3. Press the Open area.

A list of samples stored in the selected holder is displayed.



Sample list window (by holder)

b. To select the sample(s) on the sample list

b-1. Press the Functions button on the System status screen.

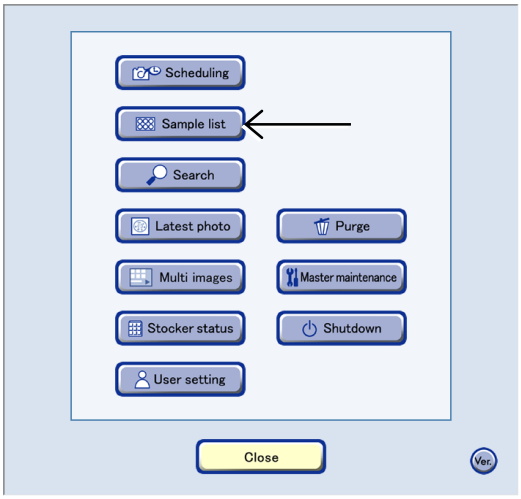
The Functions window appears.

System status screen

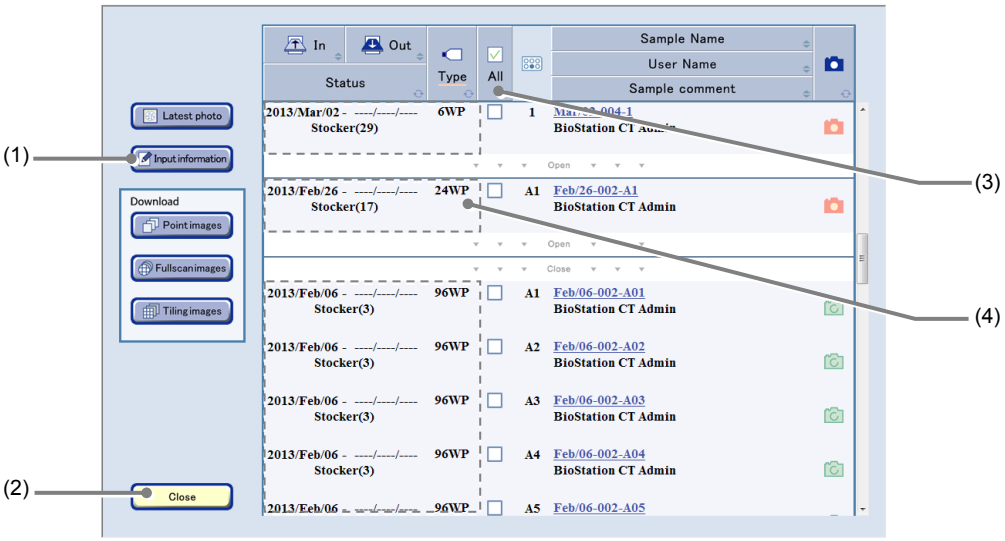
b-2. Press the Sample list button in the Functions window.

The Sample list window appears.

All samples cultured by the user are displayed in this Sample list window.



Functions window



Sample list window (all samples)

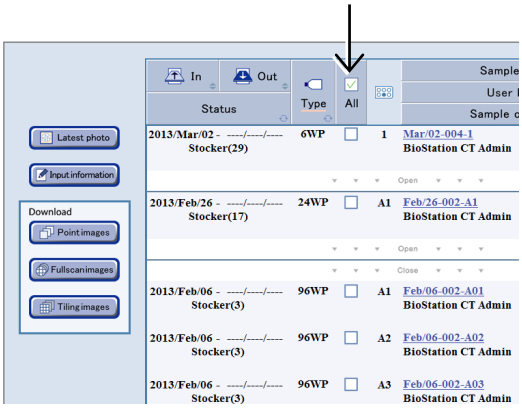
No.	Name	Function
(1)	Input information button	Press this button to display the Basic information batch input window.
(2)	Close button	Press this button to close the Sample list window.
(3)	All check button	Press this button to select all displayed samples or cancel the selection.
(4)	Stocker area	Pressing an area in the Stocker/Container field selects all samples in the holder or cancels the selection.

To display the list of the already observed culture samples in the Sample list, use the search function.
For details on the search function, see Section 4.10, “Searching for the Observation Data.”

(2) Select the samples for which basic information is to be entered in batch processing.

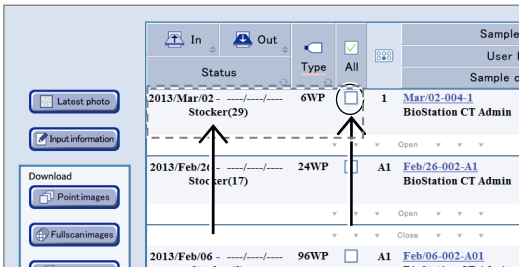
Select all samples

To select all samples in all stockers displayed in the Sample list window, select the All check button.



Select by holder

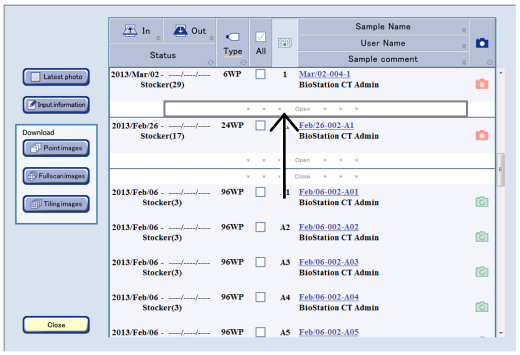
When check boxes are checked with only the first sample in the holder displayed, all samples in the holder are selected.
Also to select all samples in the holder, press the Stocker/Container field.

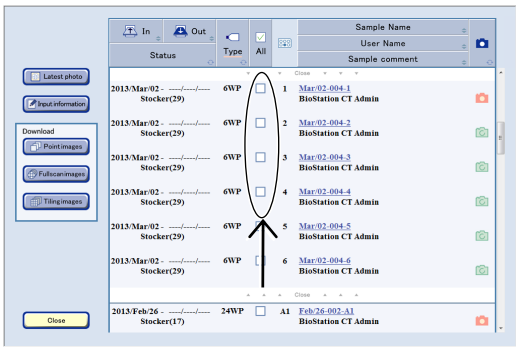


For details on vessel name abbreviations used in the Sample list window, see the list on Page 57, “Abbreviation and description for vessels.”

Select samples individually

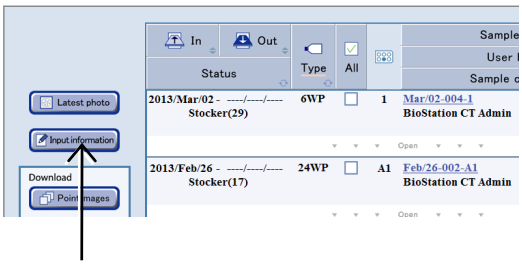
To select each sample, press the Open area to display all samples in the holder and then check the check box of a desired sample.

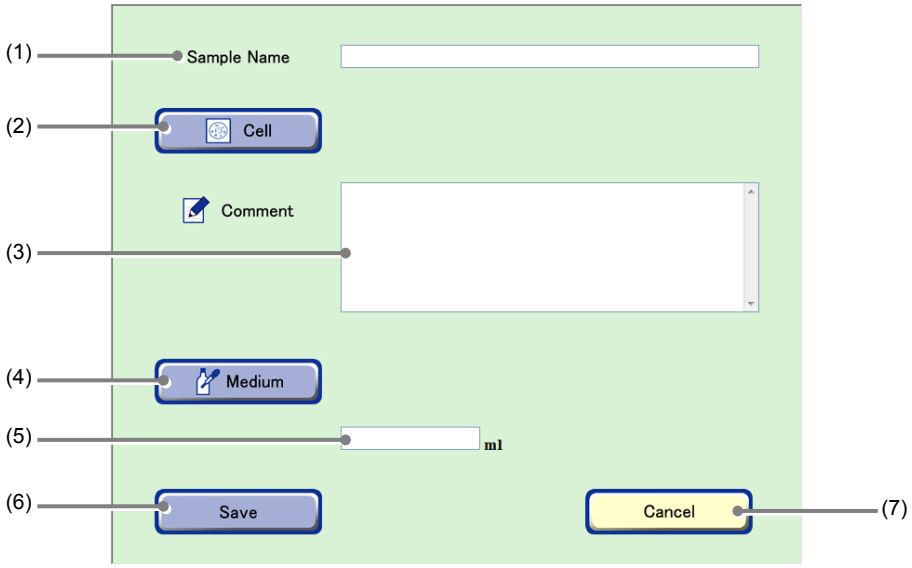




(3) Press the Input information button.

The Basic information batch input window is displayed.



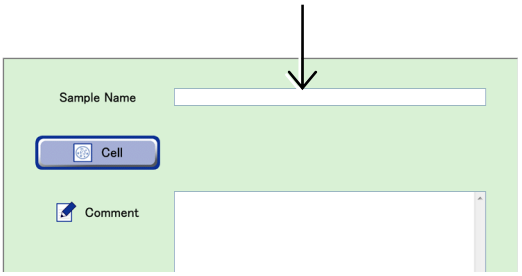


Basic information batch input window

No.	Name	Function
(1)	Sample Name field	Enter the sample name.
(2)	Cell button	Press this button to display the Cell selection window.
(3)	Comment field	Enter the comment.
(4)	Medium button	Press this button to display the Medium selection window.
(5)	Medium quantity field	Enter the culture media quantity.
(6)	Save button	Press this button to save the basic information.
(7)	Cancel button	Press this button to close the Basic information batch input window without saving.

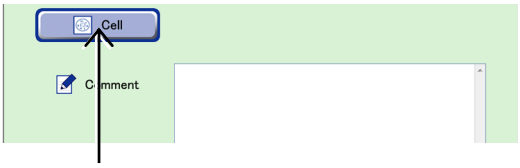
(4) Enter the sample name to be entered in batch processing.

1. **Press the Sample Name field to display the Keyboard window.**
2. **Enter the sample name in the Keyboard window and press the OK button.**



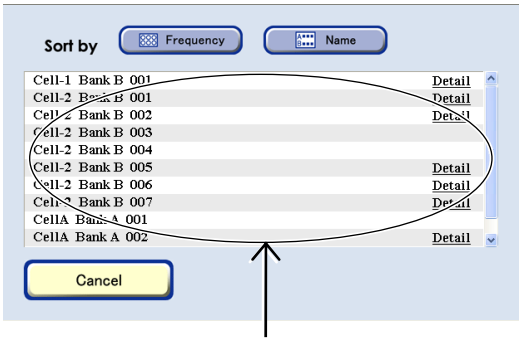
(5) Select the cell names to be entered in batch processing.

1. **Press the Cell button to display the Cell selection window.**



2. **Select the cell names from the list to be entered in batch processing.**

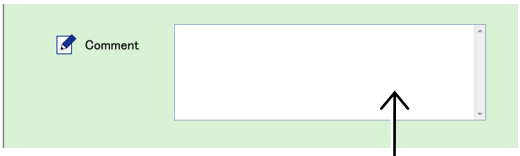
   The Basic information batch input window is displayed and the selected cell names are displayed to the side of the Cell button.



Cell selection window

(6) Enter the comment to be entered in batch processing.

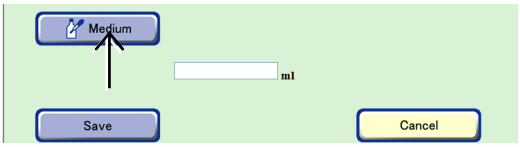
1. **Press the Comment field to display the Keyboard window.**
2. **Enter the comment in the Keyboard window and press the OK button.**



Basic information batch input window

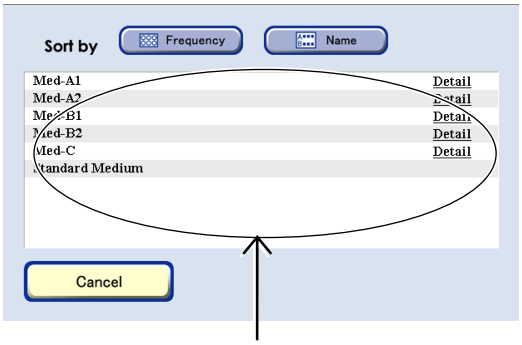
(7) Select the medium names to be entered in batch processing.

1. **Press the Medium button to display the Medium selection window.**



2. **From the list, select the medium names to be entered in batch processing.**

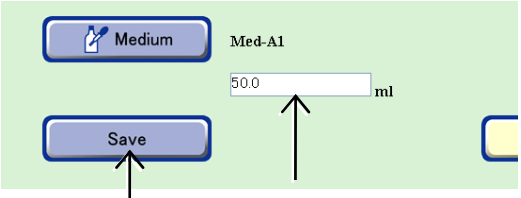
   The Basic information batch input window is displayed and the selected medium names are displayed on the side of the Medium button.



Medium selection window

(8) Enter the medium quantity to be entered in batch processing.

1. **Press the Medium quantity field to display the Keyboard window.**
2. **Enter the medium quantity in the Keyboard window and press the OK button.**



Basic information batch input window

(9) Press the Save button.

The entered basic information is entered in batch processing.

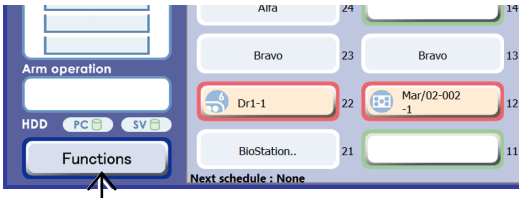
4.7.6 Displaying Thumbnails of the Latest Images

This section describes the procedure for displaying the latest images captured in scheduled observations for all samples cultured by the user.

4.7.6.1 Latest photo list of samples in all holders

(1) Press the Functions button on the System status screen.

The Functions window appears.

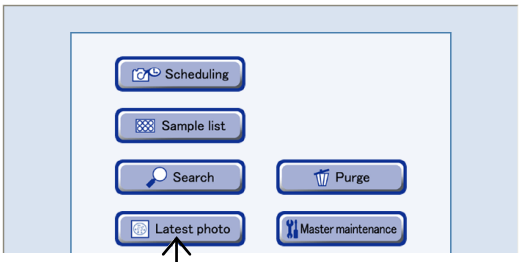


System status screen

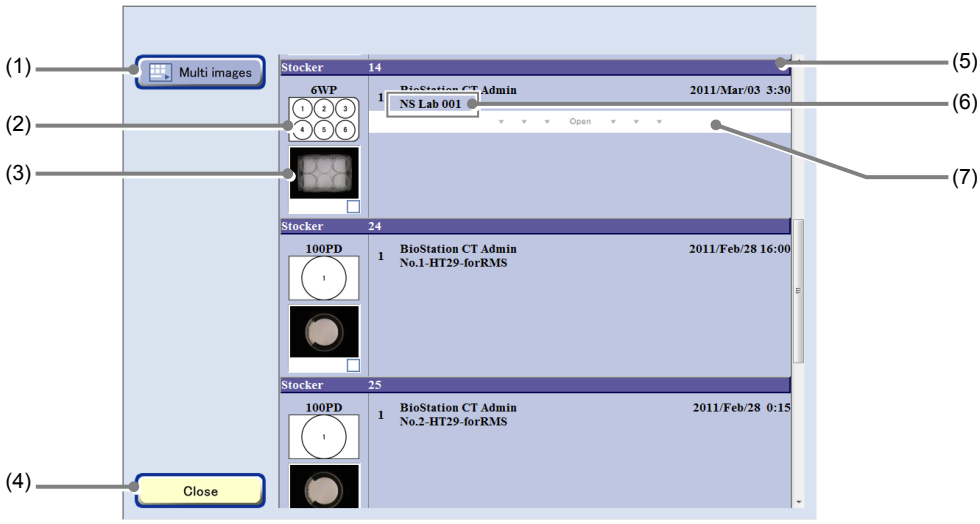
(2) Press the Latest photo button.

The Latest photo list window appears.

The Latest photo list window lists the latest images captured in scheduled observation for all samples being cultured by the user. This window does not list the images captured during a live observation or images captured during a scheduled observation in the stage exclusive mode.



Functions window



Latest photo list window (holder list)

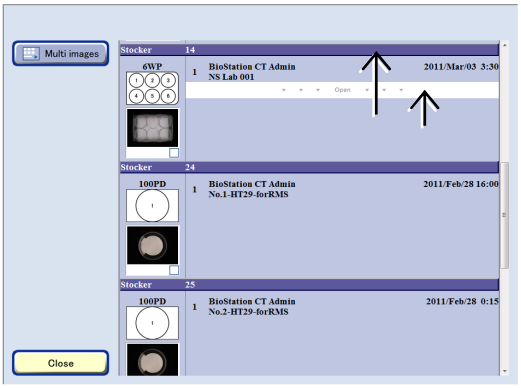
No.	Name	Function
(1)	Multi images button	Press this button to display the Multi images display window. (See Section 4.9, “Multi Images Display of Captured Images.”)
(2)	Holder information	This area displays the vessel name, the vessel shape, and the holder map number.
(3)	Macro image	This area displays the macro image thumbnail. To enlarge an image, press the thumbnail.
(4)	Close button	Press this button to close the Latest photo list window.

No.	Name	Function
(5)	Stocker number display area	This area displays the stocker number. Press the area to switch information to be displayed.
(6)	Sample name area	This area displays the sample name. Press the area to display the thumbnail of latest images for the relevant sample.
(7)	Open area	Press the area to spread the holder list and open a sample list.

(3) Switch the display to view the desired information.

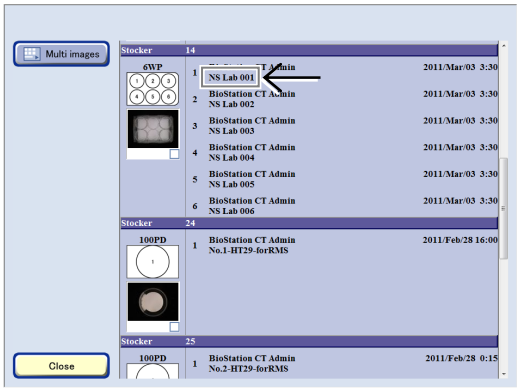
Press the Stocker number display area to change the information displayed. The display can be switched in the following order: holder list – sample list – latest photo list.

The Open area is displayed in the holder list when the relevant holder contains multiple samples. When the Open area is pressed, the holder list expands and a sample list appears.

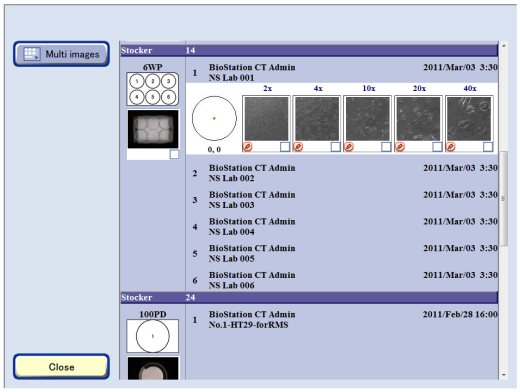


Holder list

When the Sample name area of the sample list is pressed, the latest photo list for the relevant sample appears.

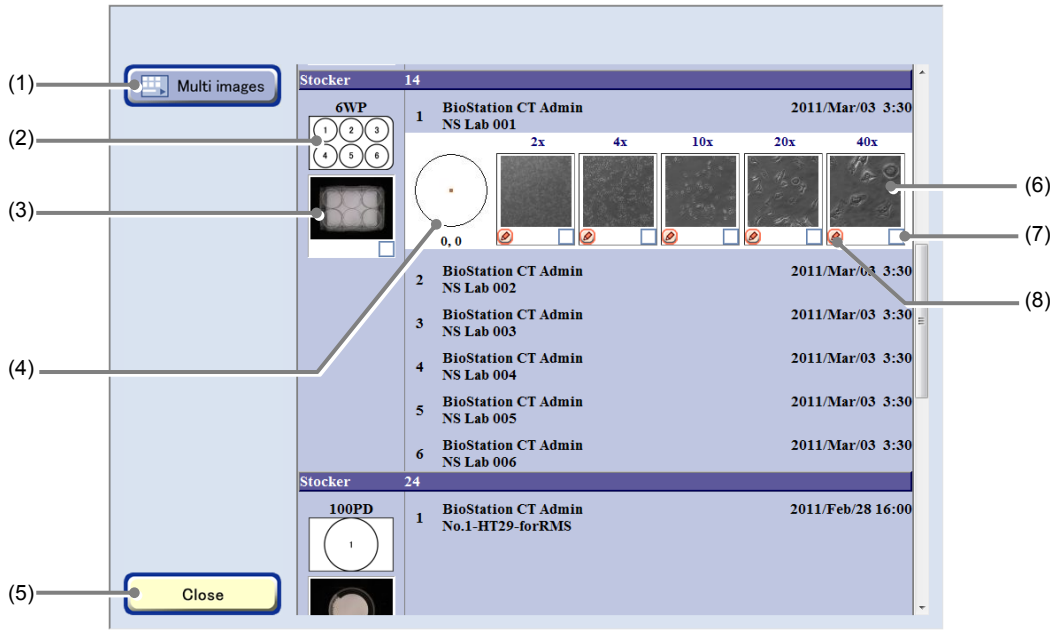


Sample list



Latest photo list window

In the Latest photo list window, the observed image thumbnails are displayed in order of the stocker number, the holder map number, and the observation position.



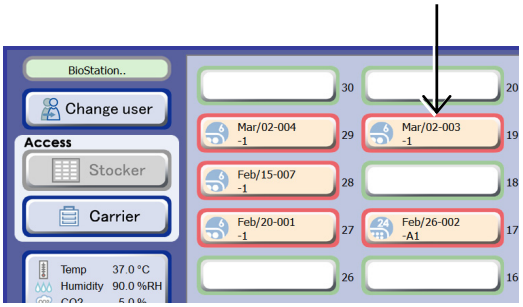
Latest photo list window

No.	Name	Function
(1)	Multi images button	Press this button to display the Multi images display window. (See Section 4.9, “Multi Images Display of Captured Images.”)
(2)	Holder information	This area displays the vessel name, the vessel shape, and the holder map number.
(3)	Macro image	This area displays the macro image thumbnail. To enlarge an image, press the thumbnail.
(4)	Observation point display	The observation position in the culture vessel is displayed.
(5)	Close button	Press this button to close the Latest photo list window.
(6)	Micro image	This area displays the micro image thumbnail. To enlarge an image, press the thumbnail.
(7)	Image selection button	Press this button to select an image to be displayed in the Multi images display window. When selected, a checked symbol appears in the check box for the image.
(8)	Comment button	To enter a comment for a micro image, press the Comment button at the bottom left of the image.

4.7.6.2 Latest photo list of samples in the specified holder

(1) To display the latest photos, press the button on the System status screen for the stocker in which the desired sample is stored.

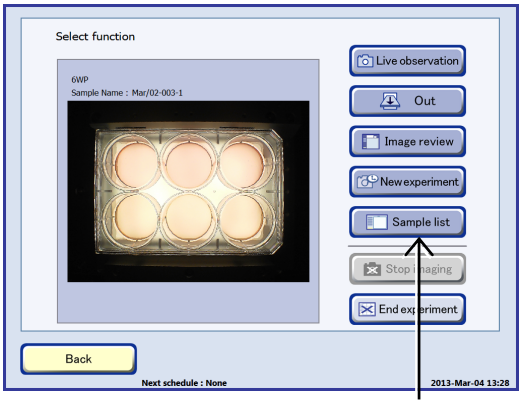
The Select function window appears.



System status screen

(2) Press the Sample list button.

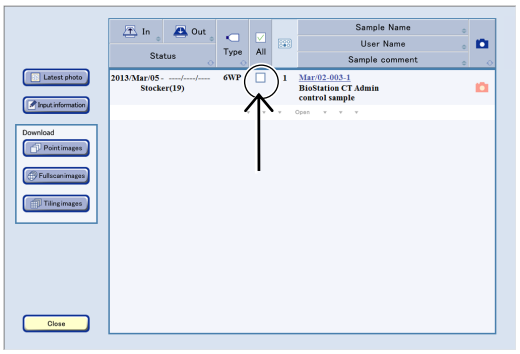
The first sample stored in the selected holder is displayed in the Sample list window.



Select function window

(3) Press the check box.

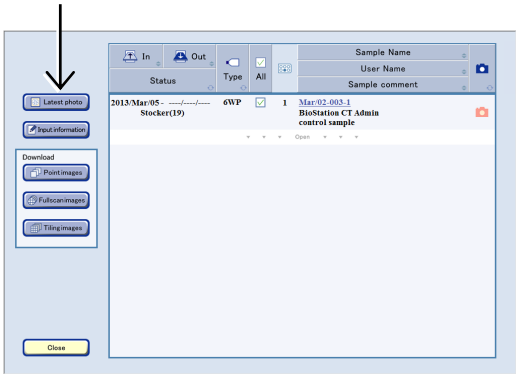
When check boxes are checked with only the first sample in the holder displayed, all samples in the holder are selected.
Also to select all samples in the holder, press the Stocker/Container field.



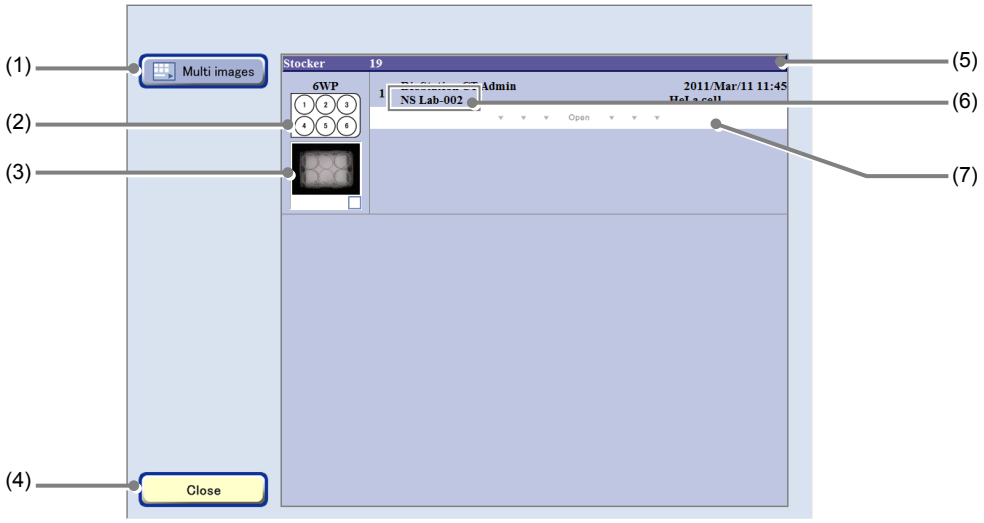
Sample list window (holder list)

(4) Press the Latest photo button.

The Latest photo list window appears.



The Latest photo list window lists the latest images captured in scheduled observations for the samples in the selected holder.
This window does not list the images captured during live observation or images captured during scheduled observation in the stage exclusive mode.

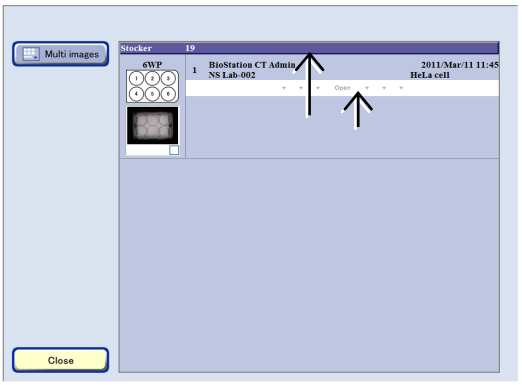


Latest photo list window (holder list)

No.	Name	Function
(1)	Multi images button	Press this button to display the Multi images display window. (See Section 4.9, “Multi Images Display of Captured Images.”)
(2)	Holder information	This area displays the vessel name, the vessel shape, and the holder map number.
(3)	Macro image	This area displays the macro image thumbnail. To enlarge an image, press the thumbnail.
(4)	Close button	Press this button to close the Latest photo list window.
(5)	Stocker number display area	This area displays the stocker number. Press the area to switch information to be displayed.
(6)	Sample name area	This area displays the sample name. Press the area to display the thumbnail of latest images for the relevant sample.
(7)	Open area	Press the area to spread the holder list and open a sample list.

(5) Switch the display to view the desired information.

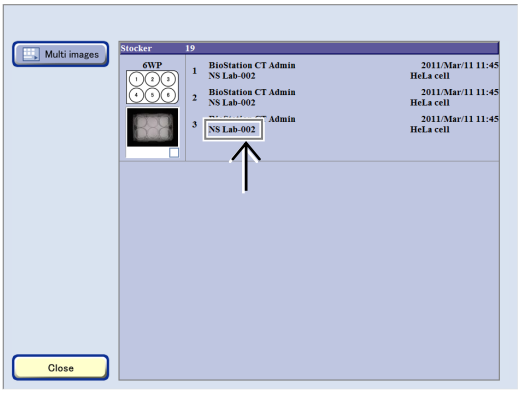
Press the Stocker number display area to change the information displayed. The display can be switched in the following order: holder list – sample list – latest photo list.



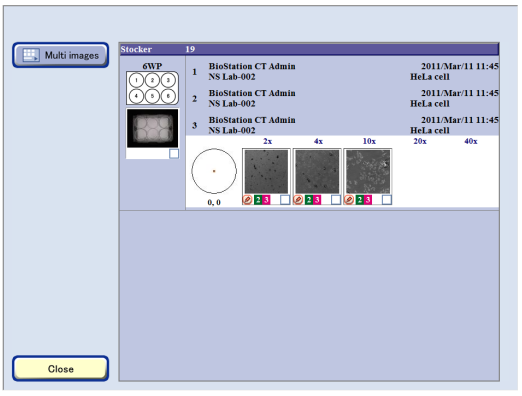
Holder list

The Open area is displayed in the holder list when the relevant holder contains multiple samples. When the Open area is pressed, the holder list expands and a sample list appears.

When the Sample name area of the sample list is pressed, the latest photo list for the relevant sample appears.

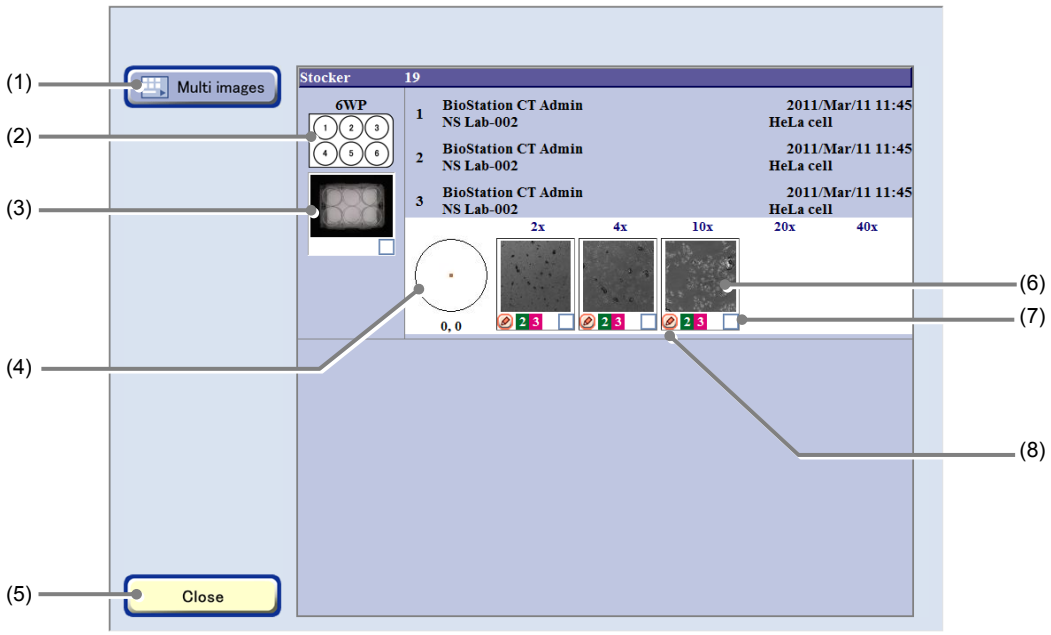


Sample list



Latest photo list window

In the Latest photo list window, the observed image thumbnails are displayed in order of the stocker number, the holder map number, and the observation position.



Latest photo list window

No.	Name	Function
(1)	Multi images button	Press this button to display the Multi images display window. (See Section 4.9, “Multi Images Display of Captured Images.”)
(2)	Holder information	This area displays the vessel name, the vessel shape, and the holder map number.
(3)	Macro image	This area displays the macro image thumbnail. To enlarge an image, press the thumbnail.
(4)	Observation point display	The observation position in the culture vessel is displayed.
(5)	Close button	Press this button to close the Latest photo list window.
(6)	Micro image	This area displays the micro image thumbnail. To enlarge an image, press the thumbnail.
(7)	Image selection button	Press this button to select an image to be displayed in the Multi images display window. When selected, a checked symbol appears in the check box for the image.
(8)	Comment button	To enter a comment for a micro image, press the Comment button at the bottom left of the image.

4.8 Analysis of Fluorescence Intensity

This section describes the functionality of and the procedures for using fluorescence intensity analysis as provided by the fluorescence unit.

Fluorescence images of the same observation points on the same sample are captured in scheduled observation, classified into target areas based on their intensity values, and then the following chart, histogram and the analysis results image are created.

To display the FL analysis results display window, select the desired channel tab in the Point image display window (FL) for the target fluorescence image and then press the FL option button.

The procedure for displaying the FL analysis results display window from the Image review window is as follows.

(1) Press the Expand button for the desired history data to display images in the Image review window.

The history area expands to display the captured thumbnail images.

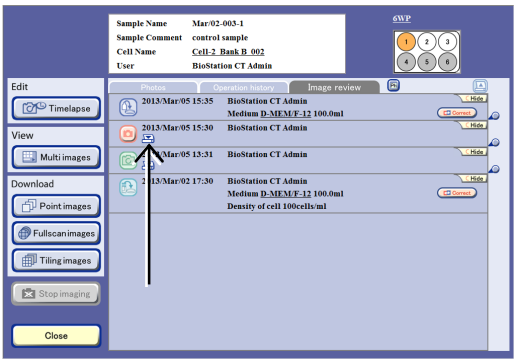


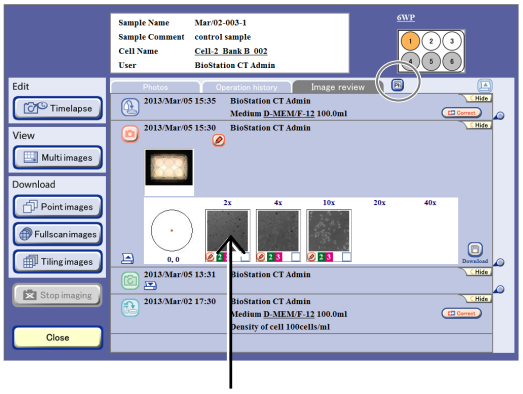
Image review window

(2) Display the FL image.

1. Change the Thumbnail display switch button to [FL].

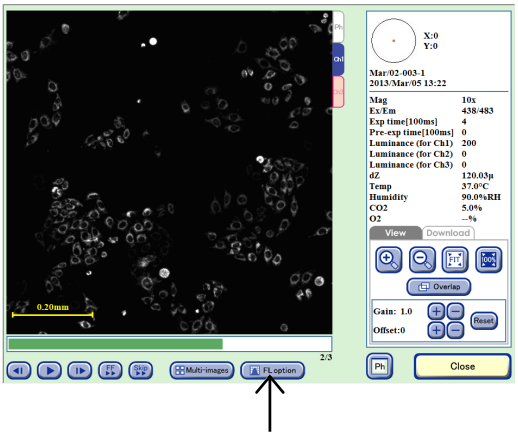
2. Press the thumbnail image of the micro image.

The Point image display window (FL) appears.



(3) Press the FL option button.

The FL analysis results display window appears.



Point image display window (FL)

4.8.1 Functions for Analysis of Fluorescence Intensity

Fluorescence intensity analysis is performed to analyze the intensity values of the fluorescence images captured in scheduled observation so they can be categorized and displayed in chronological order.

Analysis results data display area

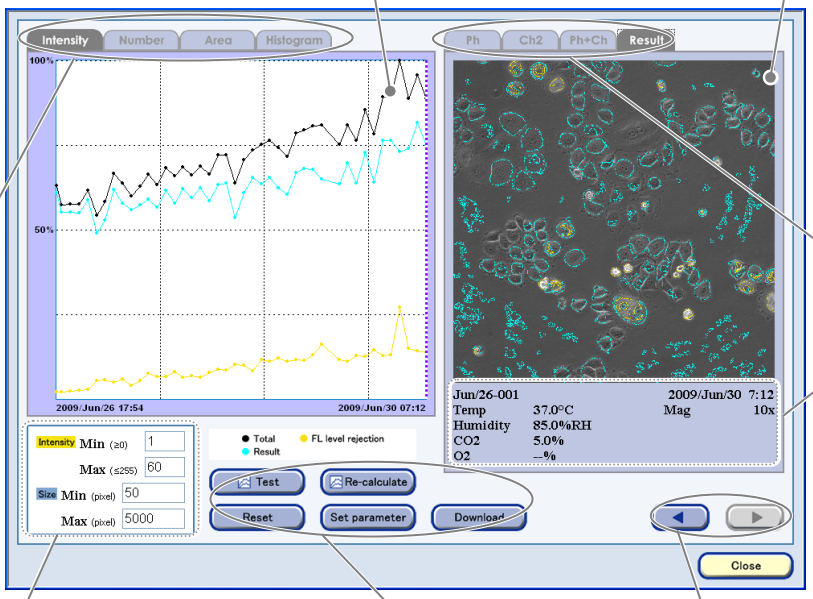
Display the following chart or histogram corresponding to the selected Display data switch tab.

- Intensity: Chart of the fluorescence intensity value
- Number: Chart of the number of fluorescence expression area
- Area: Chart of the size of fluorescence expression area
- Histogram: Histogram of the intensity values for various fluorescence images

Image display area

Display the following chart or histogram corresponding to the selected Display image switch tab.

- Ph： Phase contrast image
- Chn (n indicates a channel number): Fluorescence image to be analyzed
- Ph+Ch: Image overlaid with the fluorescence image and the phase contrast image
- Result: Analysis results image



Display data switch tabs

Display image switch tabs

Condition (Environmental information display)

Analysis parameter settings area

Execution buttons

Chronological data adjust buttons

FL analysis results display window

4.8.1.1 Analysis results

In the FL analysis results display window, the following chart, histogram, or analysis results images are created and displayed when an analysis is performed.

a. Chart of the fluorescence intensity values
Display the total intensity values of all categorized expression areas in chronological order.

b. Chart of the number of fluorescence expression areas
Display the number of categorized expression areas in chronological order.

c. Chart of the size of fluorescence expression areas
Display the total size of categorized expression areas in chronological order.

d. Histogram of the intensity values for the fluorescence images
The ratio of the generation frequency of each intensity value in the image to the entire image shown.

e. Analysis results images
Display analysis results in various colors on the image overlaid with the fluorescence image and the phase contrast image.

Histogram “d” and analysis results image “e” are created each time a fluorescence image is captured or analyzed in scheduled observation. Additionally, charts “a,” “b,” or “c” and the analysis results file (.csv) are created during analysis and at initial image capturing in scheduled observation. When analysis is performed, the data is added.

Additionally, the analysis conditions parameter can be changed, and re-calculated with the Re-calculate button in order to update charts “a”, “b”, and “c” and the analysis results files (.csv).

The analysis results file (.csv) can be downloaded in the FL analysis results display window.

For details on the procedure for using the FL analysis results display window, see Section 4.8.2.2, “Procedure for checking analysis results.”

An analysis result may be incorrect under the following conditions:

- In the case of a sample that uses a medium with strong self-fluorescence
- When, for example, the captured object is large because it is forming a colony
- When the captured object is close to the inner walls of the culture vessel
- When the object is captured at a high magnification

4.8.1.2 Analysis method

Analysis of the fluorescence images in the Scheduled settings is performed for each image. (Note that this function is not available for images captured in live observation.)

Extract a fluorescence expression area from the threshold by using the following four parameters.

a. Threshold of cell expression area (Intensity: Min. (≥0))

The intensity values lower than this parameter are excluded from the expression area.

b. Threshold of the area for the over intensity of fluorescence (Intensity: Max. (≤255))

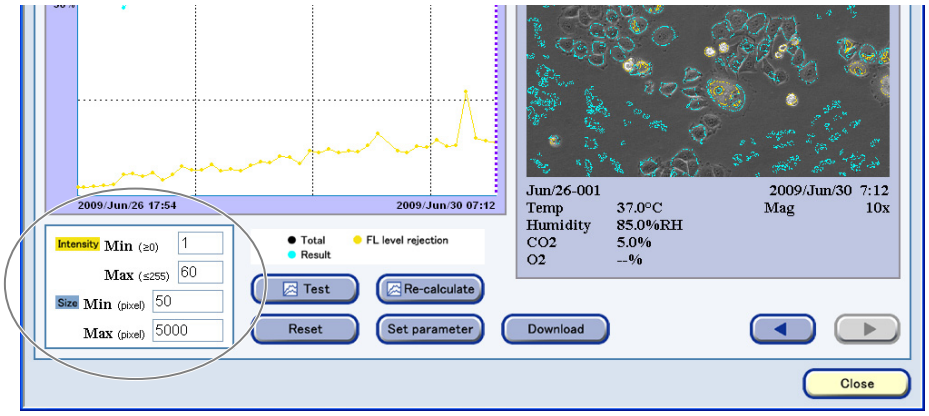
The intensity values higher than this parameter are excluded from the expression area for the over intensity of fluorescence.

c. Smallest value for expression area size (Size: Min. (pixel) (≥0))

When expression areas are extracted from the parameters “a” and “b”, the areas smaller than this parameter are excluded from these expression areas.

d. Largest value for expression area size (Size: Max. (pixel) (≤1,000,000))

When expression areas are extracted from the parameters “a” and “b”, the areas bigger than this parameter are excluded from these expression areas.



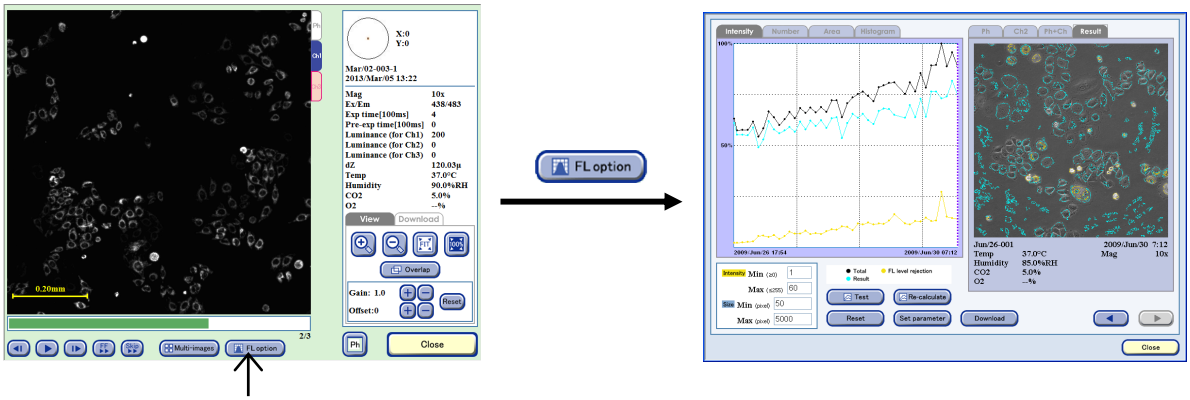
The system default values are used as the default values for these parameters when performing analysis. These default parameter values can be changed to be used as default analysis conditions for each user when checking the analysis results. For details, see Section 4.8.2.2, “Procedure for checking analysis results.”

The following two areas can be extracted using the above analysis methods.

- Fluorescence standard intensity area
- Fluorescence over intensity area

4.8.2 Using the Analysis Results Display Window

The FL analysis results display window is displayed by selecting an appropriate channel for the fluorescence image in the Point image display window (FL) with the FL option button.



Additionally, the FL option button is enabled by selecting an appropriate channel tab.

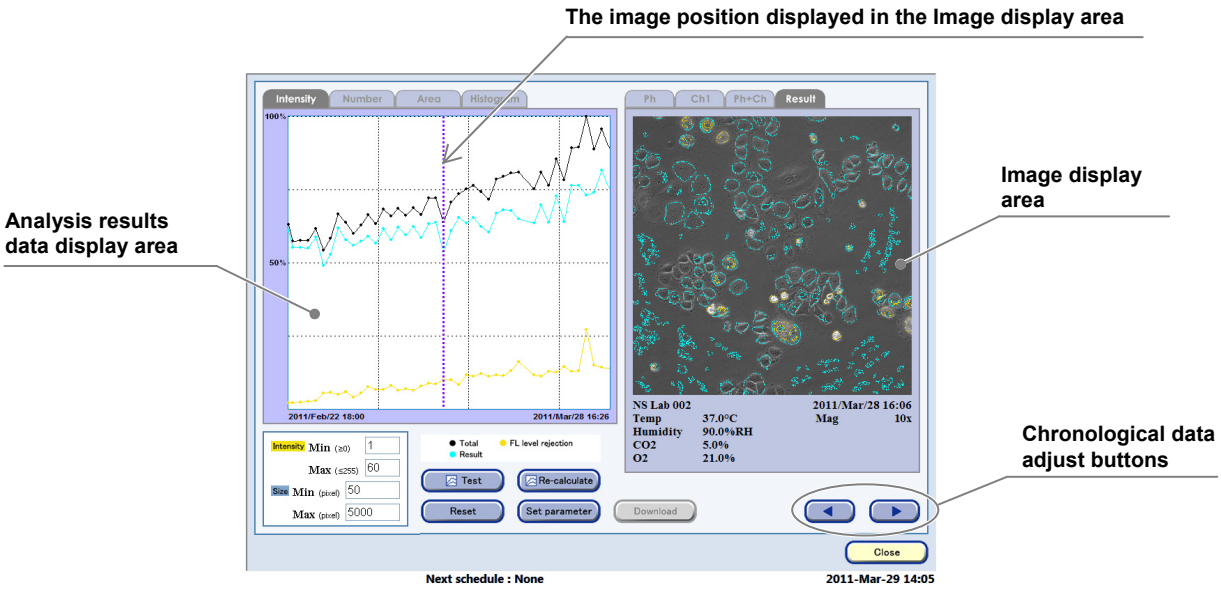
For details on displaying the Point image display window (FL), see Section 4.7.3.2, “Viewing the micro image (FL image) and preparing download.”

Check the analysis results in the FL analysis results display window.

4.8.2.1 Displaying analysis result contents

The analysis results for an image captured in scheduled observation appear in the FL analysis results display window allowing the following to be confirmed.

The analysis results data of the chart or the histogram appears in the Analysis results data display area on the left side. Additionally, the vertical purple line on the chart shows the display position (for image capture timing) of the image displayed in the Image display area. Press an appropriate position on the chart to indicate the current position or press the Chronological data adjust button to switch it.



Viewing the Analysis results data display area

The following section describes the Analysis results data display area.
The chart or histogram corresponding to the selected Display data switch tab is displayed in this area.

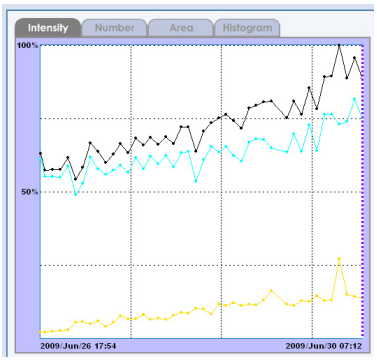
Displaying the chart

The data in the area extracted by analysis is displayed in the chart as follows:

Blue: Normal expression area

Yellow: Over-expression area

Black: The total areas in the above expressions



The horizontal axis of each chart shows time between the first observation date and time and the latest observation date and time.

The vertical axis of each chart shows the maximum value of 100 % (the maximum value of the vertical axis) to be plotted.

The data of the vertical axis for each chart varies depending on the tab selected as shown below.

a. Chart displayed in the [Intensity] tab

This is a chart of the fluorescence intensity value. The intensity value of the fluorescence expression area shows in chronological order.

The vertical axis of the chart shows the total intensity values of fluorescence expression areas extracted from each fluorescence image.

b. Chart displayed in the [Number] tab

This is a chart of the number of the fluorescence expression area. This chart displays the number of the fluorescence expression areas in chronological order.

The vertical axis of the chart shows the number of the fluorescence expression area extracted from each image.

c. Chart displayed in the [Area] tab

This is a chart of the size of the fluorescence expression area. The area size in the fluorescence expression shows in chorological order.

The vertical axis of the chart shows the total size (the number of pixels) of the fluorescence expression areas extracted from each image.

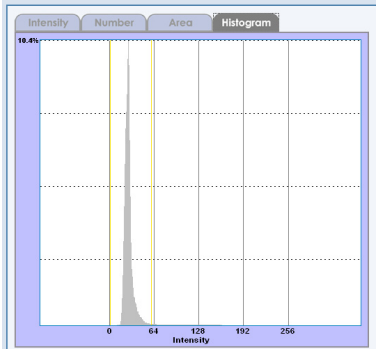
Displaying the histogram

This histogram indicates the ratio of the areas that contain each intensity value to the entire image displayed with the [Histogram] tab.

The horizontal axis of this histogram indicates the intensity value displayed in 256 (between 0 and 255) gradations.

The vertical axis of this histogram indicates the ratio of the areas (number of pixels) that contain each intensity value to the entire image (1,000,000).

Its maximum value indicates the maximum value of the vertical axis.



Viewing the Image display area

The image captured in one schedule observation and the Analysis results image appear in the Image display area. Pressing the Display image switch tab switches the display image as follows:

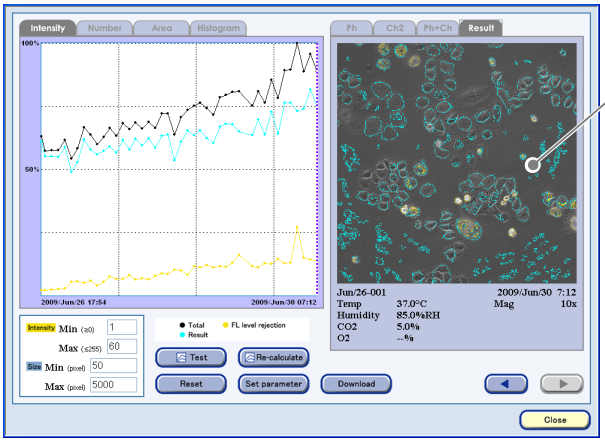


Image display area

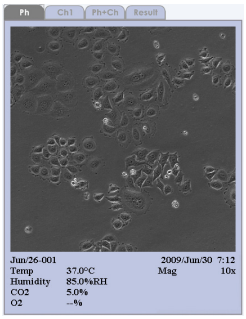
The following image compatible with the selected Display image switch tab appears.

- Ph: Phase contrast image
- Chn (n indicates a channel number): The fluorescence image for object to be analyzed.
- Ph+Ch: Image overlaid with the fluorescence image and the phase contrast image.
- Result: Analysis results image

Image display area

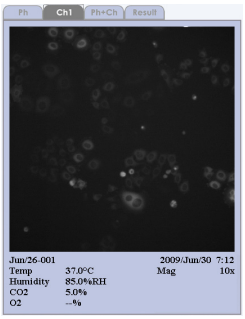
a. Image displayed with the [Ph] tab

The phase contrast image captured at the specified timing.



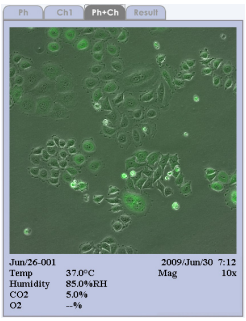
b. Image displayed with the [Chn] tab (n indicates a channel number)

The analyzed fluorescence image.



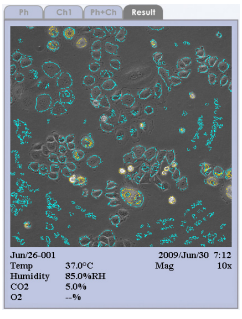
c. Image displayed with the [Ph+Ch] tab

Display the overlaid image of the fluorescence and phase contrast image captured in the specified timing.



d. Image displayed with the [Result] tab

Display the area extracted by an analysis in color over a black and white image of the overlaid image of the fluorescence and phase contrast images. The extracted area color is the same as the one displayed in the chart (the area for the standard intensity of fluorescence: Blue; the area for the over intensity of fluorescence: Yellow).



