



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

<System>

Introduction

Thank you for purchasing a Nikon product.

This instruction manual is written for the users of the Nikon Time Lapse Imaging System BioStation IMQ.

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.
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Training

You can use this product without the need of special training sessions by reading this manual thoroughly before use. Please kindly contact the distributor if you have any questions or find any errors and anything you are aware of.

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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
	Disregarding instructions marked with this symbol may lead to serious injury or death.
	Disregarding instructions marked with this symbol may lead to injury or property damage.

Warning Label and Symbols Used on the Product

The symbols used on the product indicate the need for caution during use. Refer to the instruction manual and read the relevant instructions before handling any part to which the symbols have been affixed.

Warning label/symbol	Description
▲ CAUTION ▲ 注 意	Caution for ultraviolet light irradiation
UV Light may emitted from objective. 対物レンズから紫外線が 出ている場合があります	This symbol on the upper part of the microscope calls your attention to the following:
	 Surroundings of the objective may be exposed to intense light including ultraviolet light while the illuminator is turned on.
	 The light including ultraviolet light may be irradiated outward if the slide door is opened during the fluorescent microscopy.
	 To check the surroundings of the objective during the fluorescent microscopy, open the sliding shade on the top of the microscope and look through the transparent window.
\wedge	Biohazard
	This symbol on the upper part of the microscope calls your attention to the following:
	 If a specimen is spilled onto the product, it may cause the danger of biohazard.
	 To avoid biohazard contamination, do not touch the contaminated portion with your bare hands.
	 Decontaminate the contaminated portion according to the safety standard of your facility.
\wedge	Caution for heat
	This symbol on the front of the humidifier heater inside the microscope calls your attention to the following:
	 The humidifier heater becomes very hot during operation of the product.
	• To prevent burn or fire, do not touch the humidifier heater nor place any flammable material near the product during operation.

* See Section 1.2.5, "Locations of Warning Label and Symbols" in Chapter 1, "Names of Each Part."

1. Intended use of this product (for medical care)

This product is intended primarily for microscopy and photomicrography of cells and tissues cultured in a petri dish.

Do not use this product for other purpose.

2. Intended user

It is intended for researchers and the medical professional and those who work on experimentations in the field of genetics, immunology, physiology, pharmacology, neurology, cellular biology and molecular biology.

3. Do not disassemble.

Disassembling may cause malfunction and will lead to the forfeiture of all claims against warranty. Do not disassemble any part that is not indicated in this manual. If you notice any abnormality, contact your nearest Nikon representative.

4. Read the instructions carefully.

To ensure safety, carefully read this manual and the manuals for associated devices (devices used with this product). Especially, be sure to follow the warnings and cautions indicated at the beginning of the manuals.

Note on using a light source

To perform the epi-fl microscopy with this product, a light source that has a mercury lamp is required. Take great care of the lamp in operating the light source. Read the instruction manual for the light source and follow the instructions and cautions for it.

5. Use the specified light source.

Use this product with the following light source. Do not use this product with the devices other than the following:

 Nikon INTENSILIGHT C-HGFIE HG Precentered Fiber Illuminator (light guide fiber provided)

6. Caution on the power cord

Make sure to use the specified power cord. Using a wrong power cord may result in malfunction or fire. The product is classified as subject to Class I protection against electrical shock. Make sure it is connected to an appropriate ground terminal (protective earth terminal).

For the specified power cord, see Chapter 6, "Specifications."

To avoid an electric shock, be sure to turn off the power (flip the power switch to the "O" side) of this product before plugging the power cord into the wall outlet.

7. Note on the humidifier heater

The humidifier heater becomes very hot during operation of the product.

To prevent burn or fire, do not touch the humidifier heater nor place any flammable material near the humidifier heater.

8. Ultraviolet light

When the epi-fl microscopy is performed, the surroundings of the objective are exposed to ultraviolet light that is harmful to your eyes and skin. Directly looking at ultraviolet light may cause snow blindness or, at worst, loss of vision. To avoid such injury, follow the instructions given below during the epi-fl microscopy:

• Do not open the slide door.

The surroundings of the objective are exposed to very strong light including ultraviolet light during the fluorescent microscopy. The light may be irradiated outward if the slide door is opened.

• Be sure to look through the transparent window when looking at the surroundings of the objective.

Slide open the sliding shade on the top of the microscope and look the surroundings through the transparent window.

• Securely attach the light source to the product.

Always connect the light source to the product when the light source is ready to light up. Do not turn on the light source if it is not connected to the product, and do not disconnect the light source from the product if it is lit. When disconnecting the light source from the product, turn off the power to the light source, and then unplug the power cord from the wall outlet.

9. Notes on handling the CO₂ gas

The CO₂ gas with a low concentration flows into the culture chamber to keep the cells alive for this product. Ventilate the installation area sufficiently, because the CO₂ gas flows out from this product. To handle the CO₂ mixer, regulator, and CO₂ cylinder, be sure to follow the instructions shown below.