4.8.2.2 Procedure for checking analysis results

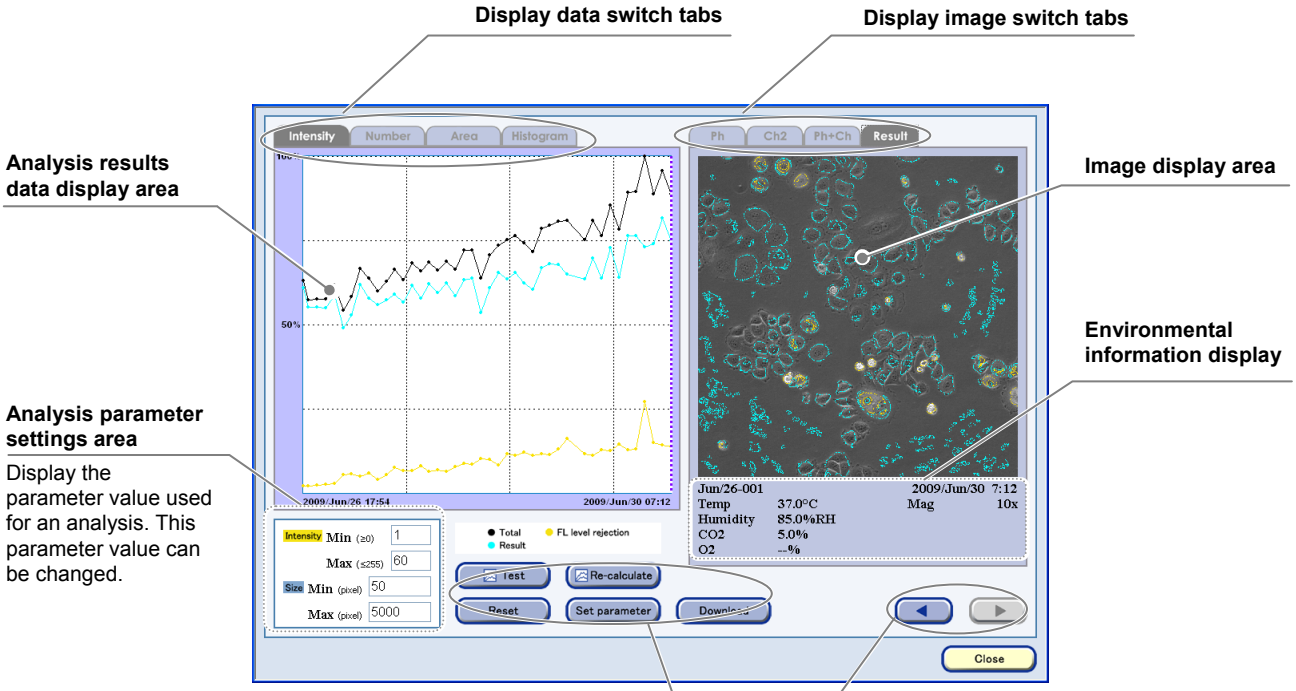
To check the analysis results, follow the procedure below.

(1) Check the content of the Analysis results data display area or Image display area.

If necessary, switch the charts, the histogram or the image to be displayed with the Display data switch tab.

Additionally, to switch the display position (for image capture timing) to check the image, use the Chronological data adjust button.

For details on checking the content of the Analysis results data display area or the Image display area, see Section 4.8.1.1, “Analysis Results.”



Execution buttons

Test	Re-calculate the data displayed in the analysis data display area using the parameter value displayed in the window.
Re-calculate	Re-calculate all data using the parameter value displayed in the window.
Reset	Reset the re-calculated-result with the Test button. When the button is pressed while the re-calculation is not performed, the parameter value is applied to be reset.
Set parameter	Display the parameter value to be registered for the analysis conditions of the logged in user.
Download	Download the analysis result file (.csv) to an external PC. This button is effective only when an external PC is operated.

Chronological data adjust button

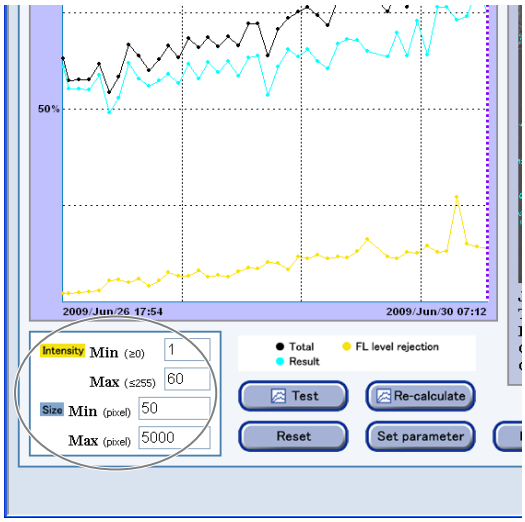
Switch the displayed image in the chronological order. Additionally, when a chart is displayed in the Analysis results data display area, the vertical axis that indicates the corresponding position of the image is also moved.

FL analysis results display window

(2) If necessary, change the analysis conditions, and then re-calculate them to extract an appropriate analysis result.

Follow the steps below.

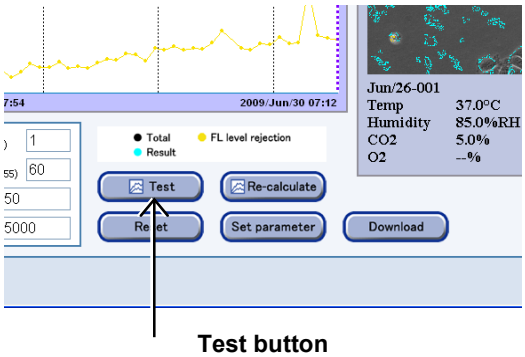
1. **Change parameters in the Analysis parameter settings area in the FL analysis results display window.**



Analysis parameter settings area

2. **Press the Test button, and then re-calculate the analysis results of the selected display position (for image capture timing).**

   The Result display and the chart in the Analysis results data display area are changed.



Test button

3. **Check the image in the Image display area.**

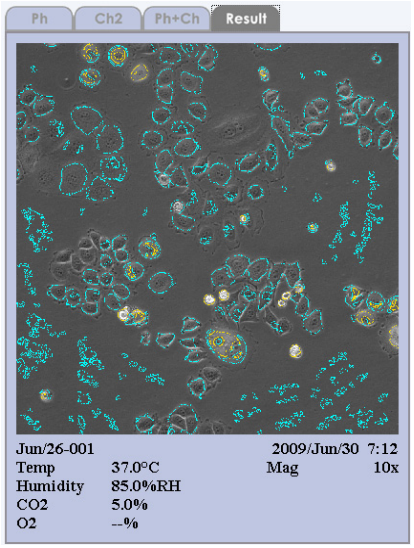
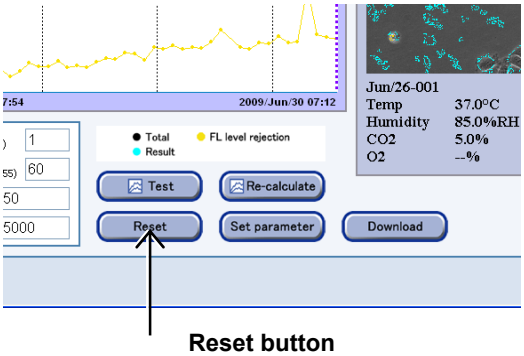


Image display area

4. **Repeat steps 1 to 3 until the appropriate image conditions are met.**

   To return the current parameter values to the default settings, press the Reset button.

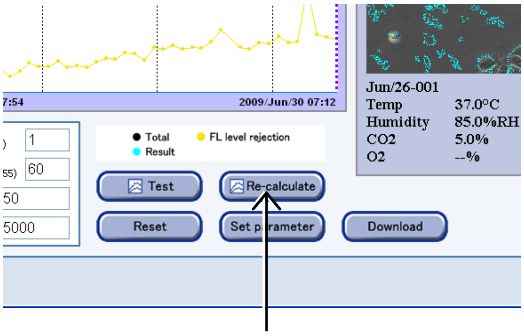


Reset button

5. **When the image in the image display area is properly adjusted, press the Re-calculate button to re-analyze all data.**

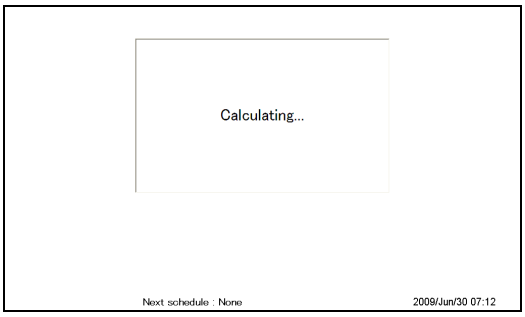
   Because it takes time for re-calculation, a message asking you to confirm whether to re-calculate all data appears.

   To continue, press the OK button.



Re-calculate button

   The dialog box indicating that the calculation is being processed appears.



6. **If necessary, check the chart in the Analysis results data display area.**

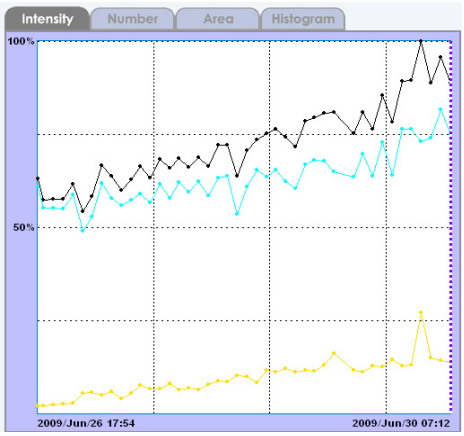
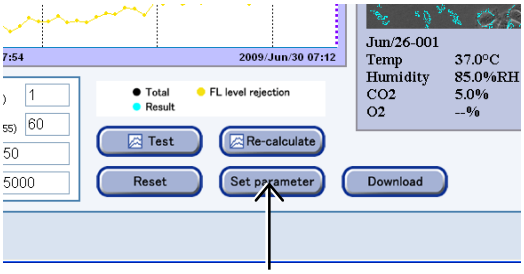


Chart in the Analysis results data display area

(3) To register the analysis conditions specified in step (2) for each user default channel, perform the following operations.

1. Press the Set parameter button.

The following Analysis conditions register window appears.



FL analysis results display window

User-defined

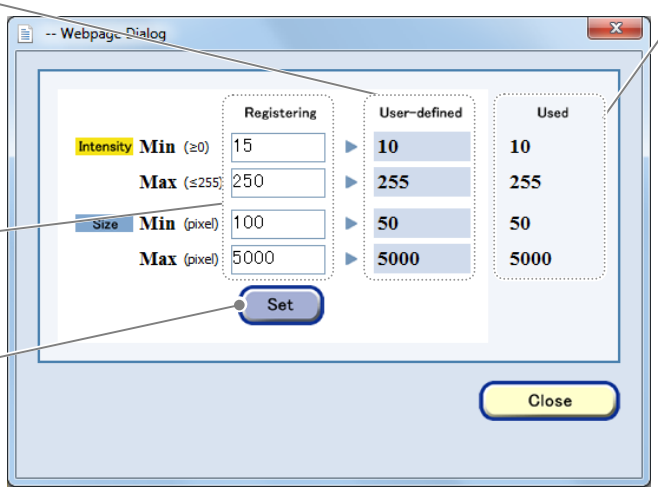
Parameter values registered as analysis conditions.

Registering

Parameters displayed in the FL analysis results display window.

Set

Register the analysis conditions displayed in the Registering field as default analysis conditions of each channel specified for each user.



Used

Parameters used for the calculation of the analysis result currently displayed. Change the parameters in the FL analysis results display window, and then press the Re-calculate button to re-calculate all data of the displayed channels.
The values in the Used field are modified. However, if only one image is re-calculated as a test the display of the field is not changed.

Analysis conditions register window (Immediately after the window is displayed)

2. To use the analysis conditions for the parameters displayed in the Registering field, press the Set button.

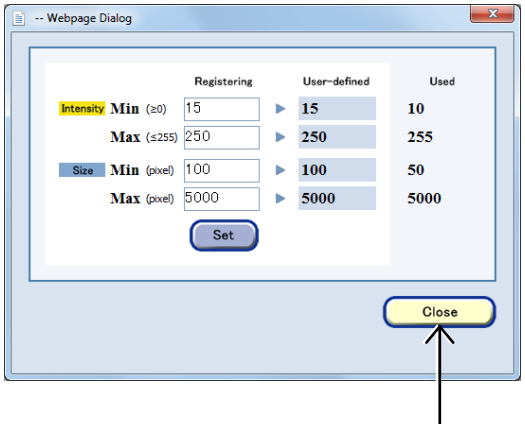
The values in the Registering field can be changed for registration.

The values in the Registering field are replaced with the values displayed on the screen. The values in the User-defined field can also be changed.

3. When the registration of the analysis conditions is completed, press the Close button.

The FL analysis results display window appears.

If you do not need to register analysis conditions in the Analysis condition register window, press the Close button without performing step (2).



Analysis conditions register window (after the conditions are set)

Analysis results can be downloaded in cvs file format.
The download operation can be performed only from an external PC. For details on the procedure for downloading analysis results, see the “BioStation CT Ver. 3.8 Instructions (PC Operations).”

4.9 Multi Images Display of Captured Images

4.9.1 Multi Images Settings

Multiple captured images can be selected, displayed, and played back side by side.
Note that an image captured with a normal scheduled observation and an image captured in the Stage exclusive modes cannot be displayed simultaneously in the Multi images display window.

(1) Open the Each image window.

a. To open the Each image window in the Image review window

a-1. Press the Expand button for the desired history data to display images in the Image review window.

The history area expands to display the captured thumbnail images.

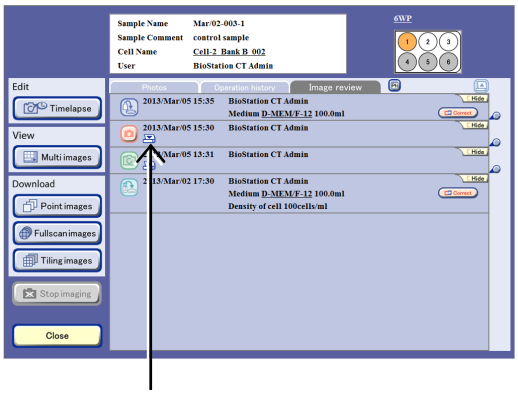
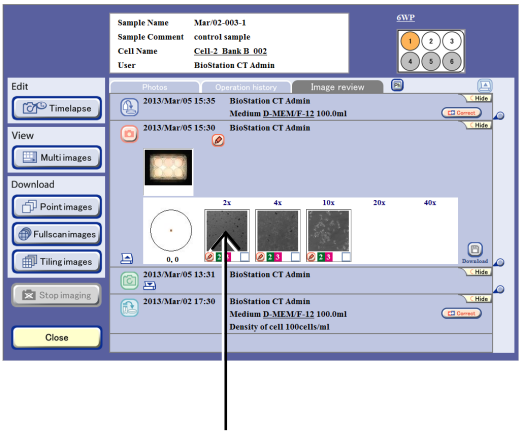


Image review window

a-2. Press the thumbnail image of various images.

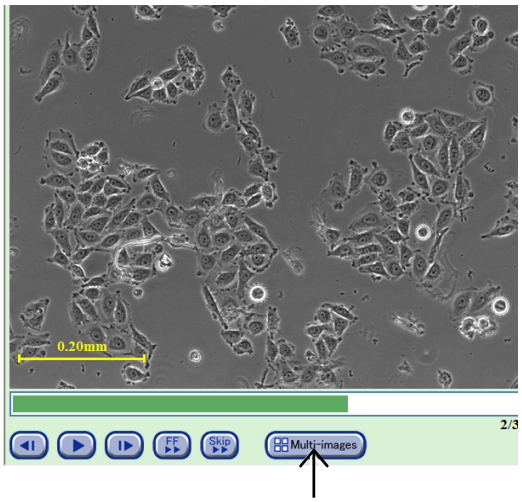
The Image display window appears.



a-3. Press the Multi-images button in the various image display window.

The Multi images display window to which the various images previously displayed in the display window were added appears.

Go to step (2).

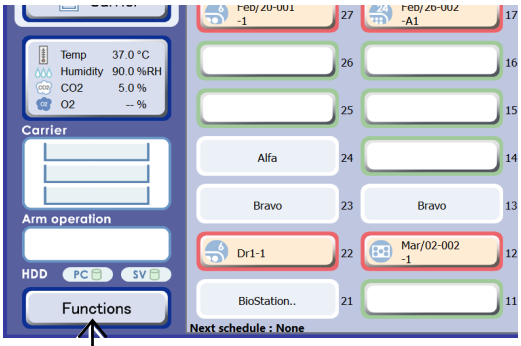


Various images display window

b. To open the each image display window in the Functions window

b-1. Press the Functions button on the System status screen.

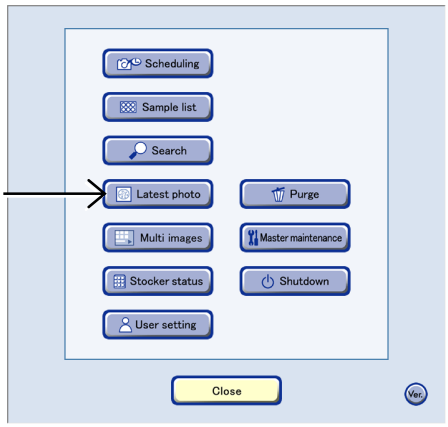
The Functions window appears.



System status screen

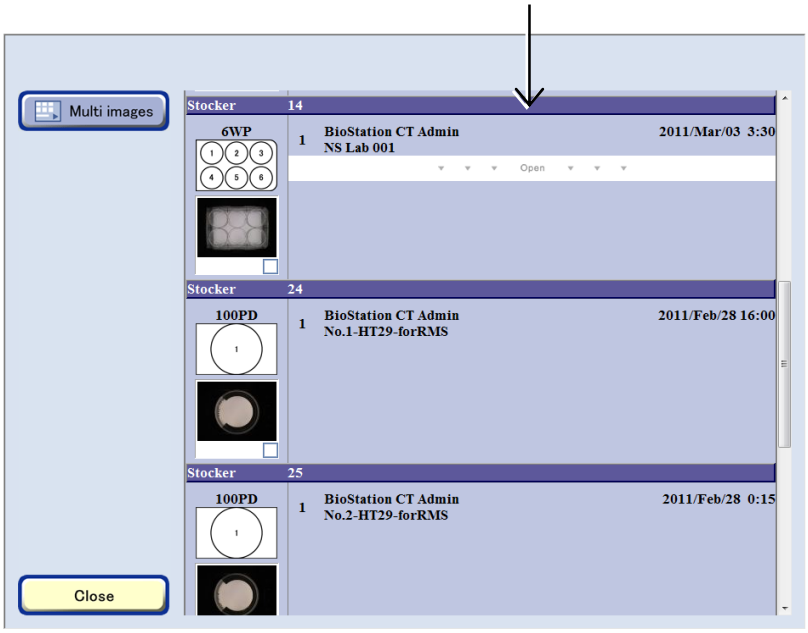
b-2. Press the Latest photo button.

The Latest photo list window appears. The latest images for all the samples cultured by the user are displayed.



Functions window

b-3. Press the Stocker number display area twice to open the Image display window.



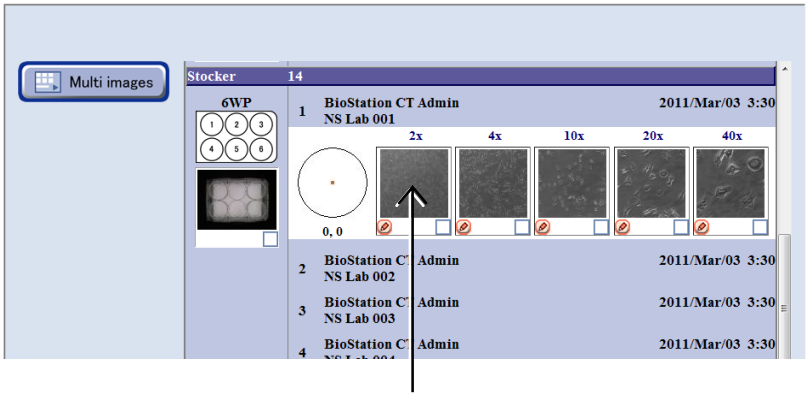
Latest photo list window (holder list)

b-4. Select an image to be displayed in the Multi images display window.

To register an image on the Multi images display from the display window of each image

Press the thumbnail image of the image to be displayed in the Multi images display window.

The various images display window appears.

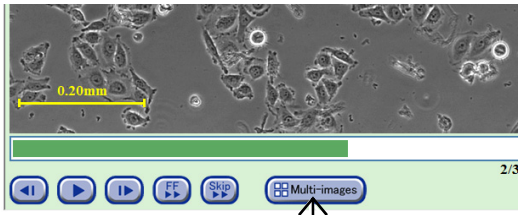


Latest photo list window (latest photo list)

Press the Multi-images button in the various image display window.

The Multi images display window to which the various images previously displayed in the display window were added appears.

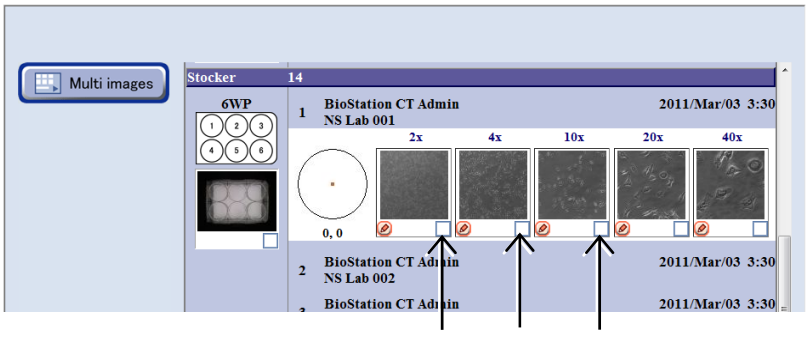
Go to step (2).



Various images display window

To register multiple images on the Multi images display in batch processing

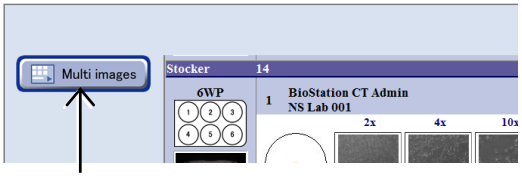
Press the image selection button below the thumbnail images to select the images to be displayed in the Multi images display window.



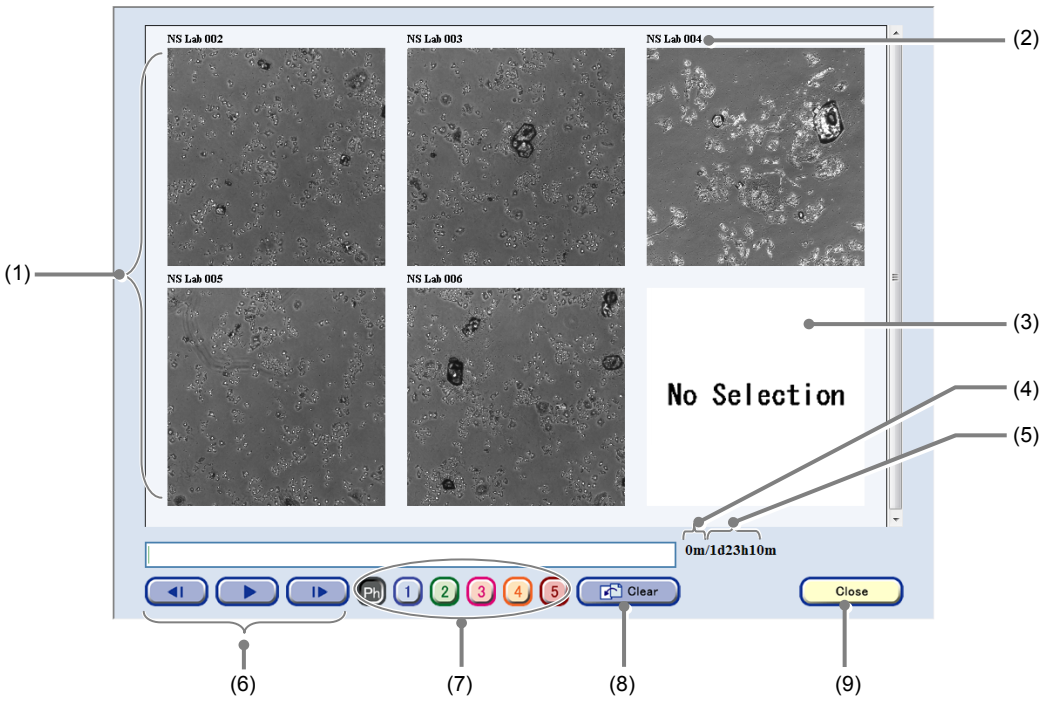
Latest photo list window (latest photo list)

Press the Multi images button.

The Multi images display window to which the selected image was added appears.



(2) To add an image to the Multi images display window, repeat step (1).



Multi images display window

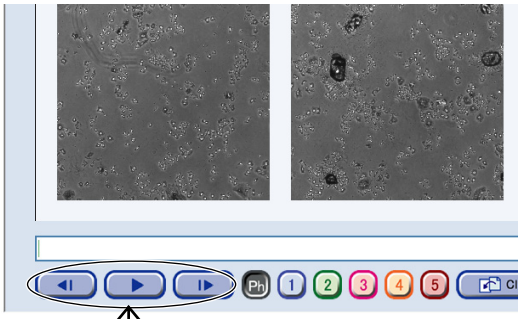
No.	Name	Function
(1)	Scheduled observation images	Press the image to display the Multi images display menu window.
(2)	Sample name area	This area displays the sample name.
(3)	No Selection area	Area not in use.
(4)	Playback time	This area displays the playback time from start to end.
(5)	Playback time for a single frame	The interval for the shortest scheduled time is defined as a single frame.
(6)	Multi images playback buttons	**► button:** Play back images. This button functions as the pause button during playback. **I►/◄I buttons:** Play one frame forward /one frame backward
(7)	Overlay display buttons	Effective for fluorescence images only. Press this button to overlay images of the selected channel.
(8)	Clear button	Press this button to clear all displayed images. To delete only a selected image, press the image, and then delete it in the Multi images display menu window.
(9)	Close button	Press this button to close the Multi images display window.

4.9.2 Multi Images Playback

Multiple selected scheduled observation images can be played back side by side.

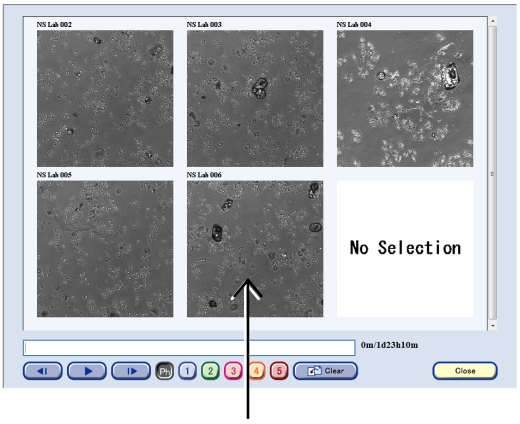
(1) Press the ► button to play back an image or the I► /◄I buttons to go forward or backward a frame.

The shortest interval for scheduled observation is played back as a single frame.

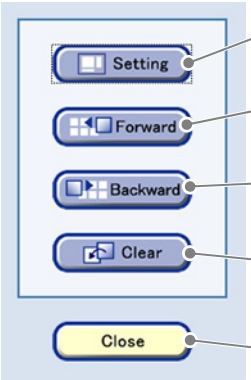


Multi images display window

(2) To set an image, press the desired scheduled observation image.



The Multi images display menu window appears.



Setting button
Display the Multi images playback setting window.

Forward button
Move the selected image forward on the Multi images display window.

Backward button
Move the selected image backward on the Multi images display window.

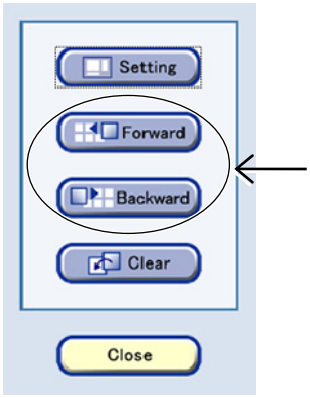
Clear button
Delete the selected image on the Multi images display window.

Close button
Return to the Multi images display window.

Multi images display menu window

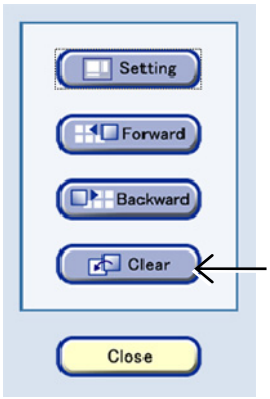
1. **Press the Forward/Backward button to move the selected images forward or backward in the Multi images display window.**

   The selected image that was moved forward or backward appears in the Multi images display window.



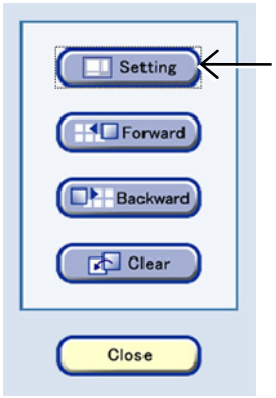
2. **To delete the selected image in the Multi images display window, press the Clear button.**

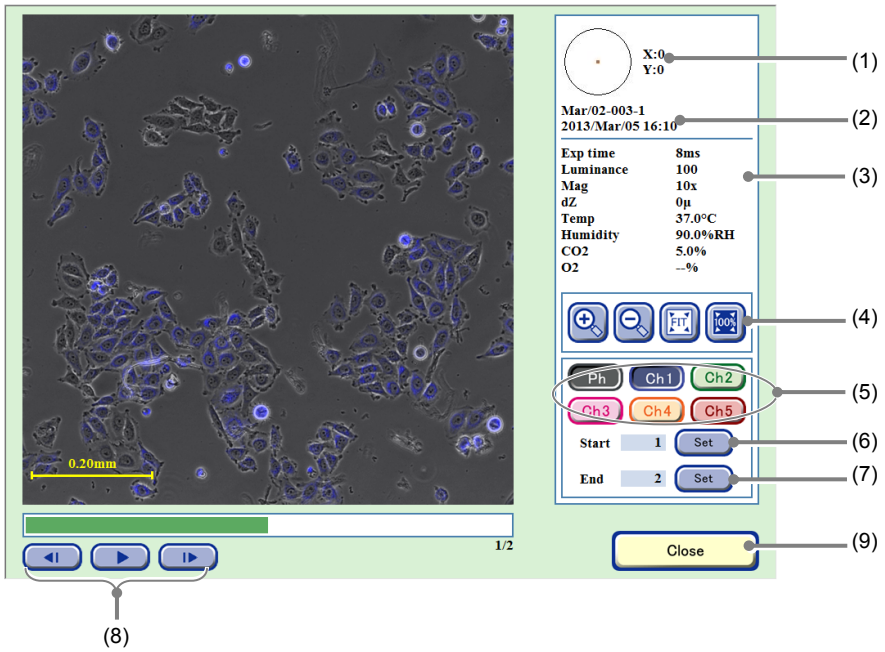
   The Multi images display window without the selected image appears.



3. **To change the start time or the end time of playback for the selected image, press the Setting button.**

   The Multi images playback setting window appears.

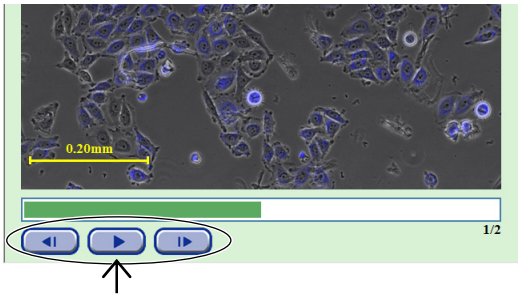




Multi images playback setting window

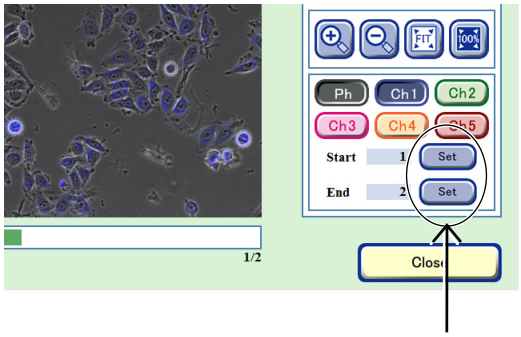
No.	Name	Function
(1)	Coordinate of the observation point	This area displays the coordinate (x,y) for the observation point where (0,0) is the center of the sample.
(2)	Sample information	This area displays the observation point, sample name, and captured date and time.
(3)	Image capture conditions and environmental information	This area displays the Image capture conditions and the temperature, humidity, and CO_2 concentration and O_2 concentration (optional) in the CO_2 incubator.
(4)	Display size change buttons	**+, - buttons:** Enlarge or reduce the image. **FIT button:** Display the image on the full screen. **100% button:** Display the image in the original size.
(5)	FL channel Display	Effective for images captured in scheduled observation only. Press a button to display the selected FL channel image.
(6)	Set button for Start	Press this button to set the playback start position.
(7)	Set button for End	Press this button to set the playback end position.
(8)	Timelapse images playback button	**► button:** Play back timelapse images continuously. During playback, this button changes to a pause button and pressing the button pauses playback. **I►/◄I button:** Plays one frame forward /one frame backward
(9)	Close button	Press this button to return to the Multi images display window.

4. **Press I►/◄I or ► button to display the image of the playback start position or the playback complete position.**



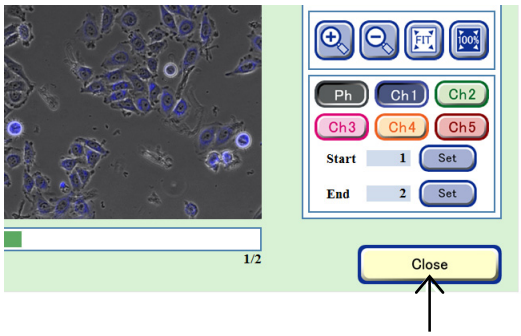
5. **For the playback start position, press the Set button for Start, or for the playback complete position, press the Set button for End.**

   The selected image is saved as the playback start position or playback complete position.

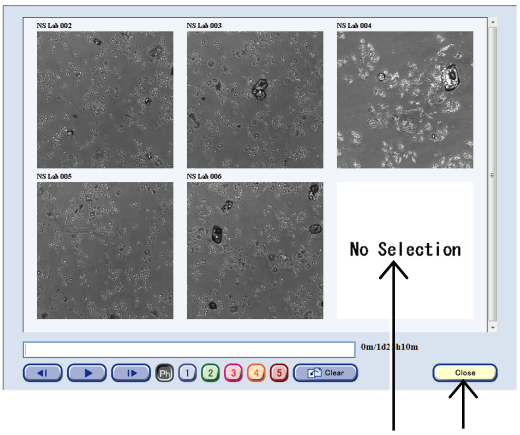


6. **Press the Close button to close the Multi images playback setting window.**

   The Multi images display window appears.



(3) **To close the Multi images display window, press the Close button or the No selection area.**



Multi images display window

4.9.3 Displaying the Multi Images Display Window

There are three methods for displaying the Multi images display window selected in Section 4.9.1, “Multi Images Settings.”
Once the image is selected, it remains until it is canceled for logout.

a. To open from the Image review window

a-1. Press the Multi images button in the Image review window.

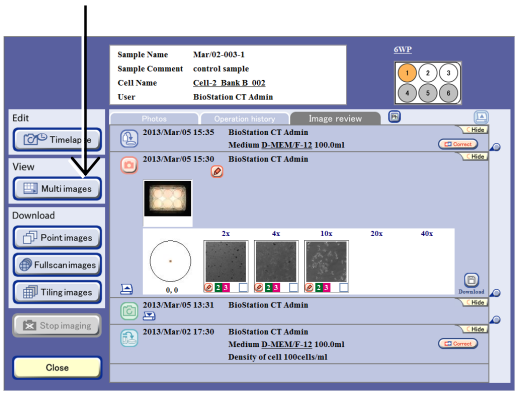
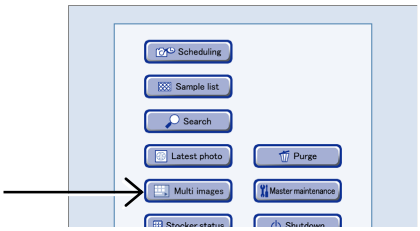


Image review window

b. To open from the Functions window

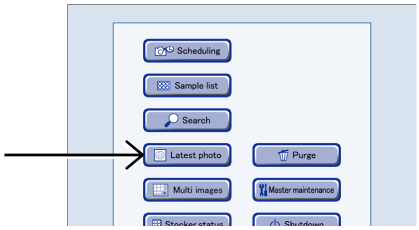
b-1. Press the Multi images button in the Functions window.



Functions window

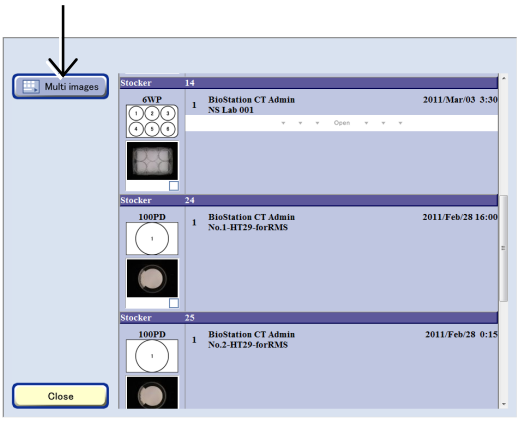
c. To open from the Latest photo window

c-1. Press the Latest photo button in the Functions window.



Functions window

c-2. Press the Multi images button in the Latest photo list window.



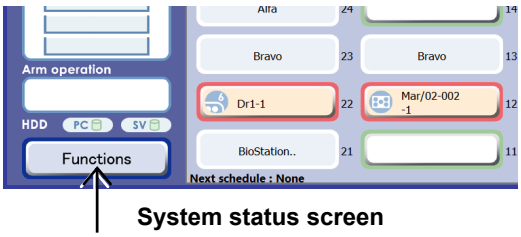
Latest photo list window

4.10 Searching for the Observation Data

This section describes the procedure for performing a keyword search for a sample in the observation data in the file server of the product. The observation data for cultured samples can be also searched for in the search window.

(1) Press the Functions button on the System status screen.

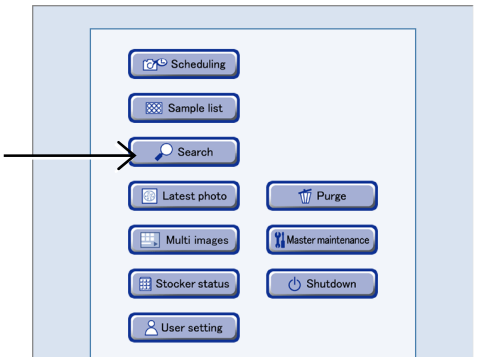
The Functions window appears.



System status screen

(2) Press the Search button.

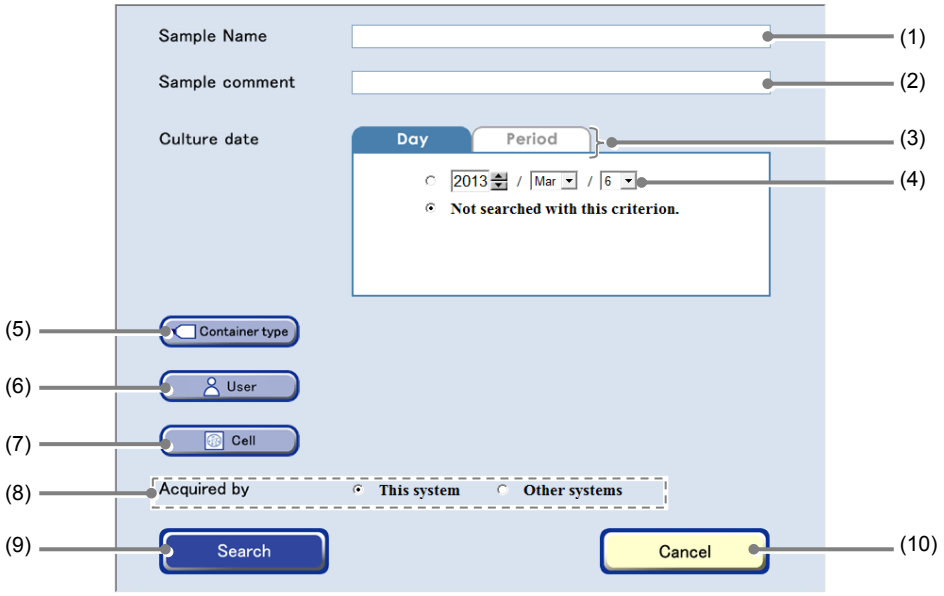
The Search window appears.



Functions window

(3) Specify the search criterion to search for a sample in the Search window.

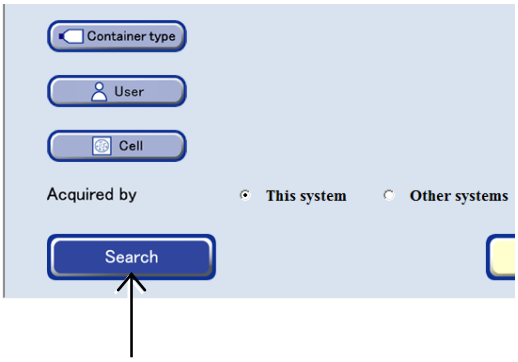
Multiple search criteria can be selected.
For details on the procedure for specifying the search criterion, see Section 4.10.1, “Entering the Search Criterion.”



Search window

No.	Name	Function
(1)	Sample Name field	Enter a sample name to search for a sample.
(2)	Sample comment field	Enter a comment registered for a sample to search for a sample.
(3)	Date selection method tab	Select the specification method between the culture date and culture period.
(4)	Culture date field	Specify a culture date to search for a sample.
(5)	Container type button	Specify a culture vessel type to search for a sample. Press this button to display the Vessel selection window.
(6)	User button	Specify a user name to search for a sample. Press this button to display the User selection window.
(7)	Cell button	Specify a cell name to search for a sample. Press this button to display the Cell selection window.
(8)	Observation equipment selection	Specify the observation equipment to search for a sample.
(9)	Search button	Press this button to start searching.
(10)	Cancel button	Press this button to close the Search window.

(4) After specifying the search criterion, press the Search button.

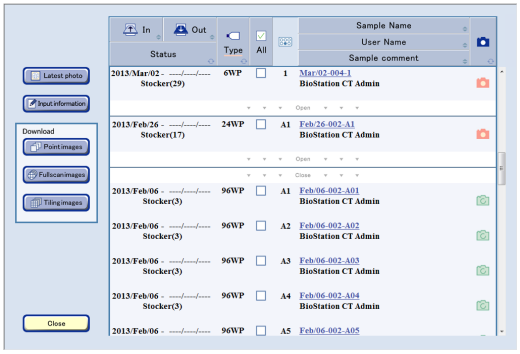


The samples that satisfy the search criterion are displayed in the Search result sample list window.

In the Search result sample list window, samples are listed in accordance with their stocker number and grouped by holder.

The display order of the sample list can be changed with the sort function at the top of the Search result sample list window.

Press the Open area to display all sample names in the holder. Press the Close area to display only the first sample.



Search result sample list window

For details on vessel name abbreviations used in the Search result sample list window, see the list on Page 57, “Abbreviation and description for vessels.”

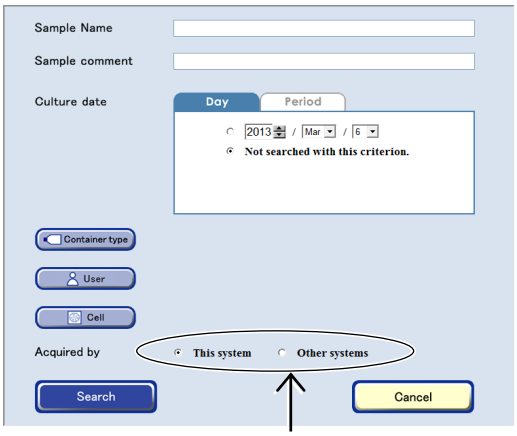
4.10.1. Entering the Search Criterion

This section describes the procedure for specifying the search criterion in the Search window.
Each search criterion can be specified separately and multiple search criteria can be combined.

(1) Select the equipment to observe.

Press the This system radio button to search for the Observation data observed by this system.

Press the Other systems radio button to search for the Observation data uploaded from other systems.



Search window

(2) Select the search criterion.

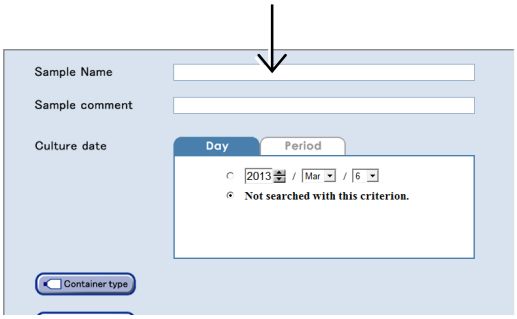
Searching by sample name

1. **Press the Sample Name field in the Search window.**

   The Keyboard window appears.

2. **Enter a full or partial sample name in the Keyboard window and press the OK button.**

   The sample name that is entered in the Sample Name field of the Search window appears.



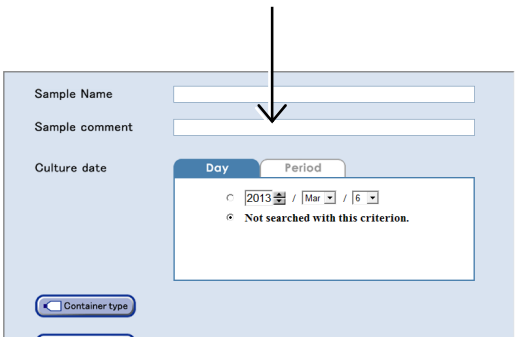
Searching by sample comment

1. **Press the Sample comment field in the Search window.**

   The Keyboard window appears.

2. **Enter a full or partial sample comment in the Keyboard window and press the OK button.**

   The sample comment entered in the Sample comment field of the Search window appears.



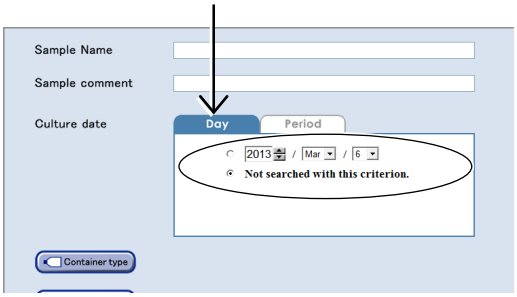
Searching by the culture date

1. **Select the Day tab in the Search window.**
2. **Specify the culture date for the search.**

   All samples that are cultured on the specified date will be searched for.

3. **Press the Radio button on the left of the date display.**

   When not searching by the culture date, turn ON the “Not searched with this criterion.” radio button.



Search window (Day tab)

Searching by specifying the culture date period

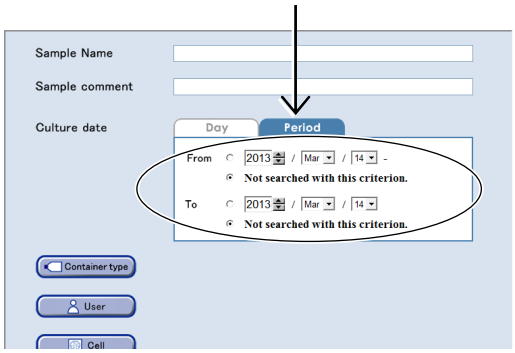
1. **Select the Period tab in the Search window.**
2. **Specify the period to search.**

   Specify the start date of the period to search in the From field.
   Specify the end date of the period to search in the To field.

   All samples that are cultured during the specified period will be searched for.

3. **Press the Radio button on the left of the date display.**

   When not specifying the date, turn ON the “Not searched with this criterion.” radio button.

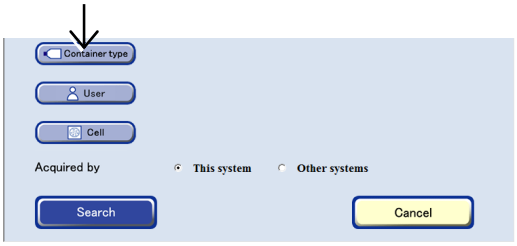


Search window (Period tab)

Searching by the culture vessel

1. **Press the Container type button in the Search window.**

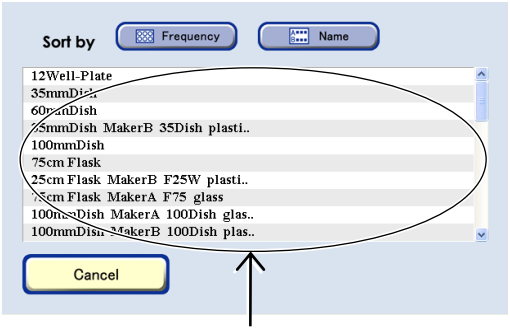
   The Vessel selection window appears.



Search window

2. **Select the culture vessel in which the targeted sample is stored.**

   The Vessel selection window is closed and the Search window appears.



Vessel selection window

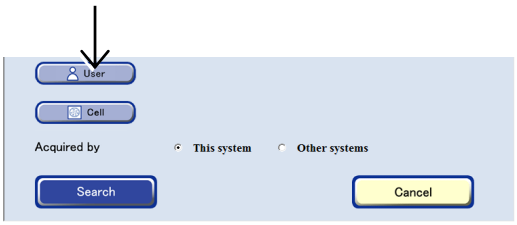
   The selected vessel name is displayed on the side of the Container type button in the Search window.

Search window

Searching by the user name

1. Press the User button in the Search window.

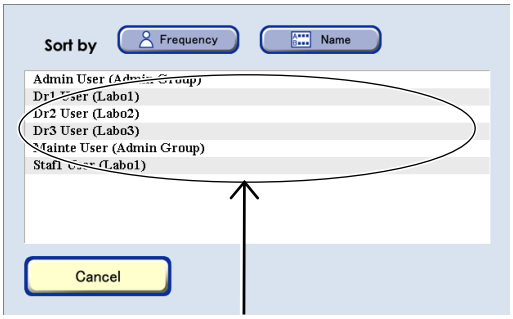
The User selection window appears.



Search window

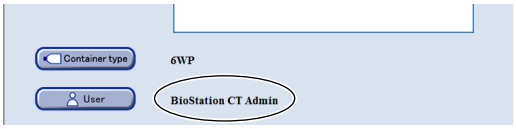
2. Select the user name for the sample to be searched for.

The User selection window closes and the Search window appears.



User selection window

The selected user name is displayed on the side of the User button on the Search window.

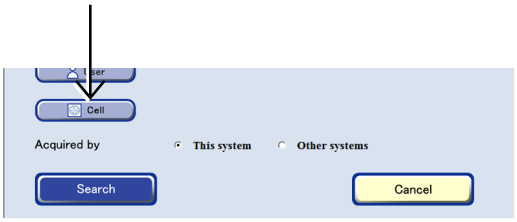


Search window

Searching by the cell name

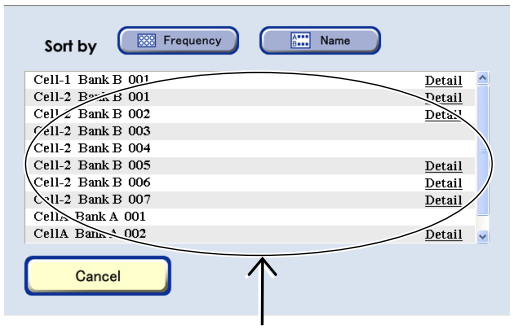
1. Press the Cell button in the Search window.

The Cell selection window appears.



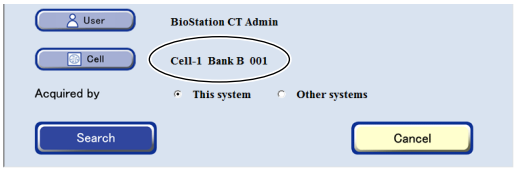
2. Select the cell name of the sample to be searched for.

The Cell selection window closes and the Search window appears.



Cell selection window

The selected cell name is displayed on the side of the Cell button on the Search window.



Search window

4.11 Downloading the Observation Data

The captured observation data (Observation image data) can be downloaded to an external PC.

There are two ways data can be downloaded:

Automatic download: (Using BioCT communicator)
By setting automatic download with the BioCT communicator beforehand, captured images are automatically downloaded to the specified folder on an external PC each time scheduled observation completes.
For a timelapse observation, the latest images will be added to the downloaded data as appropriate.

Automatic download is set on an external PC.
See the “BioStation CT Ver. 3.8 Instructions (PC Operations)” for information on how to set automatic download.

Manual download: The user manually prepares and performs download each time if required.
Download is prepared by specifying the observation data to be downloaded and then the data is downloaded to the external PC.
The procedure for download preparation is described in this section.

> To manually download the observation data, it is necessary to prepare the download in advance.
>
> Download files created by download preparation are downloaded on an external PC.
> For details on the procedure for downloading observation data on an external PC, see the “BioStation CT Ver. 3.8 Instructions (PC Operations).”

This section describes the procedure for preparing observation data (Observation data and observed image data) for download.
When download preparation is completed, a download file is created in this system.

The following methods can be used to prepare data for downloading:

To individually prepare download of currently displayed images

See the following sections for details.
Section 4.7.3.1, “Viewing the micro image (Ph image) and preparing download”
Section 4.7.3.2, “Viewing the micro image (FL image) and preparing download”
Section 4.7.3.3, “Viewing the macro image and preparing download”
Section 4.7.3.4, “Viewing the Full Scan image and preparing download”
Section 4.7.3.5, “Viewing the Tiling image and preparing download”

To prepare download of observation data specifying a specific sample

For details, see Section 4.11.1, “Download Preparation of Observation Data for Each Sample.”

To batch download preparation of observation data for multiple holder/sample

For details, see Section 4.11.2, “Batch Download Preparation of Multiple Holder/Sample Observation Data.”

To batch download preparation of images for sample

For details, see Section 4.11.3, “Batch Download Preparation of Images for Sample.”

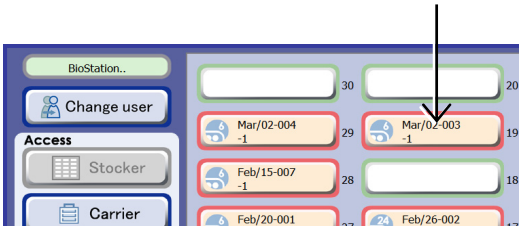
4.11.1 Download Preparation of Observation Data for Each Sample

(1) Display the Image review window.

a. To select the stocker where the sample is being cultured

a-1. On the System status screen, press the button of the stocker that contains the sample to be downloaded.

The Select function window appears.

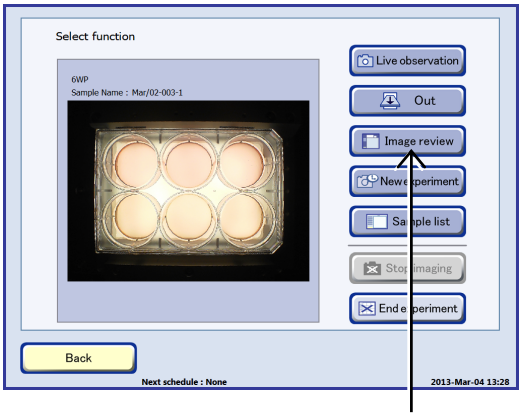


System status screen

a-2. Press the Image review button.

The Image review window of the selected sample appears.

Go to step (2).

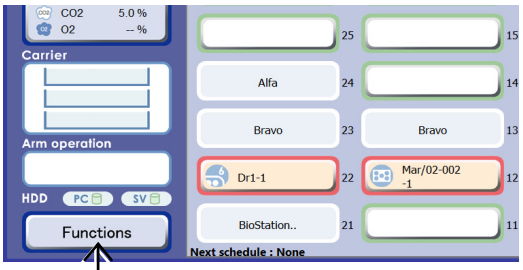


Select function window

b. To select the observation data from the sample list

b-1. Press the Functions button on the System status screen.

The Functions window appears.

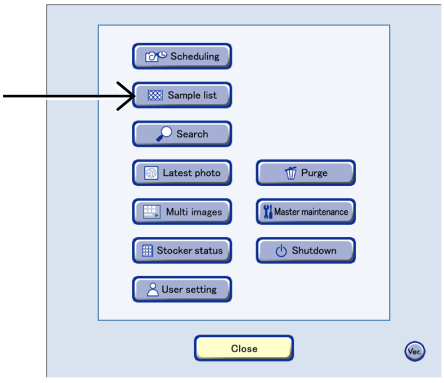


System status screen

b-2. Press the Sample list button.

All samples cultured by the user are displayed in the Sample list window.

> To display the list of the already observed culture samples onto the sample list, use the search function.
> For details on the search function, see Section 4.10, “Searching for the Observation Data.”



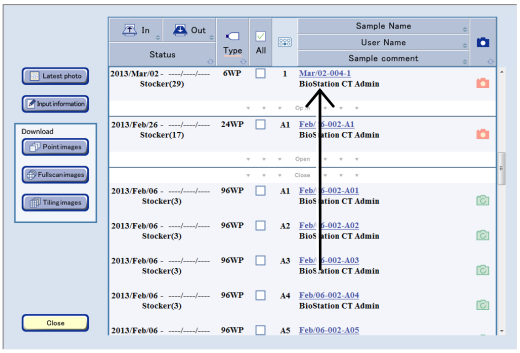
Functions window

b-3. Press the sample name to prepare for download.

The Image review window of the selected sample appears.

Press the Open area to display all sample names in the holder.

> For details on vessel name abbreviations used in the Sample list window, see the list on Page 57, “Abbreviation and description for vessels.”



Sample list window

(2) Perform download preparation of images

1. Press the Image selection button below the thumbnail images and select the image to prepare for download.

A checked symbol appears in the check box for the image corresponding to the selected Image selection button.

Multiple images can be selected.

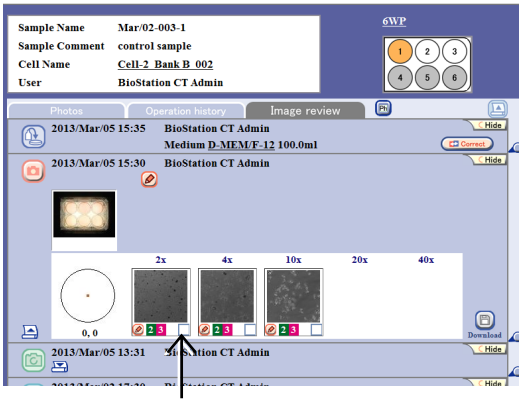
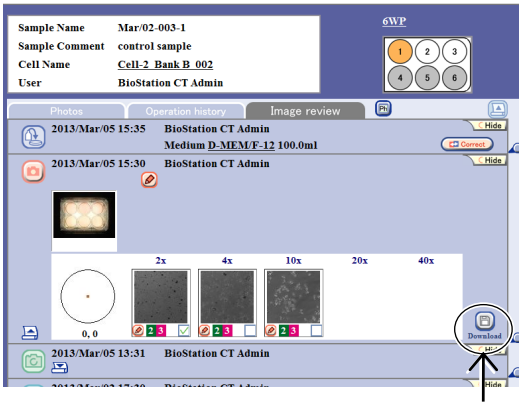


Image review window

2. Press the Download button on the right of the Observation history area.

The Image download setting window appears.

> To download a set of time-sequential images or images with different Z positions, display the original image by pressing the Enlarge button under the images for the download preparation.
> For details, see Section 4.7.3.1, “Viewing the micro image (Ph image) and preparing download.”



3. Set the format conditions of the images to be downloaded.

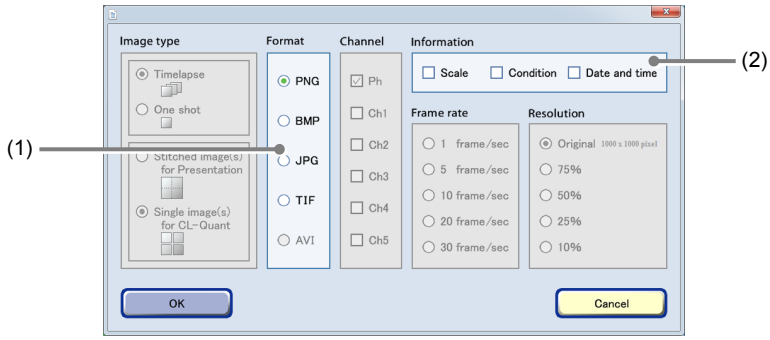


Image download settings window

No.	Name	Function
(1)	Format area	Select the format of download images.
(2)	Information area	Select the image information to be added. Three types of image information are available for Scale, Condition and Date and time.

4. Press the OK button.

After download preparation is complete, the download preparation complete dialog box appears.

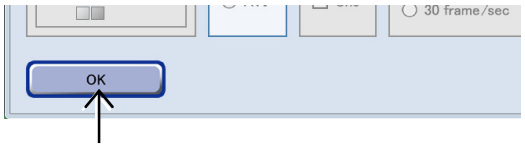
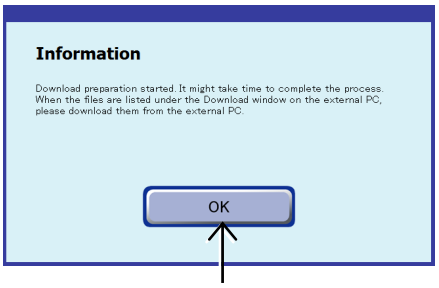


Image download setting window

5. Press the OK button.

Download preparation of the image is complete.



Complete dialog box for image download preparation

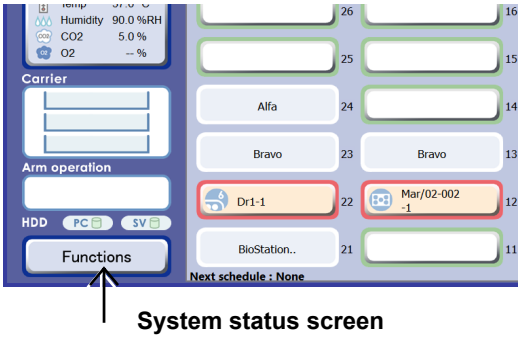
> For details on the procedure for downloading the data prepared to be downloaded to an external PC, see the “BioStation CT Ver. 3.8 Instructions (PC Operations).”

4.11.2 Batch Download Preparation of Multiple Holder/Sample Observation Data

(1) Display the Sample list window.

1. Press the Functions button on the System status screen.

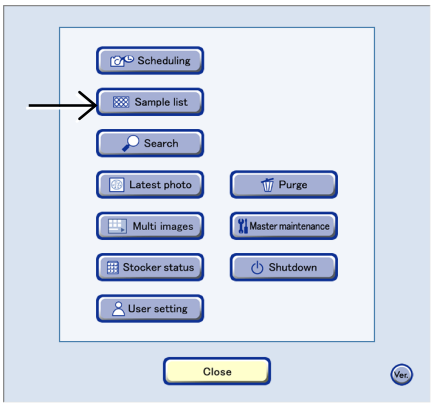
The Functions window appears.



System status screen

2. Press the Sample list button in the Functions window.

All samples cultured by the user are displayed in the Sample list window.

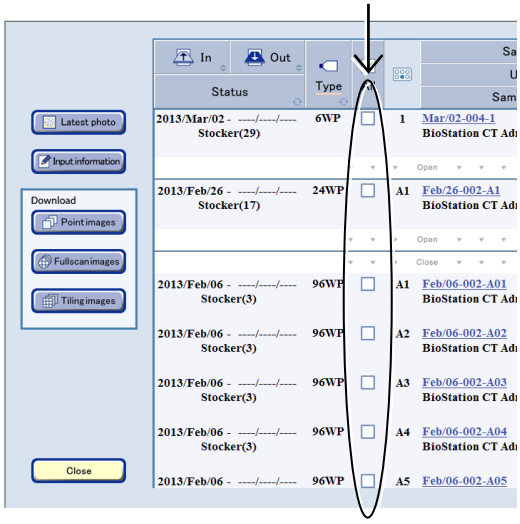


Functions window

(2) Select holders or samples for download preparation.

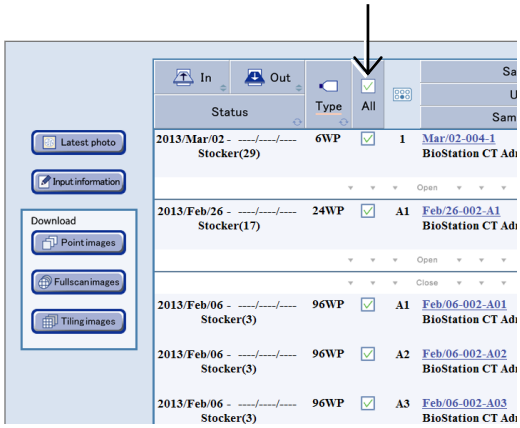
Check the check boxes in the Sample list window to select multiple holders or samples for download preparation.

> For details on vessel name abbreviations used in the Sample list window, see the list on Page 57, “Abbreviation and description for vessels.”



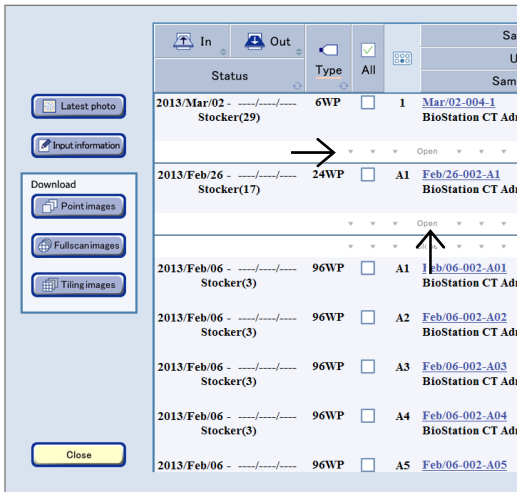
Sample list window

Pressing the All button selects all samples in all displayed holders.



Press the Open area to display all sample names in the holder. Press the Close area to display only the first sample.

When the check box is checked with only the first sample in the holder displayed, all samples in the holder are selected.



(3) Perform download preparation of images

1. **Press the Point images button to prepare download of timelapse images captured by Point observation.**

   Creation of a download file for timelapse observation images is prepared from images of the holder and sample selected in step (2).

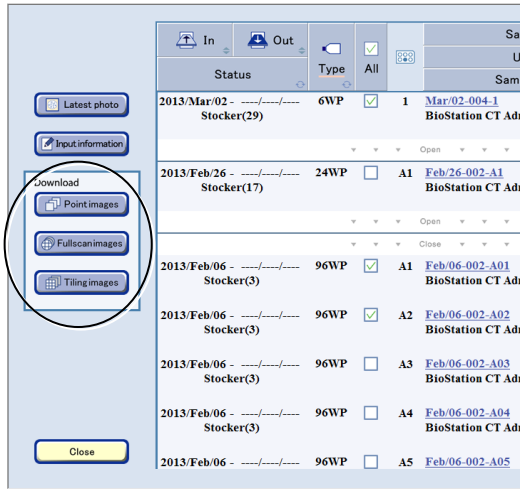
   Press the Fullscan images button to prepare download of timelapse images captured by Full Scan observation.

   Creation of a download file for Full Scan images is prepared from the images of the holder and sample selected in step (2).

   Press the Tiling images button to prepare download of timelapse images captured by Tiling observation.

   Creation of a download file for Tiling images is prepared from the images of the holder and sample selected in step (2).

   The Image download setting window appears.



Sample list window

When the Point images button is pressed, all the images captured by Point observation during scheduled observation (normal mode) are set to be downloaded.
When the Fullscan images button is pressed, all the images captured by Full Scan observation during scheduled observation (normal mode) are set to be downloaded.
When the Tiling images button is pressed, all the images captured by Tiling observation during scheduled observation (normal mode) are set to be downloaded.

Images captured during live observation and images captured during scheduled observation in stage exclusive mode are not included.
For information on download preparation of images captured during live observation, see Section 4.7.3.3, “Viewing the macro image and preparing download.”
For information on download preparation of images captured by stage exclusive mode scheduled observation, see Section 4.7.3.1, “Viewing the micro image (Ph image) and preparing download” or Section 4.7.3.2, “Viewing the micro image (FL image) and preparing download.”

2. Set the format conditions of the image for the download preparation.

The format conditions set here are applied to all images to be downloaded.

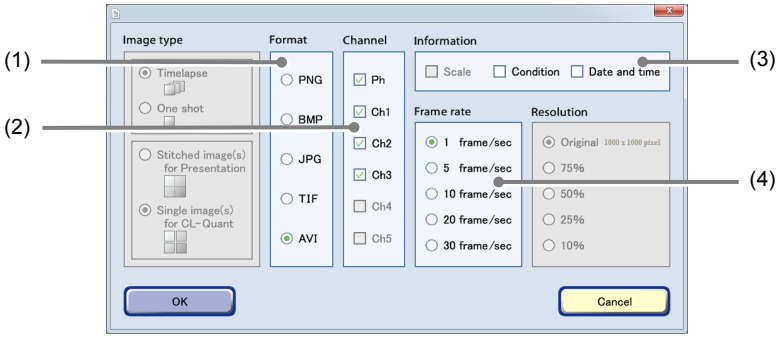


Image download setting window

No.	Name	Function
(1)	Format area	Select the format of download images.
(2)	Channel area	Select the channels to be downloaded. Enabled only when fluorescence image is selected. (Live observation data cannot be selected.)
(3)	Information area	Select the image information to be added. Three types of image information are available for Scale, Condition and Date and time. When AVI is selected for the format, Scale cannot be selected.
(4)	Frame rate area	Select the duration of playback per image. Enabled only when AVI is selected for the format.

3. Press the OK button.

After download preparation is complete, the download preparation complete dialog box appears.

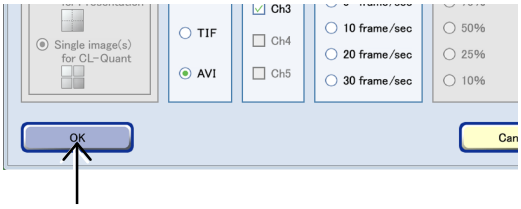
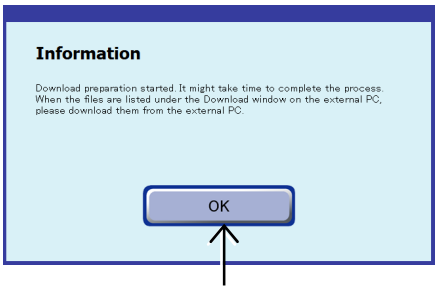


Image download setting window

4. Press the OK button.

Download preparation of the image is complete.



Complete dialog box for download preparation

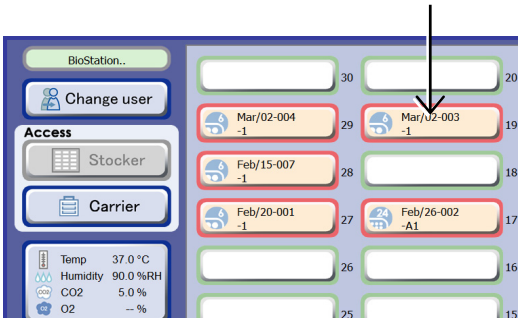
For details on the procedure for downloading the data prepared to be downloaded to an external PC, see the “BioStation CT Ver. 3.8 Instructions (PC Operations).”

4.11.3 Batch Download Preparation of Images for Sample

(1) Display the Image review window.

1. On the System status screen, press the button of the stocker that contains the sample to be downloaded.

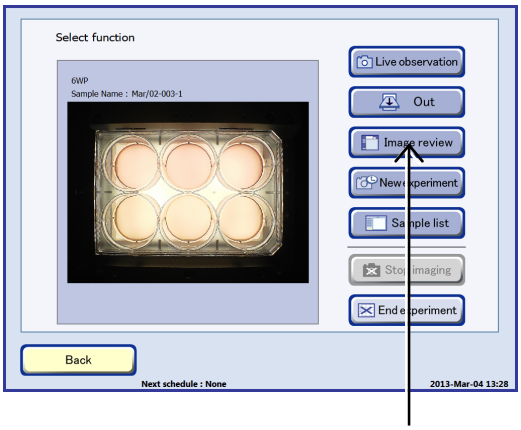
The Select function window appears.



System status screen

2. Press the Image review button.

The Image review window of the selected sample appears.



Select function window

(2) Prepare download processing.

1. Check that the right culture history is selected to be prepared for download processing, and prepare for batch download.

To prepare batch download of all Point observation timelapse images, press the Point images button.

To prepare batch download of all Full Scan observation timelapse images, press the Fullscan images button.

To prepare batch download of all Tiling observation timelapse images, press the Tiling images button.

The Image download setting window appears.

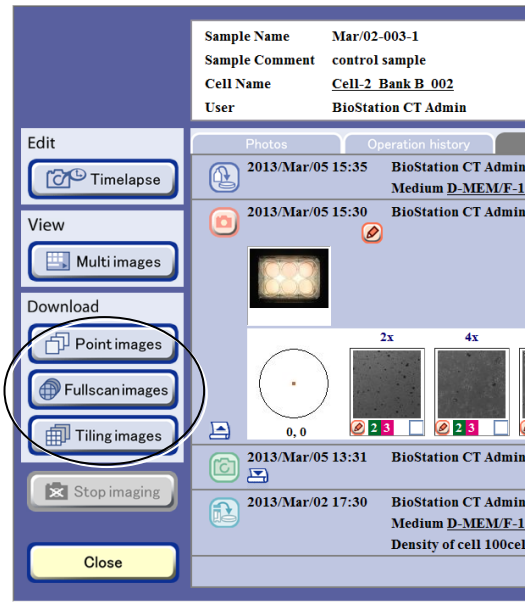


Image review window

When the Point images button is pressed, all the images captured by Point observation during scheduled observation (normal mode) are set to be downloaded.
When the Fullscan images button is pressed, all the images captured by Full Scan observation during scheduled observation (normal mode) are set to be downloaded.
When the Tiling images button is pressed, all the images captured by Tiling observation during scheduled observation (normal mode) are set to be downloaded.

Images captured during live observation and images captured during scheduled observation in stage exclusive mode are not included.
For information on download preparation of images captured during live observation, see Section 4.7.3.3, “Viewing the macro image and preparing download.”
For information on download preparation of images captured by stage exclusive mode scheduled observation, see Section 4.7.3.1, “Viewing the micro image (Ph image) and preparing download” or Section 4.7.3.2, “Viewing the micro image (FL image) and preparing download.”

2. Set the format conditions of the image for the download preparation.

The allowable conditions depend on the type of image to be downloaded.

Set the format conditions of the images to be downloaded, and then press the OK button.
After download preparation is completed, the download preparation complete dialog box appears.

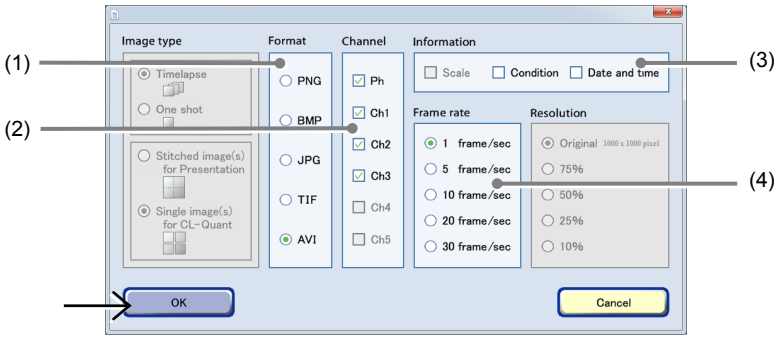
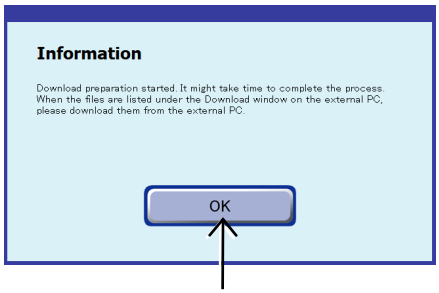


Image download setting window

No.	Name	Function
(1)	Format area	Select the format of download images. AVI is enabled only when the Point image button in the Image review window is used for download.
(2)	Channel area	Select the channels to be downloaded. Enabled only when fluorescence image is selected.
(3)	Information area	Select the image information to be added. Three types of image information are available for Scale, Condition and Date and time. When AVI is selected for the format, Scale cannot be selected.
(4)	Frame rate area	Select the duration of playback per image. Enabled only when AVI is selected for the format.

3. **Press the OK button.**

   Download preparation of the image is complete.



Complete dialog box for download preparation

> For details on the procedure for downloading the data prepared to be downloaded to an external PC, see the "BioStation CT Ver. 3.8 Instructions (PC Operations)."

Caution

The scale bar displayed in the Various image display window is not included in the original image. An image data file prepared for download includes the original image only.

The image size is calculated as shown below. The dynamic range of the CCD camera is 8 mm × 8 mm (1000 × 1000 pixels), and when the image is captured at 2x, the above size becomes 8/2 mm × 8/2 mm (4 mm × 4 mm) (1 pixel=4 μm).

The image size of each observation magnification is calculated based on the above information.

- **2x of the image size becomes 4 mm × 4 mm (1 pixel=4 μm)**
- **4x of the image size becomes 2 mm × 2 mm (1 pixel=2 μm)**
- **10x of the size becomes 0.8 mm × 0.8 mm (1 pixel=0.8 μm)**
- **20x of the image size becomes 0.4 mm × 0.4 mm (1 pixel=0.4 μm)**
- **40x of the image size becomes 0.2 mm × 0.2 mm (1 pixel=0.2 μm)**

Caution

When creating AVI files with the image download function, if the number of frames is too large, the AVI file is fragmented into multiple files because the number of frames in one AVI file is limited.

The maximum number of frames is determined by the number of pixels in the image to be downloaded.

The number of pixels for one side of the image	The maximum number of frames
1000 pixels or less	1000 frames
1001 pixels or more 2000 pixels or less	250 frames
2001 pixels or more 3000 pixels or less	110 frames
3001 pixels or more 4000 pixels or less	60 frames

* **In the Full Scan image display window (Download tab) or the Tiling image display window, if the number of vertical pixels is different from the number of horizontal pixels on a download area that has been specified with Area select area, the limitation is applied to the larger one.**

* **For standard images (single images), since the number of pixels is 1000 × 1000 pixels, the maximum number of frames is 1000 frames.**

5 Environmental Settings

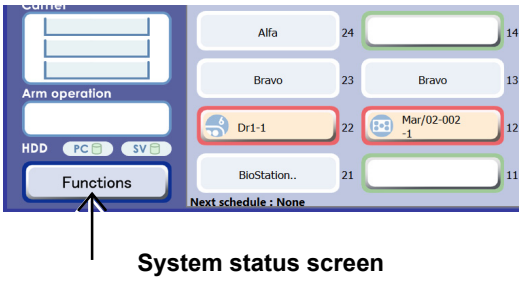
This chapter describes the procedure for setting environmental settings for this product.

5.1 User Settings

User settings window

(1) Press the Functions button on the System status screen.

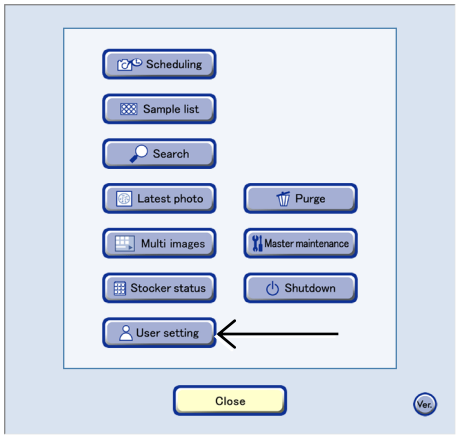
The Functions window is displayed.



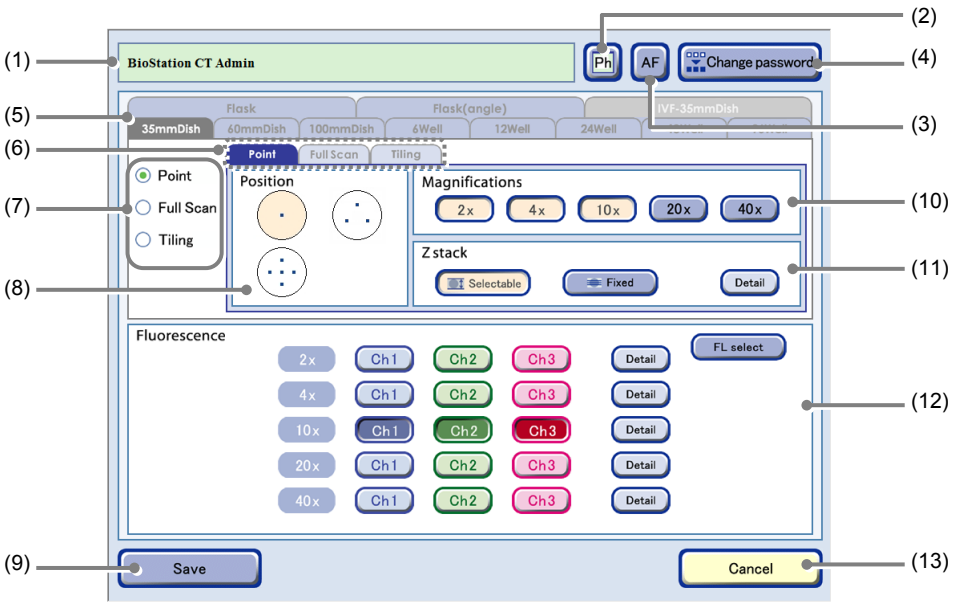
System status screen

(2) Press the User setting button.

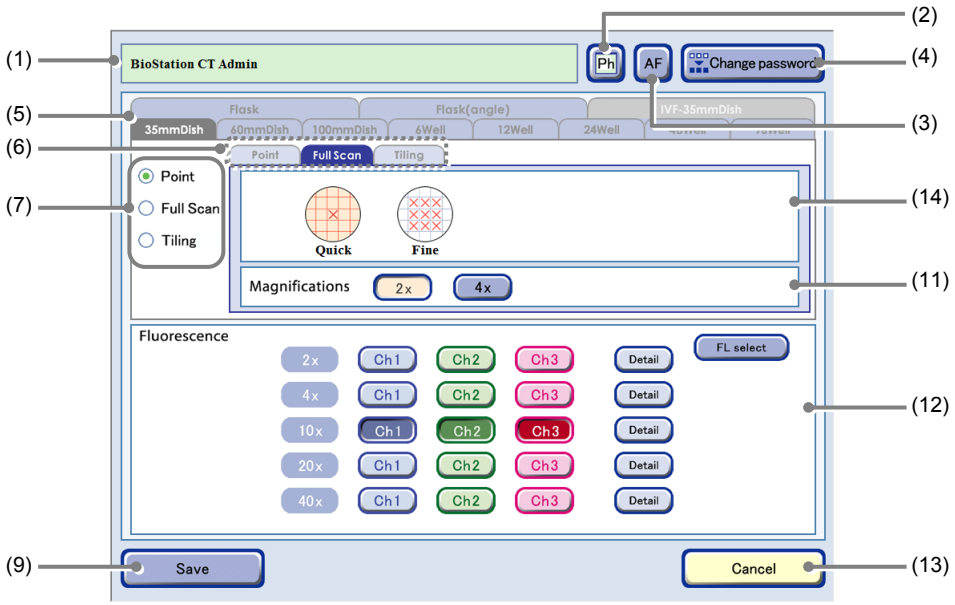
The User settings window is displayed.



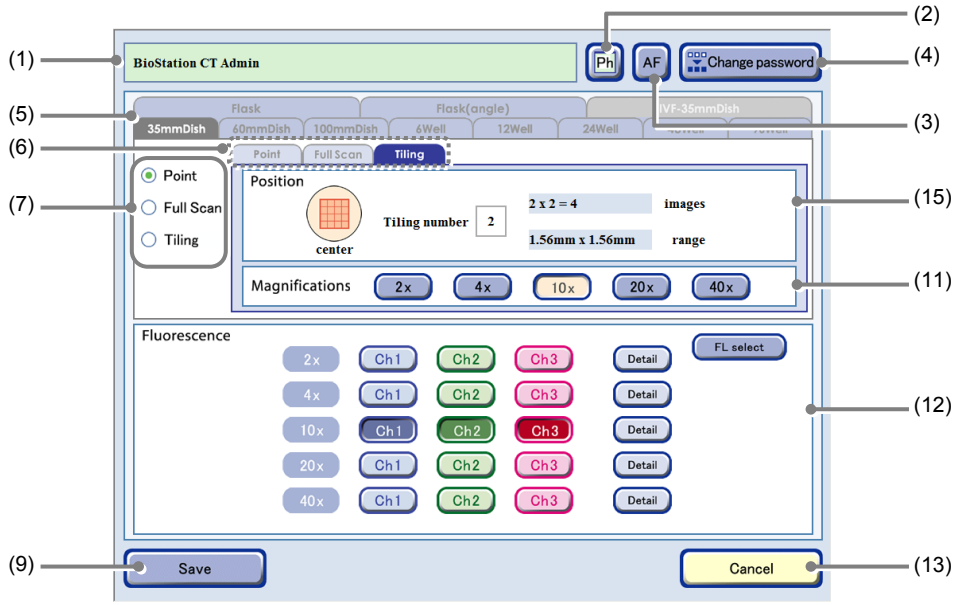
Functions window



User settings window (Point tab selected)



User settings window (Full Scan tab selected)



User settings window (Tiling tab selected)

No.	Name	Function
(1)	User name	This area displays the current user name.
(2)	Thumbnail image display switch button 	Set the type of thumbnail image to be displayed in the Image review window. If the displayed thumbnail images are phase contrast images, [Ph] is displayed, and [FL] is displayed for fluorescence images.
(3)	AF button	Enable or disable autofocus when starting observation of the Live observation window. (Convex): AF is enabled. (Concave): AF is disabled.

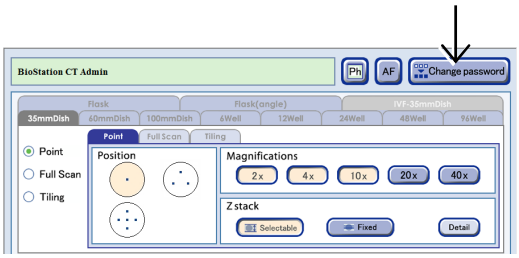
No.	Name	Function
(4)	Change password button	Press this button to change the login password of the current user. Open the Password change window. (See Section 5.1.1.)
(5)	Culture vessel tabs	Select the culture vessel for which the default settings for scheduled observation are to be set up.
(6)	Observation method tabs	Set the observation conditions for each of the observation methods. Display the Observation condition setting window for each observation method by switching over the tab. Each Observation method tab has its own setting procedure: Point: See “Default Setting for Point Tab.” Full Scan: See “Default Setting for Full Scan Tab.” Tiling: See “Default Setting for Tiling Tab.”
(7)	Default display selection for the Observation method selection tabs	Set the Observation method selection tabs initially displayed when the Observation condition setting window is opened. (See Section 5.1.2.)
(8)	Position area	Set the default observation point initially displayed when the Point tab in the Observation condition setting window is opened.
(9)	Save button	Press this button to save the default settings for scheduled observation.
(10)	Magnifications area	Set the observation magnification initially displayed in the Observation condition setting window. Multiple observation magnifications cannot be selected for Tiling observation. The selectable observation magnifications depend on each vessel for Full Scan observation.
(11)	Z stack setting area	Set the Z stack condition initially displayed when the Point tab in the Observation condition setting window is opened.
(12)	FL channel settings area (optional)	With the Fluorescence unit connected, settings in the FL channel settings field for setting the default observation conditions of the fluorescence image can be edited in the User settings window. Multiple channels can be selected for each magnification as default settings in Point tab. When any channel from FL channel button(s) is selected for the Fluorescence observation, the magnification select button is automatically selected, and the phase contrast image is captured as a set. When the Tiling tab or Full Scan tab is selected, multiple FL channels can be selected only within the observation magnification selected in the Magnifications area. The values set here are applied when the Default button is pressed in the Observation condition setting window. (See Section 4.6.2.)
(13)	Cancel button	Press this button to cancel the settings and close the window.
(14)	Full Scan observation AF position setting area	Select the AF position initially displayed when the Full Scan tab in the Observation condition setting window is opened. **Quick:** Autofocus is performed at the center of the sample. **Fine:** Autofocus is performed at multiple points. Disabled when a 96-/48-well plate is used. Depending on the selected vessel, this may not be displayed because it is not applicable for observation.
(15)	Tiling observation position setting area	Set a numeric value for the tiling area initially displayed when using Center to set the observation conditions for Tiling observation. Select a tiling area from the number selection box showing a number from 1 to 20 which is displayed by pressing the Tiling number field. (Selecting 5 captures 25 images in a 5×5 grid.)

5.1.1 Changing the Password

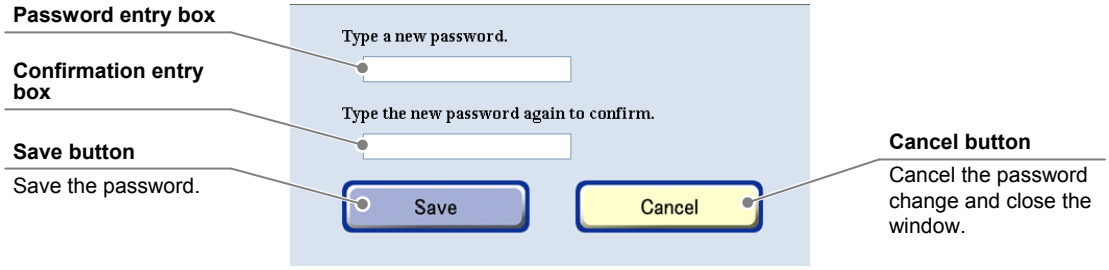
To change the login password of the current user, perform the following:

(1) Press the Change password button in the User settings window.

The Password change window is displayed.



User settings window

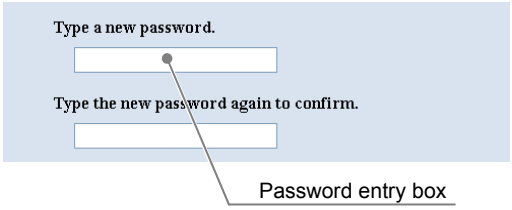


Password change window

(2) Enter a new password into the password entry box.

1. Press the password entry box.

The Keyboard window for entering a password is displayed.



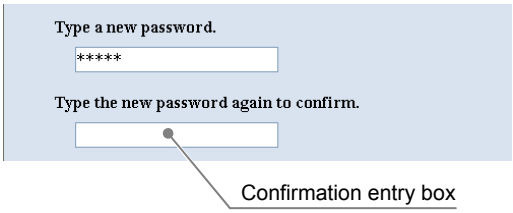
2. Enter a new password and press the OK button.

(3) Enter the new password into the confirmation entry box to confirm it.

1. Press the confirmation entry box.

The Keyboard window for entering the password is displayed.

2. Enter the new password again and press the OK button.



(4) Press the Save button.

The new password is saved and the Password change window closes.

5.1.2 Scheduled Observation Default Settings

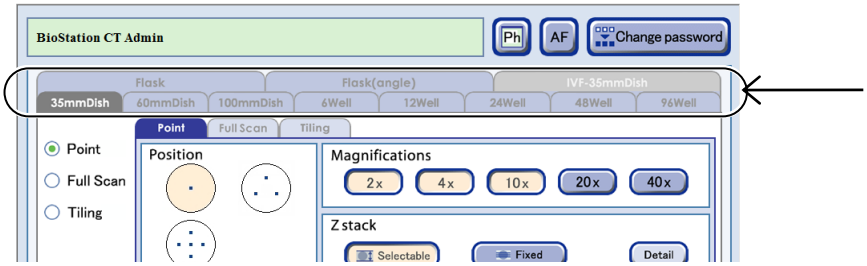
This section describes the procedures for setting the initial screen of the Observation condition setting window.

Setting frequent observation conditions as default settings reduces the time required for setting the scheduled observation settings. Values that are set here will be displayed on the initial screen of the Observation condition setting window. The values can be edited in the scheduled observation settings.

(1) Press the Culture vessel tab in the User settings window and then select the culture vessel for which the default settings for scheduled observation are to be set up.

In the case of a flask type culture vessel, settings can be made for slant neck type and straight angle neck type flasks. To use a slant neck type flask, select the Flask tab. To use a straight angle neck type flask, select the Flask (angle) tab.

Default scheduled observation settings can be set for each culture vessel.

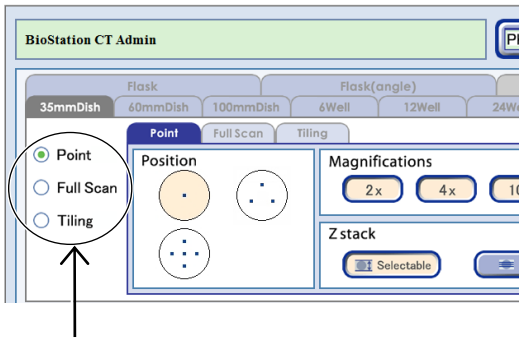


User settings window

(2) Set the Default window from the Observation method selection tab.

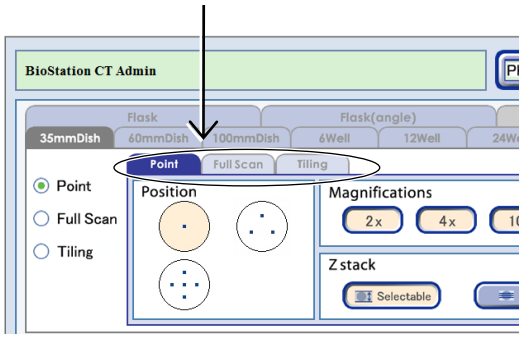
Select one of the three radio buttons; Point, Full Scan or Tiling.

The observation method set here is the default displayed on the Observation method selection tab when displayed from the Observation condition setting window.



(3) Set the observation conditions for each of the observation methods.

Display the Observation condition setting window for each observation method by switching over the tab.



User settings window (Point setting tab)

Each Observation method tab has its own setting procedure:

Point: See “Default Setting for Point Tab.”

Full Scan: See “Default Setting for Full Scan Tab.”

Tiling: See “Default Setting for Tiling Tab.”

Each procedure is explained below.

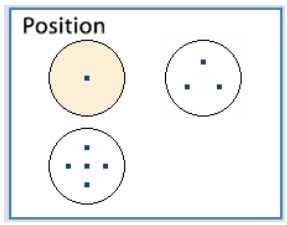
Default Setting for Point Tab

Set the default observation position, observation magnification, and Z stack that are initially displayed when opening the Point tab in the Observation condition setting window.

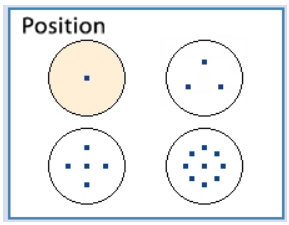
(1) Select a scheduled observation position.

Each culture vessel has several observation position layouts.

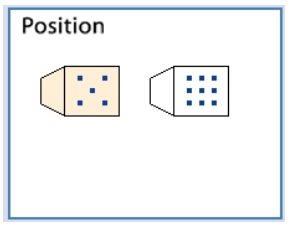
Observation positions are indicated as small blue squares in each culture vessel.



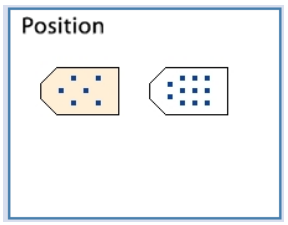
35 mm Dish/ 60 mm Dish/ 6 Well/12 Well/24 Well /48 Well/96 Well



100 mm Dish



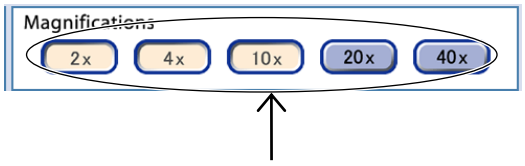
Flask



Flask(angle)

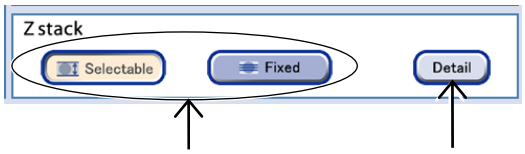
(2) Select the observation magnification to be used for scheduled observation.

Press a magnification button to select it.



(3) Configure the Z stack settings.

There are two Z stack settings: Selectable pitch and Fixed pitch. Press the Selectable or Fixed button to select the setting.



a. When Selectable pitch is selected

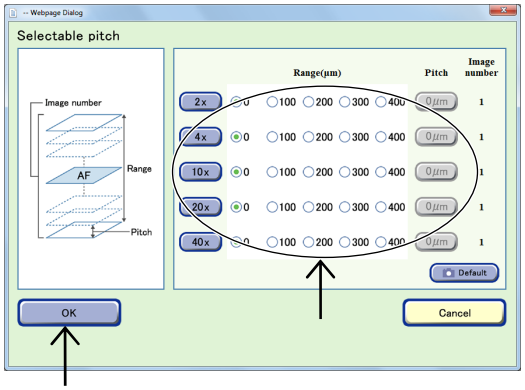
a-1. Press the Selectable button and then press the Detail button.

The Selectable pitch setting window appears.

a-2. Select a Range and Pitch for each magnification.

In accordance with the selected Range and Pitch, the number of capturing images is determined and displayed on the right side of the window.

a-3. Press the OK button.



Selectable pitch setting window

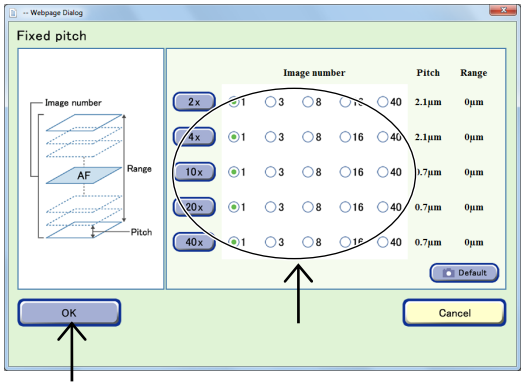
b. When Fixed pitch is selected

b-1. Press the Fixed button and then press the Detail button.

The Fixed pitch setting window appears.

b-2. Directly select the number of images to be captured (1, 3, 8, 16, or 40).

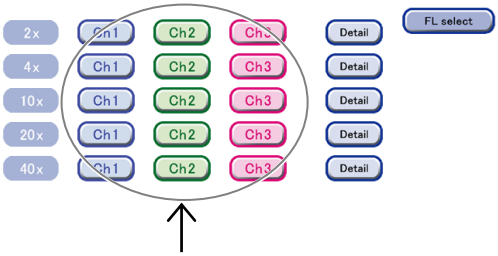
b-3. Press the OK button.



Fixed pitch setting window

(4) Select the desired channel button for each observation magnification used in scheduled observation.

Multiple FL channels can be selected.

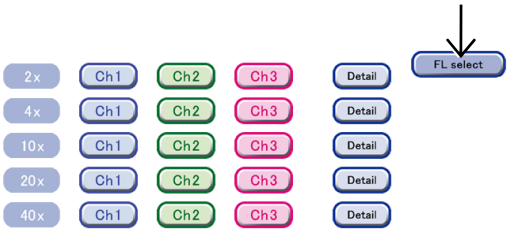


User settings window (Fluorescence image capturing conditions)

When up to 5 types of fluorescence filter cube are mounted, channels (Ch1 to Ch3) to be displayed by default can be changed to the added fluorescence filter channels (Ch4, Ch5).

1. Press the FL select button in the FL channel settings area in the User settings window.

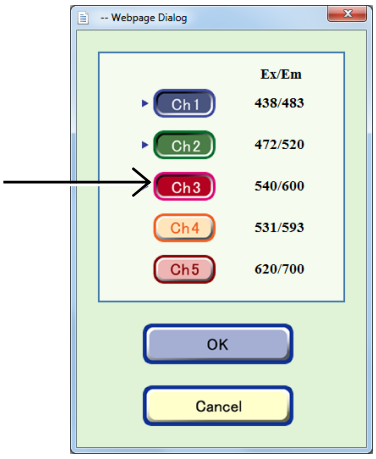
The FL channel selection window appears.



FL channel settings area

2. Select the button for the fluorescence channel not to be used.

The channel is deselected and the surface becomes convexed.



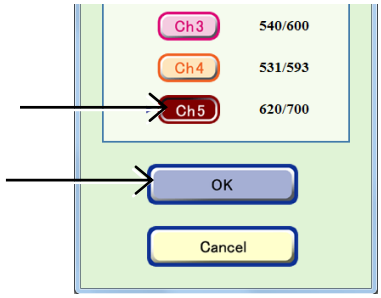
FL channel selection window

3. **Select the desired fluorescence channel button.**

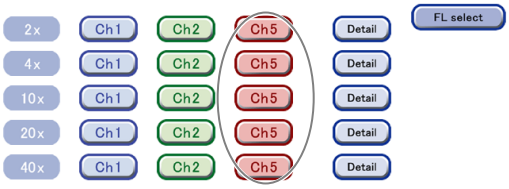
   The channel is selected and the surface becomes concaved.

4. **Press the OK button.**

   The User setting window appears again.



The selected channel is applied to the FL channel settings area.

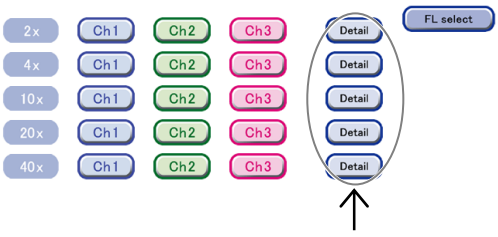


FL channel settings area

(5) Set the exposure time and the intensity of each excitation light source for the observation.

1. **Press the Detail button for the observation magnification to be specified.**

   The Fluorescence image capturing conditions setting window appears.

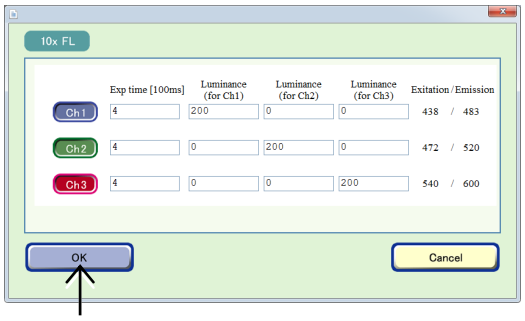


2. **Press the Exp time field and the Luminance field on the right side of the FL channel button to enter the exposure time and the intensity of each excitation light source.**

   These values are used for image exposure conditions for scheduled observation and default observation conditions of live observation.

   The unit of the exposure time is [100ms], but up to the 2nd decimal digit (i.e. unit: 1 ms) can be entered.
   And the maximum value of the intensity of excitation light source is 240.

   Channels can be selected also by pressing the FL channel buttons on the left side in the window.



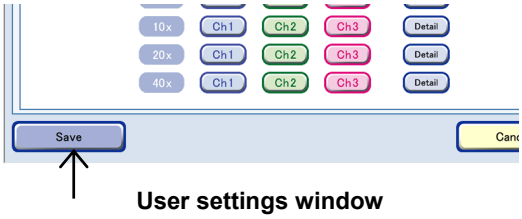
Fluorescence image exposure conditions setting window

3. **Press the OK button.**

   The exposure time and the intensity of each excitation light source are set and the Fluorescence image exposure conditions setting window is closed.

(6) Press the Save button in the User settings window.

The default settings are saved and the User settings window closes.



User settings window

Default Setting for Full Scan Tab

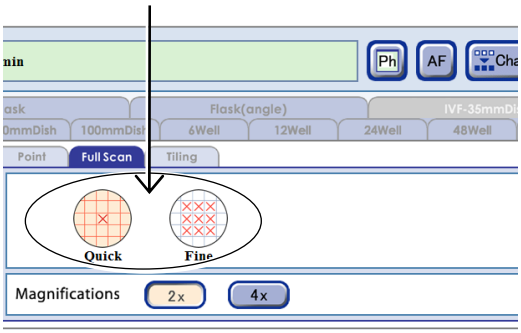
Set the default AF position, observation magnification, and FL channel that are initially displayed when the Full Scan tab is opened in the Observation condition setting window.

(1) Select the AF position.

Select the autofocus position.

Quick: Autofocus is performed at the center of the sample.

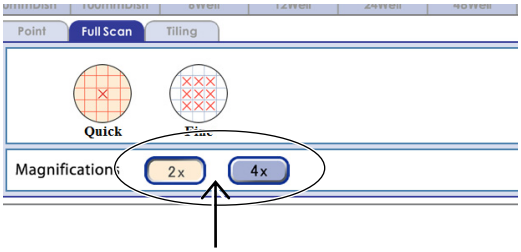
Fine: Autofocus is performed at multiple points. Disabled when a 96-/48-well plate is used.



User settings window (Full Scan setting tab)

(2) Select an observation magnification to be used for scheduled observation.

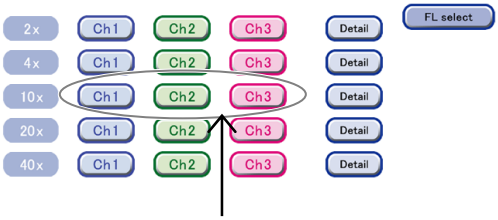
The observation magnification displayed in the Magnification area depends on the combination of the type of culture vessel and Quick or Fine in the AF position setting area.



(3) Select the FL channel button for the observation magnification selected in step 2.

Multiple channels can be selected for the same magnification.

Selecting an FL channel button other than the observation magnification selected in the Magnifications area does not affect the default display of the Observation condition setting window.



User settings window (Fluorescence image capturing conditions)

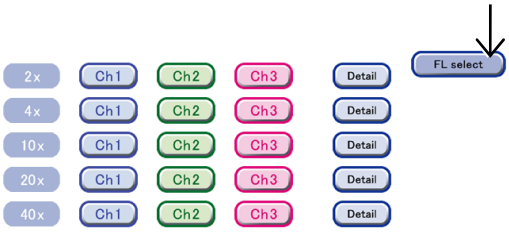
When up to 5 types of fluorescence filter cube are mounted, channels (Ch1 to Ch3) to be displayed by default can be changed to the added fluorescence filter channels (Ch4, Ch5).

1. **Press the FL select button in the FL channel settings area in the User settings window.**

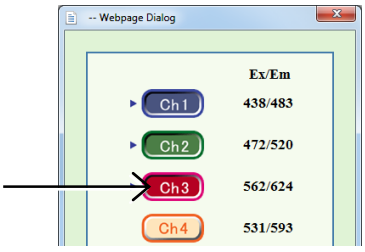
   The FL channel selection window appears.

2. **Select the button for the fluorescence channel not to be used.**

   The channel is deselected and the surface becomes convexed.



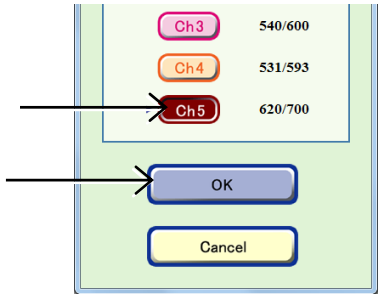
FL channel settings area



FL channel selection window

3. Select the desired fluorescence channel button.

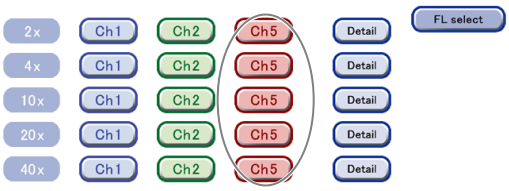
The channel is selected and the surface becomes concaved.



4. Press the OK button.

The User setting window appears again.

The selected channel is applied to the FL channel settings area.

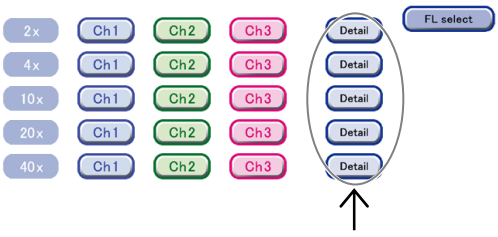


FL channel settings area

(4) Set the exposure time and the intensity of each excitation light source for the observation.

1. Press the Detail button for the observation magnification to be specified.

The Fluorescence image exposure conditions setting window appears.

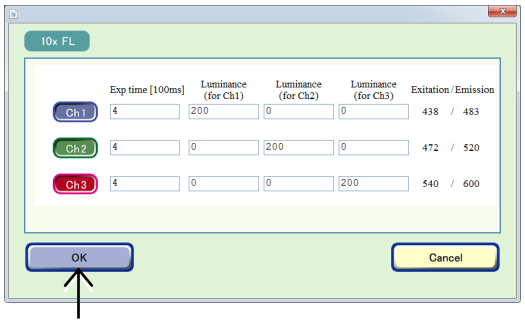


2. Press the Exp time field and the Luminance field on the right side of the FL channel button to enter the exposure time and the intensity of each excitation light source.

These values are used for image exposure conditions for scheduled observation and default observation conditions of live observation.

The unit of the exposure time is [100ms], but up to the 2nd decimal digit (i.e. unit: 1 ms) can be entered.
And the maximum value of the intensity of excitation light source is 240.

Channels can be selected also by pressing the FL channel buttons on the left side in the window.



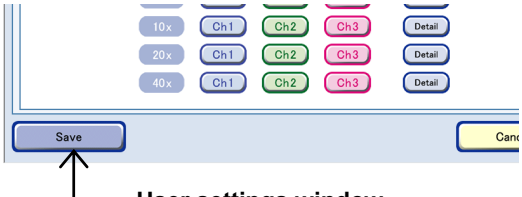
Fluorescence image exposure conditions setting window

3. Press the OK button.

The exposure time and the intensity of each excitation light source are set and the Fluorescence image exposure conditions setting window is closed.

(6) Press the Save button in the User settings window.

The default settings are saved and the User settings window closes.



User settings window

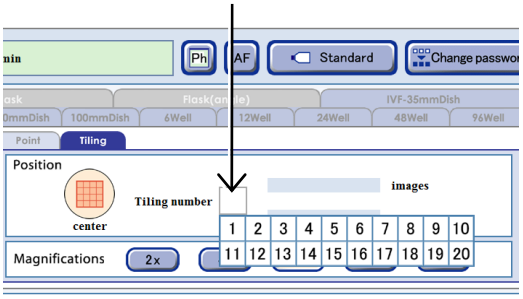
Default Setting for Tiling Tab

Set the Tiling number, observation magnification, and FL channel that are initially displayed when the Tiling tab is opened in the Observation condition setting window and Center is selected as the observation position

(1) Set the area for tiling observation in the Tiling number column.

Select an area for tiling observation from a number selection box (1 to 20) that pops up when the Tiling number column is pressed.

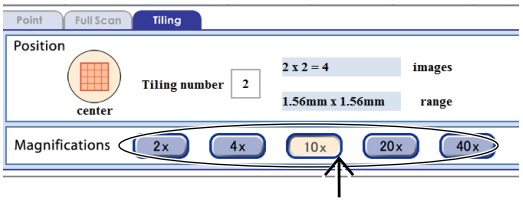
Select “5” to capture 25 images in 5×5 cells.



User settings window (Tiling setting tab)

(2) Select an observation magnification to be used for scheduled observation.

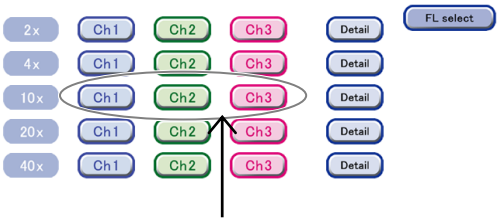
Selecting more than one magnification is not possible for Tiling observation (Tiling).



(3) Select the FL channel button for the observation magnification selected in Step 2.

Multiple channels can be selected for the same magnification.

Selecting an FL channel button other than the observation magnification selected in the Magnifications area does not affect the default display of the Observation condition setting window.



User settings window (Fluorescence image capturing conditions)

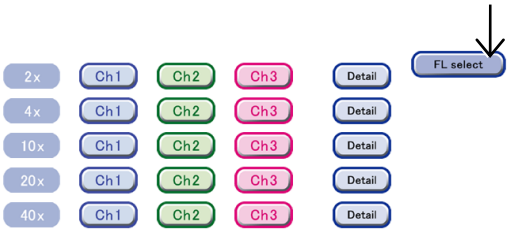
When up to 5 types of fluorescence filter cube are mounted, channels (Ch1 to Ch3) to be displayed by default can be changed to the added fluorescence filter channels (Ch4, Ch5).

1. **Press the FL select button in the FL channel settings area in the User settings window.**

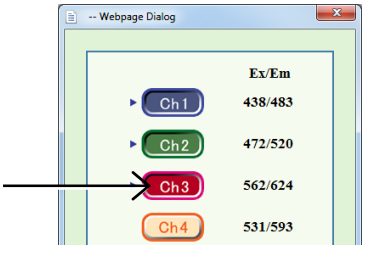
   The FL channel selection window appears.

2. **Select the button for the fluorescence channel not to be used.**

   The channel is deselected and the surface becomes convexed.



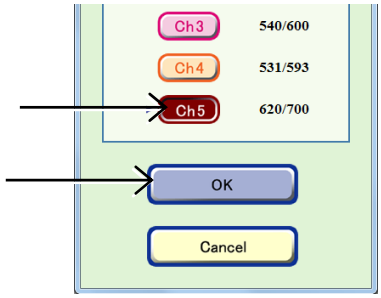
FL channel settings area



FL channel selection window

3. **Select the desired fluorescence channel button.**

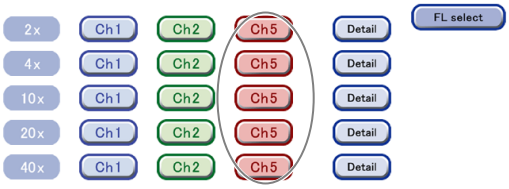
   The channel is selected and the surface becomes concaved.



4. **Press the OK button.**

   The User setting window appears again.

   The selected channel is applied to the FL channel settings area.

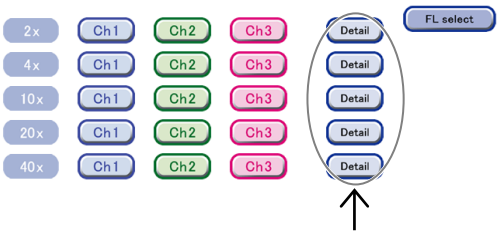


FL channel settings area

(4) Set the exposure time and the intensity of each excitation light source for the observation.

1. **Press the Detail button for the observation magnification to be specified.**

   The Fluorescence image exposure conditions setting window appears.

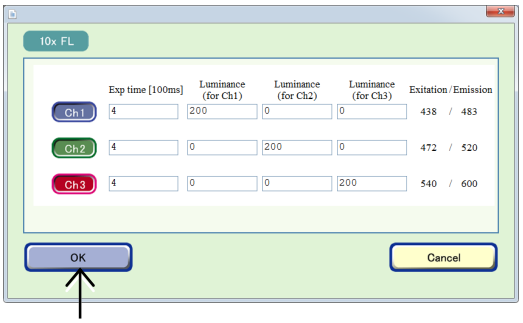


2. **Press the Exp time field and the Luminance field on the right side of the FL channel button to enter the exposure time and the intensity of each excitation light source.**

   These values are used for image exposure conditions for scheduled observation and default observation conditions of live observation.

   The unit of the exposure time is [100ms], but up to the 2nd decimal digit (i.e. unit: 1 ms) can be entered.
   And the maximum value of the intensity of excitation light source is 240.

   Channels can be selected also by pressing the FL channel buttons on the left side in the window.



Fluorescence image exposure conditions setting window

3. **Press the OK button.**

   The exposure time and the intensity of each excitation light source are set and the Fluorescence image exposure conditions setting window is closed.

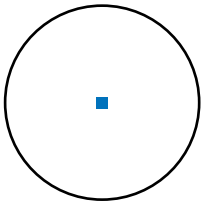
(5) Press the Save button in the User settings window.

The default settings are saved and the User settings window closes.

5.1.3 Default Observation Position

Dishes, well plates

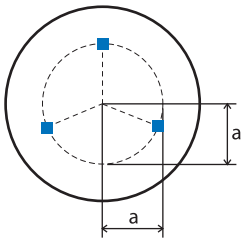
1-point observation



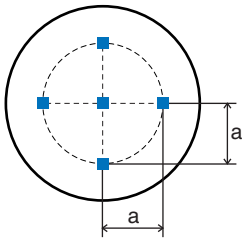
This is the default point for single point photography.

> In observation of the vessel center area on a 24-well, 48-well or 96-well plate using an observation magnification of 2x or 4x, the acquired image might be unclear. See Section 4.4.1, “Observation Area.”

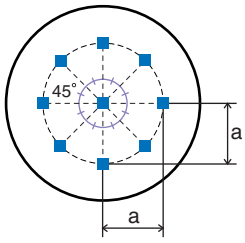
3-point observation



5-point observation



9-point observation

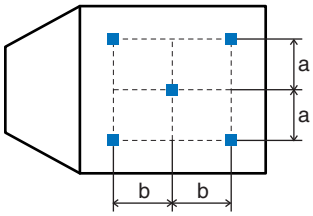


	35 mm dish 6-well plate 12-well plate	24-well plate	48-well plate	96-well plate	60 mm dish	100 mm dish
a	4 mm	2 mm	1.5 mm	0.8 mm	10.2 mm	23.125 mm

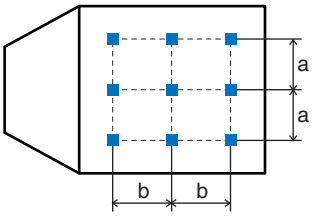
> Note that the default observation positions for 35 mm dishes may produce unclear images with an observation magnification of 2x. Likewise, the default observation positions for the 12-well, 24-well, 48-well and 96-well plates may produce unclear images with an observation magnification of 2x or 4x. See Section 4.4.1, “Observation Area.”
>
> When using a Nunc 4-well multidish, load it as a 100 mm dish because there is no corresponding vessel selection button in the Vessel selection window when loading. Note that the default observation position for a 100 mm dish cannot be used in this case.

Flasks (slant neck)

5-point observation



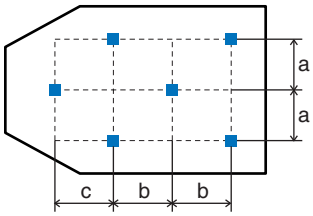
9-point observation



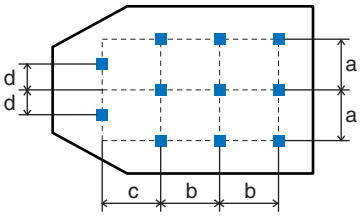
	25 cm^2 culture flask	75 cm^2 culture flask
a	5 mm	20 mm
b	8 mm	22 mm

Flasks (straight angle neck)

6-point observation



11-point observation



	25 cm^2 culture flask	75 cm^2 culture flask
a	5 mm	20 mm
b	8 mm	22 mm
c	14 mm	20 mm
d	2.5 mm	10 mm

6 Daily Maintenance

This chapter describes the procedures for supplying or replacing the consumables required for operating the product and the daily maintenance required for maintaining the proper product condition.

6.1 Distilled Water

Distilled water must be supplied to the humidifier. This section describes the procedures for checking the amount of distilled water in the humidifier water tank and for supplying water.

Checking the amount of distilled water

The amount of distilled water in the humidifier water tank can be checked through the window on the tank replacement door. When the amount is lower than a certain level, the blue lamp blinks (the lamp is lit when the amount is sufficient).
When the amount of distilled water is low, supply it in accordance with the following procedure.

Supplying distilled water

Supply distilled water in accordance with the following procedure.

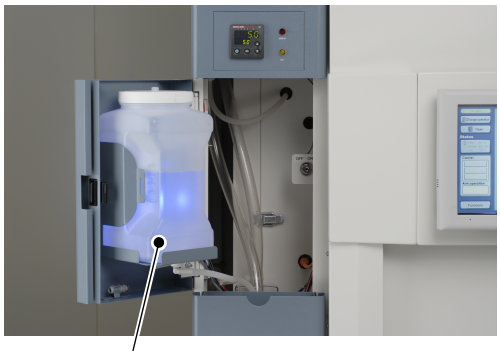
(1) Open the humidifier water tank door.

(2) Open the lid of the humidifier water tank and supply sterilized distilled water.

(3) Attach the lid and close the door.

Be careful not to pinch the distilled water piping tube.

> Chlorinated water and water supplied from a water purifier with chlorine may deteriorate the stainless steel or cause rust, voiding the warranty.



Supplying distilled water

Distilled water

For best operation of the CO_2 incubator, sterilized, distilled, demineralized or de-ionized water should be used in the humidifier water tank. See ASTM Standard D5391-93 or D4195-88 for measuring water purity.

Distillation systems and reverse osmosis water purity systems produce water that is neutral in pH (approximately 7) and is the preferred water to use for humidification. High purity, ultra pure or milli-q water is considered to be an aggressive solvent and slightly acidic. While it may be used, it is not preferred. Chlorinated tap water, or additives containing chlorine, should not be used as chlorine can deteriorate the stainless steel. Tap water may also have a high mineral content, which would produce a build-up of scale in the reservoir.

Even high purity water can contain bacteria and organic contaminants. Water should always be sterilized or treated with a decontaminant, in order to protect stainless steel and other parts of the product, prior to being introduced into the humidifier water tank.

6.2 Replacing/Connecting CO_2 Cylinders

For proper operation of this product, a liquid CO_2 cylinder (CO_2 cylinder) must be installed.
Check the amount of CO_2 gas and replace the cylinder in accordance with the following procedure if necessary.

To install or replace the cylinder, follow the instructions in respective manuals for the CO_2 cylinder, regulator and pressure gauge.

The CO_2 cylinder and the pressure regulator must be prepared separately.

Caution

Be sure that the CO_2 cylinder is standing vertically when using it. If the cylinder is laid on its side, the inside will freeze.

Carefully read the instruction manual for the CO_2 cylinder and install the specified equipment such as the regulator and the pressure indicator for the cylinder.

Checking the amount of CO_2 gas in the cylinder

If a gas meter is attached to the valve of the CO_2 cylinder, check the amount of gas with the gas meter.

If no gas meter is attached, check the value on the primary pressure indicator on the cylinder side of the regulator. If the indicated pressure value is low, replace the CO_2 cylinder.

Connecting the CO_2 hose

(1) Quickly open the valve on the CO_2 cylinder with the H-shaped rod, blow off the dust adhered to the connection of the CO_2 cylinder, and then, close the valve.

(2) Attach the regulator to the connection of the CO_2 cylinder with packing.

If the CO_2 cylinder is new and unused, the primary pressure will be approximately 5.9±0.5 MPa (approximately 60 atmospheres). Be sure to attach the regulator directly to the connection of the CO_2 cylinder. The regulator to be attached must display both a secondary pressure between 0 to 0.2 MPa and a maximum flow rate of 25 liter/min.

Be sure to use a spanner (or a monkey wrench) to securely attach the regulator.

(3) Using the hose provided, connect the regulator to this product and secure the regulator side of the hose with the hose clamp.

If necessary, cut the hose to an appropriate length.

(4) Check that there is no gas leakage from the hose connection in accordance with the following:

A Close the pressure control valve by rotating it counterclockwise to prevent damage to the regulator.

B Open the flow control valve by rotating it counterclockwise.

C Open the valve on the cylinder by half to pass the gas.

D Increase the pressure by rotating the pressure control valve clockwise. When the secondary pressure reaches a value between 0.02 and 0.03 MPa, check that there is no leakage of the gas by providing soap water onto the connection of the hose.

(5) Specify the secondary pressure of the regulator valve for the CO_2 cylinder to less than 0.063 MPa (0.05 MPa recommended).

Note on handling the CO_2 gas

CO_2 gas of high concentration can cause asphyxiation. The Occupation Safety and Health Administration standards specify that employees must not be exposed to carbon dioxide exceeding 5000 ppm average (0.5% CO_2) if working for eight hours a day, that is, 40 hours a week. The upper limit of exposure for the short-time working (15 minutes or less) is 30,000 ppm (3% CO_2). Carbon dioxide monitors are recommended for confined areas where the concentration of CO_2 gas increases easily.

CO_2 gas (reference)

The product is designed to be operated with CO_2 gas only.
Connecting a flammable or toxic gas can result in a hazardous condition.
Gases other than CO_2 should not be connected to the product.

CO_2 gas cylinders have a UN1013 label on the cylinder and are equipped with a CGA 320 outlet valve. Check the gas cylinder for the proper identification labels.

The CO_2 gas supply being connected to the product should be industrial grade, 99.5% pure (nominal). Do not use CO_2 gas cylinders equipped with siphon tubes. A siphon tube is used to extract liquid CO_2 from the cylinder which can damage the pressure regulator. Consult your gas supplier to ensure that the CO_2 cylinder does not contain a siphon tube.

Gas cylinders should also be secured to a wall or other stationary object to prevent them from tipping.

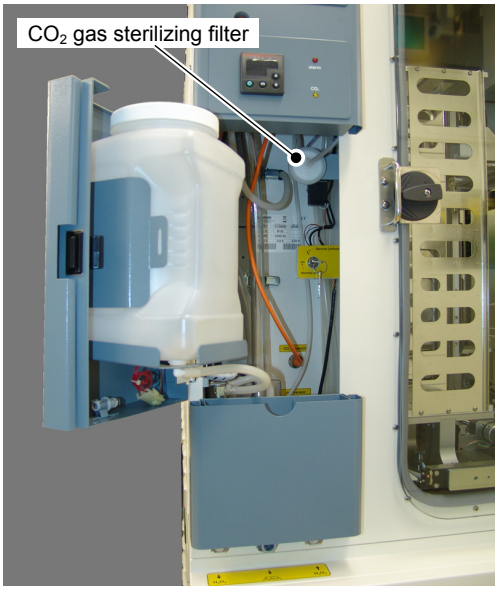
A two-stage CO_2 pressure regulator is required to be installed on the outlet valve of the gas cylinder.

6.3 Replacing CO_2 Gas Sterilizing Filters

The sterilizing filter is inserted into the back of the humidifier water tank inside this product. CO_2 gas supplied from the cylinder contains foreign materials or bacteria that can cause contamination. This filter prevents contamination from entering this product.

It is recommended that the filter is replaced once a year.

For details, contact your nearest Nikon representative.



CO_2 gas sterilizing filter

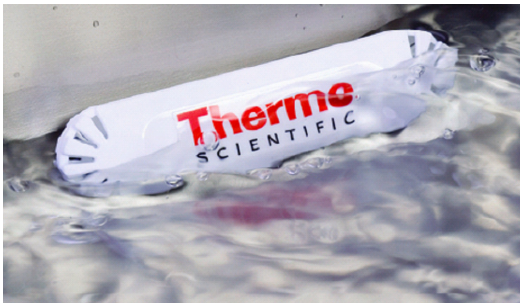
6.4 Replacing Water Preservative Agent for CO_2 Incubator

This product uses Aqua Tec as a water preservative agent.

It is recommended that it is replaced every six months.

For details, contact your nearest Nikon representative.

* AquaTec is a trademark of Thermo Scientific.



6.5 Cleaning/Decontaminating Inside the CO_2 Incubator

Warning

Keep your hands out of the culture chamber except when operating the carrier slider to avoid the risk of pinching fingers with the movable part inside the culture chamber.

Caution

If you spill a sample in the product accidentally, take note of the risk presented by the sample. If the sample is hazardous, follow the standard procedures of your facility.

Residue in the equipment might cause contamination. If there is any residue, the inside of the equipment must be decontaminated to maintain the proper condition. For details on decontaminating the inside of the equipment, contact your nearest Nikon representative.

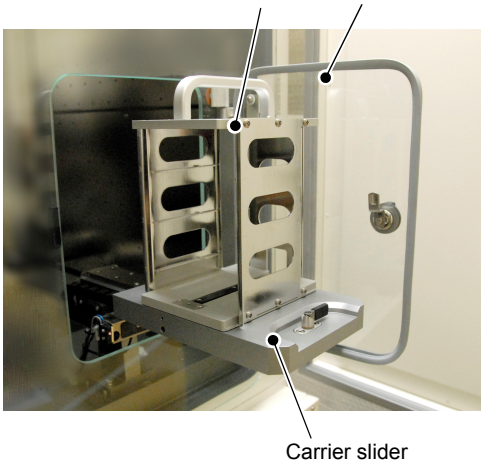
6.5.1 Cleaning/Decontaminating the Carrier and the Carrier Slider

Use the special carrier and carrier slider to load the culture vessels into the CO_2 incubator.

Those parts are directly exposed to outer air. Therefore, clean or decontaminate them on a regular basis in accordance with the following procedure.

The cleaning schedule must be drawn up taking into consideration the culture schedules and the frequency of culture vessel usage.

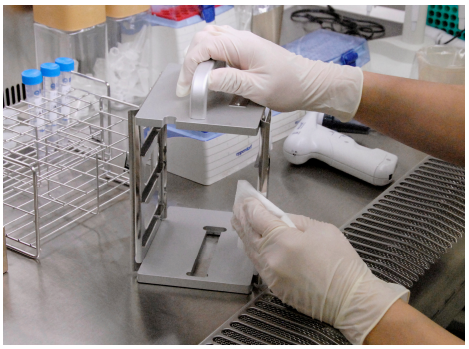
Wear gloves for safety during the cleaning task.



Carrier and carrier slider

Cleaning the carrier

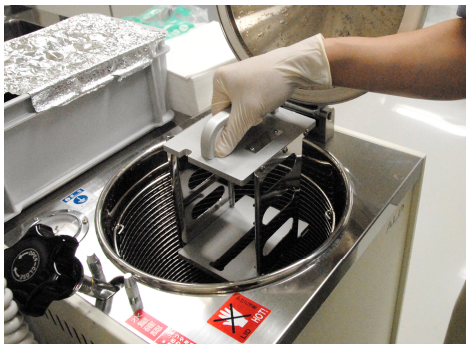
Clean the carrier with disinfectant alcohol in a clean environment such as a safety cabinet.



Cleaning the carrier

Sterilizing the carrier with an autoclave

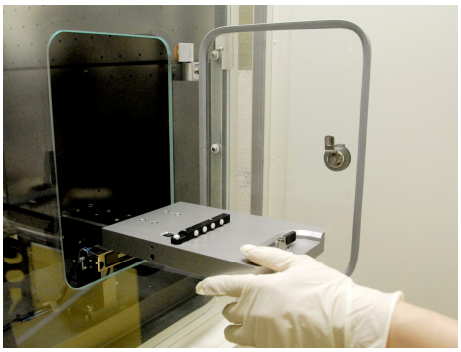
The carrier can be inserted directly into an autoclave for sterilization.



Sterilizing the carrier with an autoclave

Cleaning the carrier slider

Pull the carrier slider forward and clean the area exposed to outside-air such as the carrier setting position with disinfectant alcohol.



Cleaning the carrier slider

6.5.2 Cleaning/Decontaminating the Stockers and the Loading Unit

The product is designed for round-the-clock operation. The stockers and the loading unit cannot be touched during operation. Therefore, to clean the stockers or the loading unit, the power to the entire product must be turned off.

For the procedure for turning off the power, see Section 3.2.3, “Shutdown Procedure in Normal Case.”

Stockers

Cleaning the stockers and the loading unit

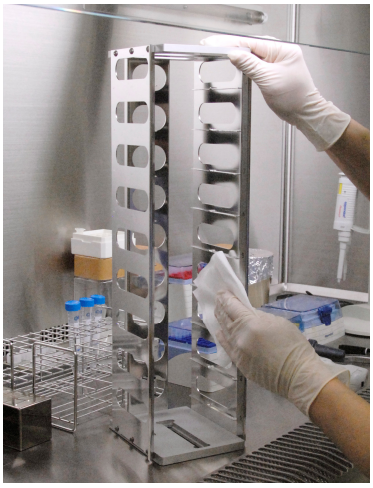
(1) Turn off the product completely in accordance with the procedure in 3.2.3, “Shutdown Procedure in Normal Case.”

(2) Check that the power is turned off and the product is stopped completely. Open the main glass door of the culture chamber.

(3) Remove the stockers.

Each row of stockers can be removed separately.

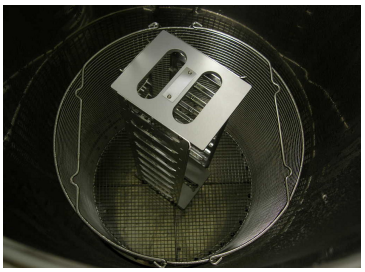
(4) Clean the stockers with disinfectant alcohol in a clean environment such as a safety cabinet.



Cleaning the stockers

Sterilizing the stockers with an autoclave

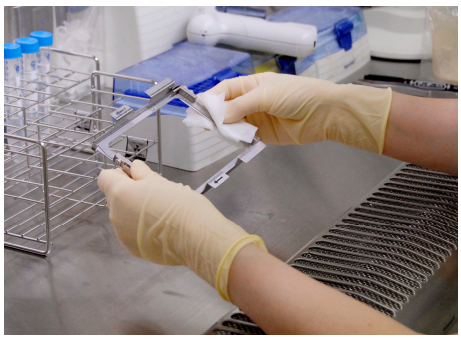
The stockers can be inserted directly into an autoclave for sterilization.



Sterilizing the stockers with an autoclave

6.5.3 Cleaning/Decontaminating the Holder

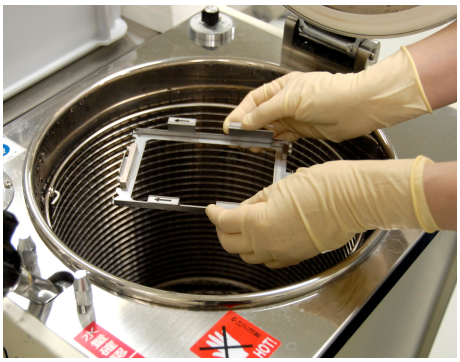
Clean the holder with disinfectant alcohol in a clean environment such as a safety cabinet.



Cleaning the holder

Sterilizing the holder with an autoclave

The holder can be inserted directly into the autoclave for sterilization.



Sterilizing the holder with an autoclave

6.6 Action to be Taken if Contamination Occurs

Although this product is designed and manufactured to be completely safe during use, incorrect usage or failure to follow the safety instructions provided may cause personal injury or property damage.
Mold contamination or similar can occur inside the product depending on the sample in use or how the product is used.
To prevent contamination, use the product correctly observing "Notes on Handling the Product" (see p. xvii).

Generally, mold entering from outside contaminates inside the product.
In the same way as ordinary cell cultures, be very careful not to introduce any substances that can cause contamination in the product during use.

Any contamination in the product can prevent the desired cell culture from being obtained.
This product includes a precision instrument in a cell culture space and is configured so that the customer is not able to clean/cleanse the product.
Ordinary alcohol is used to clean/cleanse the product and hydrogen peroxide is used to decontaminate it.

To request internal cleaning/cleansing or decontamination of the product, contact your nearest Nikon representative. Note that there will be a cleaning charged.

6.7 Cleaning the Exterior

Caution

Do not use organic solvents such as alcohol, ether, or paint thinner on painted components, plastic components, or printed components. Using organic solvents may result in discoloration of the plastic parts or removal of printed letters.

Clean the exterior of this product with dry gauze.

For persistent dirt, dampen a piece of gauze with neutral detergent and wipe gently.

BioStation CT

6.8 Removing Dew Condensation

Due to the properties of this product, dew condensation can occur even under the recommended use environment during continuous use.
Note that dew, if left to drip and remain in the unit, can affect the performance of the product.
Follow the procedure below to remove the dew condensation periodically. Monthly removal is recommended.

Preparation

- **Turn OFF the power of the product before starting. (See Section 3.2.3, "Shutdown Procedure in Normal Case" for details.)**
- **Prepare a clean disposable towel, such as a wiping cloth.**

Wipe-off

(1) Open the CO_2 incubator outer door.

(2) Wipe off any water drops inside the outer door.

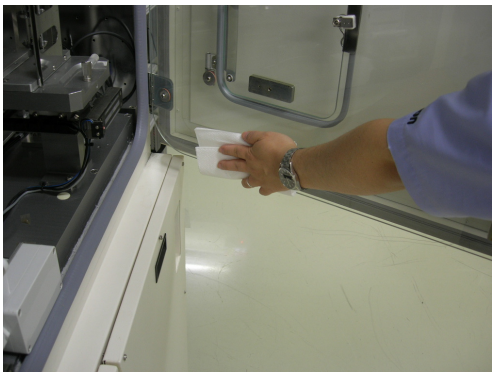
(3) Wipe off any water drops in the area shown in the photo on the right.

If the product is equipped with a water catch tray, wipe off water drops inside the tray.

(4) Open the main glass door.

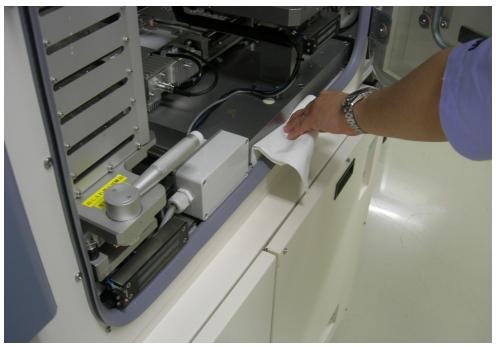
Slowly open the main glass door while supporting the lower part of the glass with a wiping cloth, as shown in the photo on the right, so as to catch any water drops that may be inside the door.

(5) Wipe off the inside of the main glass door.



(6) Wipe up any water that may have accumulated in the lower CO_2 incubator.

Wipe off the inner wall of the CO_2 incubator if any dew condensation is seen.



This completes the removal of dew condensation.

Close the main glass door and outer door, and then start the system. (See Section 3.2.2, "Startup Procedure in Normal Case" for details.)

6.9 Regular Inspections (Charged)

The performance of the product for continuous usage is specified based on the assumption that regular inspection is performed. Regular inspection (charged) is required once a year to maintain the performance of this product. For details, contact your nearest Nikon representative.

Regular inspection consists of two categories, the cleanliness inside the CO_2 incubator and the performance of the product.

Cleanliness inside the CO_2 incubator

If daily cleaning is performed properly and the product is operating normally, no problem with CO_2 incubator cleanliness will occur in the standard application of the product. However, if an accident occurs that might cause contamination inside the product, immediately stop operation and clean the inside the CO_2 incubator.

For details on cleaning the CO_2 incubator, contact your nearest Nikon representative.

Performance of the product

Regular inspections for product performance fall into the following categories:

1 Loading unit alignment and parts replacement

This product is equipped with a loading unit for transporting culture vessels. To maintain the performance of the product, it is necessary to align parts of the loading unit and replace consumables.

2 CO_2 incubator

2-1 Filters

This product contains a CO_2 gas filter and an air filter to keep the inside of the incubator clean. Depending on the gas used and the installation environment, filter clogging may occur and reduce product performance. Filters must be replaced regularly.

2-2 Silicone gaskets

Silicone gaskets are used at the main glass door and the access gate to shield the inside of the incubator from the outside. These silicone gaskets may deteriorate with age. If any deterioration or abnormality is found in gaskets during a regular inspection, replace them.

2-3 CO_2 hose

The CO_2 hose that connects the CO_2 incubator and the CO_2 cylinder may deteriorate with age. If any deterioration or abnormality is found in the hose during a regular inspection, replace it.

2-4 Humidifier unit

Humidity is controlled with the active humidity method using an ultrasonic transducer. If any deterioration or abnormality is found during a regular inspection, replace the humidifier unit as a whole.

2-5 Water leakage sensor

To alert the user to water leakages within the product, a water leakage sensor is installed. The sensor battery needs regular replacement.

In addition, some models may not have a water leakage sensor. For details, contact your nearest Nikon representative.

3 Microscope unit

3-1 XY stage inspection

The stage of the microscope unit may deteriorate with age and may fail to maintain the required performance for observation of cell cultures. Conduct regular inspection of each part and system operation to maintain the proper performance.

3-2 Brightness inspection of the micro illumination and the macro illumination

The illumination units will not be affected by age because long-life LEDs are used. However, depending on the state of the CO_2 incubator where the illumination units are installed, deterioration may occur. Perform adjustment during regular inspection to maintain the proper performance.

3-3 Cleanliness and operation of each part

It is possible to maintain the proper performance of the product by cleaning the optical devices within the CO_2 incubator, inspecting the operation of the electronic devices, and cleaning them.

4 General

This product is equipped with a UPS (uninterruptible power supply) as a backup power supply in case of blackout. The battery of the UPS has a limited life and if the battery is used longer than the life, an accident may occur. Replace the battery at a regular interval. (For replacement timing of the UPS battery, see Section 8.5, “Troubleshooting the UPS (Uninterruptible Power Supply).”)

5 Consumables

The following consumables (or items to be inspected) can be replaced as the result of the regular inspection:

Consumables	Replacement timing
Loading unit timing belt	1 year
Micro switch on each axis of the loading unit	1 year
CO_2 gas filter	1 year
Air filer	1 year
Silicone gaskets	When deterioration or abnormality is found
CO_2 hoses	When deterioration or abnormality is found
Humidifier unit	When deterioration or abnormality is found
UPS battery	When deterioration or abnormality is found
Water leakage sensor battery	1 year

Only trained personnel must perform the regular inspections.
Ask your nearest Nikon representative to perform the regular inspections.

7 Administrative Functions

This chapter describes the administrative functions of the system. The functions can be used by administrators only.

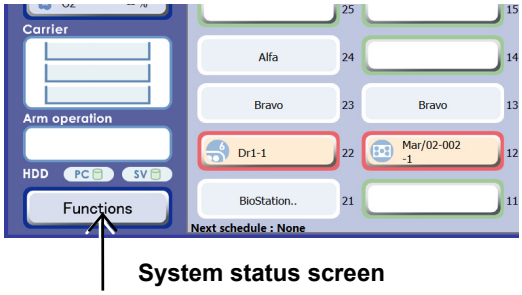
7.1 Functions Window for Administrators

When an administrator logs in to the product, buttons for administrators are displayed in the Functions window. This section describes the Functions window for administrators.

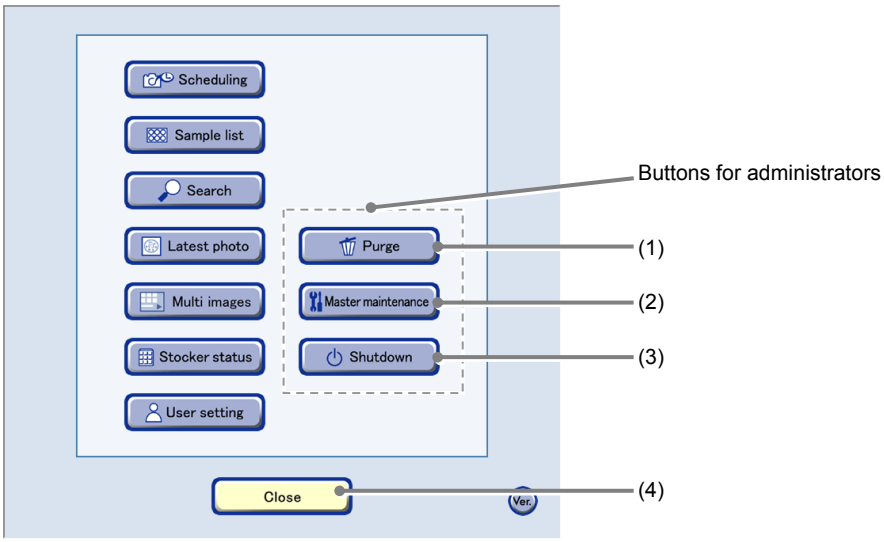
(1) Log in the product as a user who has administrative privileges.

(2) Press the Functions button on the System status screen.

The Functions window for administrators appears.



System status screen



Functions window (for administrators)

No.	Name	Function
(1)	Purge button	Press this button to display the Purge window. (See Section 7.2.)
(2)	Master maintenance button	Press this button to display the Master data maintenance window. (See Section 7.3.)
(3)	Shutdown button	Press this button to shut down the system of the product. (See Section 7.4.)
(4)	Close button	Press this button to close the Functions window.

7.2 Deleting the Observation Data

If the Full icon is displayed on the System status screen, the free space on the file server is insufficient. Delete the observation data in accordance with the following procedure to increase free space on the file server.

When the free space on the file server is insufficient, old observation data will be automatically deleted in the order it was saved. Therefore, download the observation data of cultured samples to a PC to delete unnecessary observation data in the file server. For the procedure for downloading the observation data from the file server, see the “BioStation CT Ver. 3.8 Instructions (PC Operations).”

Caution

Once the culture history is deleted without an appropriate download file, it cannot be restored.

Before executing the deletion, be sure that the data to be deleted is no longer necessary, and if the data is necessary, it must be downloaded.

Caution

The observation data of samples being cultured cannot be deleted.

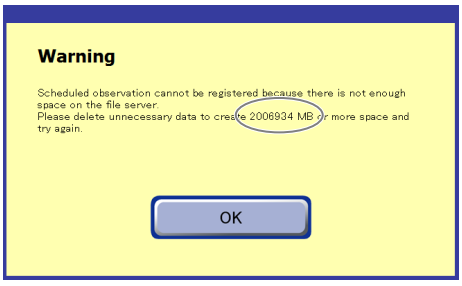
Note that if a sample is unloaded for the purpose of “Medium change”, the sample can be manually deleted.

When schedule cannot be set:

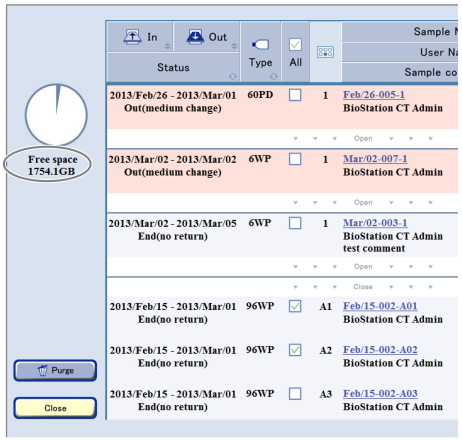
If sufficient space to save the observation data cannot be allocated on the file server, scheduled observations cannot be set. In this case, unnecessary observation data must be deleted so that sufficient space can be allocated.

The data space shown in the message of the Insufficient data space warning dialog box is the estimated amount of observation data to be deleted. Delete the observation data so that amount of space on the file server shown on the left of the Purge window exceeds the amount of space shown in the Insufficient data space warning dialog box.

In addition, the data space shown in the Insufficient data space warning dialog box is in MB (megabytes) while the data space shown in the amount of space display area on the Purge window is in GB (gigabytes). When deleting images, use the formula GB = MB/1024 to calculate required amount.



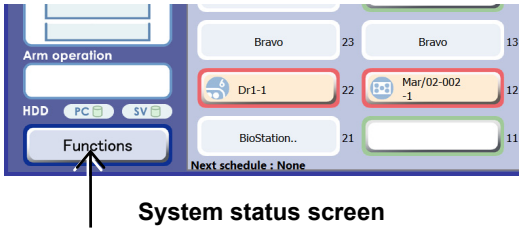
Insufficient data space warning dialog box



Purge window

(1) Press the Functions button on the System status screen.

The Functions window is displayed.

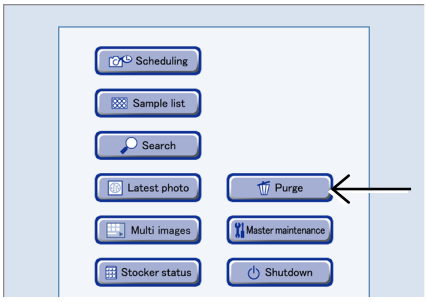


System status screen

(2) Press the Purge button in the Functions window.

The list of the cultured samples is displayed.

> The data of samples in culture and the data uploaded after downloaded are not displayed in the Purge window.

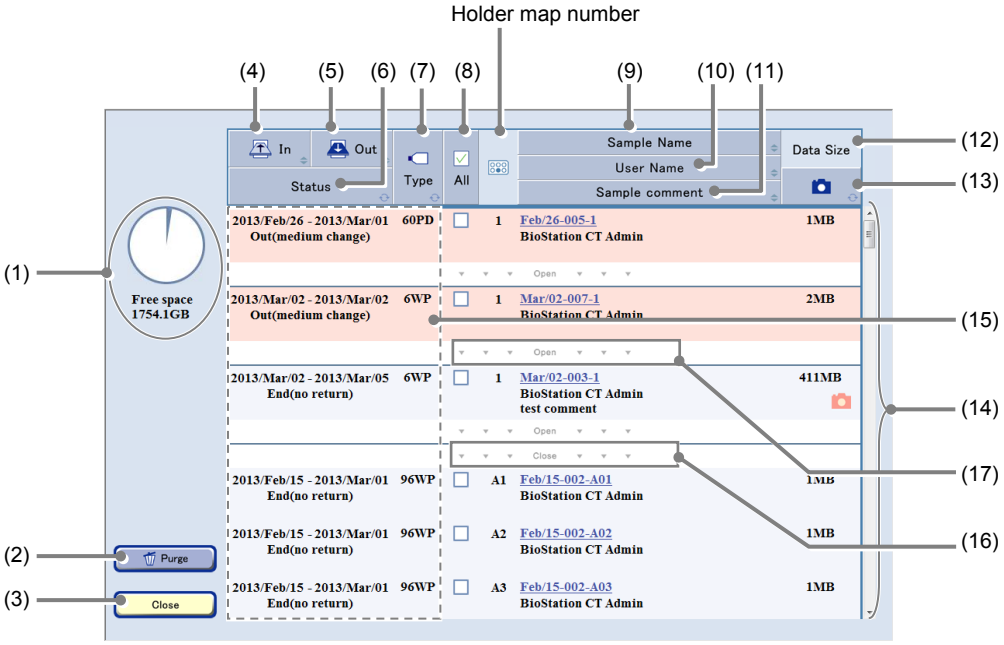


Functions window

mark ··· Switches between the ascending order and descending order to display the sorted result.

mark ··· Displays the sorted result from the top sequentially as a loop.

* The most recently used Sort button is underlined in pink.



Purge window

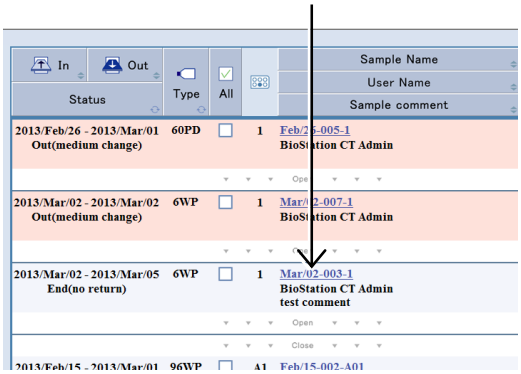
No.	Name	Function
(1)	Free space indicator	The free space on the file server is displayed. The blue area of the pie chart shows the used area of the file server and the white area shows the free space of the file server.
(2)	Purge button	Press this button to delete the selected data.
(3)	Close button	Press this button to close the Purge window.
(4)	In sort button	Press this button to sort the list in order of loaded date.

No.	Name	Function
(5)	Out sort button	Press this button to sort the list in order of unloaded date.
(6)	Status sort button	Press this button to sort the list in order of status.
(7)	Type sort button	Press this button to sort the list by the type of culture vessels.
(8)	All check button	Press this button to select or cancel all displayed or selected samples.
(9)	Sample Name sort button	Press this button to sort the list in order of sample name.
(10)	User Name sort button	Press this button to sort the list in order of user name.
(11)	Sample comment sort button	Press this button to sort the list in order of sample comment.
(12)	Data Size area	Press this button to sort the list in order of data size.
(13)	Observation status sort button	Press this button to sort the list in order of observation status. (Pink) : Scheduled observation images are included (Green) : Live observation images are included (No icon) : No observation images
(14)	Sample list	The user name and the sample name of the cultured samples or samples being unloaded for a medium change are listed.
(15)	Stocker/Container area	Press this area to select all samples in the holder or cancel the selection.
(16)	Close area	Press this area to display only the first sample in the holder.
(17)	Open area	Press this area to display all sample names in the holder.

(3) Verify the observation data to be deleted.

1. Press the sample name to check its observation data.

The Image review window is displayed.



2. Verify the contents of the observation data to be deleted.

3. Press the Close button.

The Image review window is closed and the Purge window is displayed.

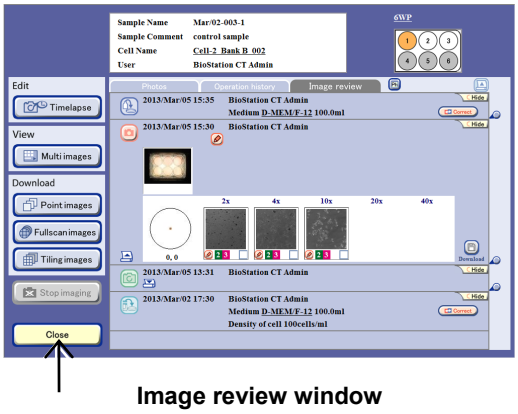


Image review window

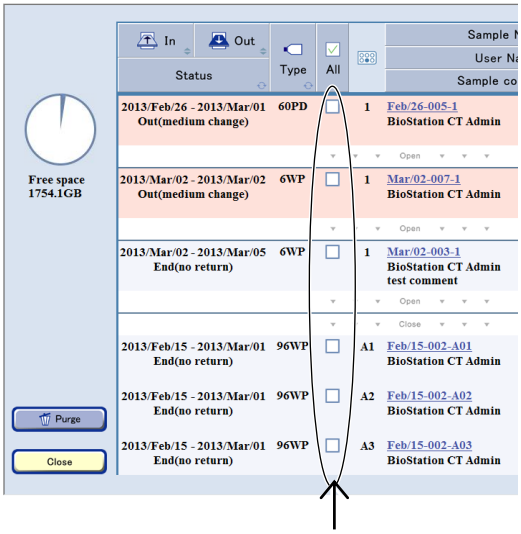
(4) Select the check box of the observation data to be deleted.

When check boxes are checked with only the first sample in the holder displayed, all samples in the holder are selected.

Also to select all samples in the holder, press the Stocker/Container area.

To select each sample, press the Open area to display all samples in the holder and then check the check box of a desired sample.

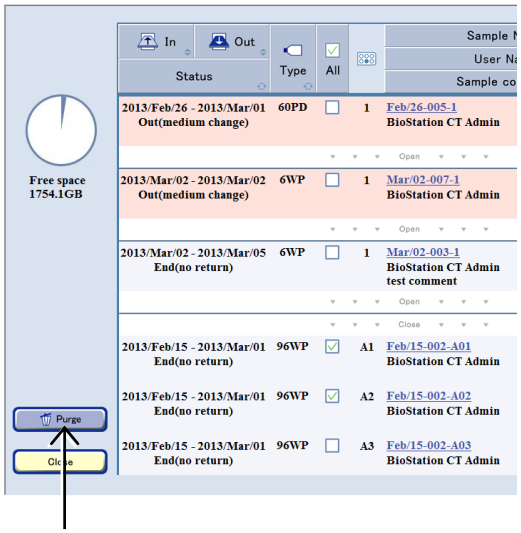
To select all observation data, press the All check button. All the data in the Purge window is selected.



Purge window

(5) Press the Purge button.

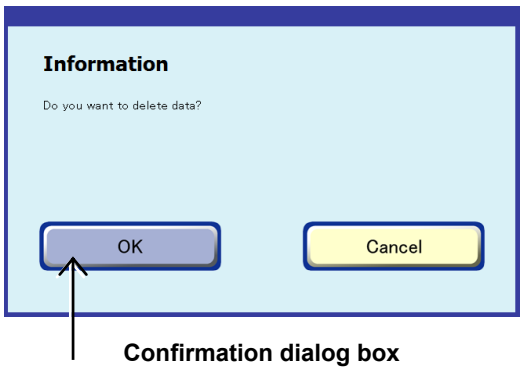
A delete confirmation dialog box appears.



(6) Press the OK button.

The selected observation data is deleted.

When the free space on the file server is 200 GB or more, the Full icon disappears from the System status screen.



Confirmation dialog box

7.3 Master Data Maintenance

Before starting the culture observation with the product, register the master data in accordance with the following instructions.

7.3.1 Master Data Maintenance Window

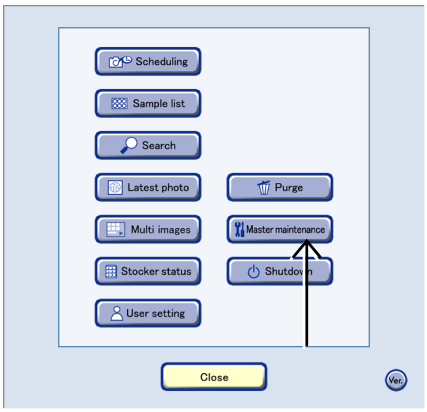
(1) Press the Functions button on the System status screen.

The Functions window is displayed.

System status screen

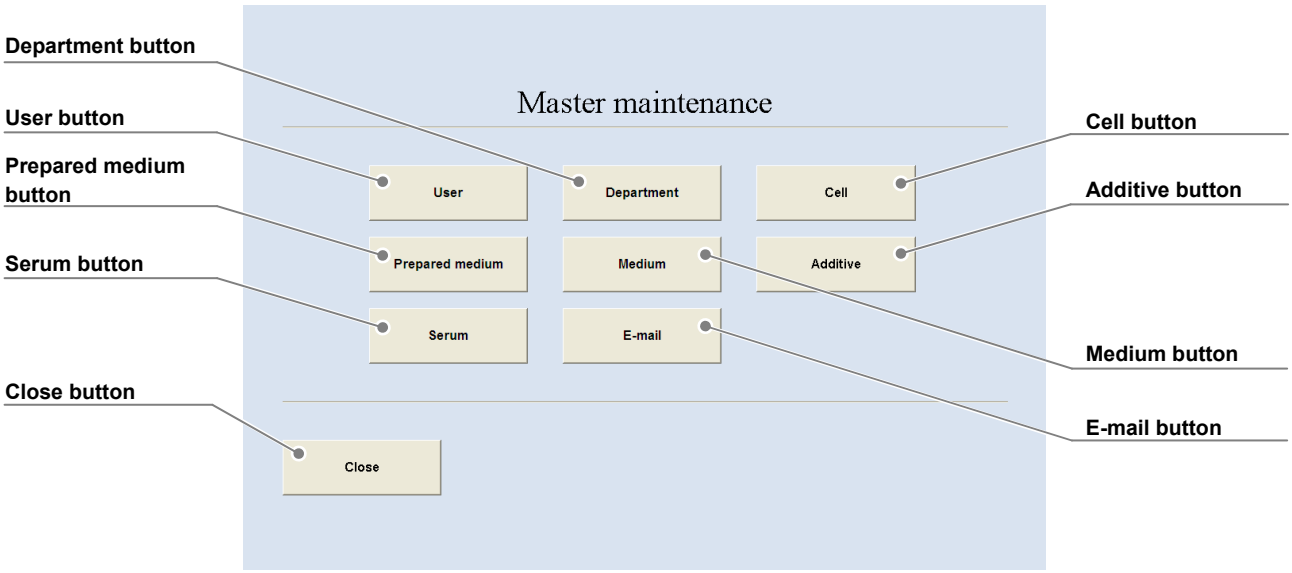
(2) Press the Master maintenance button in the Functions window.

The Master data maintenance window is displayed.



Functions window

Press the buttons in the Master data maintenance window to perform master data maintenance.



Master data maintenance window

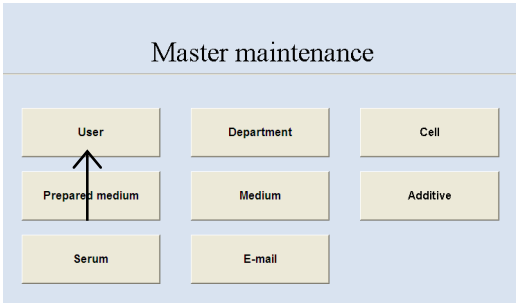
7.3.2 User Master Data

This section describes the procedure for registering, editing, or deleting a user master data.
If the user master data is registered, it is possible to log in to the product using the registered name. To enter the department name into the user master data, register the department name master data in advance.

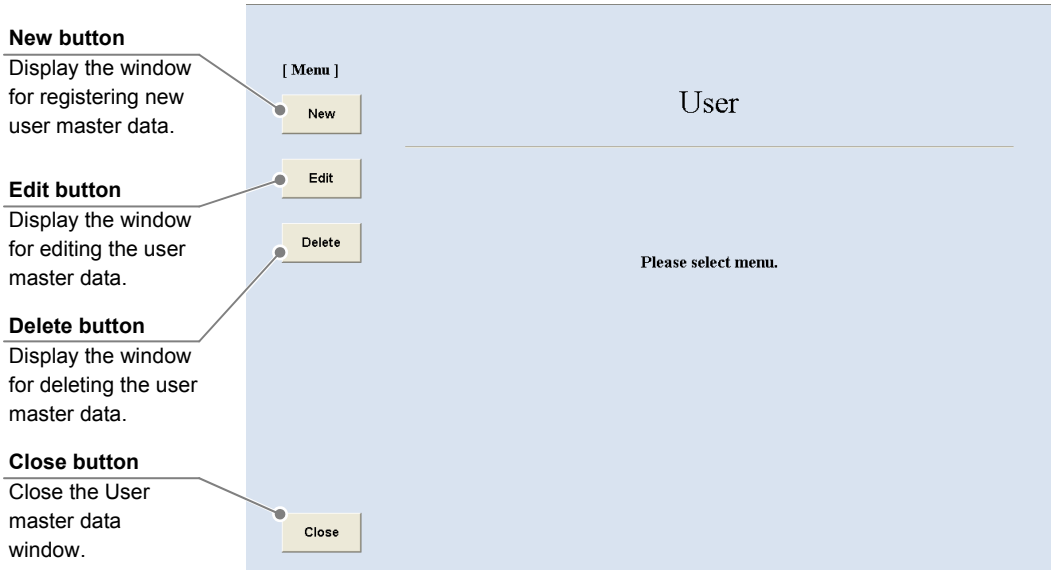
7.3.2.1 Registering new user master data

(1) Press the User button in the Master data maintenance window.

The User master data window is displayed.



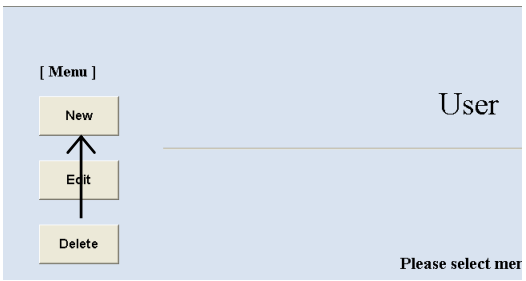
Master data maintenance window



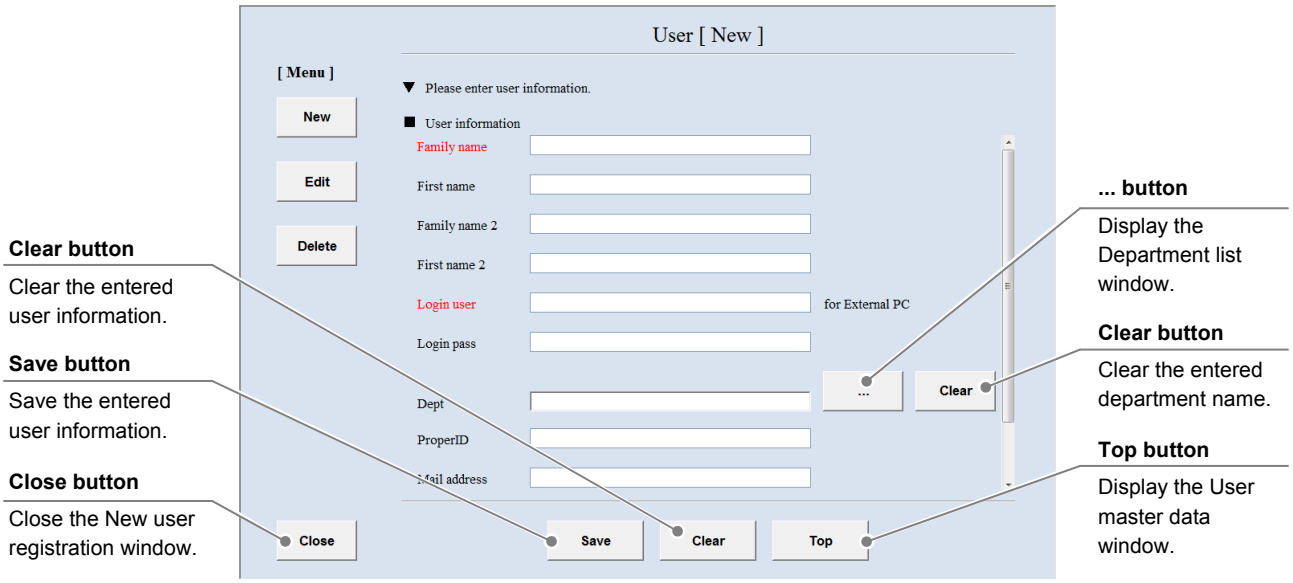
User master data window

(2) Press the New button.

The New user registration window is displayed.



Fill in the items displayed in red. For other items, fill in if necessary.

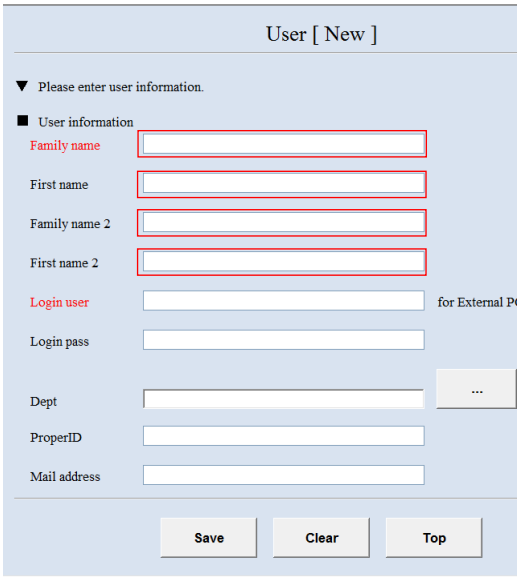


New user registration window

(3) Enter the user name.

1. Enter the user name into the Family name field and the First name field.

The user name entered here is added to the user list displayed in the Select User window.
Enter the login name of the user in the Login name field.
A user who does not have a login name is not displayed in the user list.



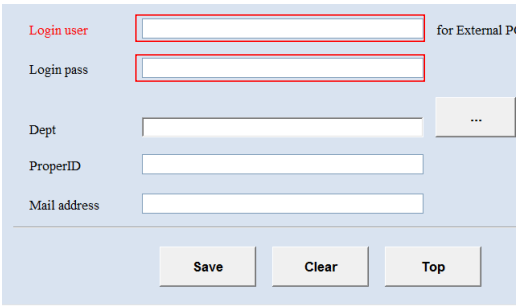
2. Enter the middle name or an alias into the Family name 2 field and the First name 2 field if necessary.

When the Family name 2 field and the First name 2 field are filled, the Select User window display users in a order sorted by Family name 2, First name 2, Family name, and First name.

> If a name already registered as a combination of the First name and Family name is entered, it cannot be saved in the master data.

(4) Enter the login name into the Login user field.

If the login name is not entered, the person cannot be recognized as a user to operate the BioStation either from an external PC or directly on this product.

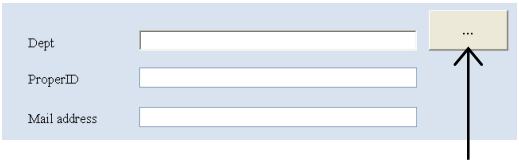


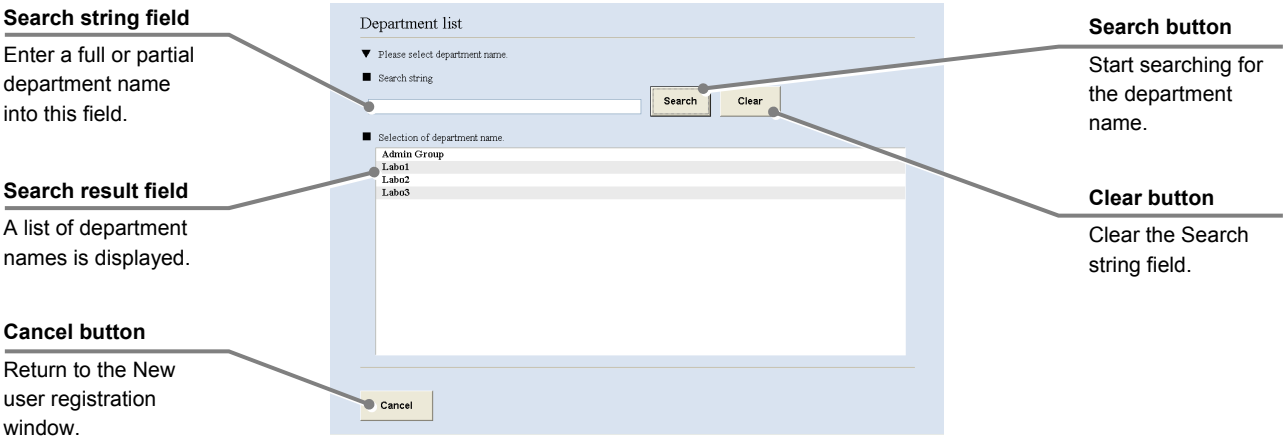
(5) Enter the login password into the Login pass field.

The login name and the login password must be composed of alphanumeric characters.
When the login password is entered, the Password window is displayed in the login procedure.

(6) Enter the department name into the Dept field.

1. Press the ··· button to display the Department list window.

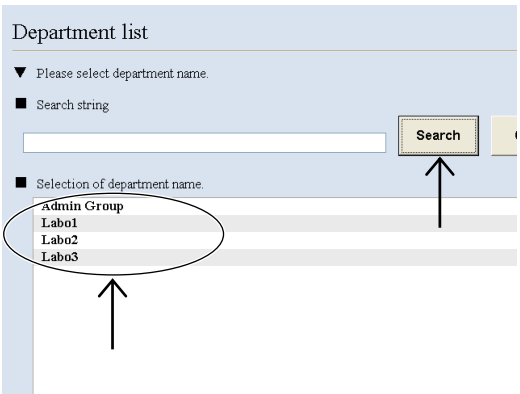




Department list window

2. Enter a partial department name into the Search string field and press the Search button.
If you do not know any characters of the search department name, press the Search button without data entry.

The found department names are displayed in the Search result field.



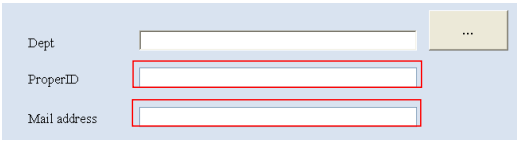
> Department names registered in the department master data are displayed. If there is no department name to select, register the department name in accordance with the procedure in 7.3.3.1, “Registering new department master data” in advance.

3. Press the department name to be entered.

The New user registration window is displayed and the selected department name is displayed in the Dept field.

(7) Fill in the Proper ID field.

(8) Fill in the Mail address field.

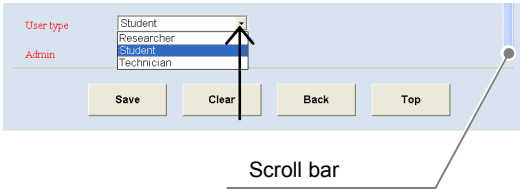


(9) Select the user type.

1. **Scroll down the window by pressing the scroll bar on the right side of the New user registration window.**

   The User type field and the Admin field are displayed.

2. **Select a user type (Researcher, Student, or Technician) from the list box displayed by pressing the User type field.**

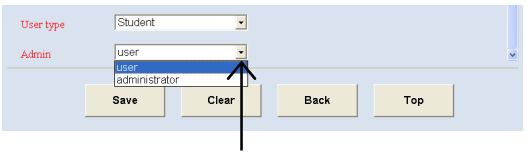


(10) Select the authorization of the user.

1. **Select the authorization of the user (user or administrator) from the list box displayed by pressing the Admin field.**

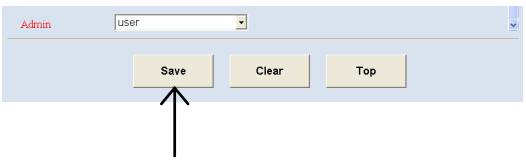
   user:
   Select “user” to use the general user’s functions only.

   administrator:
   Select “administrator” to use all functions including administrative functions.



(11) Press the Save button.

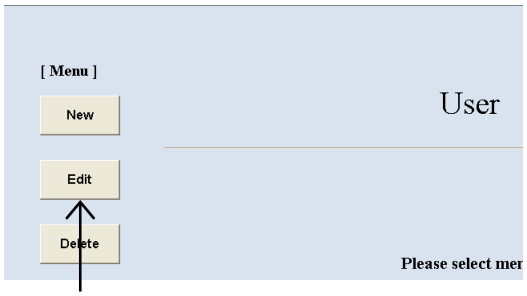
The entered user information is saved into the master data.



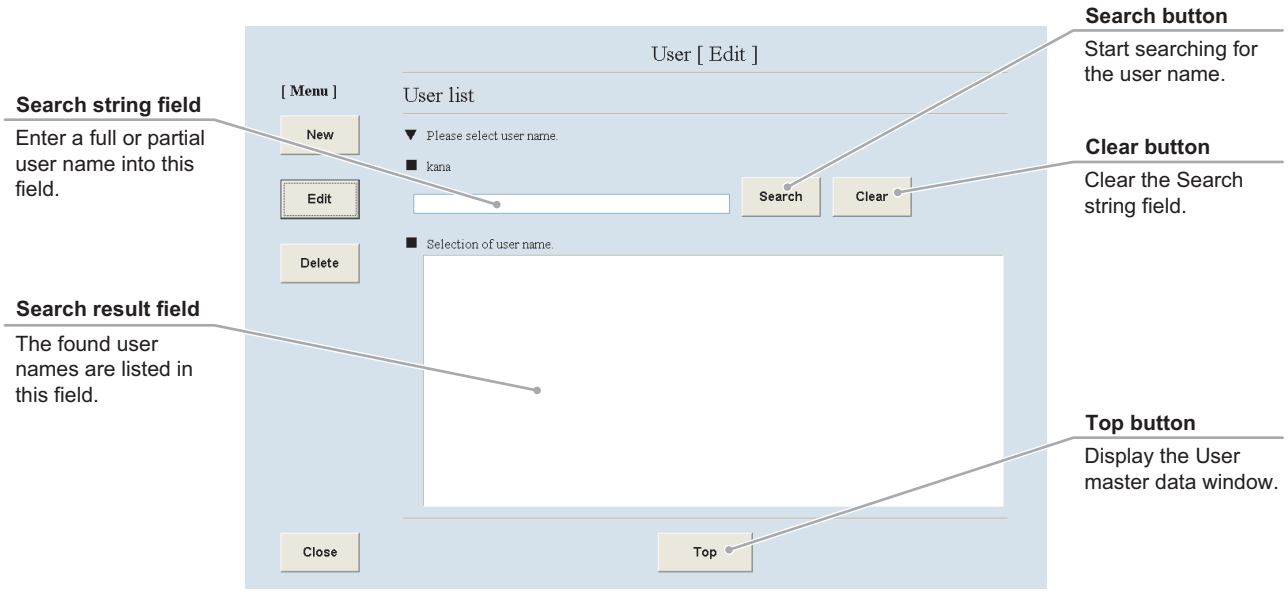
7.3.2.2 Editing the user master data

(1) Press the Edit button in the User master data window.

The User edit search window is displayed.



User master data window



User edit search window

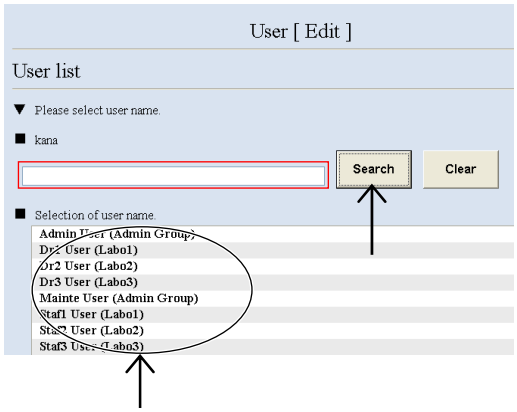
(2) Search for the user master data to be edited.

1. Enter a full or partial user name in the search string field and press the Search button. If you do not know any characters of the search user name, press the Search button without data entry.

The found user names are listed in the Search result field.

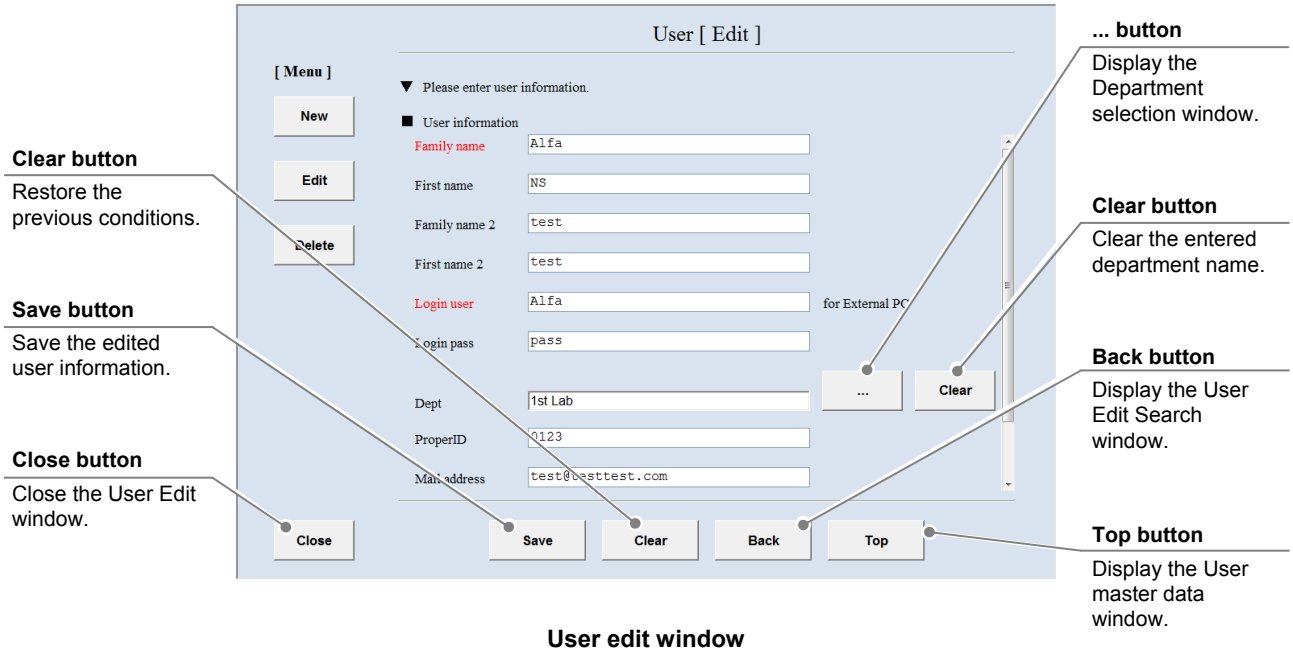
2. Select a user name from the Search result field.

The selected user master data is displayed in the User edit window.



(3) Edit the user master data.

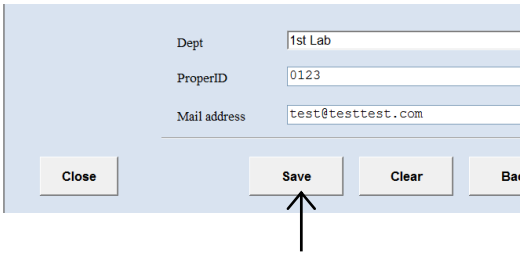
The procedure for editing the user master data is the same as the procedure for registering new user master data. For the procedure for editing each item, see Section 7.3.2.1, “Registering new user master data.”



User edit window

(4) Press the Save button.

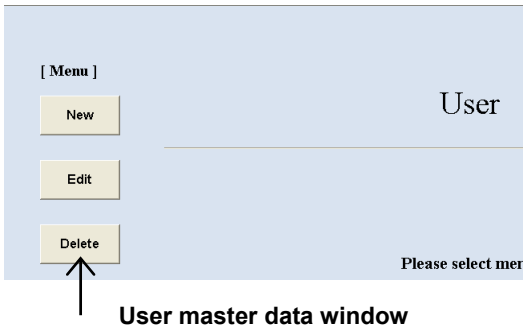
The edited user information is saved into the master data.



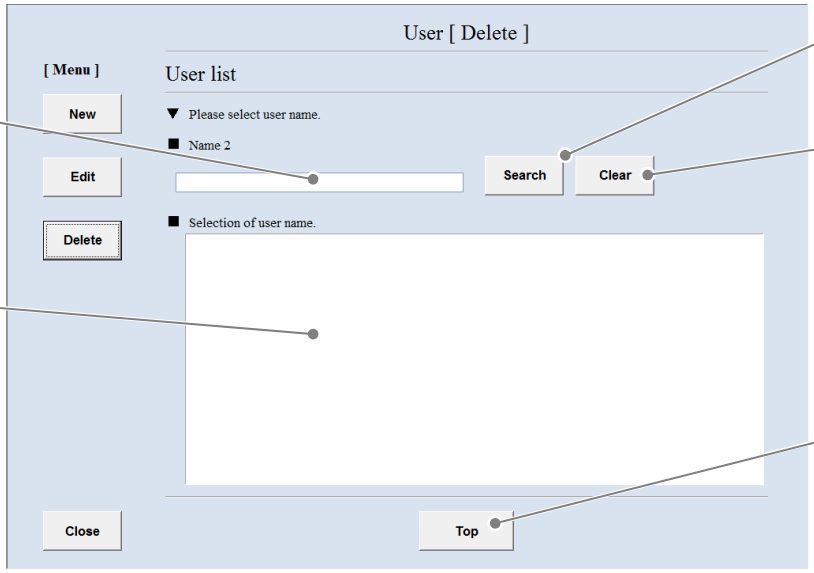
7.3.2.3 Deleting the user master data

(1) Press the Delete button in the User master data window.

The User delete search window is displayed.



User master data window



Search string field

Enter a full or partial user name into this field.

Search result field

The found user names are listed in this field.

Search button

Start searching for the user name.

Clear button

Clear the Search string field.

Top button

Display the User master data window.

User delete search window

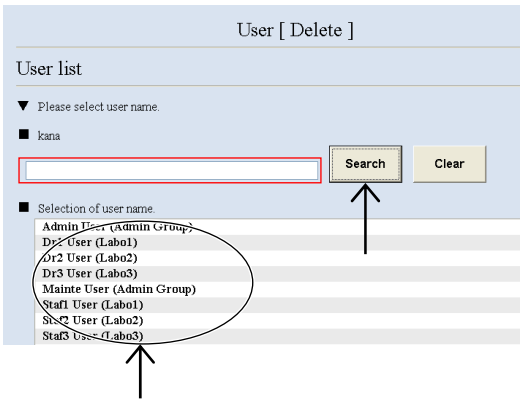
(2) Search for the user master data to be deleted.

1. Enter a full or partial user name into the Search string field and press the Search button.
If you do not know any characters of the user name, press the Search button without data entry.

The found user names are listed in the Search result field.

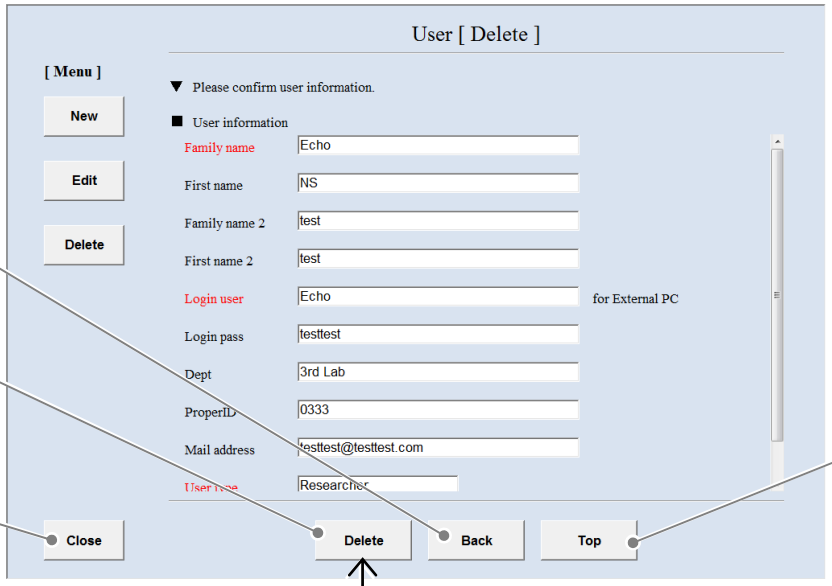
2. Select the user name to be deleted from the Search result field.

The selected user master data is displayed in the User delete window.



(3) Press the Delete button.

The displayed user information is deleted from the master data.



Back button

Display the User delete Search window.

Delete button

Delete the displayed user master data.

Close button

Close the User delete window.

Top button

Display the User master data window.

User delete window

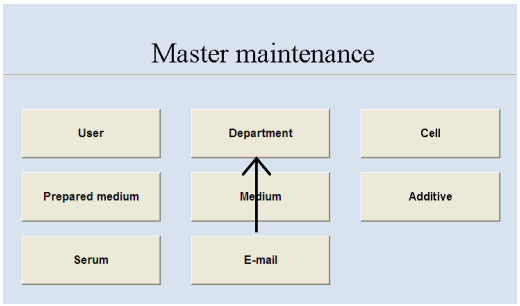
7.3.3 Department Master Data

This section describes the procedure for registering, editing or deleting a department master data. If the department name is registered into the department master data, it can be selected in the User master data registration window.

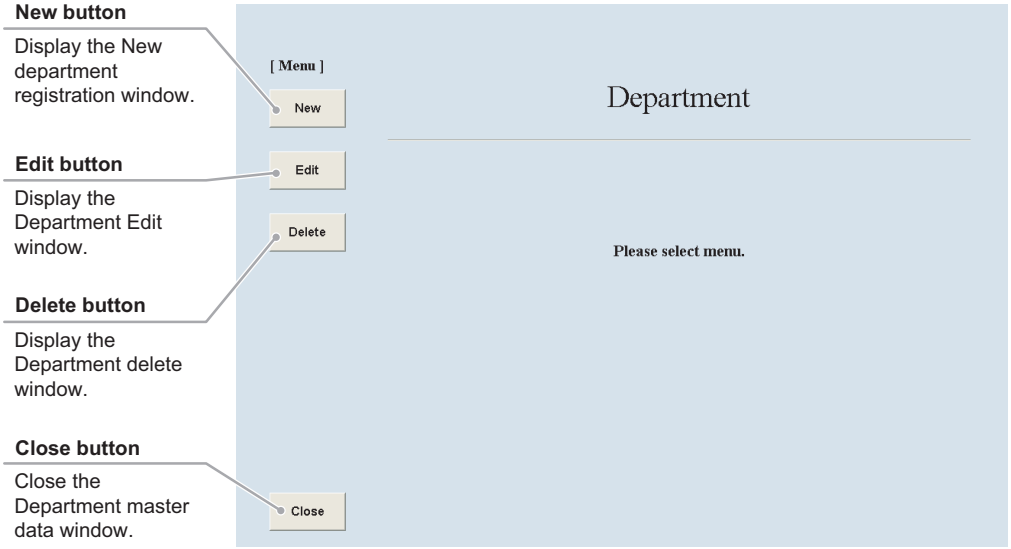
7.3.3.1 Registering new department master data

(1) Press the Department button in the Master data maintenance window.

The Department master data window is displayed.



Master data maintenance window

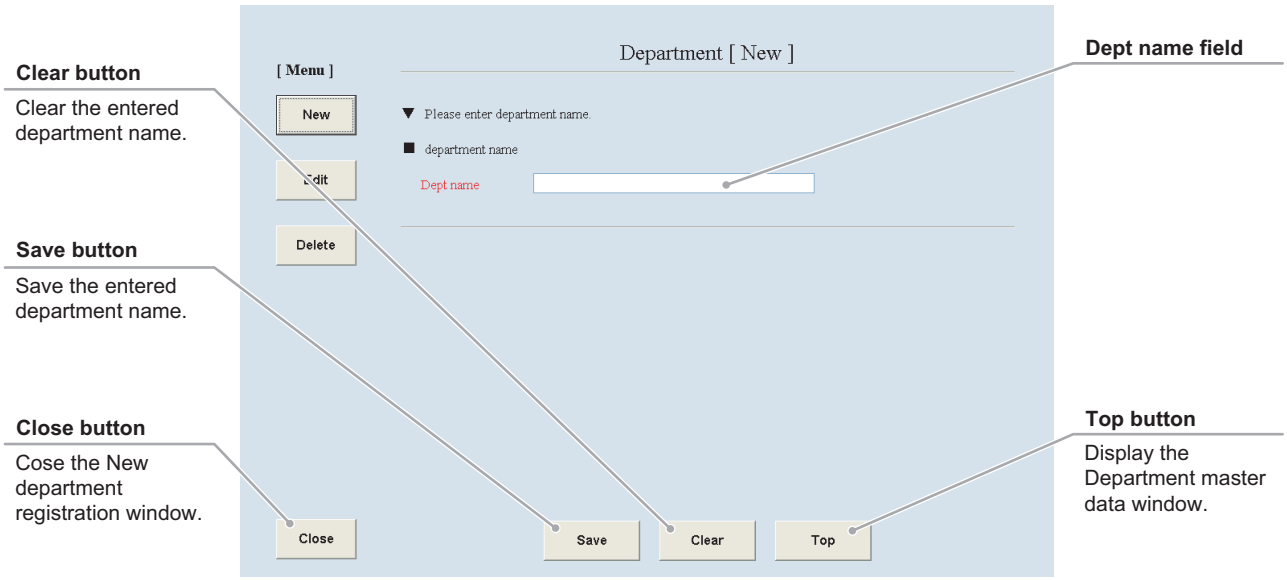


Department master data window

(2) Press the New button.

The New department registration window is displayed.

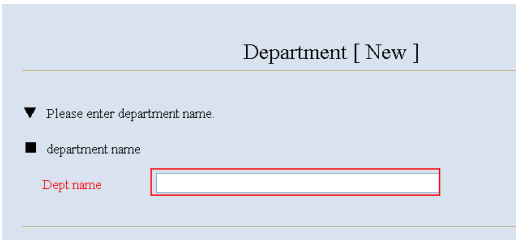
Fill in the Dept name field.



New department registration window

(3) Enter the department name into the Dept name field.

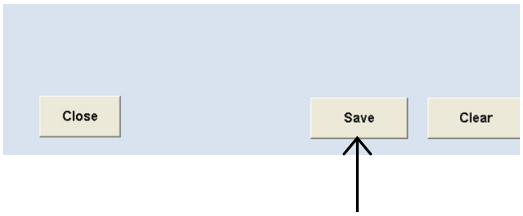
The entered department name can be selected in the User master data registration window.



> When the Dept name is already registered, it cannot be saved in the user master data.

(4) Press the Save button.

The entered department name is saved into the master data.

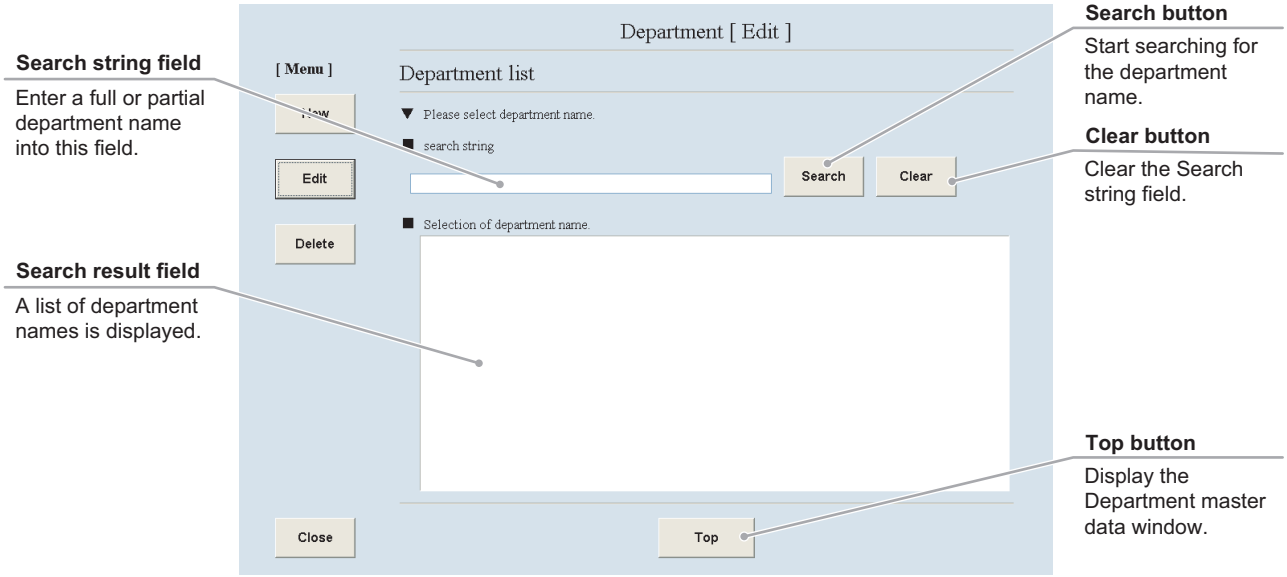


7.3.3.2 Editing department master data

(1) Press the Edit button in the Department master data window.

The Department edit search window is displayed.

Department master data window

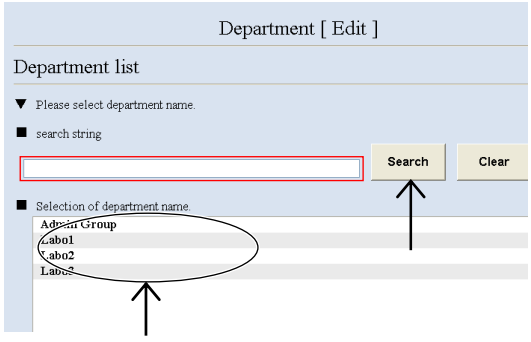


Department edit search window

(2) Search for the department master data to be edited.

1. Enter a full or partial department name into the Search string field and press the Search button.
If you do not know any characters of the department name, press the Search button without data entry.

The found department names are listed in the Search result field.

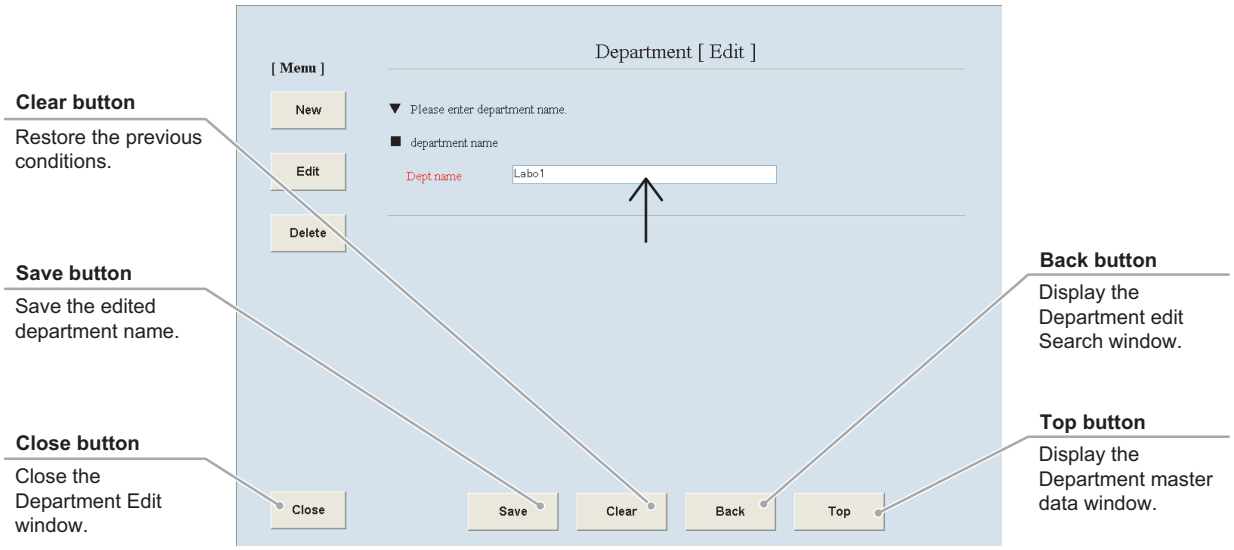


2. Select a department name from the Search result field.

The selected department master data is displayed in the Department edit window.

(3) Press the Dept name field and edit the department name.

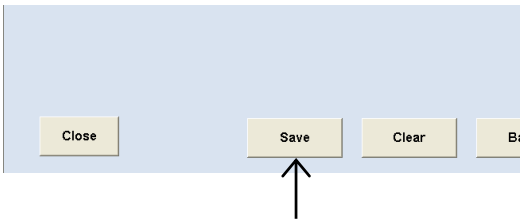
When the Dept name is already registered, it cannot be saved in the user master data.



Department edit window

(4) Press the Save button.

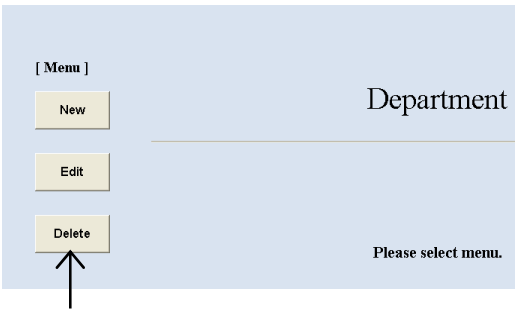
The edited department name is saved into the master data.



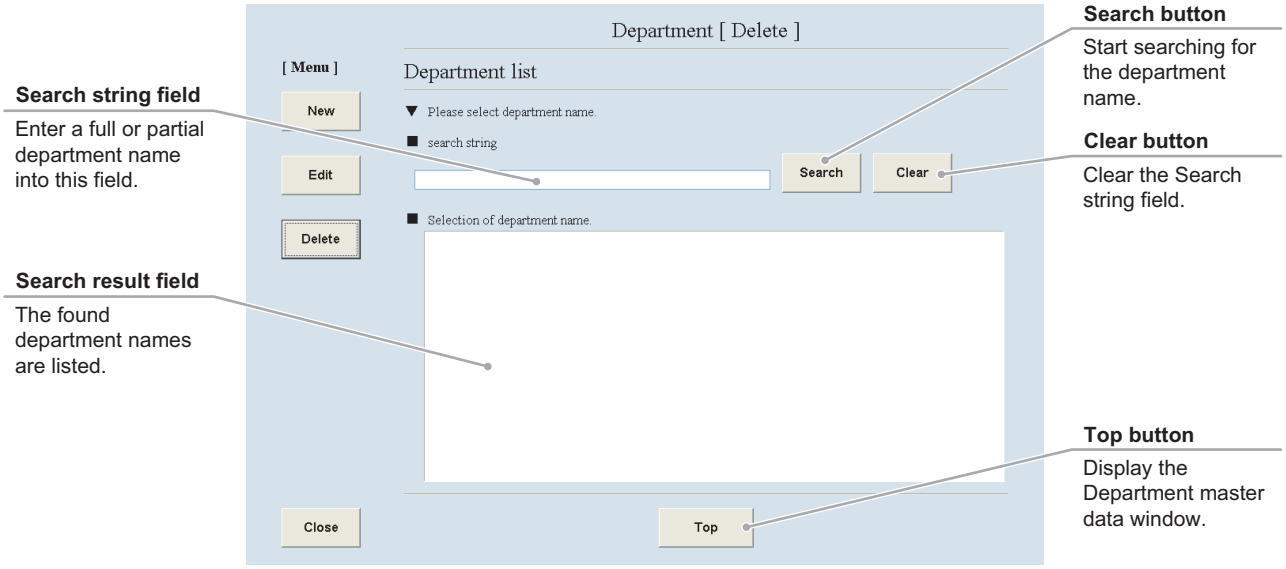
7.3.3.3 Deleting the department master data

(1) Press the Delete button in the Department master data window.

The Department delete search window is displayed.



Department master data window

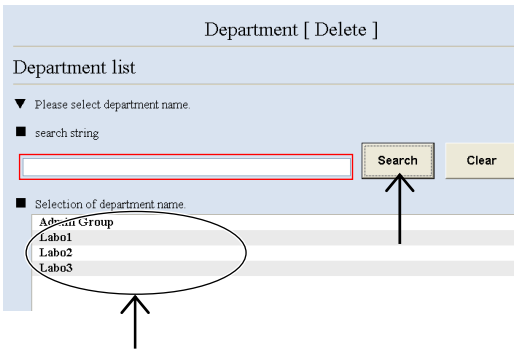


Department delete search window

(2) Search for the department master data to be deleted.

1. Enter a full or partial department name into the Search string field and press the Search button.
If you do not know any characters of the department name, press the Search button without data entry.

The found department names are listed in the Search result field.

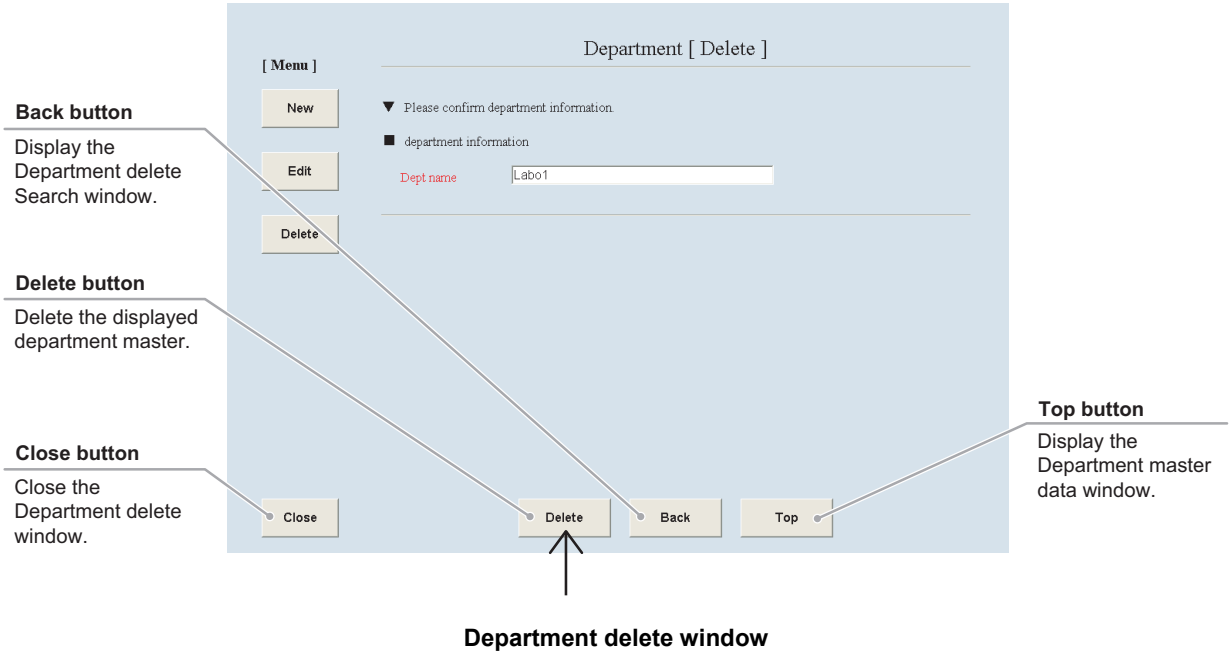


2. Select a department name from the Search result field.

The selected department master data is displayed in the Department delete window.

(3) Press the Delete button.

The displayed department name is deleted from the master data.



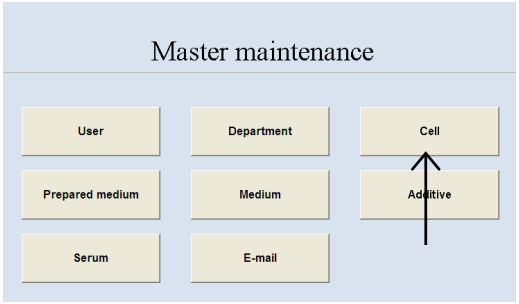
7.3.4 Cell Master Data

This section describes the procedure for registering, editing or deleting cell master data.

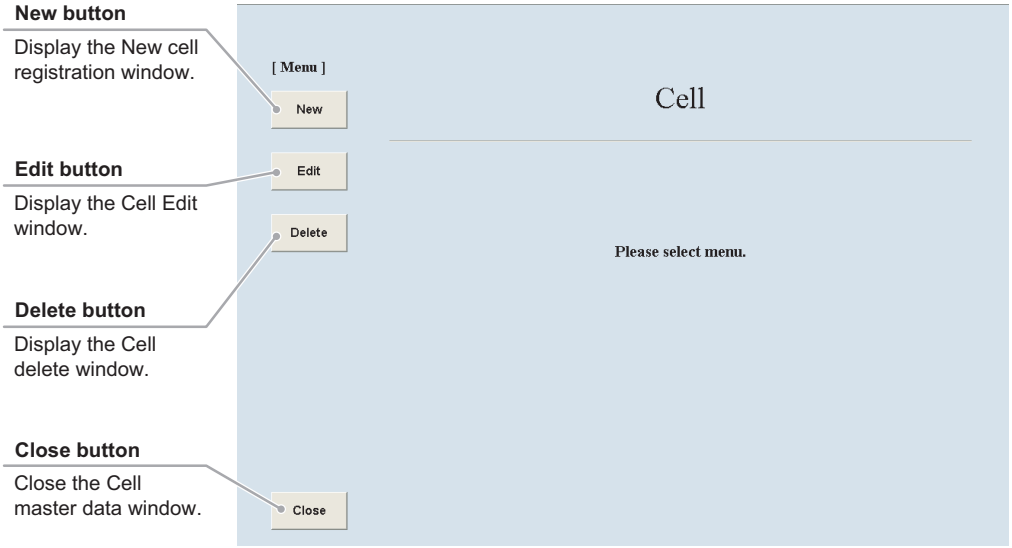
7.3.4.1 Registering new cell master data

(1) Press the Cell button in the Master data maintenance window.

The Cell master data window is displayed.



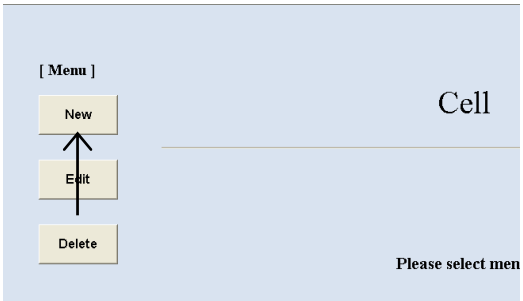
Master data maintenance window



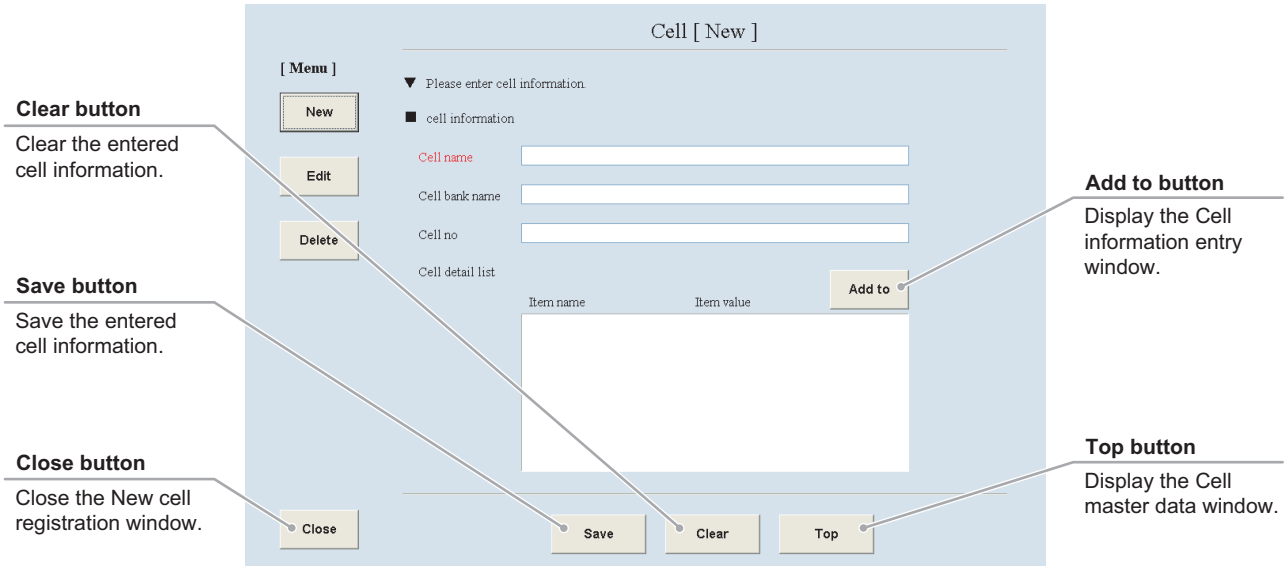
Cell master data window

(2) Press the New button.

The New cell registration window is displayed.



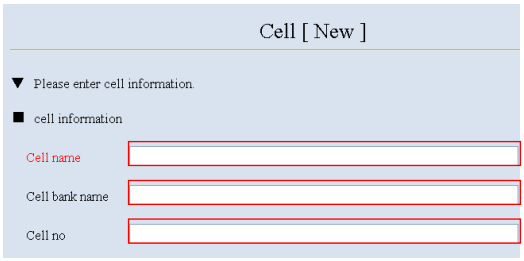
Fill in the items displayed in red. For other items, fill in if necessary.



New cell registration window

(3) Enter the cell name into the Cell name field.

> When the Cell name is already registered, it cannot be saved in the user master data.

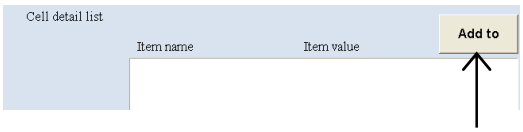


(4) Enter the cell bank name into the Cell bank name field.

(5) Enter the cell number into the Cell no field.

(6) Enter the cell information.

1. Press the Add to button to display the Cell information entry window.

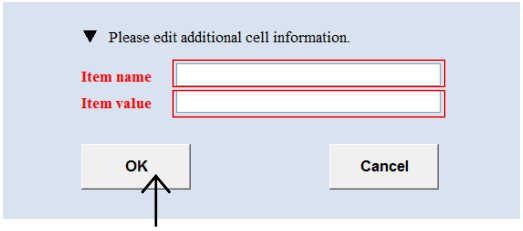


2. Enter the item name of the cell information into the Item name field.

3. Enter the item value of the cell information into the Item value field.

4. Press the OK button.

The entered cell information is saved and the New cell registration window is displayed.

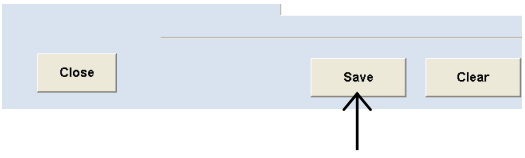


Cell information entry window

5. To enter other cell information, repeat steps 1 to 4.

(7) Press the Save button.

The entered cell information is saved into the master data.

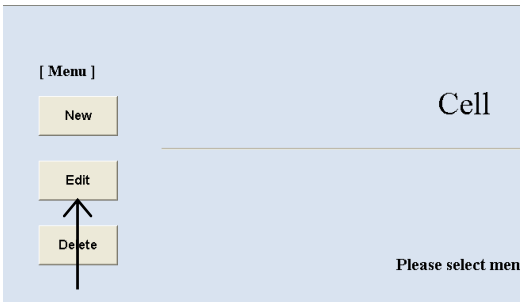


New cell registration window

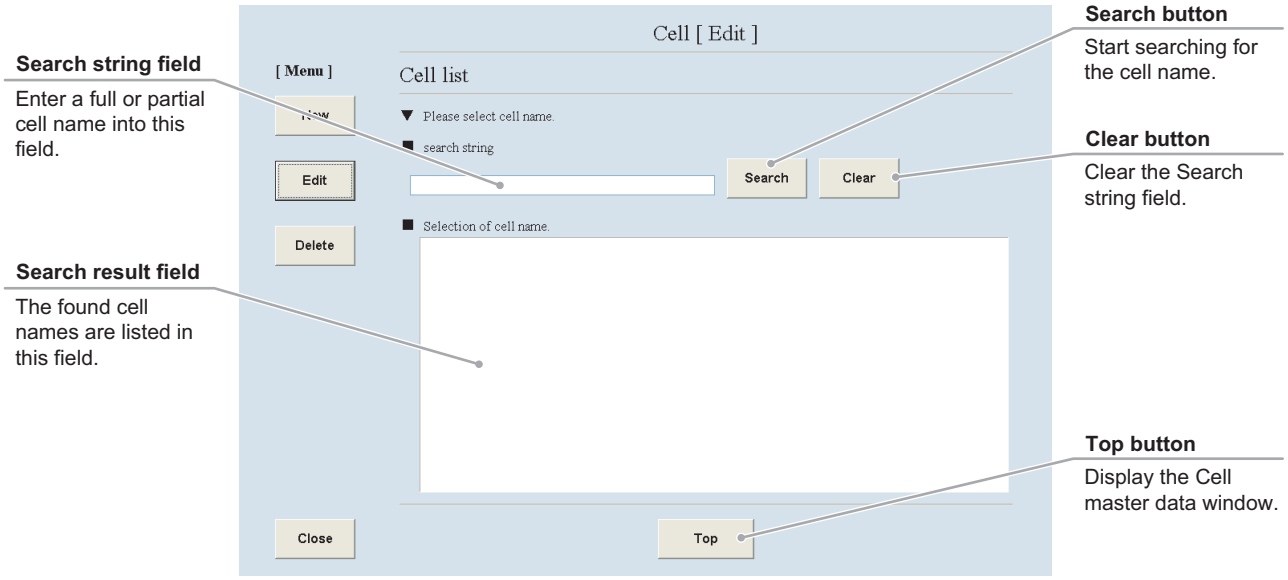
7.3.4.2 Editing the cell master data

(1) Press the Edit button in the Cell master data window.

The Cell edit search window is displayed.



Cell master data window



Cell edit search window

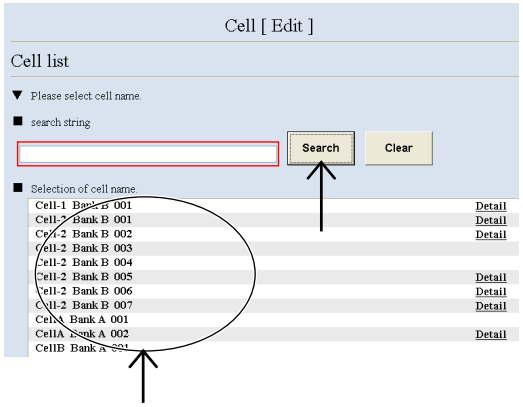
(2) Search for the cell master data to be edited.

1. **Enter a full or partial cell name into the Search string field and press the Search button.**
   If you do not know any characters of the cell name, press the Search button without data entry.

   The found cell names are listed in the Search result field.
   If the details of the cell information are registered, it can be displayed by pressing the Detail button on the right side of the Search result field.

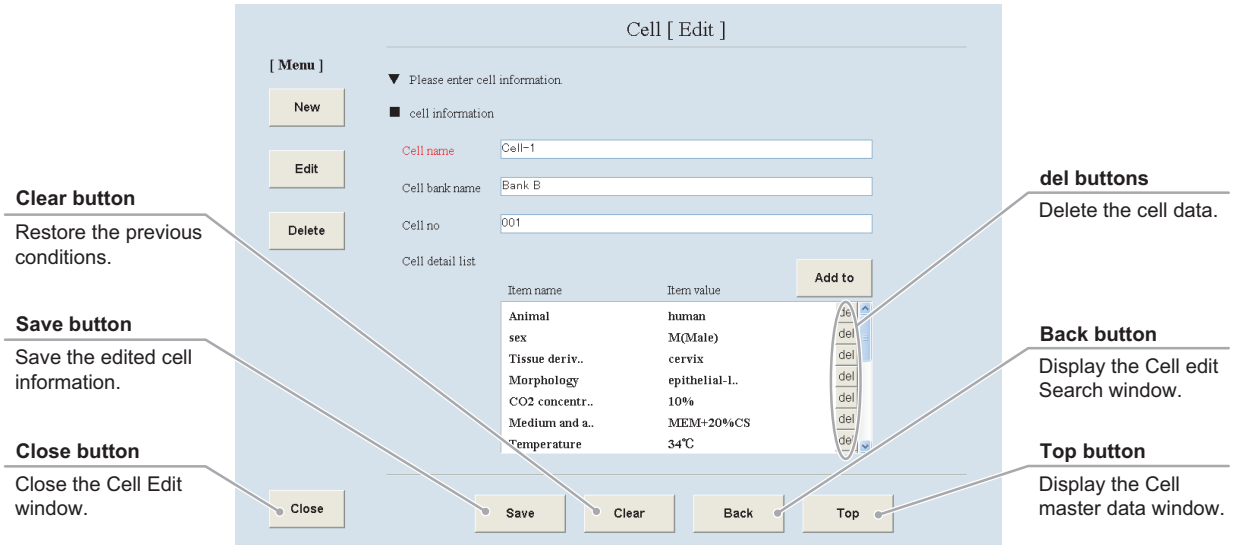
2. **Select a cell name from the Search result field.**

   The selected cell master data is displayed in the Cell edit window.



(3) Edit the cell master data.

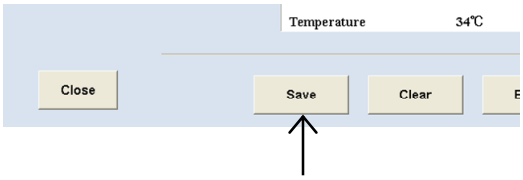
The procedure for editing the cell master data is the same as the procedure for registering the new cell master data. For the procedure of editing each item, see Section 7.3.4.1, “Registering new cell master data.”



Cell edit window

(4) Press the Save button.

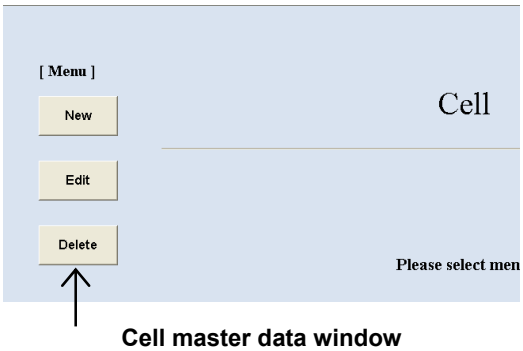
The edited cell information is saved into the master data.



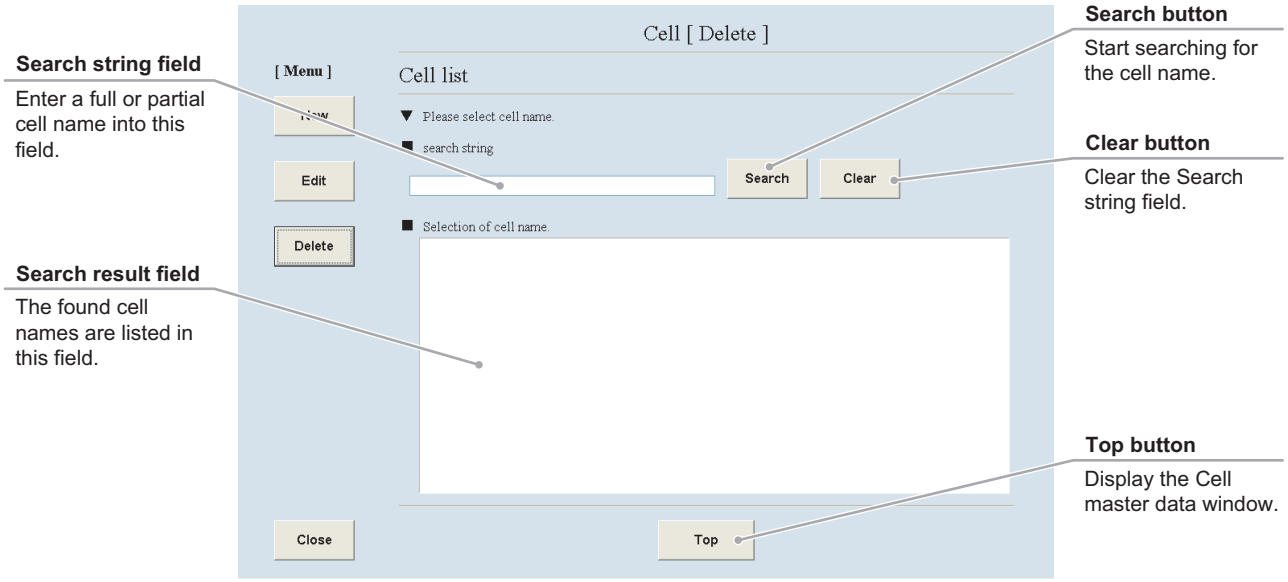
7.3.4.3 Deleting the cell master data

(1) Press the Delete button in the Cell master data window.

The Cell delete search window is displayed.



Cell master data window



Cell delete search window

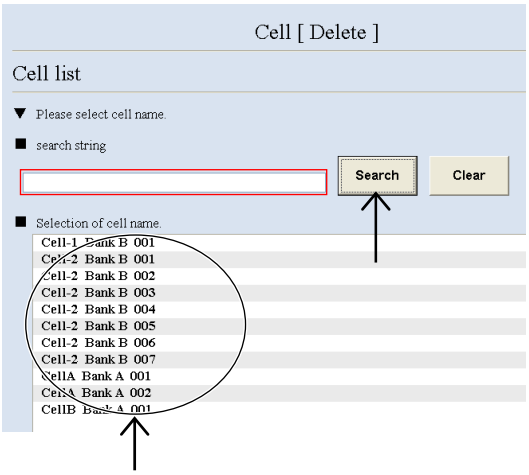
(2) Search for the cell master data to be deleted.

1. **Enter a full or partial cell name into the Search string field and press the Search button.**
   If you do not know any characters of the cell name, press the Search button without data entry.

   The found cell names are listed in the Search result field.

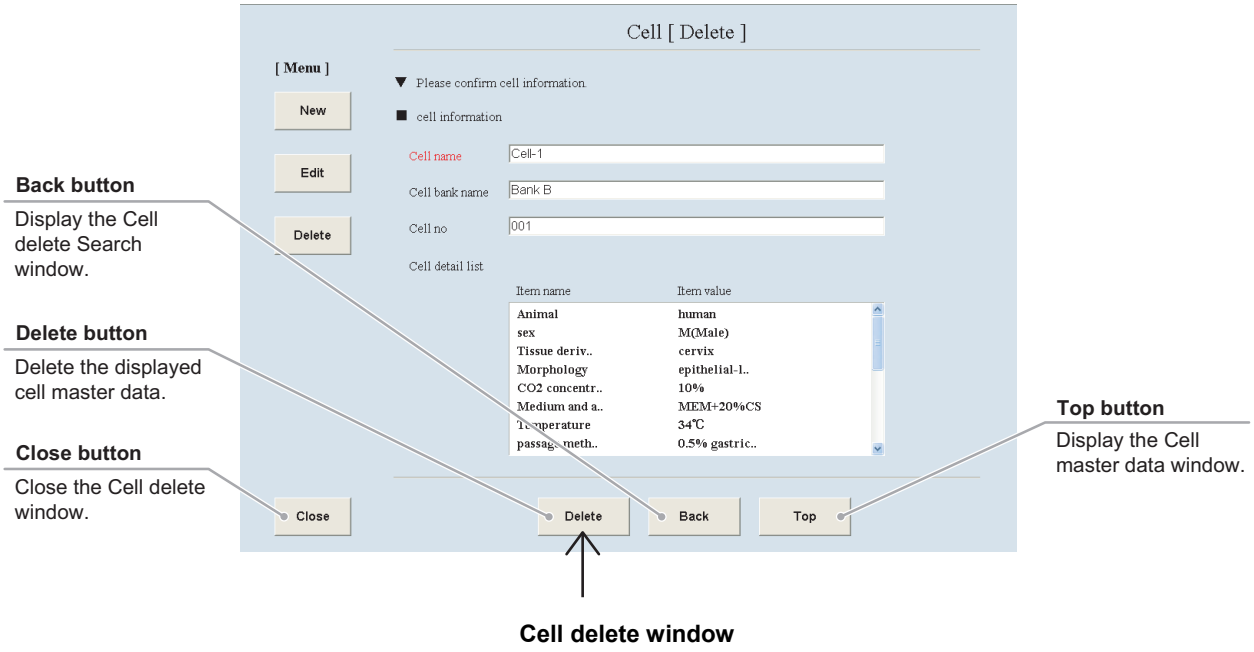
2. **Select the cell name to be deleted from the Search result field.**

   The selected cell master data is displayed in the Cell delete window.



(3) Press the Delete button.

The displayed cell name is deleted from the master data.



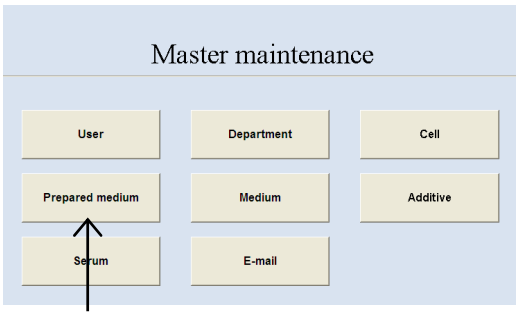
7.3.5 Prepared Medium Master Data

This section describes the procedure for registering, editing or deleting the prepared medium master data. To enter the medium, the additive or the serum in the prepared medium master data, register the medium master data, the additive master data, and the serum master data in advance.

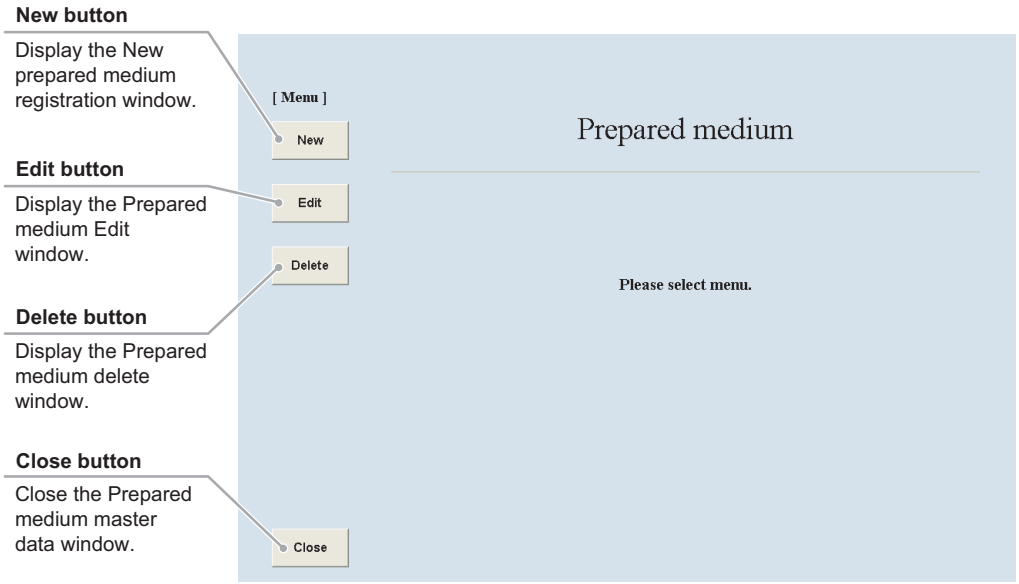
7.3.5.1 Registering new prepared medium master data

(1) Press the Prepared medium button in the Master data maintenance window.

The Prepared medium master data window is displayed.



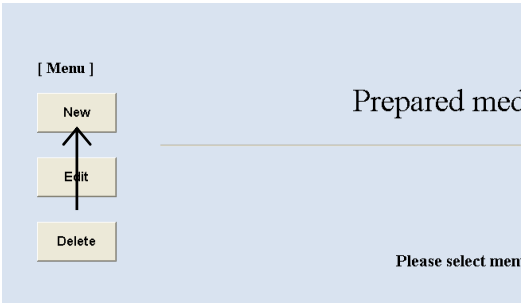
Master data maintenance window



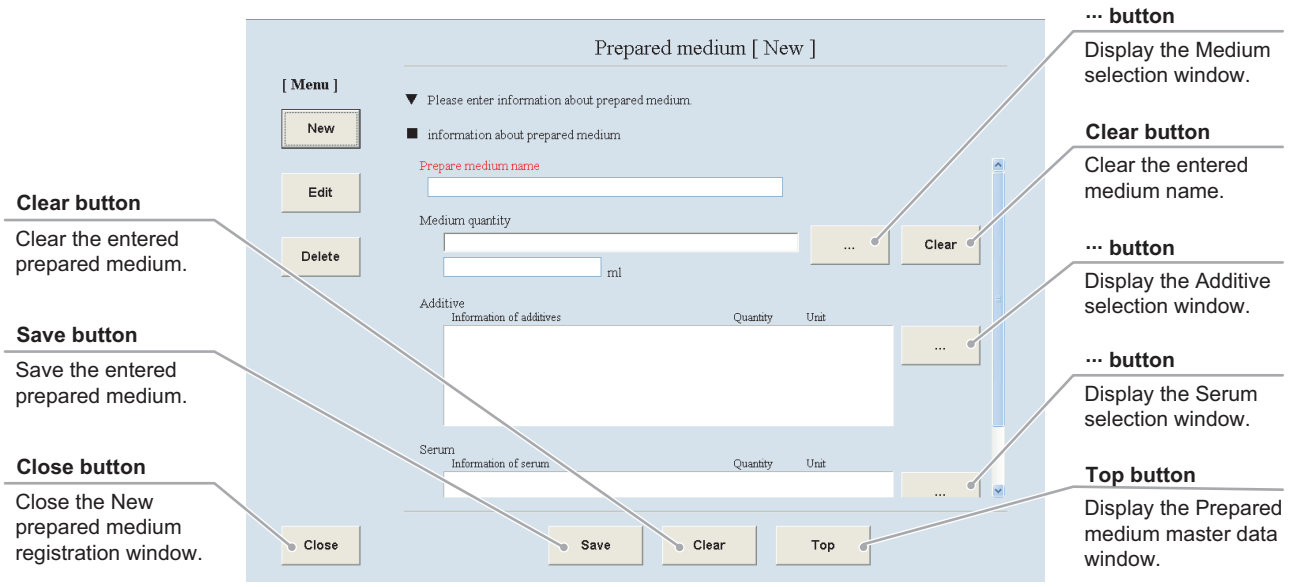
Prepared medium master data window

(2) Press the New button.

The New prepared medium registration window is displayed.

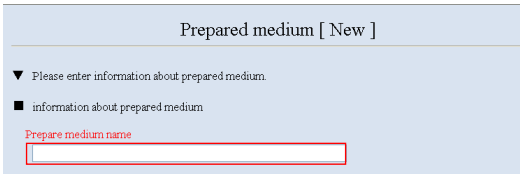


Fill in the items displayed in red. For other items, fill in if necessary.



New prepared medium registration window

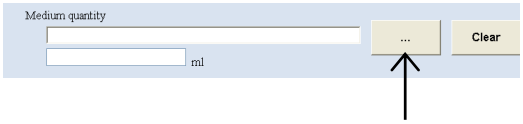
(3) Enter the prepared medium name into the Prepare medium name field.

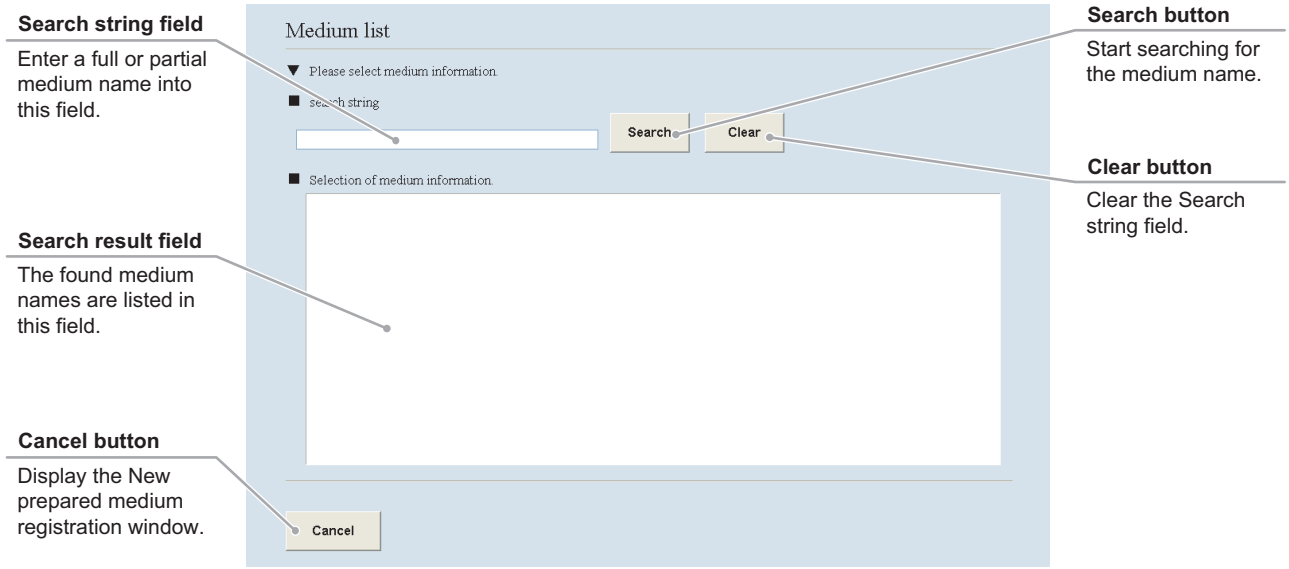


> The prepared medium, whose name has already been registered in the Prepare medium name, cannot be saved in the user master data.

(4) Enter the medium name and the medium quantity.

1. Display the Medium list window by pressing the ··· button on the right side of the Medium quantity field.

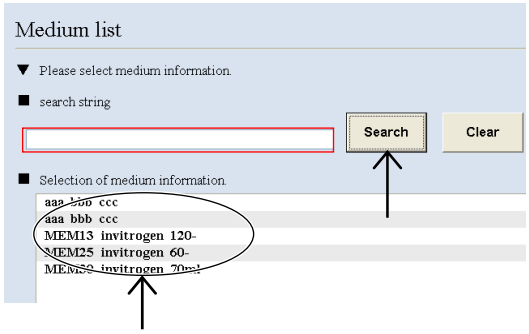




Medium list window

2. **Enter a full or partial medium name into the Search string field and press the Search button.**
   If you do not know any characters of the medium name, press the Search button without data entry.

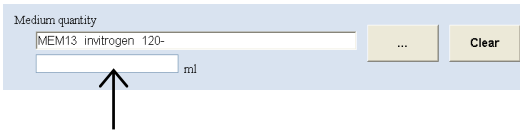
   The found medium names are listed in the Search result field.



3. **Select a medium name from the Search result field.**

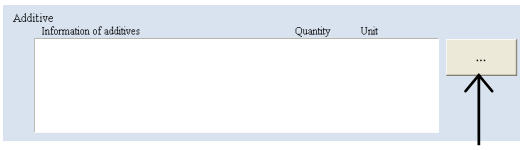
   The selected medium name is displayed in the New prepared medium registration window.

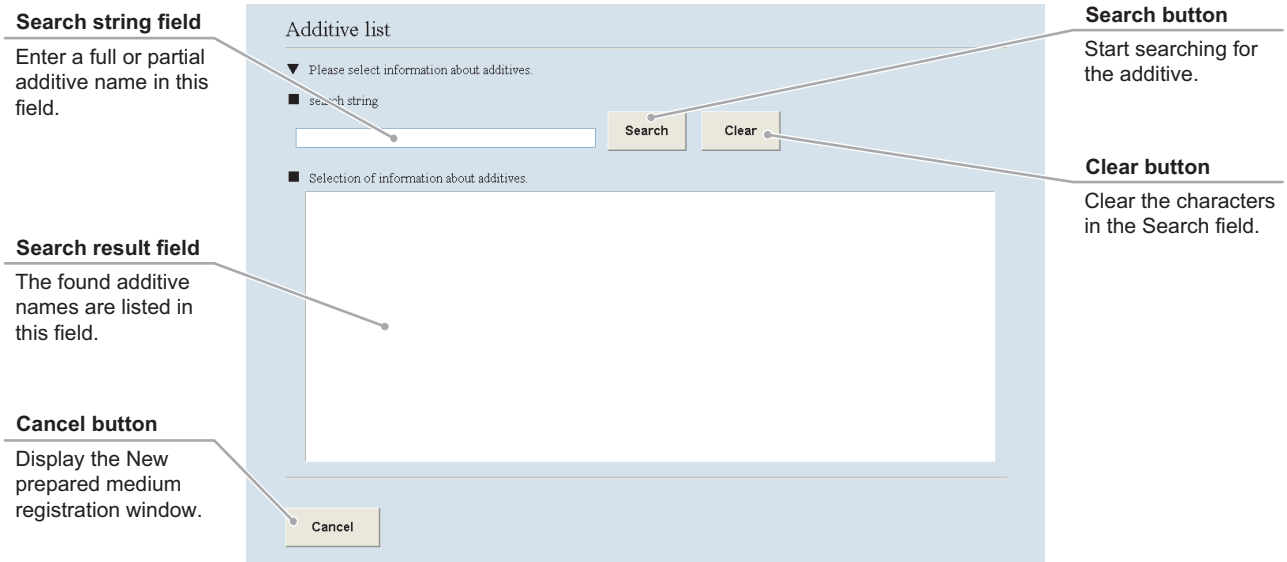
4. **Enter the medium quantity into the Medium quantity field.**



(5) Enter the information of the additive used for the prepared medium.

1. **Display the Additive list window by pressing the ··· button on the right side of the Additive field.**





Additive list window

2. **Enter a full or partial additive name into the Search string field and press the Search button.**
   If you do not know any characters of the additive name, press the Search button without data entry.

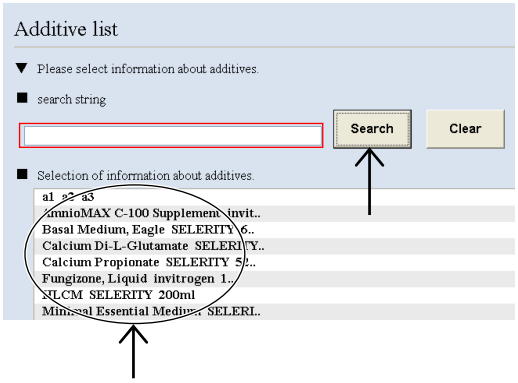
   The found additive names are listed in the Search result field.

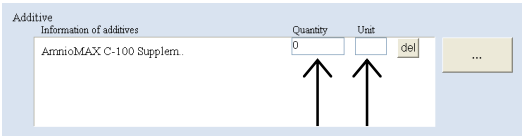
3. **Select an additive name from the Search result field.**

   The selected additive name is displayed in the New prepared medium registration window.

4. **Enter the quantity and the unit of the additive into the Quantity field and the Unit field.**

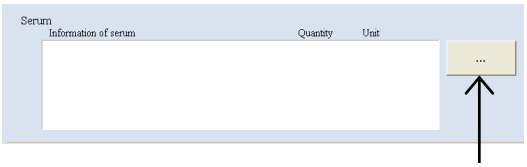
5. **To enter other additive information, repeat steps 1 to 4.**

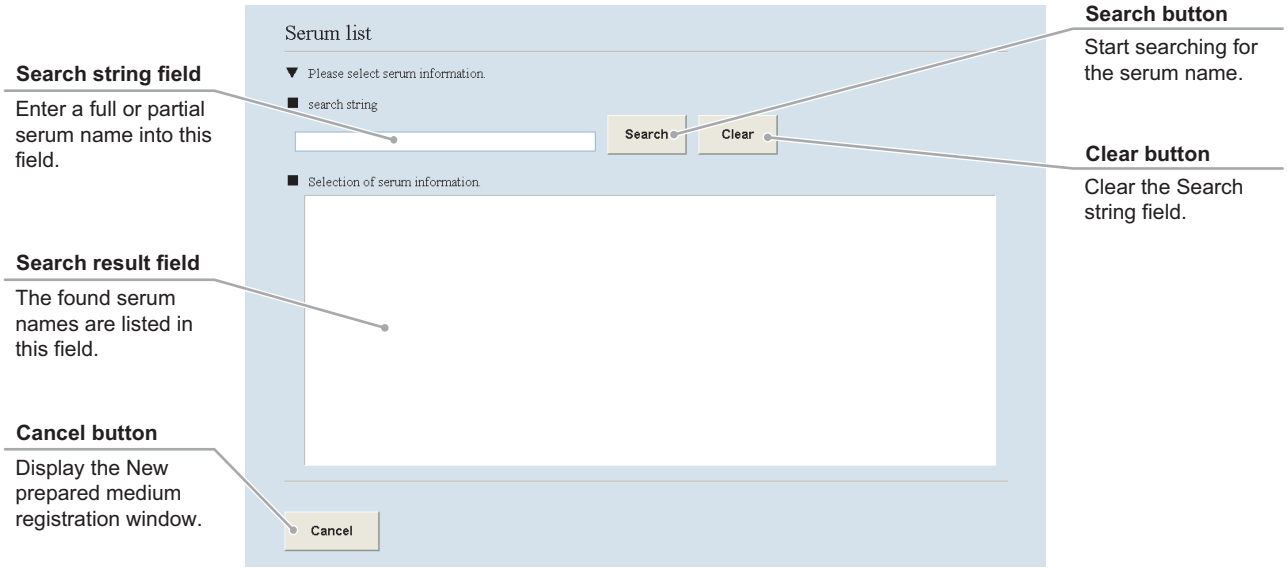




(6) Enter the serum name used for the prepared medium.

1. **Display the Serum list window by pressing the ··· button on the right side of the Serum field.**

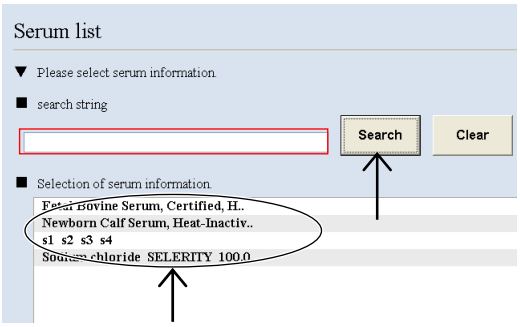




Serum list window

2. **Enter a full or partial serum name into the Search string field and press the Search button.**
   If you do not know any characters of the serum name, press the Search button without data entry.

   The found serum names are listed in the Search result field.

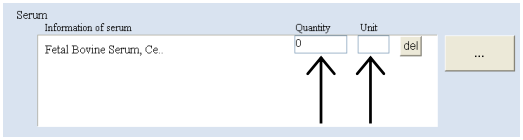


3. **Select a serum name from the Search result field.**

   The selected serum name is displayed in the New prepared medium registration window.

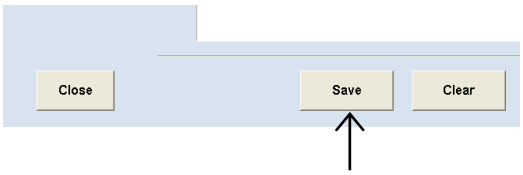
4. **Enter the quantity and the unit of the serum into the Quantity field and the Unit field.**

5. **To enter other serum information, repeat steps 1 to 4.**



(7) Press the Save button.

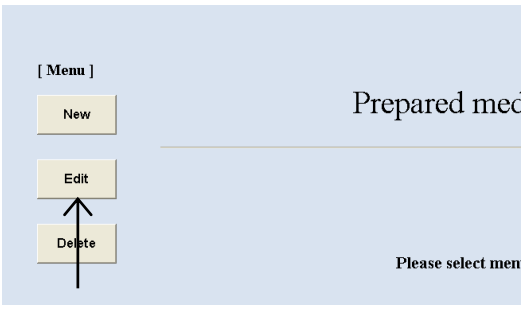
The entered prepared medium information is saved into the master data.



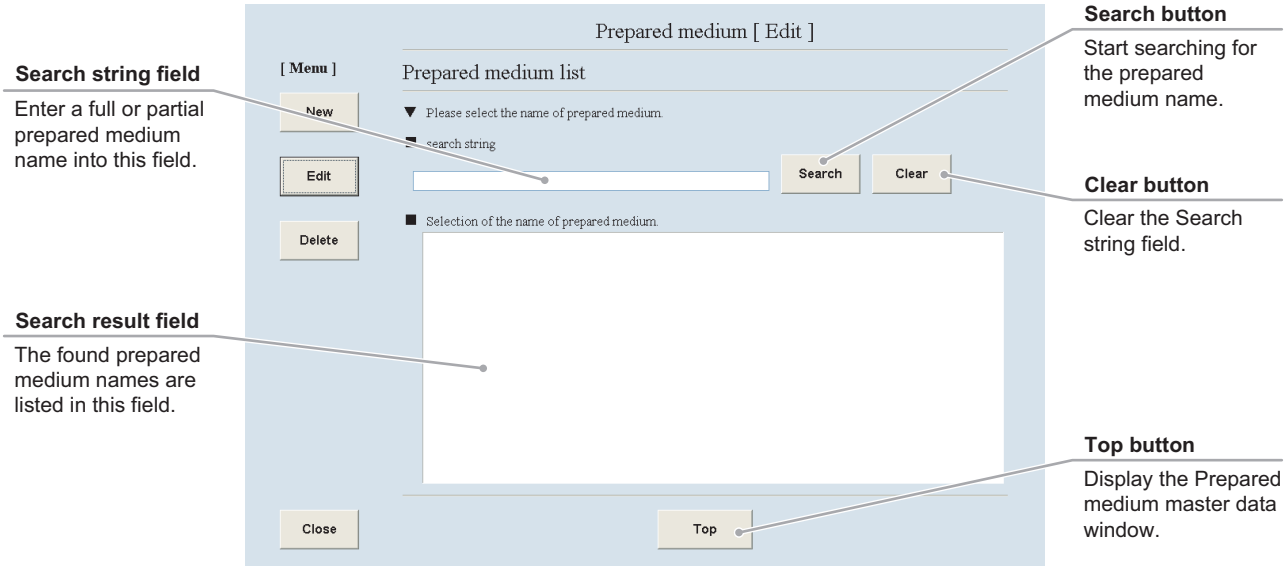
7.3.5.2 Editing the prepared medium master data

(1) Press the Edit button in the Prepared medium master data window.

The Prepared medium edit search window is displayed.



Prepared medium master data window



Prepared medium edit search window

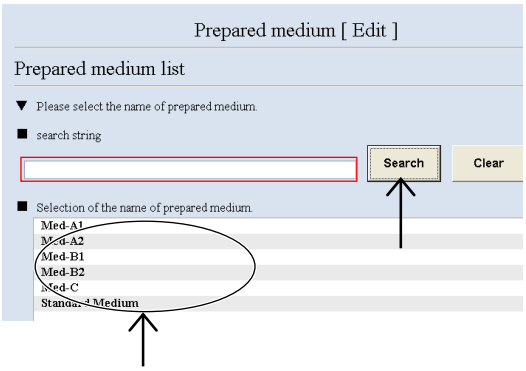
(2) Search for the prepared medium master data to be edited.

1. Enter a full or partial prepared medium name into the Search string field and press the Search button.
If you do not know any characters of the prepared medium name, press the Search button without data entry.

The found prepared medium names are listed in the Search result field.

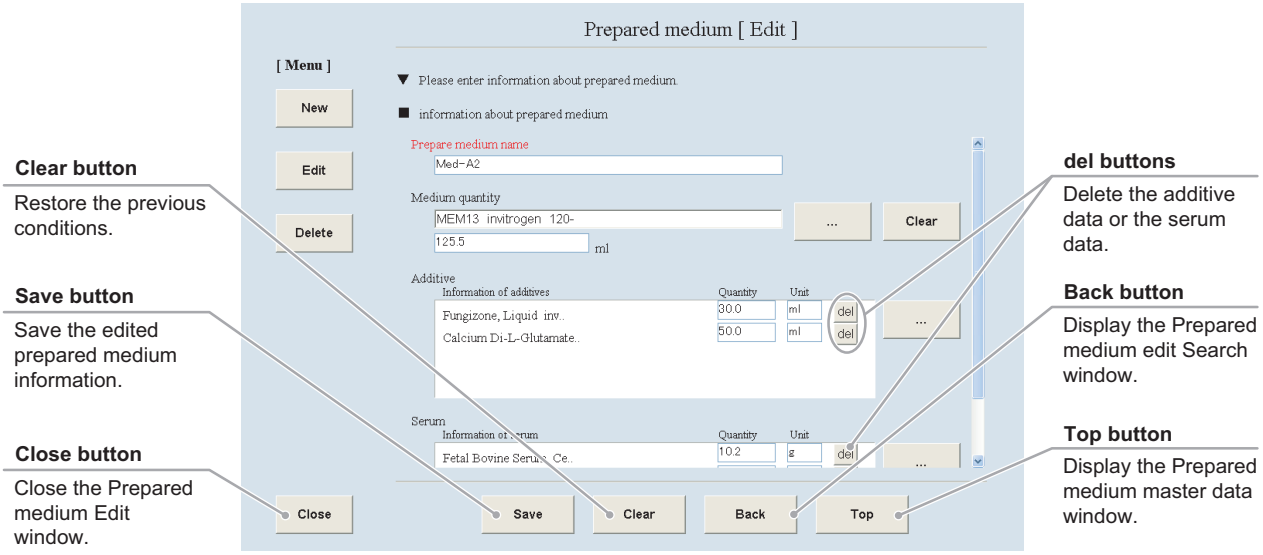
2. Select a prepared medium to edit from the Search result field.

The selected prepared medium master data is displayed in the Prepared medium edit window.



(3) Edit the prepared medium master data.

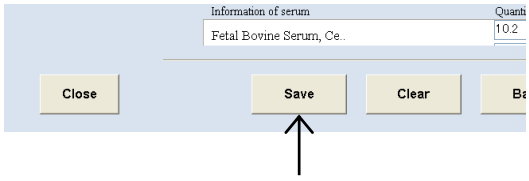
The procedure for editing the prepared medium master data is the same as the procedure for registering the new prepared medium master data. For the procedure for editing each item, see Section 7.3.5.1, “Registering new prepared medium master data.”



Prepared medium edit window

(4) Press the Save button.

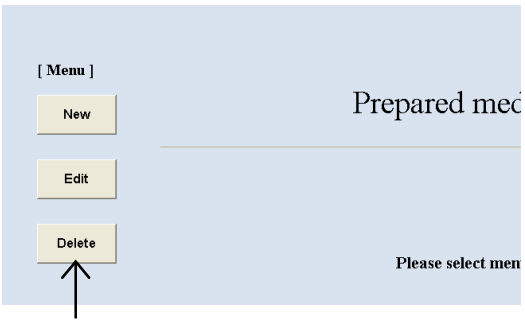
The edited prepared medium information is saved into the master data.



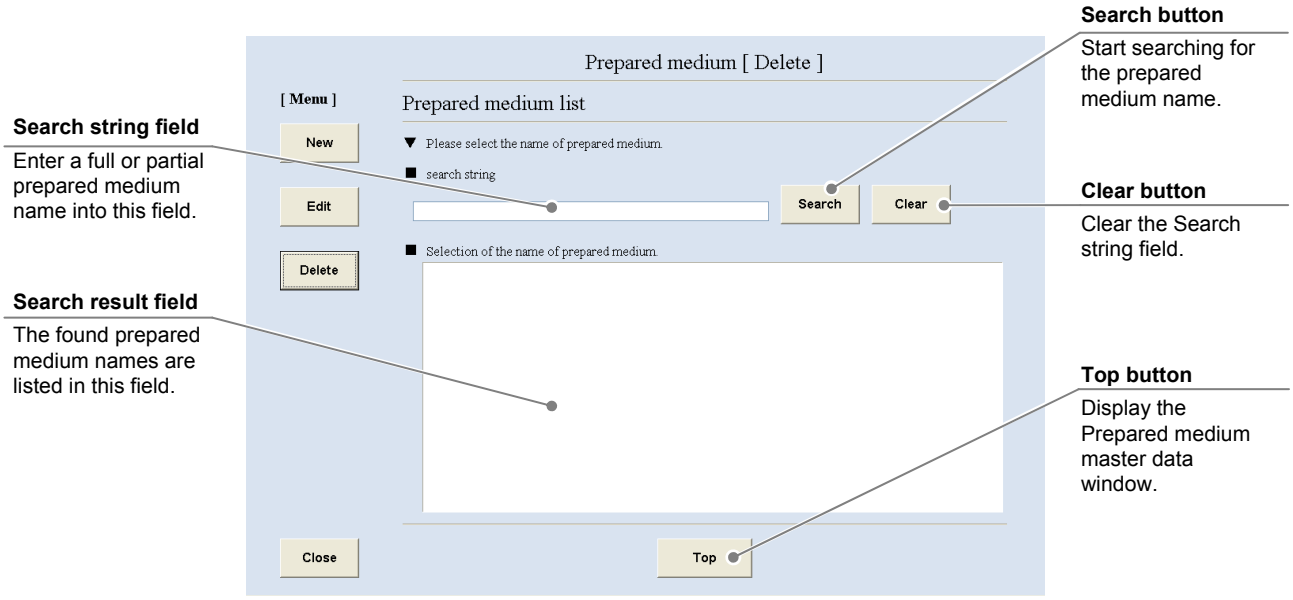
7.3.5.3 Deleting the prepared medium master data

(1) Press the Delete button in the Prepared medium master data window.

The Prepared medium delete search window is displayed.



Prepared medium master data window



Prepared medium delete search window

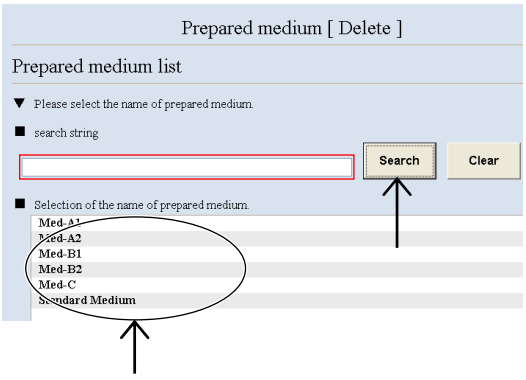
(2) Search for the prepared medium master data to be deleted.

1. Enter a full or partial prepared medium name into the Search string field and press the Search button.
If you do not know any characters of the prepared medium name, press the Search button without data entry.

The found prepared medium names are listed in the Search result field.

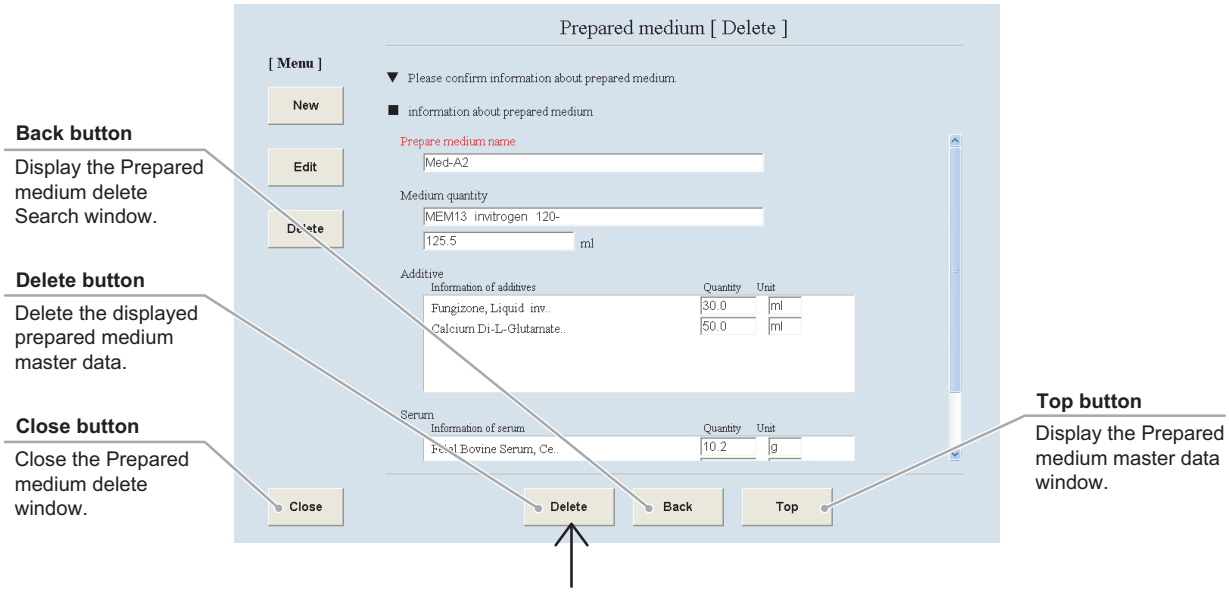
2. Select a prepared medium name to be deleted from the Search result field.

The selected prepared medium master data is displayed in the Prepared medium delete window.



(3) Press the Delete button.

The displayed prepared medium information is deleted from the master data.



Prepared medium delete window

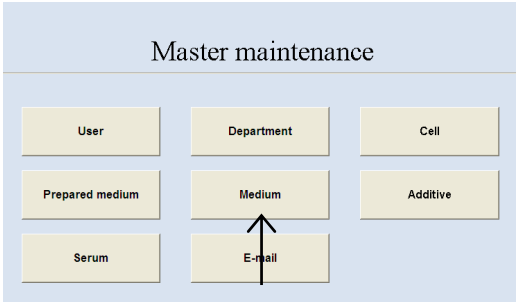
7.3.6 Medium Master Data

This section describes the procedure for registering, editing or deleting the medium master data.

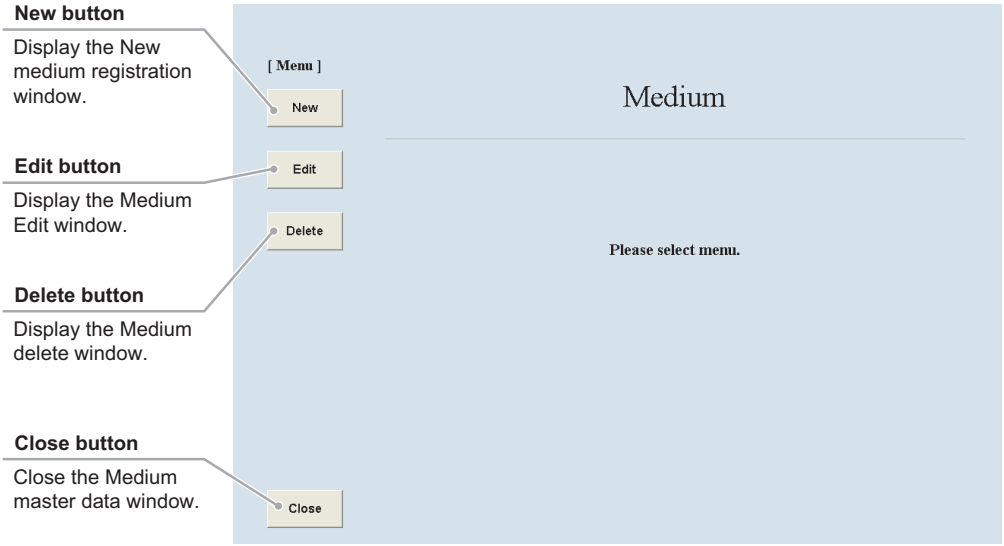
7.3.6.1 Registering new medium master data

(1) Press the Medium button in the Master data maintenance window.

The Medium master data window is displayed.



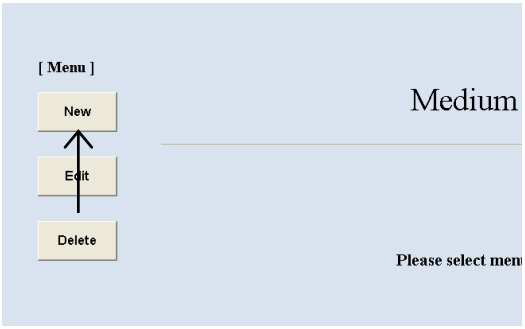
Master data maintenance window



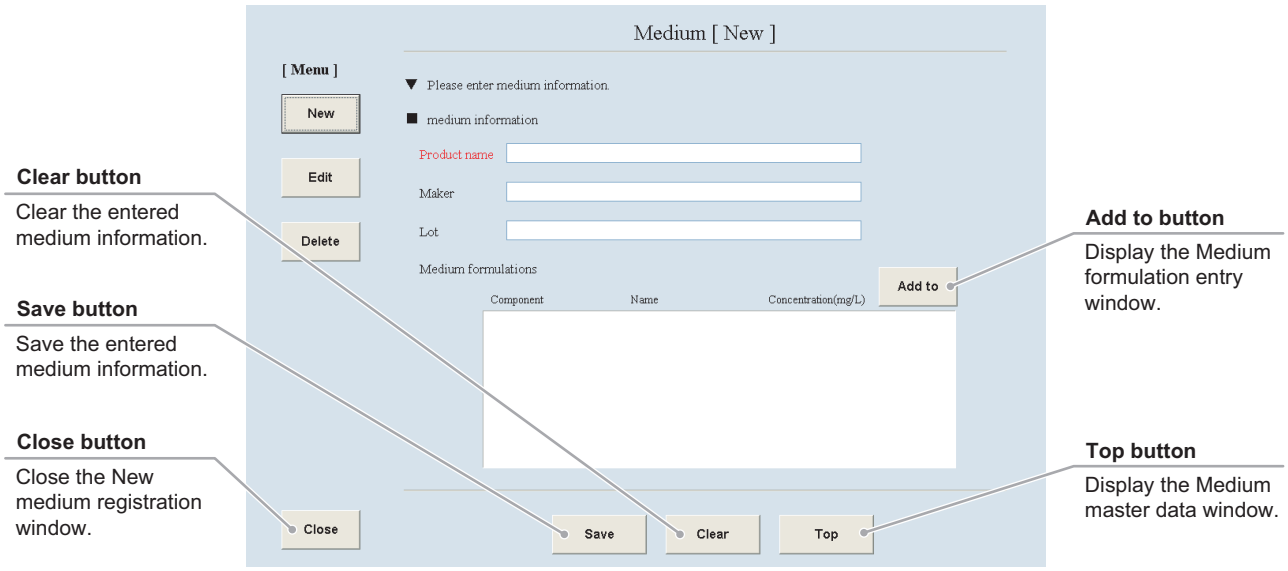
Medium master data window

(2) Press the New button.

The New medium registration window is displayed.



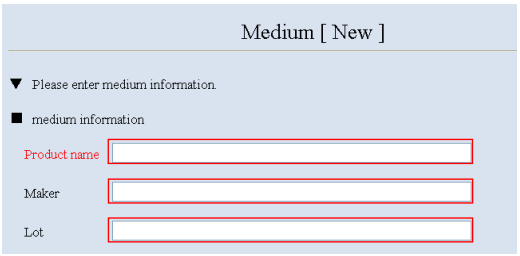
Fill in the items displayed in red. For other items, fill in if necessary.



New medium registration window

(3) Enter the medium name into the Product name field.

> When the Product name is already registered, it cannot be saved in the user master data.

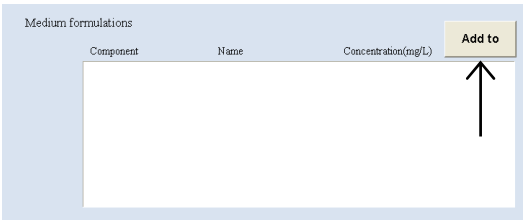


(4) Enter the supplier name of the medium into the Maker field.

(5) Enter the lot number into the Lot field.

(6) Enter the medium formulation information.

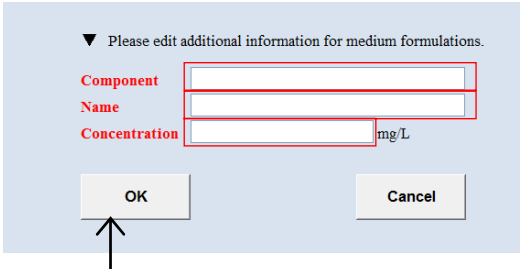
1. Press the Add to button to display the Medium formulation entry window.



2. Enter the medium formulation information into the Component, the Name, and the Concentration field.

3. Press the OK button.

The entered medium formulation information is saved and the New medium registration window is displayed.



Medium formulation entry window

4. To enter other medium formulation information, repeat steps 1 to 3.

(7) Press the Save button in the New medium registration window.

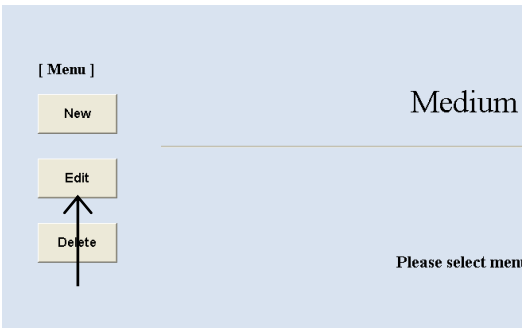
The entered medium information is saved into the master data.

New medium registration window

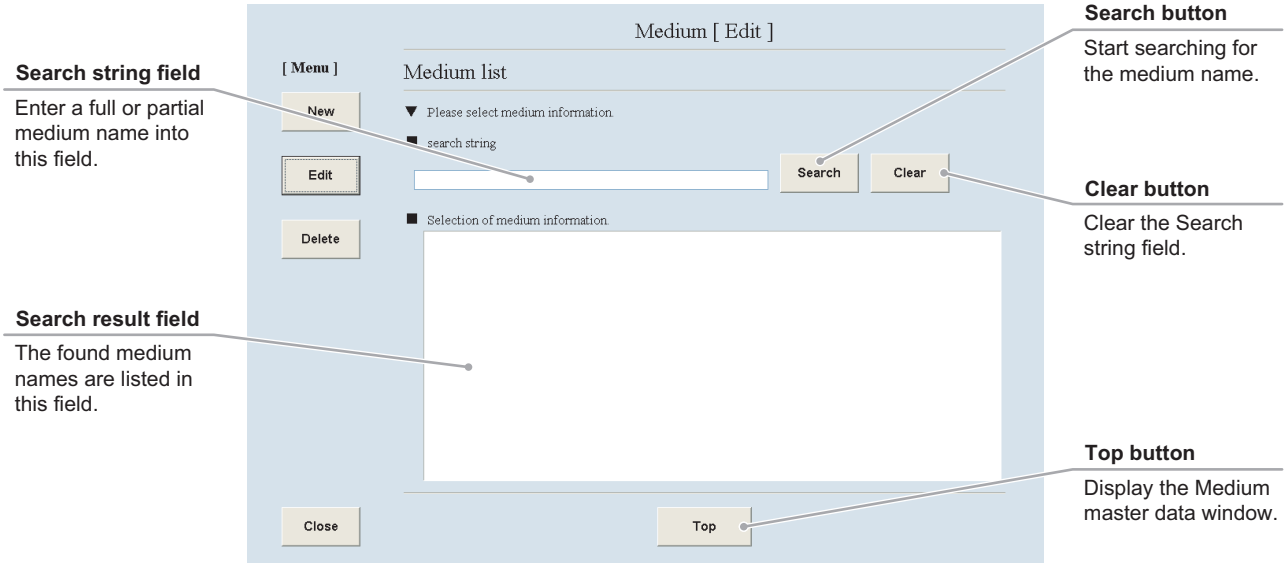
7.3.6.2 Editing the medium master data

(1) Press the Edit button in the medium master data window.

The Medium edit search window is displayed.



Medium master data window



Medium edit search window

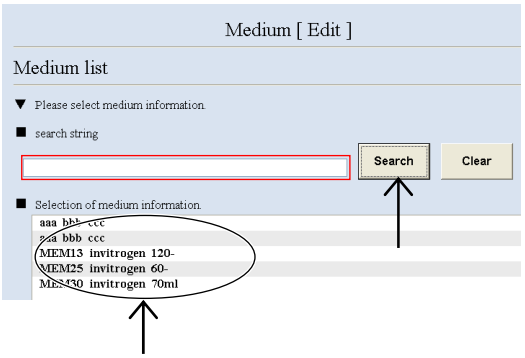
(2) Search for the medium master data to be edited.

1. Enter a full or partial medium name into the Search string field and press the Search button.
If you do not know any characters of the medium name, press the Search button without data entry.

The found medium names are listed in the Search result field.

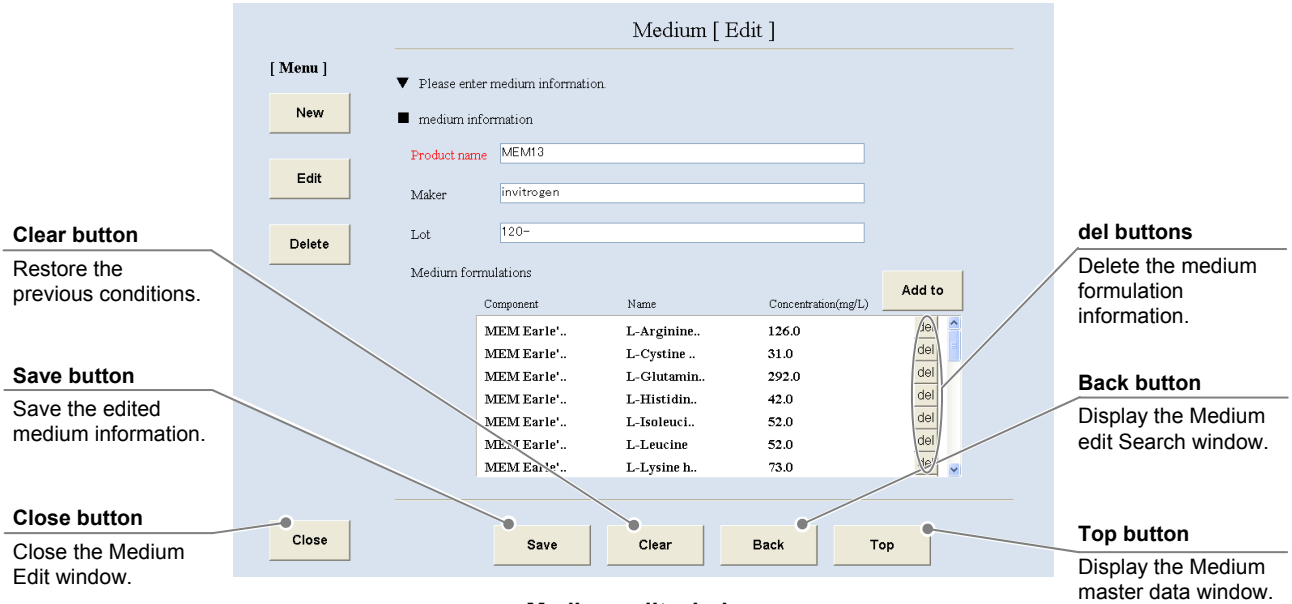
2. Select a medium name from the Search result field.

The selected medium master data is displayed in the Medium edit window.



(3) Edit the medium master data.

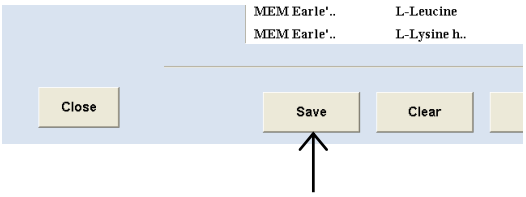
The procedure for editing the medium master data is the same as the procedure for registering the new medium master data. For the procedure for editing each item, see Section 7.3.6.1, “Registering new medium master data.”



Medium edit window

(4) Press the Save button.

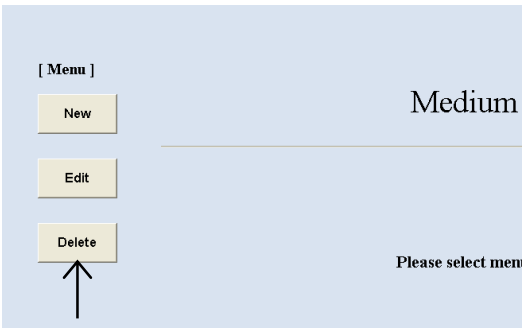
The edited medium information is saved into the master data.



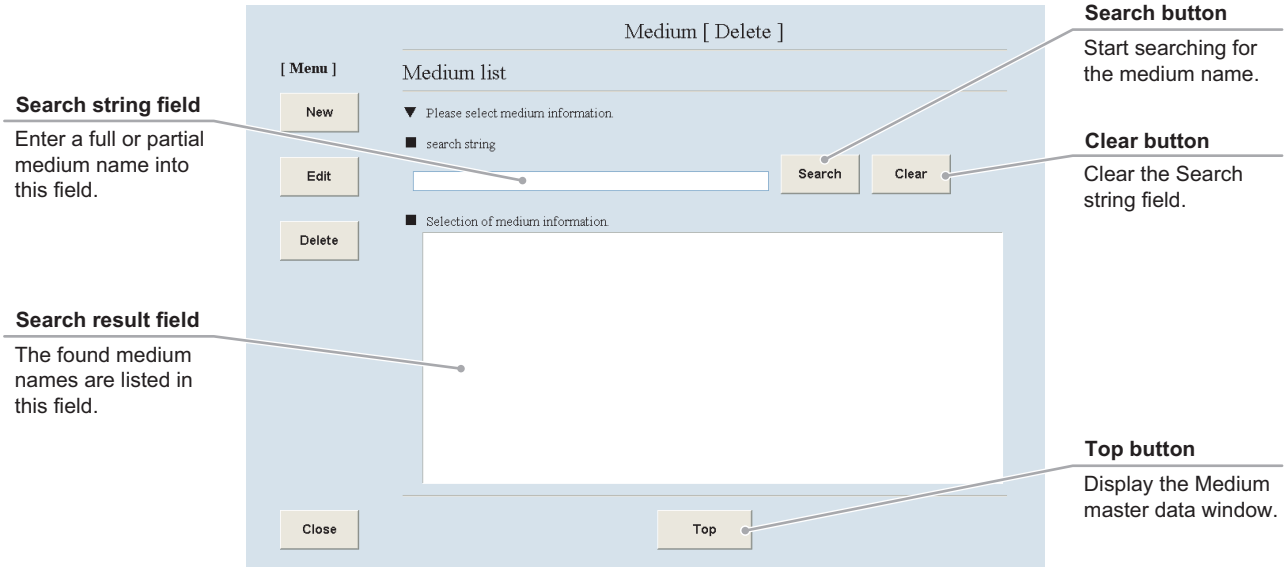
7.3.6.3 Deleting the medium master data

(1) Press the Delete button in the Medium master data window.

The Medium delete search window is displayed.



Medium master data window



Medium delete search window

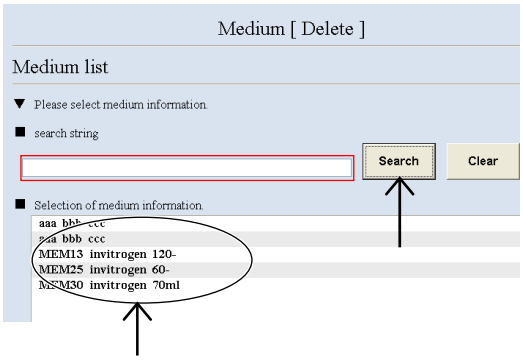
(2) Search for the medium master data to be deleted.

1. Enter a full or partial medium name into the Search string field and press the Search button.
If you do not know any characters of the medium name, press the Search button without data entry.

The found medium names are listed in the Search result field.

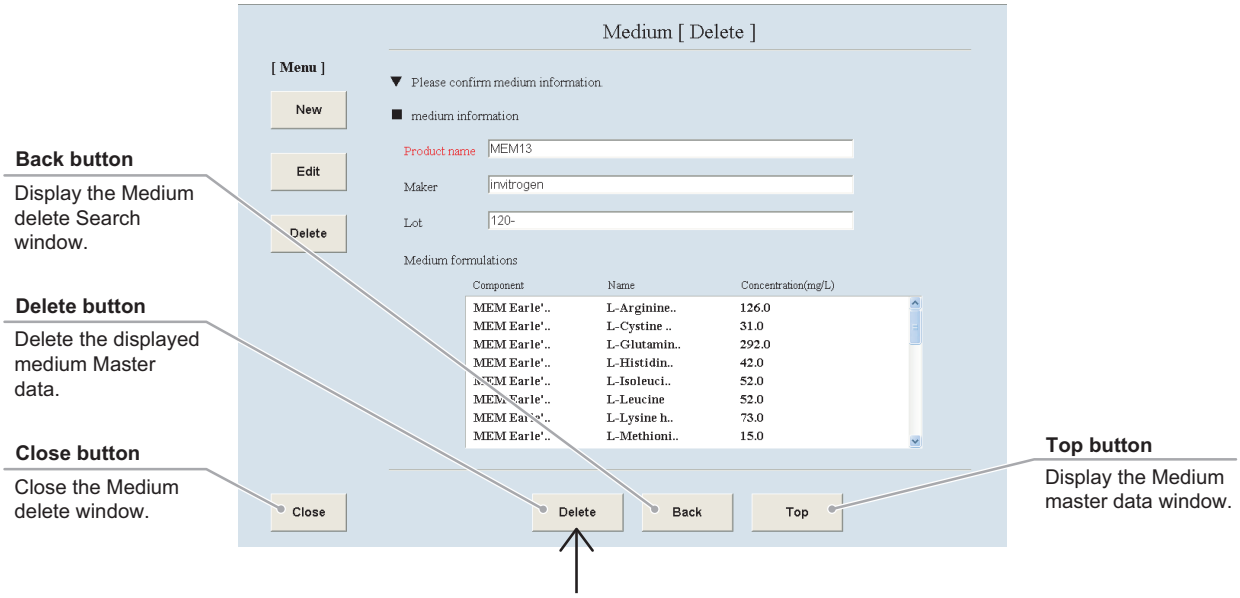
2. Select a medium name to be deleted from the Search result field.

The selected medium master data is displayed in the Medium delete window.



(3) Press the Delete button.

The displayed medium name is deleted from the master data.



Back button

Display the Medium delete Search window.

Delete button

Delete the displayed medium Master data.

Close button

Close the Medium delete window.

Top button

Display the Medium master data window.

Medium delete window

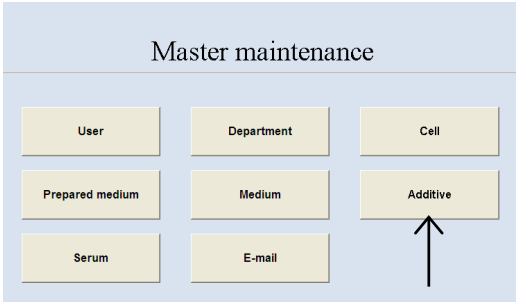
7.3.7 Additive Master Data

This section describes the procedure for registering, editing or deleting the additive master data.

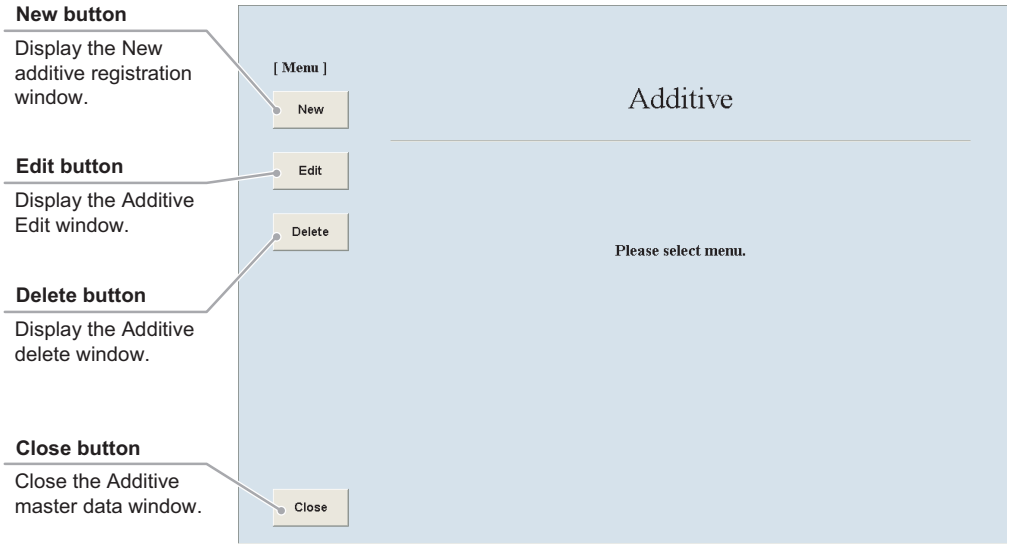
7.3.7.1 Registering new additive master data

(1) Press the Additive button in the Master data maintenance window.

The Additive master data window is displayed.



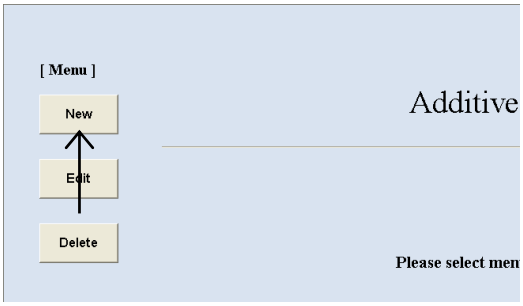
Master data maintenance window



Additive master data window

(2) Press the New button.

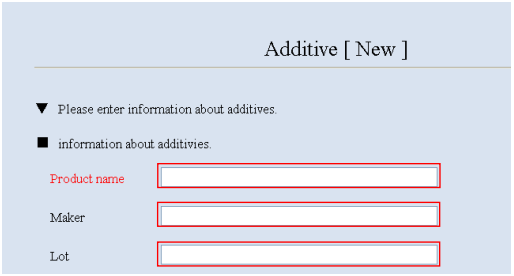
The New additive registration window is displayed.



Fill in the items displayed in red. For other items, fill in if necessary.

New additive registration window

(3) Enter the additive name into the Product name field.



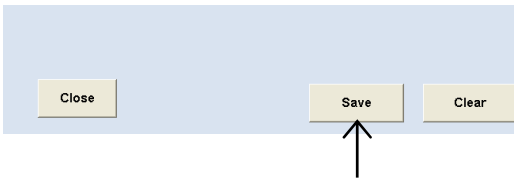
> When the Product name is already registered, it cannot be saved in the additive master data.

(4) Enter the supplier name of the medium into the Maker field.

(5) Enter the lot number into the Lot field.

(6) Press the Save button.

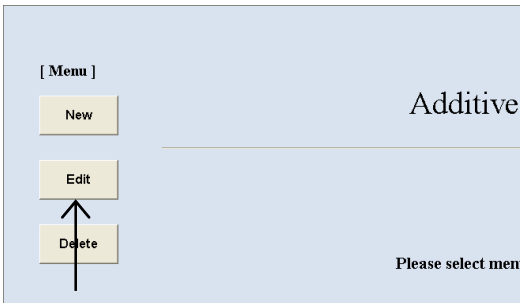
The entered additive information is saved into the master data.



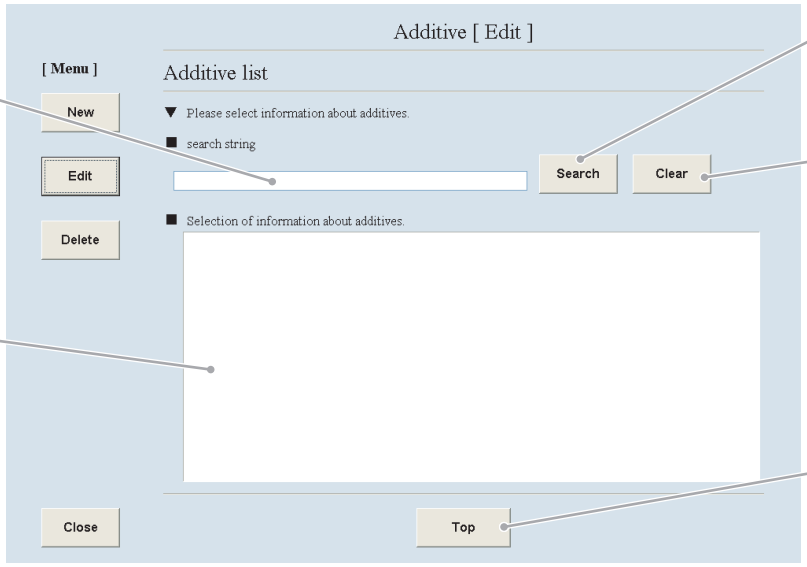
7.3.7.2 Editing the additive master data

(1) Press the Edit button in the Additive master data window.

The Additive edit search window is displayed.



Additive master data window



Search string field
Enter a full or partial additive name into this field.

Search result field
The found additive names are listed in this field.

Search button
Start searching for the additive.

Clear button
Clear the Search string field.

Top button
Display the Additive master data window.

Additive edit search window

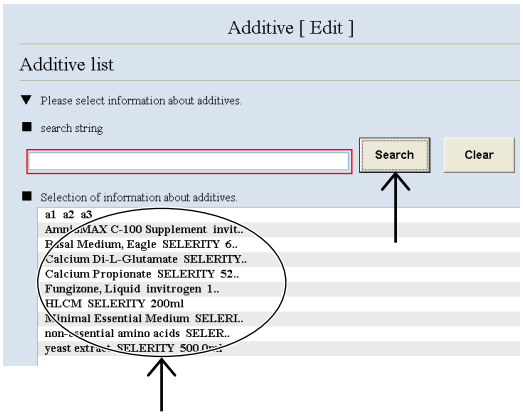
(2) Search for the additive master data to be edited.

1. Enter a full or partial additive name into the Search string field and press the Search button.
If you do not know any characters of the additive name, press the Search button without data entry.

The found additive names are listed in the Search result field.

2. Select an additive name from the Search result field.

The selected additive master data is displayed in the Additive edit window.



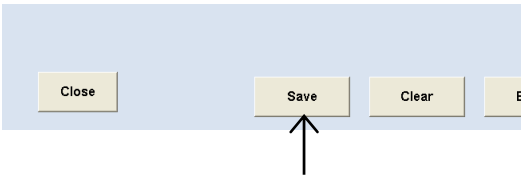
(3) Edit the additive master data.

The procedure for editing the additive master data is the same as the procedure for registering the new additive master data. For the procedure for editing each item, see Section 7.3.7.1, “Registering new additive master data.”

Additive edit window

(4) Press the Save button.

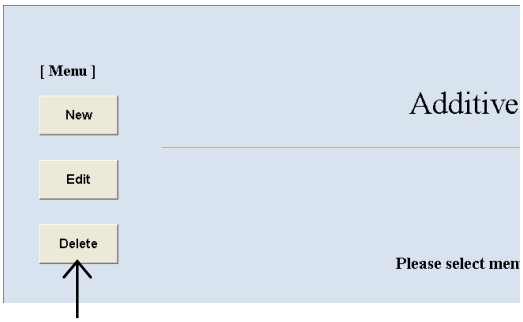
The edited additive information is saved into the master data.



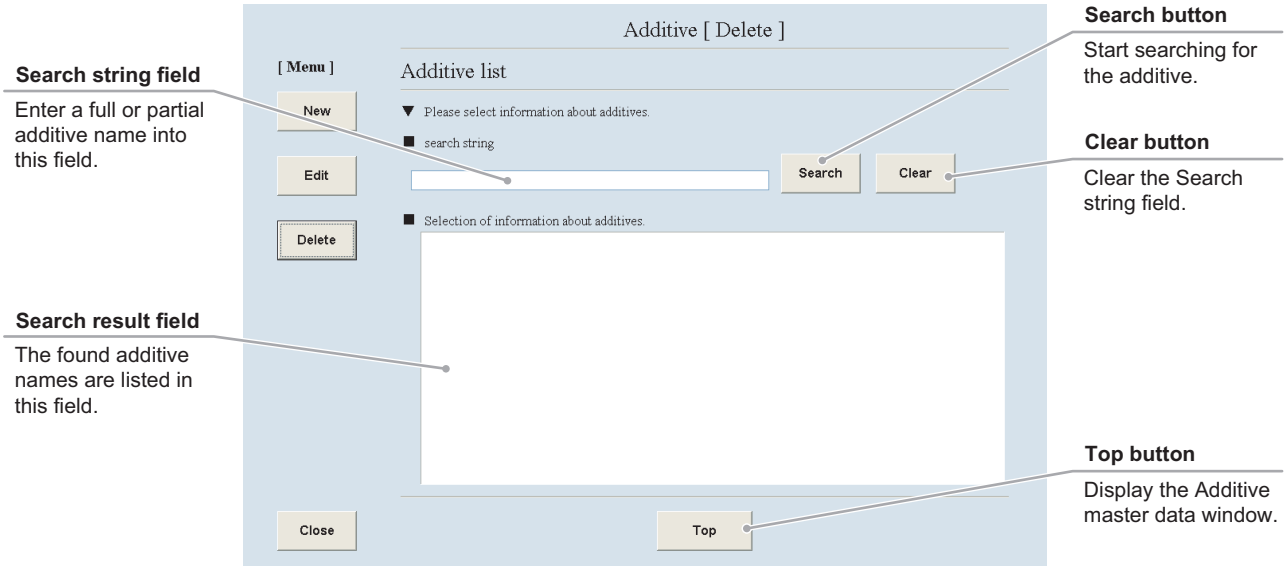
7.3.7.3 Deleting the additive master data

(1) Press the Delete button in the Additive master data window.

The Additive delete search window is displayed.



Additive master data window



Additive delete search window

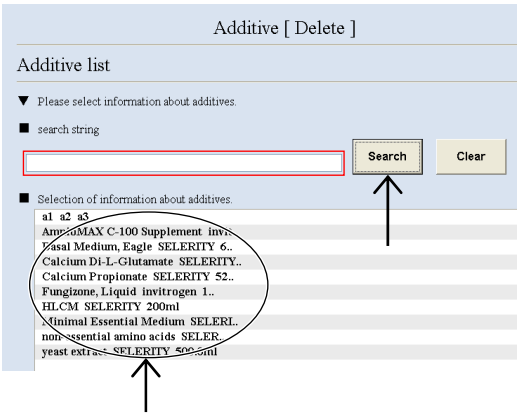
(2) Search for the additive master data to be deleted.

1. Enter a full or partial additive medium name into the Search string field and press the Search button.
If you do not know any characters of the additive name, press the Search button without data entry.

The found additive names are listed in the search result field.

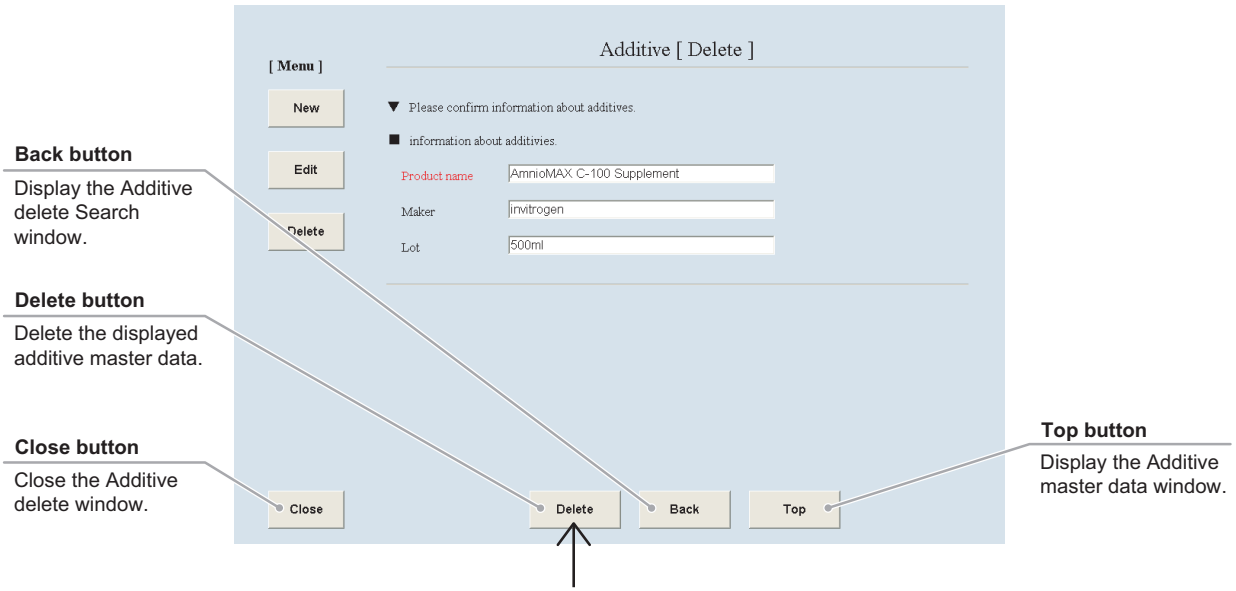
2. Select an additive name to be deleted from the search result field.

The selected additive master data is displayed in the Additive delete window.



(3) Press the Delete button.

The displayed additive name is deleted from the master data.



Additive delete window

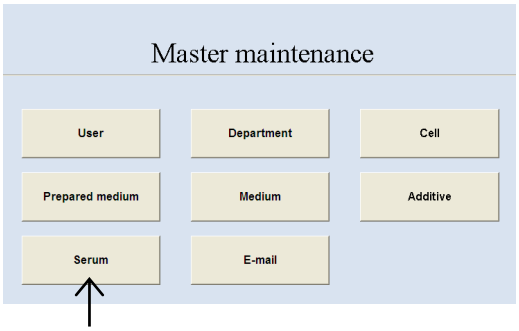
7.3.8 Serum Master Data

This section describes the procedure for registering, editing or deleting the serum master data.

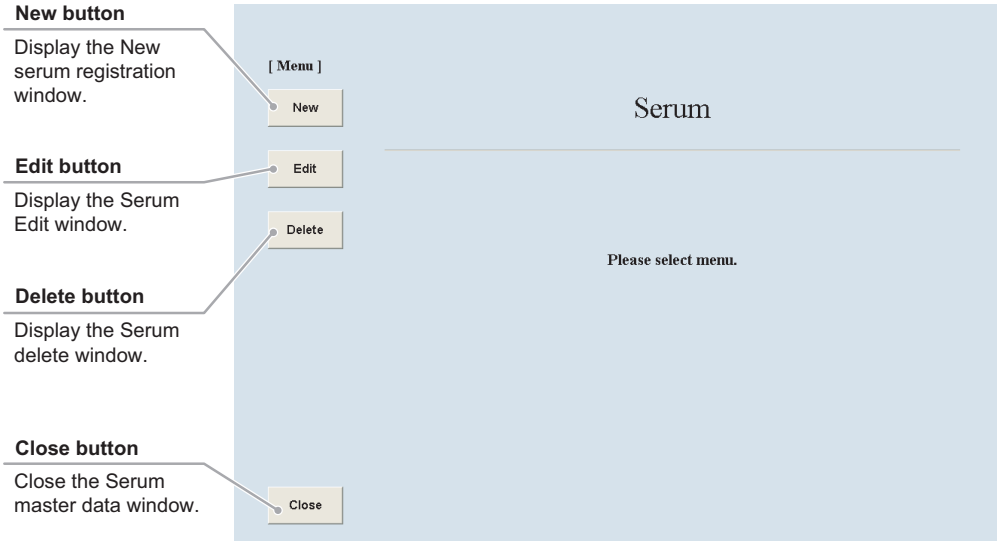
7.3.8.1 Registering new serum master data

(1) Press the Serum button in the Master data maintenance window.

The Serum master data window is displayed.



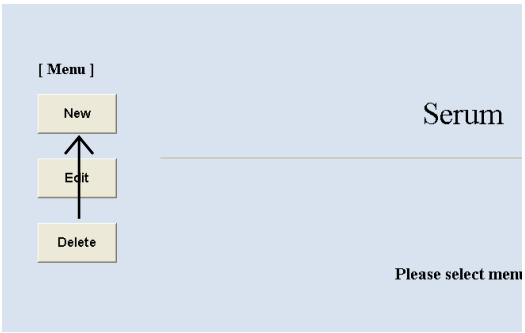
Master data maintenance window



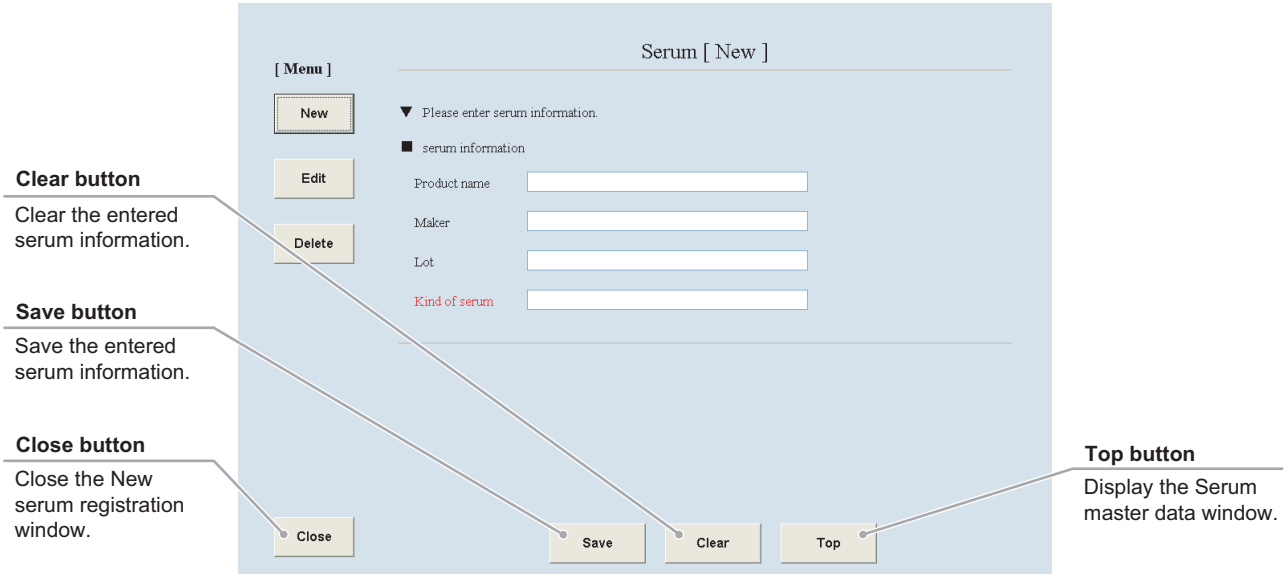
Serum master data window

(2) Press the New button.

The New serum registration window is displayed.



Fill in the items displayed in red. For other items, fill in if necessary.



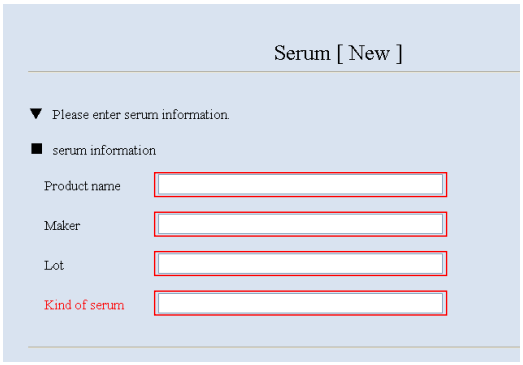
New serum registration window

(3) Enter the serum name into the Product name field.

(4) Enter the supplier name of the serum into the Maker field.

(5) Enter the lot number into the Lot field.

(6) Enter the kind of the serum into the Kind of serum field.



> When the Kind of serum is already registered, it cannot be saved in the user master data.

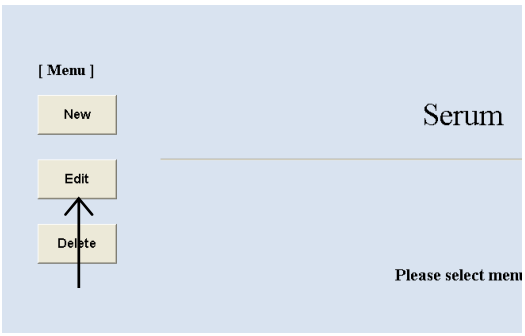
(7) Press the Save button.

The entered serum information is saved into the master data.

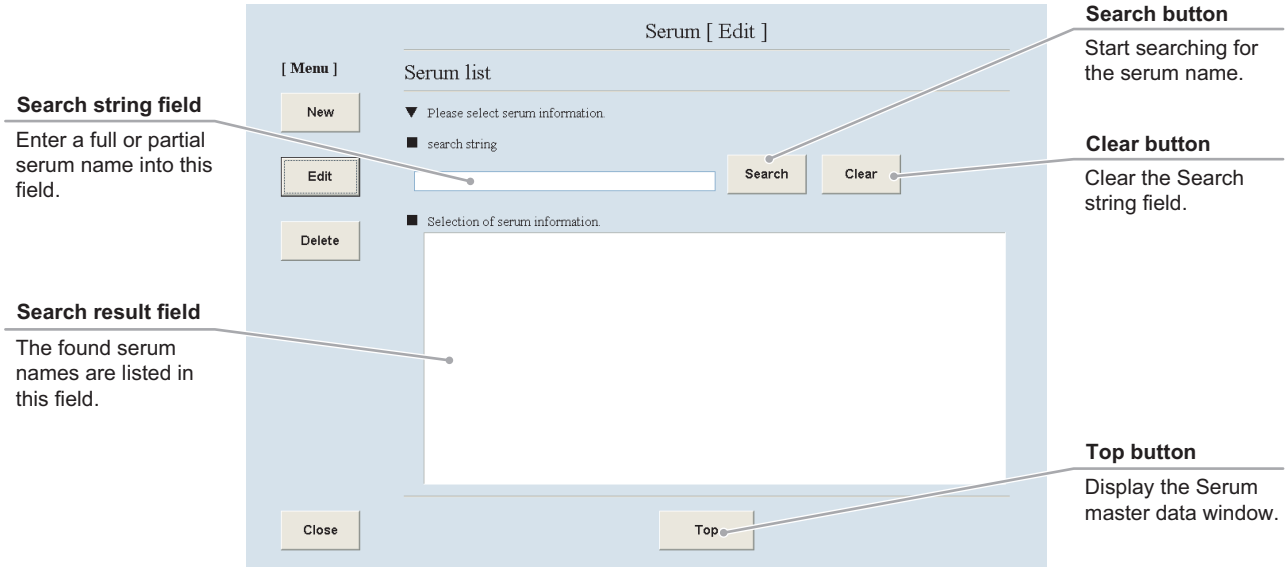
7.3.8.2 Editing the serum master data

(1) Press the Edit button in the Serum master data window.

The Serum edit search window is displayed.



Serum master data window



Serum edit search window

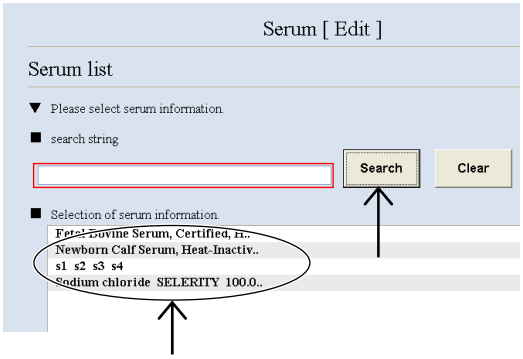
(2) Search for the serum master data to be edited.

1. Enter a full or partial serum name into the Search string field and press the Search button.
If you do not know any characters of the serum name, press the Search button without data entry.

The found serum names are listed in the Search result field.

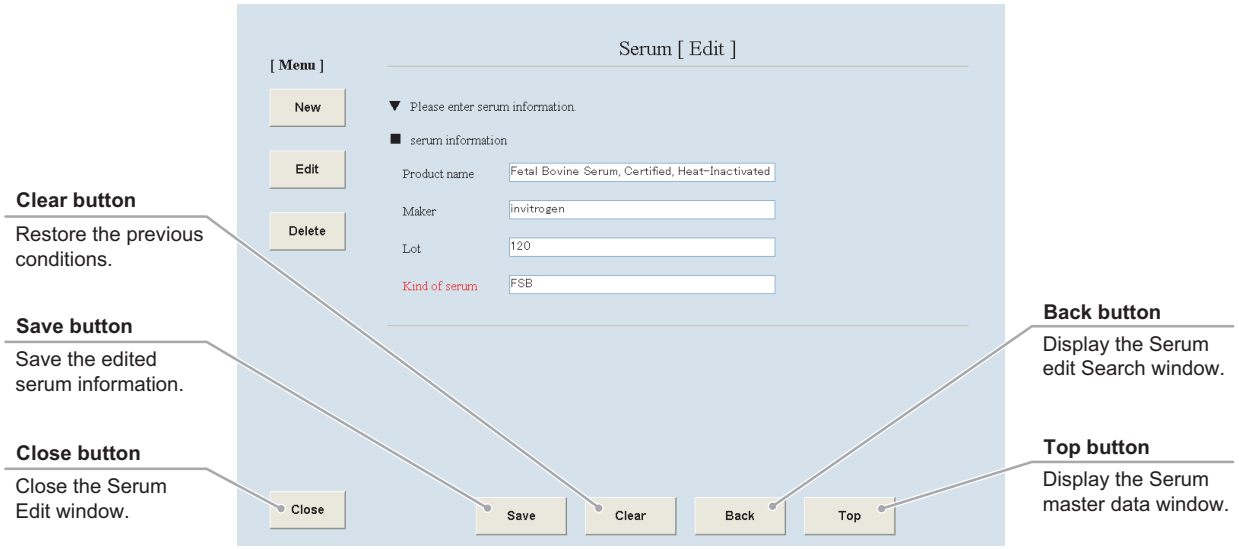
2. Select a serum name from the Search result field.

The selected serum master data is displayed in the Serum edit window.



(3) Edit the serum master data.

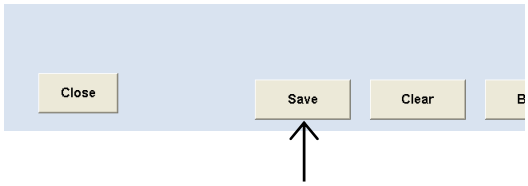
The procedure for editing the serum master data is the same as the procedure for registering the new serum master data. For the procedure for editing each item, see 7.3.8.1 “Registering new serum master data.”



Serum edit window

(4) Press the Save button.

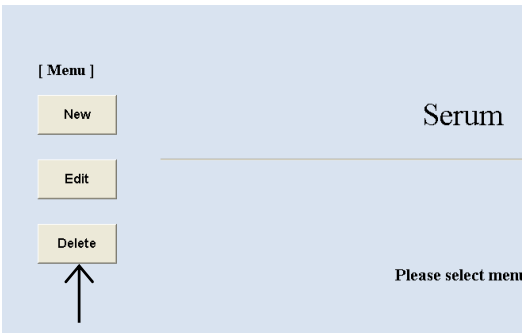
The edited serum information is saved into the master data.



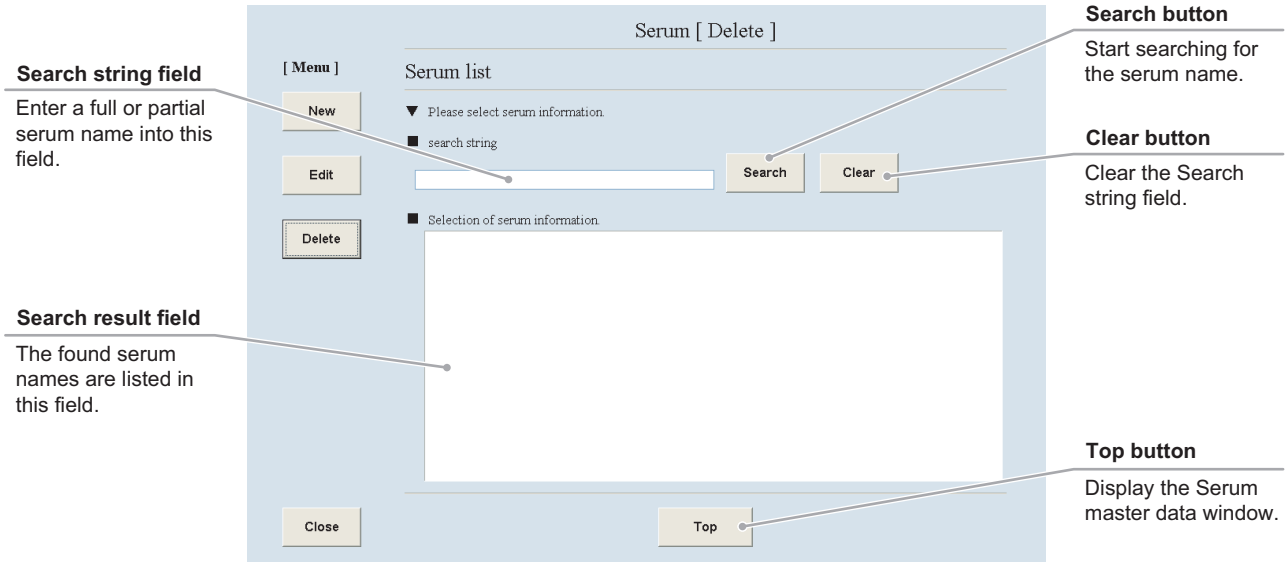
7.3.8.3 Deleting the serum master data

(1) Press the Delete button in the serum master data window.

The Serum delete search window is displayed.



Serum master data window



Serum delete search window

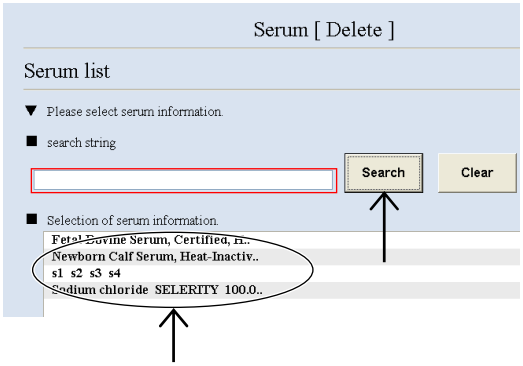
(2) Search for the serum master data to be deleted.

1. Enter a full or partial serum name into the Search string field and press the Search button.
If you do not know any characters of the serum name, press the Search button without data entry.

The found serum names are listed in the Search result field.

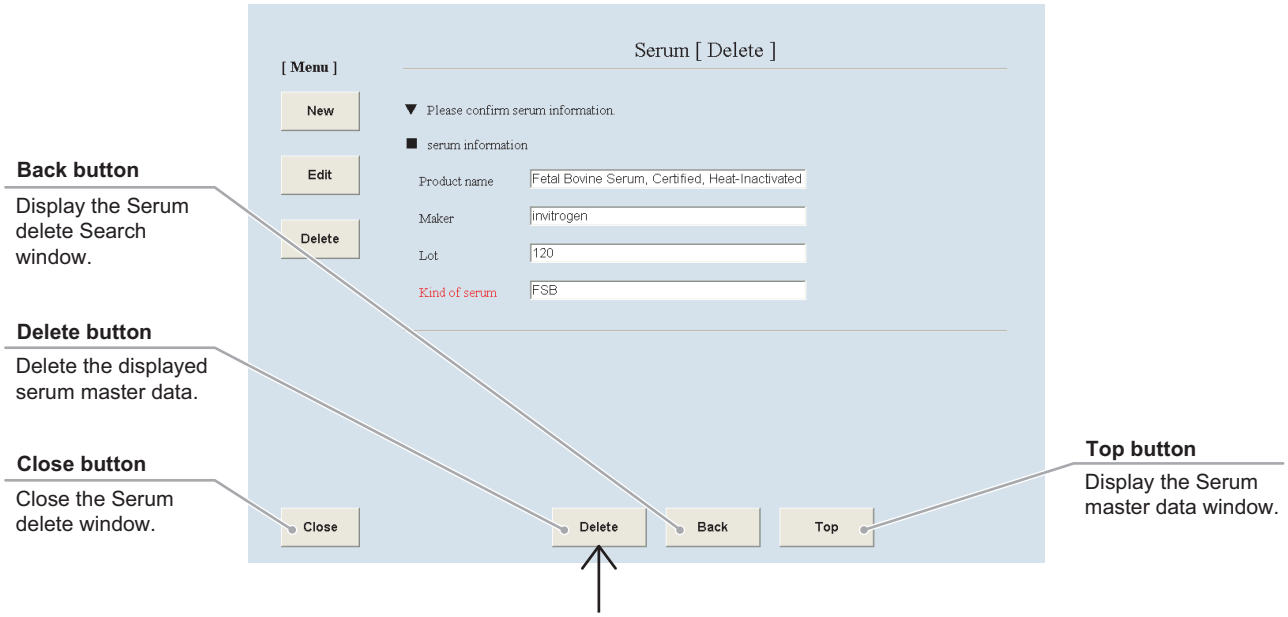
2. Select a serum name to be deleted from the Search result field.

The selected serum master data is displayed in the Serum delete window.



(3) Press the Delete button.

The displayed serum name is deleted from the master data.



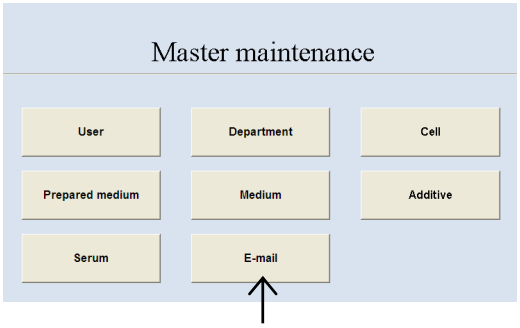
Serum delete window

7.3.9 E-mail Notification Setting

This section describes the procedure for configuring the e-mail notification function, which provides notification of the occurrence of problems on the equipment via e-mail.

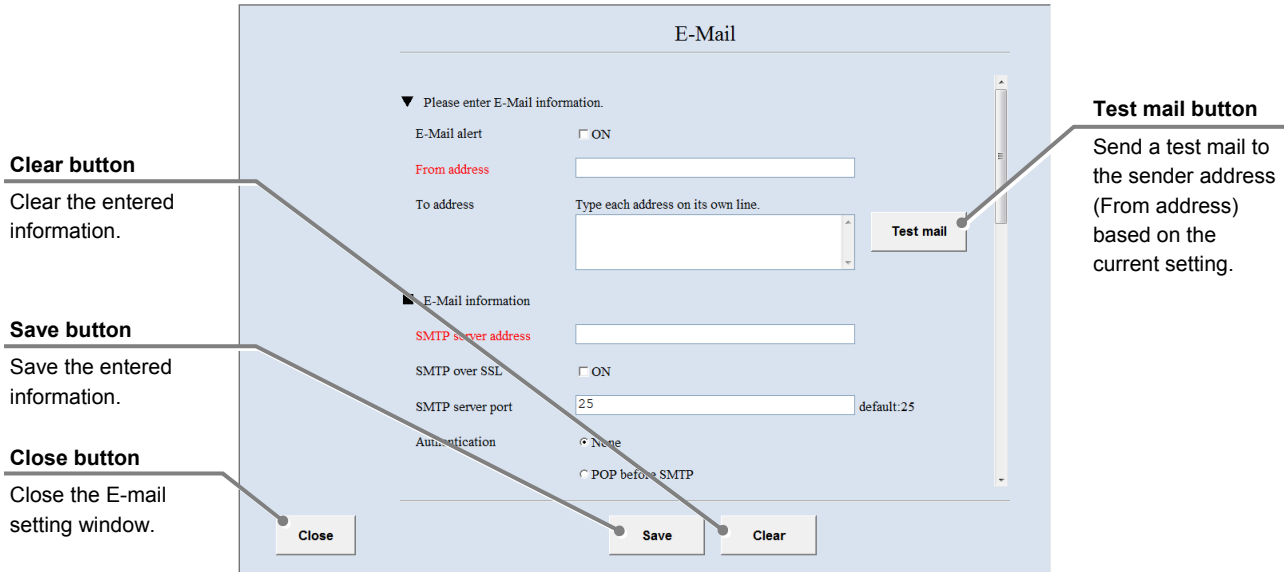
(1) Press the E-mail button in the Master data maintenance window.

The E-mail setting window is displayed.



Master data maintenance window

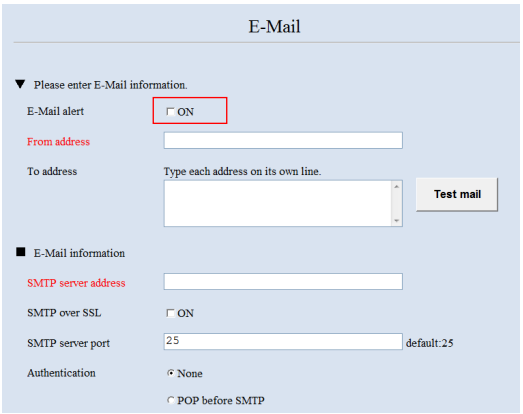
Fill in the items displayed in red. For other items, fill in if necessary.



E-mail setting window

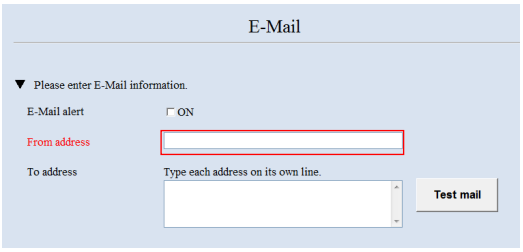
(2) Select ON for the E-Mail alert to enable the E-mail notification setting.

OFF is selected by default.



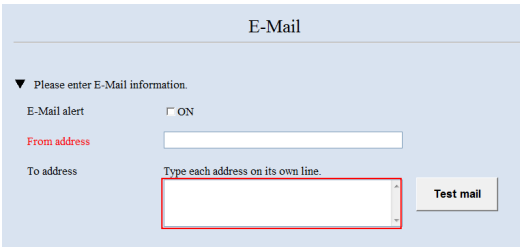
(3) Enter the e-mail address of the sender.

The From address must be entered.



(4) Enter the destination e-mail address(es) in the To address field.

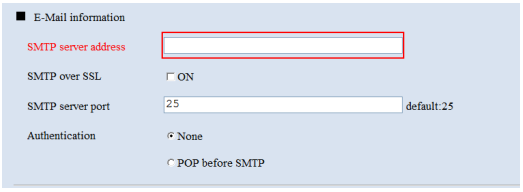
To enter more than one destination address, enter a line break between addresses.



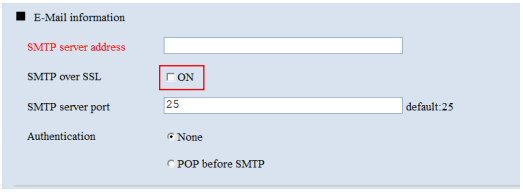
(5) Enter the address of the e-mail (SMTP) server.

The SMTP server address must be entered.

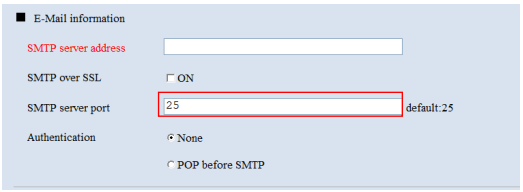
For details on the setting of the e-mail (SMTP) server, ask the network administrator of your facility.



(6) Check the SMTP over SSL check box when using SSL to encrypt the communication between a user and the mail server.



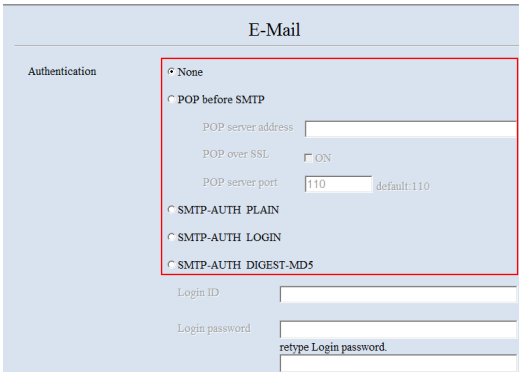
(7) Enter the port number of the e-mail (SMTP) server.



(8) Select an SMTP user authentication method.

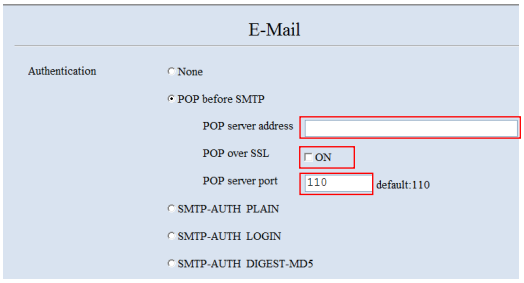
Select it from POP before SMTP, SMTP-AUTH PLAIN, SMTP-AUTH LOGIN, and SMTP-AUTH DIGEST-MD5.

Select None if the user authentication method is not used.



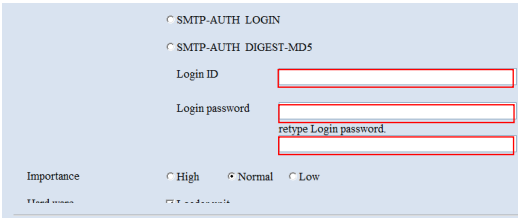
If POP before SMTP is selected, enter the address and port number of the POP server.

Check the SMTP over SSL check box when using SSL to encrypt the communication between the mailer and the mail server when receiving an email.



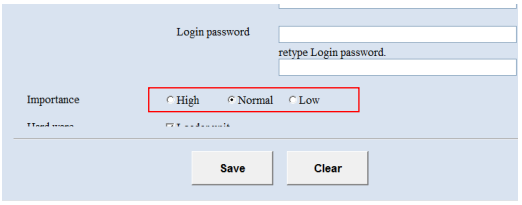
(9) Enter the user ID and password for the SMTP server and the POP server.

> When the SMTP AUTH (SMTP Authentication) feature is used on the SMTP server, the password must be entered.



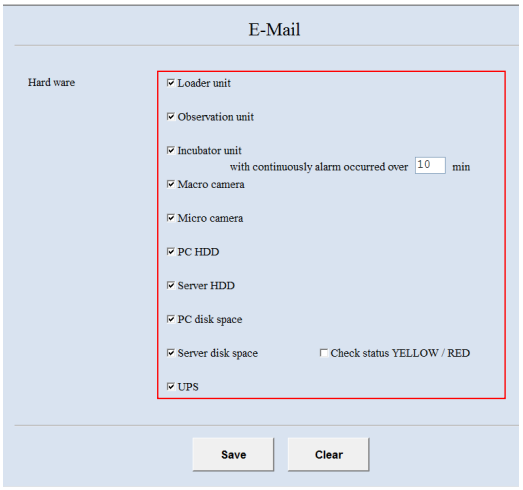
(10) Select the importance (High, Normal, or Low) of the notification mail.

Normal is selected by default.



(11) Select item(s) to be monitored for e-mail notification.

If an error occurs on the selected item, notification is sent via e-mail.



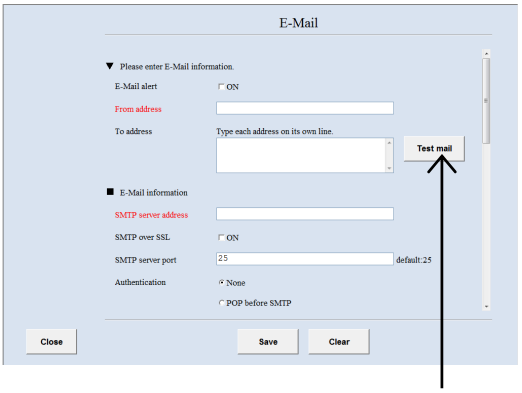
Monitored items

Loader unit:	The loader unit
Observation unit:	The observation unit
Incubator unit:	The environmental unit The value for the “Incubator unit with continuously alarm occurred over” can be specified in minutes. Default value: 10
Macro camera:	The macro camera
Micro camera:	The micro camera
PC HDD:	The hard disk drive of the control PC
Server HDD:	The hard disk drive of the file server
PC disk space:	The disk space in the hard disk drive of the control PC
Server disk space:	The disk space in the hard disk drive of the file server
UPS:	The uninterruptible power system

(12) Press the Test mail button to send a test mail to check that the settings are correctly configured.

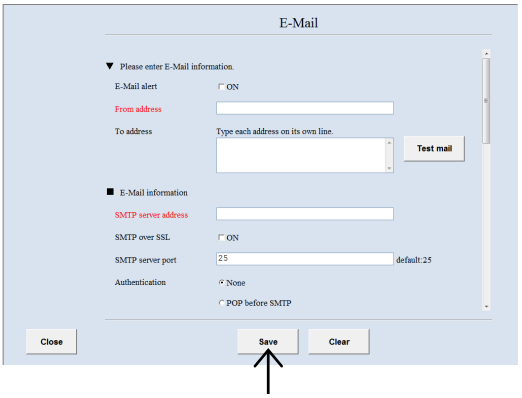
A notification e-mail is sent to the mail address of the sender.

If the notification e-mail is not sent, check the e-mail settings.



(13) Press the Save button.

The entered e-mail notification settings are saved.

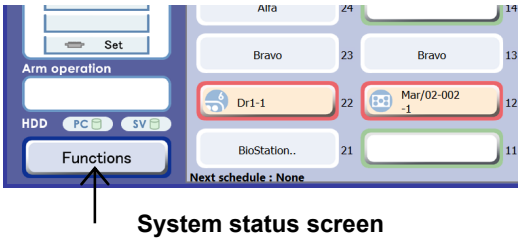


7.4 Shutdown

This section describes the procedure for shutting down the power of the control PC mounted on this system. Read this section along with the procedure for shutting down the product.

(1) Press the Functions button in the System status screen.

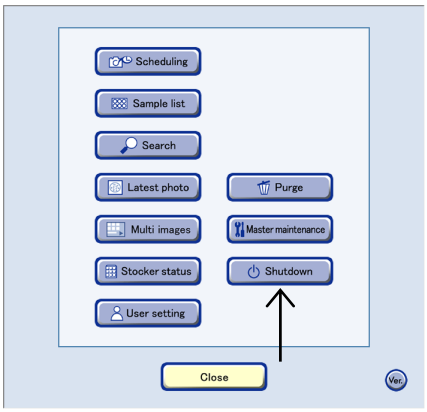
The Functions window is displayed.



System status screen

(2) Press the Shutdown button.

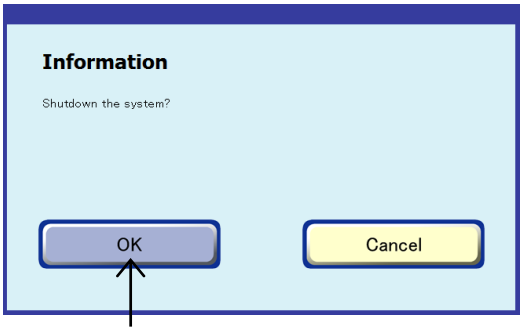
A control PC shutdown confirmation dialog box is displayed.



Functions window

(3) Press the OK button on the dialog box.

The control PC shuts down.



Confirmation dialog box

8 Troubleshooting and Tips

This chapter describes troubleshooting and tips for capturing a better image.

Misuse of the product may adversely affect performance, even if the product is not damaged. If any of the following problems occurs, be sure to check the following table for possible causes before requesting service.

If the problems cannot be resolved by taking the following measures, contact your nearest Nikon representative.

8.1 General Troubleshooting

Problem	Cause	Countermeasure	See also
The product cannot be turned on.	The power cord is not connected.	Connect the power cord correctly.	-
	The local supply voltage is unsuitable for the product.	Check the local voltage and connect the product to a power supply of the proper voltage.	Section 9.2
	The ground fault breaker is turned off.	Turn on the ground fault breaker. If it turns off during operation, suspend use of this product and contact your nearest Nikon representative.	Section 3.2
The product can be turned on but nothing is displayed on the touch panel.	The UPS is turned off.	Turn on the UPS.	Section 3.2
	The power to the touch panel display is turned off.	Turn on the touch panel display.	Section 3.2
	The power to the control PC is turned off.	Turn on the control PC.	Section 3.2
The power to the CO_2 incubator does not turn on.	The CO_2 incubator breaker switch or the CO_2 incubator power switch is turned off.	Turn on the CO_2 incubator breaker switch and the CO_2 incubator power switch.	Section 3.2
System initialization does not finish.	The power supply for some device is not being supplied.	Check all power supplies. Turn on all devices and restart the system. If initialization fails again, contact your nearest Nikon representative.	Section 3.2
File server is beeping.	The hard disk or related apparatus has failed.	Contact your nearest Nikon representative.	-
The control PC's power LED is flashing (red) and/or beeping.	The control PC has failed.	Contact your nearest Nikon representative.	-
Turning on the control PC's power, the program freezes when the Windows startup screen displays. The System status screen does not appear even after waiting.	The control PC has not booted up normally.	Press and hold the power switch on the control PC for several seconds until the control PC shuts down. Turn the control PC back on. If the System status screen still does not appear, contact your nearest Nikon representative.	-
The control PC shuts down automatically.	Automatic restart due to monthly maintenance at 0:30 a.m. on the 2nd day of each month (midnight on the 1st day).	Please wait. The control PC will restart immediately after being shut down. If a scheduled observation is scheduled during automatic restart (0:20 a.m. to 0:40 a.m.), the PC will not restart and maintenance will be postponed to the next month. In addition, only the control PC will restart. The culture conditions are not affected.	-

8.2 Troubleshooting on the CO_2 Incubator

Problem	Cause	Countermeasure	See also
The temperature or the humidity in the CO_2 incubator is unstable.	The warm-up stage in the start-up sequence has not been completed yet.	Wait until the warm-up stage is completed.	-
	The CO_2 incubator door or the access gate is open, or has frequently been opened/closed.	Close the CO_2 incubator door, minimize frequency of opening the access gate, and wait until the temperature and humidity become stable.	Section 3.3.1
	Distilled water in the humidifier water tank is insufficient.	Refill the tank with distilled water.	Section 6.1
	The ambient temperature changes widely.	Improve the condition of the environment the product is used in so that the environment is suitable.	Section 9.3
	The temperature controller, humidifier heater, temperature sensor, or the humidity sensor is damaged.	Contact your nearest Nikon representative.	-
CO_2 concentration in the CO_2 incubator is unstable.	The CO_2 incubator door or the access gate is open, or has frequently been opened/closed.	Close the CO_2 incubator door, minimize the frequency of opening the access gate, and wait until the CO_2 concentration becomes stable.	Section 3.3.1
	The CO_2 cylinder is not connected.	Connect the CO_2 cylinder correctly.	Section 6.2
	The pressure in the CO_2 cylinder decreases.	Replace the CO_2 cylinder.	Section 6.2
	The valve of the CO_2 cylinder or the regulator (primary pressure or secondary pressure) is closed.	Adjust the flow of the CO_2 gas.	Section 6.2
The pH of the culture medium changes significantly.	The CO_2 incubator door or the access gate is open, or has frequently been opened/closed.	Close the CO_2 incubator door, minimize the frequency of opening the access gate, and wait until the CO_2 concentration becomes stable.	Section 3.3.1
	The CO_2 cylinder is not connected.	Connect the CO_2 cylinder correctly.	Section 6.2
	The pressure in the CO_2 cylinder decreases.	Replace the CO_2 cylinder.	Section 6.2
	The valve of the CO_2 cylinder or the regulator (primary pressure or secondary pressure) is closed.	Adjust the flow of the CO_2 gas.	Section 6.2
Cells die unexpectedly.	The condition is unsuitable for the culture.	Correct the temperature, humidity, and CO_2 concentration to be suitable for culture.	Section 3.3
	The temperature, humidity, or CO_2 concentration in the CO_2 incubator is unstable.	See “The temperature or the humidity in the CO_2 incubator is unstable” and “CO_2 concentration in the CO_2 incubator is unstable.”	-
	There is a problem with the cell or the medium.	Nikon does not guarantee against cell problems and medium problems. It is the responsibility of the user to verify that the conditions for cells and mediums are suitable and that the equipment is set up properly.	-

8.3 Troubleshooting on the Loading Unit

Problem	Cause	Countermeasure	See also
The sample position is shifted each time the sample is transported to the observation unit.	The wrong holder is being used.	Use a holder suitable for the culture vessel.	Section 3.4.2
	The bottom of the culture vessel or the stage of the observation part is dirty or wet.	Clean the culture vessel and the stage.	Section 6.9
	The setting orientation of the sample in the stocker is wrong.	Unload the sample from the CO_2 incubator. Correct the orientation of the sample. And then, reload the sample.	Section 3.4.3
	The wrong culture vessel setting is specified for loading samples into the CO_2 incubator.	Unload the sample from the CO_2 incubator. Correct the culture vessel setting. And then, reload the sample.	Section 4.3
	The culture vessel is not fixed on the holder correctly.	Fix the culture vessel on the holder correctly. See “Holders” in Chapter 3 and the separated instruction manual for holders.	Section 3.4.3
	A vessel that cannot be firmly set on the holder is being used.	See “Unavailable Culture Vessels” for details.	Section 3.4.1.1
The culture vessel tips over when being transported.	The wrong holder is being used.	Prepare a holder suitable for the culture vessel.	Section 3.4.2
	The wrong culture vessel setting is specified for loading samples into the CO_2 incubator.	Unload the sample from the CO_2 incubator. Correct the culture vessel setting. And then, reload the sample.	Section 4.3
	The setting orientation of the sample in the stocker is wrong.	Unload the sample from the CO_2 incubator. Correct the orientation of the sample. And then, reload the sample.	Section 3.4.3
	Some vessel types are not available.	See “Unavailable Culture Vessels” for details.	Section 3.4.1.1
A vessel cannot be mounted on the holder.	A holder that does not match the culture vessel is being used.	Use a holder that matches the culture vessel being used.	Section 3.4.2
The holder cannot be slid into the carrier normally.	The direction of the holder is wrong.	Insert the holder in a correct direction.	Section 3.5.2
	The insertion position of the holder is wrong.	Align the holder position with the carrier shelf and insert the holder straight into the carrier.	Section 3.5.2
The carrier cannot be placed on the carrier normally.	The orientation of the carrier is wrong.	Place the carrier on the carrier slider so that the carrier opening faces to the left.	Section 4.3.2.2
	Foreign matter adheres to the lower surface of the carrier or the upper surface of the carrier slider.	Remove the foreign matter from the lower surface of the carrier or the upper surface of the carrier slider, and then put the carrier on the carrier slider again.	-
The medium spilled onto the carrier slider.	The carrier slider was moved roughly.	Move the carrier slider gently.	Section 4.3.2.2
The carrier cannot be recognized. **The Carrier button is disabled.**	The carrier mount position is misaligned.	Insert the carrier correctly.	Section 4.3.2.2

8.4 Troubleshooting on the Observation Part and Image Data

Problem	Cause	Countermeasure	See also
The field of view is vignetting or uneven in brightness. The image is invisible or dark.	The objective is contaminated with dirt or dust.	Contact your nearest Nikon representative.	-
	The sample is placed on a nonstandard vessel.	Place the sample on the specified vessel.	Section 3.4.1
	The side wall of the culture vessel reflects or refracts the illumination.	This phenomenon occurs depending on the observation magnification used or when the observation position is not suitable. See Chapter 4, “Operation” and change the culture vessel, magnification, or observation position.	Chapter 4
	Dew condenses on the vessel immediately after medium change or loading.	Wait for about 30 minutes after loading before starting observation or keep the top and bottom of the vessel from becoming cold before loading.	-
Dirt or dust is seen in the field of view.	The objective is contaminated with dirt or dust.	Contact your nearest Nikon representative.	-
The sample does not come into focus.	No cell is found at the center of the field of view of the microscope.	Move the stage to place cells at the center of the field of view.	Section 4.5.1
	The culture vessel is not fixed to the holder correctly.	Fix the culture vessel to the holder correctly. See “Holders” in Chapter 3 and the separated instruction manual for the holder.	Section 3.4.3
	Dew condenses on the vessel immediately after medium change or loading.	Wait for about 30 minutes after loading before starting observation or keep the top and bottom of the vessel from becoming cold before loading.	-
	A scheduled observation has been set for wells on which cells are not seeded on a well plate.	Set the scheduled observation only for wells on which cells are seeded.	Section 4.6.2
	A culture insert is being used.	Some types of culture insert may not be able to focus. Press the Long button in the AF area in the Ph live observation window to see if it can focus. If it cannot, a clear image cannot be captured using the vessel.	Section 4.5.1
	The settings for using a 96-well plate are not correct.	See “Obtaining Good Observations When Using 96-well Plates” for details.	Section 8.9
	A round-bottom 96-well plate is being used that has a culture plane outside the AF range.	Some round-bottom 96-well plates have a culture plane outside the AF range of this product, resulting in a possible defocus. Contact your nearest Nikon representative for details.	Section 3.4.1.1
An FL image cannot be in focus.	Since a FL image has a different focal plane than a Ph image, the FL image is defocused.	Correct the Z position for each FL channel in the FL live observation window.	Section 4.5.2.2

Problem	Cause	Countermeasure	See also
Some areas are defocused during Full Scan observation.	The vessel is distorted.	When setting the Full Scan observation conditions, select Fine in the AF point setting area in the Observation condition setting window.	Section 4.6.2.2
	The vessel is tilted.	Mount the vessel correctly.	Section 3.4.3
The contrast of a captured fluorescence image with Ch2 is low.	The contrast is low due to the autofluorescence caused by the medium, etc.	To reduce the autofluorescence of the medium, perform fluorescence pre-exposure prior to fluorescence image capturing.	Section 4.6.2.4
The sample position shifts each time when capturing images.	The bottom of the culture vessel or the stage of observation part is dirty or wet.	Clean the culture vessel and the stage.	Section 6.9
	The culture vessel is not fixed to the holder correctly.	Fix the culture vessel to the holder correctly. See “Holders” in Chapter 3 and the separated instruction manual for the holder.	Section 3.4.3
The image is not clear.	The bottom of the culture vessel or the stage of the observation part is dirty or wet.	Clean the culture vessel and the stage.	Section 6.9
	The culture vessel is not fixed to the holder correctly.	Fix the culture vessel to the holder correctly. See “Holders” in Chapter 3 and the separated instruction manual for the holder.	Section 3.4.3
	Dew condenses on the vessel immediately after medium change or loading.	Wait for about 30 minutes after loading before starting observation or keep the top and bottom of the vessel from becoming cold before loading.	-
	A scheduled observation has been set for wells on which cells are not seeded on a well plate.	Set the scheduled observation only for wells on which cells are seeded.	Section 4.6.2
	A round-bottom 96-well plate is being used that has a culture plane outside the AF range.	Some round-bottom 96-well plates have a culture plane outside the AF range of this product, resulting in a possible defocus. Contact your nearest Nikon representative for details.	Section 3.4.1.1
Misalignment/defocus due to an abnormal observation method	A slide glass or cover glass is being used.	Do not use vessels other than those designated for this system.	Section 3.4.1
The image under the phase contrast microscopy is extremely dark.	The LED for the diascopic illumination is broken.	Contact your nearest Nikon representative.	-
	The culture vessel is not fixed to the holder correctly.	Fix the culture vessel to the holder correctly. See “Holders” in Chapter 3 and the separated instruction manual for the holder.	Section 3.4.3
Old image data is deleted unexpectedly.	The file server does not have free disk space.	Remove any unnecessary image data from the file server to increase available free disk space.	Section 7.2
The data space is insufficient, making the scheduled observation impossible.	The file server does not have sufficient data space enough to save the observation data.	Remove any unnecessary image data from the file server to increase available free disk space.	Section 7.2

8.5 Troubleshooting the UPS (Uninterruptible Power Supply)

When the UPS experiences a problem, an alarm will sound as follows to indicate the corresponding type of problem:

Problem	Cause	Countermeasure
A warning alarm sounds 4 times with 30-second intervals. **(The On Battery LED will light up.)**	The power supply to the product has stopped.	Confirm that the power supply is properly connected.
A warning alarm sounds continuously. **(The Overload LED will light up.)**	The power voltage is overloaded.	Immediately turn off the control breaker and contact your nearest Nikon representative.
A warning alarm sounds every 4 hours. **(The Battery Replacement LED will light up.)**	The battery needs to be replaced or recharged.	Charge the battery for 24 hours and then redo the self-test. If the problem persists, it is necessary to replace the battery. Contact your nearest Nikon representative.
A short warning alarm sounds every 2 seconds. **(The Battery Replacement LED will light up.)**	The battery is not properly connected.	Connect the battery properly.

8.6 Troubleshooting on the External PC

Problem	Cause	Countermeasure	
This product cannot be accessed through a network from an external PC.	The network cable is not connected correctly or the connected network cable is broken.	Connect this product correctly to the network.	Section 2.1 in “BioStation CT Ver. 3.8 Instructions (PC Operations)”
	A network hub is used to connect this product to the network but the connected cable is a cross-wired type.	Consult with the administrator of your network and use a correct cable.	
	The IP address setting is wrong.	Consult with the administrator of your network and set the IP address correctly.	
	The proxy setting is wrong.	Consult with the administrator of your network and set the proxy correctly.	
	The firewall setting is wrong.	Consult with the administrator of your network and set the firewall correctly.	
An error message is displayed during automatic download using the Downloader (BioStation CT Downloader). Downloading failed.	The downloader's storage location path contains a “%”.	Change the Downloader's storage location path to one that does not contain a “%”.	Section 3.9.3.2 in “BioStation CT Ver. 3.8 Instructions (PC Operations)”

8.7 Error Information Sent by E-mail

Monitored item	Actions
Loader Unit	If an error occurs in the loader unit, an e-mail with the subject “ERROR (Loader unit)” is sent. Contact your nearest Nikon representative.
Observation Unit	If an error occurs in the Observation unit, an e-mail with the subject “ERROR (Observation unit)” is sent. Contact your nearest Nikon representative.
Incubator Unit	If an alarm setting item exceeds the specified value in the environmental unit, an e-mail with the subject “WARNING (Incubator unit)” is sent. Check the water level of the humidifier water tank, operation of the environmental unit, and whether the specified alarm setting values are correct. If the possible cause of the error might be something other than the above, contact your nearest Nikon representative.
Macro Camera	If an error occurs in the macro camera, an e-mail with the subject “ERROR (Macro camera)” is sent. Contact your nearest Nikon representative.
Micro Camera	If an error occurs in the micro camera, an e-mail with the subject “ERROR (Micro camera)” is sent. Contact your nearest Nikon representative.
PC HDD	If an error occurs in the hard disk drive of the control PC, an e-mail with the subject “ERROR (PC HDD)” is sent. Contact your nearest Nikon representative.
Server HDD	If an error occurs in the hard disk drive of the file server, an e-mail with the subject “ERROR (Server HDD)” is sent. Contact your nearest Nikon representative.
PC disk Space	If there is a problem with the free disk space in the hard disk drive of the control PC, an e-mail with the subject “ERROR (PC disk space)” is sent. Contact your nearest Nikon representative.
Server Disk Space	If the free disk space of the hard disk drive in the file server is insufficient, an e-mail with the subject “WARNING (Server disk space)” is sent. Then, the older data will be automatically deleted in chronological order allowing the newly captured image data to be stored. Download important data to an external PC.
UPS	If this product receives a shutdown request from the UPS, an e-mail with the subject “WARNING (System shutdown)” is sent. If a blackout continues for more than five minutes, the product automatically shuts down. After the power supply is restored, restart the system.

8.8 Power Outage Response and Recovery

How the Product Responds to a Power Outage

When the power supply to the product stops for five minute, the product automatically performs the power outage process in the following order:

(1) Abort any scheduled observation and live observation and return the sample to its original position.

(2) Move the loading unit and observation part to their normal waiting position.

(3) Shut down the control PC and file server.

(4) Shut down the UPS.

Recovery Procedures after a Power Outage

Once the power supply has come back on and the battery housed in the UPS has reached 90%, the UPS will return to operation state and the main power switch's OFF button will light up. Perform the following procedures after the main power switch's OFF button lights up.

(1) Turn on the UPS.

The OFF button of the main power switch lights.

(2) Press the main power switch's ON button.

The product will be supplied with power and begin operating.

(3) Turn on the file server.

1. **Release the file server's front lock and then remove the front cover.**
2. **Turn on the power switch.**
3. **Reattach the front cover and then lock it.**

(4) Turn on the control PC.

(5) Wait until the environment inside the CO_2 incubator stabilizes.

Confirm that the CO_2 incubator's internal temperature, humidity and CO_2 concentration are at the set level prior to loading a sample.

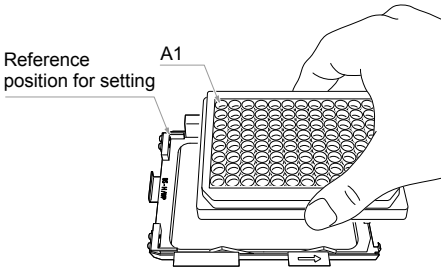
8.9 Optimal Observation Using 96-well Plates

Because 96-well plates are smaller than other vessels, the samples must be loaded and the observation conditions set correctly. Otherwise, observation can fail due to misaligned vessels.

Note the following when using 96-well plates:

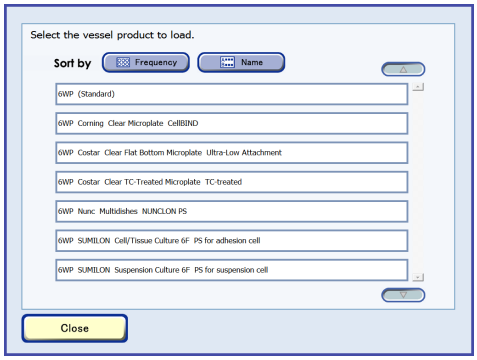
Setting to the holder

- Attach the plate to the holder in the correct direction (with the A1 well on the top left).
- Follow the correct procedure to set it to the holder. (See Section 3.4.3, “Setting the Culture Vessel onto the Holder.”)



Selecting the vessel type

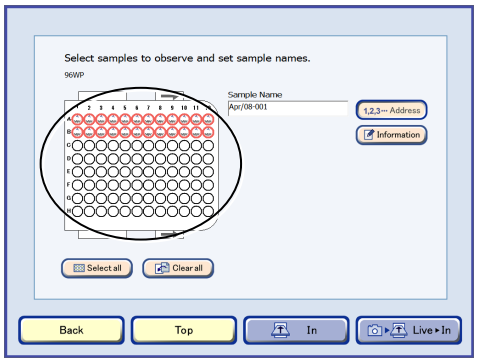
- Check the product actually being used in the Vessel selection window for the 96-well plate and select the corresponding vessel from the vessel selection list. (See Step 14 in Section 4.3.2.3, “Loading a new sample.”)



Vessel selection window

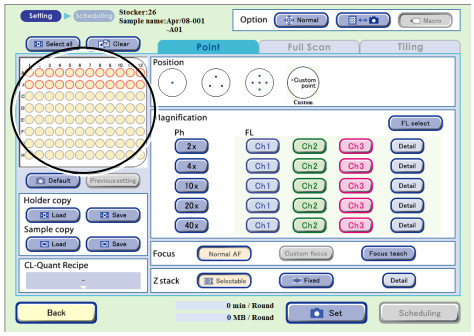
Registering samples

- Register only wells on which cells are seeded. Do not register an empty well on which cells are not seeded and a well that contains only a medium. (See Step 15 in Section 4.3.2.3, “Loading a new sample.”)



Well plate sample selection window (96-well)

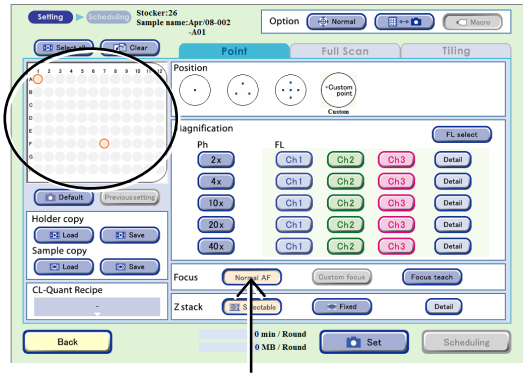
- If a well on which cells are not seeded has been registered, select only wells on which cells are seeded when setting the observation conditions and make the relevant settings. (See Step 3 in Section 4.6.2, “Setting the Scheduled Observation Conditions.”)



Observation condition setting window

Setting the observation conditions

As shown on the right, if there is a large empty space between the wells to be observed (5 or more rows or columns), AF may fail with the settings of normal observation conditions using Normal AF.



Ex.) Setting the observation conditions only for A1 and F7 wells

Take the following two measures for the observation if there is a large empty space between the wells to be observed:

a. Using Custom focus for each sample

This is effective for Point observations.

a-1. Before setting the observation conditions, perform a live observation in the Ph live observation window.

a-2. Focus the first well and press the Custom point button to register a custom observation position.

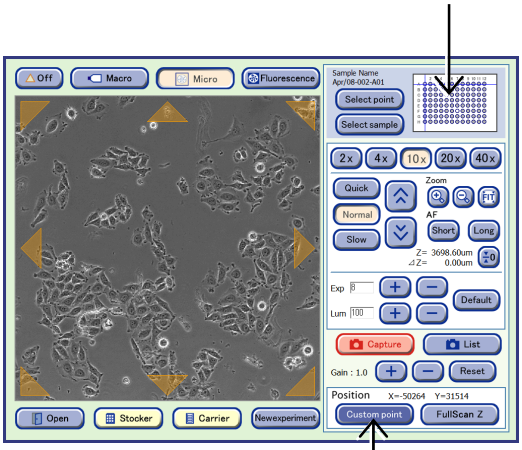
a-3. Press the holder map to display the Select sample window, and then select a second well.

a-4. Focus the second well and press the Custom point button to register a custom observation position.

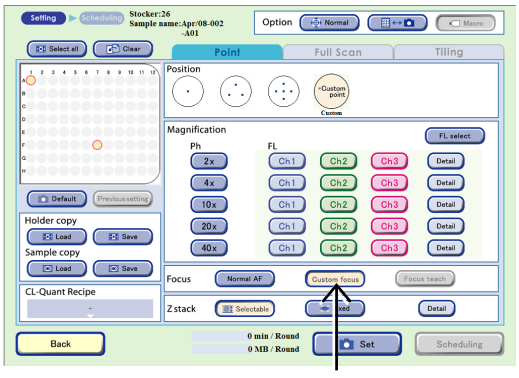
Repeat steps a-3 to a-4 for each well, if any.

a-5. Select Custom focus on the Observation condition setting window.

Finally, set the observation conditions and perform a scheduled observation using the normal procedure.



Ph live observation window

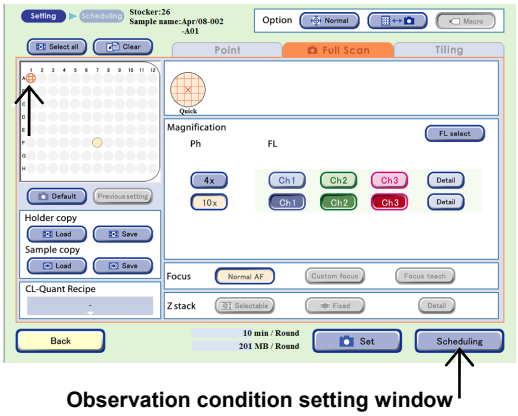


Observation condition setting window

b. Setting a scheduled observation for each well

This is effective for FullScan and Tiling observations.

b-1. Select the first well in the Sample selection area and set the observation conditions.

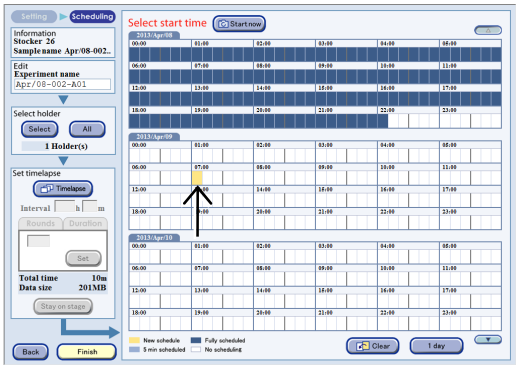


Observation condition setting window

b-2. Schedule the first well in the Scheduling window.

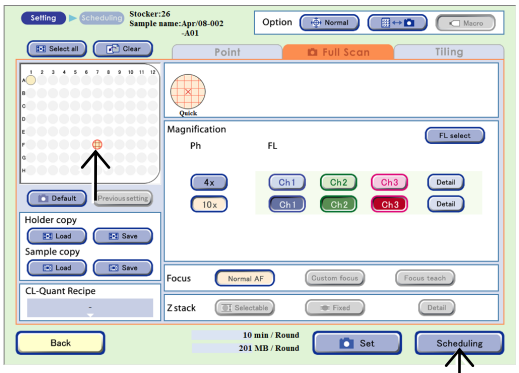
b-3. Press the Finish button to go back to the Select function window.

b-4. Press the New experiment button in the Select function window to display the Observation conditions window.



Scheduling window

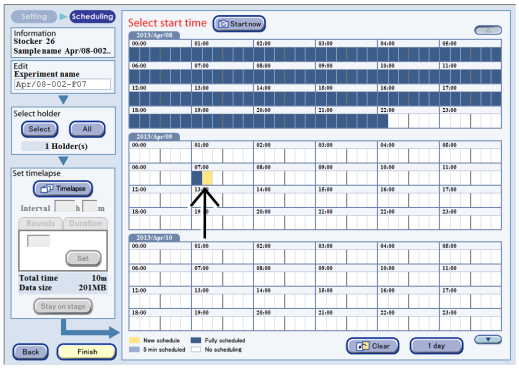
b-5. Select the second well in the sample selection area and set the observation conditions.



Observation condition setting window

b-6. Schedule the second well in the Scheduling window.

Repeat steps b-3 to b-6 for each well, if any.



Scheduling window

9 Specifications

9.1 Operational Basis

Observes or photographs cells with the microscope built in the system while culturing cells loaded into a CO_2 incubator using the touch-panel screen of the system or an external PC.

9.2 Performance Characteristics

Model	BioStation CT	
Operation method	Center control with the touch panel LCD monitor Operation can be performed via a network (with Internet Explorer 8 or Internet Explorer 9. However, do not enlarge the screen display with functions such as the zoom function of Internet Explorer.	
Incubator	Type: Capacity: A door with an electromagnetic lock mechanism is equipped.	CO_2 incubator 460 liters
Environmental condition control	Temperature, humidity, and CO_2 concentration are controlled in the incubator.	
Temperature control	Control method: Controllable range: Stability:	direct method with a heater panel 5°C added to the environmental temperature to 42°C, 0.1°C steps (initial setting: 37°C) ±0.1°C (environmental temperature: 23°C, temperature setting: 37°C)
Humidity control	Control method: Controllable range: Conditions for no condensation: Stability: Humidifier water tank:	air spray humidifier 70 to 95% RH, 1% steps (initial setting: 90% RH) environmental temperature: 15 to 28 °C, temperature setting: 37°C, humidity setting: 90% RH) ±2% (environmental temperature: 23°C, humidity setting: 90%) equipped at the front of the product
CO_2 concentration control	CO_2 supplying method: Controllable range: Stability: Ventilation:	with an external CO_2 cylinder (coupler: ø8 mm) 0 to 20%, 0.1% steps (initial setting: 5%) ±0.1% (environmental temperature: 23°C, concentration setting: 5%) ventilating air to outside of the product through air vents
Culture vessel	Flasks: Dishes: Well plates:	75 cm^2, 25 cm^2 35 mm, 60 mm, 100 mm 6-well, 12-well, 24-well, 48-well and 96-well * Nunc 4-well multidish conditionally available.
Sample loading mechanism	Setting samples with the special carrier, passing through the access gate Special holders are used for various vessels.	
Sample loading mechanism	Movable axes: Travelling route:	X, Y, Z, and rotation axes between the carrier and the stocker between the stocker and the observation part (XY stage) between the carrier and the observation part (XY stage)

Sample stocker	3 rows by 10 columns
Macro observation	Capturing the entire sample with a special camera (macro image) Camera head: color CCD camera (1280 × 960 pixels) Illumination: white backlight illumination
Micro observation	Magnification: 2x, 4x, 10x, 20x, and 40x Objective: Plan Apo DLL 4x, Plan Fluor ADL 10x
Digital camera for micro observations	Equipped model: DS-1QM Camera head: 2/3 inch cooled CCD camera (1-megapixel) Frame rate: 15 fr/s
Microscopy method	Phase Contrast microscopy under diascopic illumination: automatically switching the phase ring
Light source	Light source for diascopic illumination: high intensity red LED
Stage	X-Y axis moving range: 120 × 120 mm
Focusing method	Detection of a focal point: phase difference detection method with Z-axis scanning Shift of a focal point: by moving the Z-axis direction
Observation method	With a PC monitor or so on (No eyepiece is equipped.)
Power supply	Power ratings: 100, 115, 230 VAC ±10% 1300 VA Frequency: 50/60 Hz
Provided power cord	For countries where the supply voltage is 100V to 120V excluding Japan: UL Listed Attachable cord (3 conductor grounding Type SJT, No.14 AWG, 3 m long maximum, rated at 125 VAC minimum.) For countries where the supply voltage is 220 V to 240 V: EU/EN-approved three-conductor power cord (3 conductor grounding Type HO5VV-F, 1.00 mm^2, 3 m long maximum, rated at 250 VAC minimum) For Japan: Power cord conforming with the Electrical Appliance and Material Safety Law (with PSE mark) (3 conductor grounding Type VCTF3 × 2.0 mm^2, 3 m long maximum, rated at 125 VAC minimum)

Model	BS-FL Fluorescence unit (Option)			
External dimensions	122 mm (W) × 288 mm (D) × 192.5 mm (H) (Excluding protrusions)			
Mass	Approximately 2.0 kg			
Components	Fluorescence filter turret	Mechanism	Filter change with turret rotation	
		Driver device	DC motor with an encoder	
		Sensor	Six address sensors and a position sensor	
		Interface	Power supply	Supplied from the BioStation CT body
			Control	Controlled from the BioStation CT body
	Fluorescence filter cube [Standard]	Ch1 (CFP)	Model Number	CFP-2432C (Semrock)
			Excitation	438 nm (Center value)/ 24 nm (Half-value width)
			Emission	483 nm (Center value)/ 32 nm (Half-value width)
			Dichroic	458 nm
		Ch2 (GFP)	Model Number	GFP-3035D (Semrock)
			Excitation	472 nm (Center value)/ 30 nm (Half-value width)
			Emission	520 nm (Center value)/ 35 nm (Half-value width)
			Dichroic	495 nm
		Ch3 (RFP)	Model Number	DsRed2 42005 (Chroma)
			Excitation	540 nm (Center value)/ 40 nm (Half-value width)
			Emission	600 nm (Center value)/ 50 nm (Half-value width)
			Dichroic	570 nm

Components (continued)	Fluorescence filter cube [Additionally mount on Ch4 and Ch5 (*)]	(1)	Model Number	ET-EYFP-49003 (Chroma)
			Excitation	500 nm (Center value)/ 20 nm (Half-value width)
			Emission	535 nm (Center value)/ 30 nm (Half-value width)
			Dichroic	515 nm
		(2)	Model Number	YFP-2427B (Semrock)
			Excitation	500 nm (Center value)/ 29 nm (Half-value width)
			Emission	542 nm (Center value)/ 32 nm (Half-value width)
			Dichroic	528 nm
		(3)	Model Number	CY3-4040C (Semrock)
			Excitation	531 nm (Center value)/ 45 nm (Half-value width)
			Emission	593 nm (Center value)/ 46 nm (Half-value width)
			Dichroic	565 nm
		(4)	Model Number	TxRed-4040C (Semrock)
			Excitation	562 nm (Center value)/ 46 nm (Half-value width)
			Emission	624 nm (Center value)/ 46 nm (Half-value width)
			Dichroic	601 nm
		(5)	Model Number	ET-Cy5-49006 (Chroma)
			Excitation	620 nm (Center value)/ 60 nm (Half-value width)
			Emission	700 nm (Center value)/ 75 nm (Half-value width)
			Dichroic	660 nm
	Excitation light source (LED's)	Light source (1)	Peak Wavelength	458 nm
		Light source (2)	Peak Wavelength	475 nm
		Light source (3)	Peak Wavelength	To 620 nm
LED power rating (for each LED)	Max. voltage: 12 VDC Max. current: 1.1 A			

(*) Lists (1) to (5) are filters registered by default in the equipment for Ch4 and Ch5. Up to two types of fluorescence filter cube specified during purchase may also be mounted.

9.3 Physical Characteristic

Model name	BioStation CT
External dimensions	1120 mm (width) × 920 mm (990 mm when the touch panel is tilted) (depth) × 1850 mm (height)
Mass	Approximately 470 kg
Operating conditions	Temperature: +15 to +28°C Humidity: 60% RH maximum (no condensation) Altitude: 2000 m maximum Pollution degree: Degree 2 Overvoltage category: Category II Short-term temporary overvoltage: 1,440V (up to 5 seconds) Long-term temporary overvoltage: 490V (longer than 5 seconds) Electrical shock protection class: Class I Indoor use only
Storage conditions	Temperature: -5 to +50°C Humidity: 60% RH maximum (no condensation) Altitude: 2000 m maximum Pollution degree: Degree 2 Overvoltage category: Category II Short-term temporary overvoltage: 1,440V (up to 5 seconds) Long-term temporary overvoltage: 490V (longer than 5 seconds) Electrical shock protection class: Class I Indoor use only
Safety standards	This class A digital apparatus complies with Canadian ICES-003(A) / NMB-003(A). Cet appareil numérique de la classe A est conforme à la norme NMB-003(A). du Canada. • This product complies with Australian AS/NZS CISPR11 Group 1 Class B. CE marking • This product meets EU IVD Directive requirements. • EN61010-1, EN61010-2-101, EN591, EN60825-1 • This product meets EU Low Voltage Directive requirements. (EN61010-1) • This product meets EN61326 (EN55011 Group1 Class B EN61000-3-2/3-3/4-2/4-3/4-4/4-5/4-6/4-8/4-11.) • This product meets FCC Part 15B Class A requirements. This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant Part 15 of the FCC Rules. These limits are designed provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference own expense.

A Hazardous Substances List

A.1 Hazardous Substances List

Warning

Do not use any explosives, inflammables, or substances that contain them with this product.

A.1.1 Explosive Substances

Explosive substances

(1) Nitroglycol, nitroglycerine, nitrocellulose, and other explosive nitrate esters.

(2) Trinitrobenzene, trinitrotoluene, picric acid, and other explosive nitro compounds.

(3) Peracetic acid, methyl ethyl ketone peroxides, benzoyl peroxide, and other organic peroxides.

A.1.2 Combustible Substances

Combustible substances

Metallic lithium, metallic potassium, metallic sodium, yellow phosphorus, phosphorus sulfide, red phosphorus, celluloids, calcium carbonate (also called carbide), calcium phosphate, magnesium powder, aluminum powder, other metallic powders, and sodium dithionite (also called sodium hydrosulfite).

Oxidizing agents

(1) Potassium chlorate, sodium chlorate, ammonium chlorate, and other chlorates.

(2) Potassium perchlorate, sodium perchlorate, ammonium perchlorate, and other perchlorates.

(3) Potassium peroxide, sodium peroxide, barium peroxide, and other inorganic peroxides.

(4) Potassium nitrate, sodium nitrate, ammonium nitrate, and other nitrates.

(5) Sodium chlorite and other chlorites.

(6) Calcium hypochlorite and other hypochlorites.

Ignitable substances

(1) Ethyl ether, gasoline, acetaldehyde, propylene chloride, carbon disulfide, and other substances with an ignition point below -30°C.

(2) Normal hexane, ethylene oxide, acetone, benzene, methyl ethyl ketone, and other substances with an ignition point above -30°C and below 0°C.

(3) Methanol, ethanol, xylene, pentyl acetate (also called amyl acetate), and other substances with an ignition point above 0°C and below 30°C.

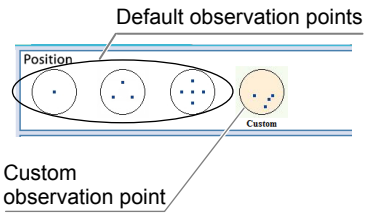
(4) Kerosene, light oil, turpentine oil, isopentyl alcohol (also called isoamyl alcohol), acetic acid, and other substances with an ignition point above 30°C and below 65°C.

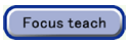
Combustible gases

Hydrogen, acetylene, ethylene, methane, ethane, propane, butane, and other combustible substances that are in a gaseous state at a temperature of 15°C and at a pressure of 1 atmosphere.

(Referred from table 1 on article 6 of Industrial Safety and Health Law in Japan.)

B Glossary

Access gate	A small glass door used when a carrier is loaded/unloaded into/from the product. Using this door minimizes the change to the environment due to opening and closing the door.
User	A person who operates this product to manipulate samples.
Observation point	**Default observation point:** An observation point preregistered in this system by default. **Custom observation point:** An observation point newly registered for a live observation, scheduled observation, or in the Full scan image display window. 
Carrier	A transportation case for loading/unloading samples to/from the product. A carrier can contain up to three holders containing culture vessels.
Scheduled observation (Automatic observation)	An observation method that allows automatic capturing of microscope images of multiple observation points at specified times in accordance with the observation schedule specified for each sample. This makes it possible to record changes in cellular behavior over time.
Stocker	Shelf with three columns and ten rows inside the product, used to store samples in culture flasks, dishes, and well plates.
Timelapse image capturing	A method for capturing sample images using a specified observation method at a specific time interval.
Download	The task of transferring obtained observation image data from BioStation CT to an external PC. There are two types of download: “automatic download” for transferring captured images to an external PC each time scheduled observation is completed, and “manual download” performed by the user each time scheduled observation is completed.
Download preparation (Manual download)	The task of creating downloadable files on the hard disk drive of BioStation CT by selecting the observation image data to be downloaded to an external PC.
Detail button 	**Fluorescence:** Pressing the Detail button in the FL channel setting area of the Observation condition setting window displays the Fluorescence image exposure conditions setting window. The Fluorescence image exposure conditions setting window allows you to select channels, and adjust the exposure time and the intensity of each excitation light source. **Z stack:** Pressing the Detail button after selecting the Selectable  or Fixed  button in the Z stack area of the Observation condition setting window displays each window for Z stack settings. When the Selectable button is selected, the Selectable pitch setting window in which the number of Z stack capture images can be specified by selecting capture ranges (Range) and pitches (Pitch) appears. When the Fixed button is selected, the Fixed pitch setting window in which the number of Z stack capture images can be selected directly appears. **Tiling capture setting area:** Pressing the Detail button in the Tiling capture setting area field in the Full Scan image display window displays the Tiling observation condition modification window. In this window, the magnification, number of tiled images and capture area can be changed.
Loading	Loading samples means loading into the culture chamber new samples or samples that were removed so the culture medium could be changed. Loading the carrier means loading a carrier through the access gate into the culture chamber.

Unloading	Unloading samples means to remove samples from the culture chamber to, for example, change the culture medium. Unloading the carrier is to take out a carrier through the access gate.
Focus type	**Normal AF:**  When a default observation point is selected in the Observation condition setting window, the Focus type is automatically changed to Normal AF. Each observation image is captured at the position where autofocus is performed at the default observation point (specified by X and Y coordinates). **Custom focus:**  When a custom observation point is selected in the Observation condition setting window, the Focus type is automatically changed to Custom focus. Each observation image is captured at a registered custom observation point specified by X, Y, and Z coordinates. At the Full scan observation, if the Full Scan Z button is pressed in the Ph live observation window, auto-focusing is performed near the registered custom observation point. **Focus teach:**  In the Observation condition setting window, when this button is pressed, autofocus is performed for the selected observation point and that position is newly registered as a custom observation point. (Enabled only when Normal AF is used)
Holder	Holders are used as adapters to make various types of culture vessels usable in this product.
Macro image	An entire sample image captured with the color camera. This image is useful for recording the color of the medium and some letter information written on the vessel.
Multi image display	In the Multi image display window, this feature displays observation images captured at the same observation point, replacing one image to another in chronological order.
Micro image (Ph image/FL image)	An image of cells captured at a selected magnification (2x, 4x, 10x, 20x, or 40x). This type of image can record images of cells.
Observation mode	When setting scheduled observation, either the Normal mode or Stage exclusive mode can be selected. **Normal mode:**  Every time a scheduled observation is completed, the sample is returned to the stocker. Select this mode to perform a scheduled observation for multiple samples. **Stage exclusive mode:**  After a scheduled observation, the sample remains on the stage. Select this mode to efficiently perform a scheduled observation for one sample.
Live observation (Manual observation)	A manual observation method for observing samples in a carrier or stocker through the touch panel display.
Log In	A user operation of selecting the user's name through the touch panel display to enable the user to handle samples. A user cannot handle samples if the user is not authorized as a user for the sample.
Log Out	A user operation of canceling the selected logged in user through the touch panel display. Immediately after start up of this product, it is in logged out status. In the logged out status, no samples can be operated.
Full Scan (Entire sample area observation)	A function for capturing the entire sample area as tiled images in a scheduled observation. Full Scan observation is not possible when a 75 cm^2 culture flask or Nunc 4-well multi dish is used. The image data captured during the Full Scan observation can be analyzed by CL-Quant.