- To use the CO₂ mixer, regulator, and the CO₂ cylinder, carefully read the instruction manuals provided by the manufacturers and make sure to follow the instructions.
- Install the CO₂ mixer, regulator, and CO₂ cylinder, and connect the tubes securely so
 that there is no leakage. Check periodically if any cracks or looseness of the tubes and
 connected areas exist.

If the CO_2 gas is released in large quantities in the room, it may cause suffocation. Take the following measures immediately:

• Evacuate immediately and stay away from the room.

• Take the predetermined procedure such as calling the security or fire department.

The Occupational Safety & Health Administration (OSHA) specifies that exposure to carbon dioxide must be less than 5,000 ppm (0.5% CO₂) on average in the case of 40-hour week (8-hour day). Short time exposure within 15 minutes must be 30,000 ppm (3% CO₂) or less. It is recommended to install the CO₂ concentration monitor or the like against leakage.

10. Notes on handing a hazardous specimen

Before handling a biological specimen, check whether or not the specimen is hazardous.

When handling a hazardous specimen with this product, follow the safety standard of your facility. When handling a hazardous, potentially infectious specimen, wear rubber gloves to avoid direct touch. If such specimen is spilled onto this product, the portion must be decontaminated in a safety manner. Follow the safety standard of your facility.

1. Isolate this product from the power source during assembly, connecting/disconnecting cords, lamp replacement, and maintenance.

To prevent electric shock and malfunctions, be sure to turn off the power (flip the power switch to the "O" side) of this product and associated devices, and unplug the power cords from the wall outlet before assembling, connecting or disconnecting cables, or cleaning the microscope and the objectives.

2. Do not wet the product.

Do not wet this product. If this product gets wet, a short circuit may occur resulting in a malfunction or an abnormal heating. If you wet this product, immediately turn off the power (flip the power switch to the "O" side) of this product and associated devices, and unplug the power cords from the wall outlet. Then, wipe off the water with a piece of dry cloth. If water enters the product, stop using the product, and contact your nearest Nikon representative.

3. Do not place any object on the product.

Do not place any object on the product. The slide door and plate rail become deformed, resulting in a malfunction.

4. Do not block the opening of the cooling fan on the back.

This product is equipped with the cooling fan on the rear side to cool down the inside of the product. Do not block the opening of the cooling fan covering the product with a piece of cloth, etc. The temperature inside of the system rises, resulting in a malfunction.

5. Do not use the product covered with a piece of cloth.

Do not use the product covered with a piece of cloth, etc. Heat release is interfered, resulting in an abnormal heat and fire.

6. Weak electromagnetic waves

This product emits weak electromagnetic waves. Keep this product and associated devices away from precision electronic equipment so as not to affect the accuracy of nearby precision electronic equipment. If the product or associated devices affects TV or radio reception, move the radio or TV a little further from the product (or associated devices).

7. Dispose of the product according to the standard procedure specified for your facility.

To prevent biohazard risks, dispose of the product as contaminated equipment according to the standard procedure specified for your facility.

Notes on Handling the Product

1. Handling the Product

This product is a precision optical instrument. Handle the product carefully, and do not apply any vibration or shock.

In particular, objectives may lose accuracy when exposed to even a weak physical shock.

Cautions on moving the product

- The product weighs approximately 30 kg. The product must be carried by at least two people.
- Remove the specimen and the humidifier water tank, and disconnect the connected cables and the tubes before carrying the product.
 - If other devices are attached to the product, also remove them before carrying the product.
- When carrying the product, hold the recess at the base on the front side and the handle on the rear side.
- Secure the movable parts before carrying the product. For details, refer to Section 7.1, "Assembly and Connection."
- This product uses a floating structure to sustain vibration or impact. If this product is transported without the floating unit secured, it may be considerably damaged.
 For details on securing the floating units, see Section 7.1, "Assembly and Connection."

Cautions on assembling and installing the product

- Avoid getting your finger or hand pinched.
- Scratches or fouling such as fingerprints on the optical parts (such as lenses or filters) will adversely
 affect the microscope image.
 Be careful not to scratch or touch the lenses and filters when assembling the optical parts.

2. Installation location and storage location

This product is a precision optical instrument. The product may get damaged or lose accuracy if it is used or stored under unsuitable conditions. When selecting the installation or storage location, note the following:

- Installation and storage conditions: Temperature: 0 to 40°C/relative humidity: 85% or less To prevent condensation, avoid the place where rapid temperature change occurs. When the product is installed or stored in hot, humid places, mold formation or condensation may occur, resulting in performance degradation or malfunctions.
- Operating conditions: Temperature: 18 to 28°C; Use the product in an air-conditioned room. Do not install the product in the place where rapid temperature change occurs such as the places close to the door or directly blown by the air-stream from the air conditioner.
- Avoid the locations where the product is exposed to direct sunlight.
- Install in a place with little dust and dirt.
- Install in a place with little vibrations.
 Do not install the CO₂ mixer in the same place as the microscope. The CO₂ mixer causes a vibration, resulting in performance deterioration.
- Install and store the product on a level, sturdy desk or table.
- Install the product in the location of 10 cm or more away from the surrounding walls.
- Install the product in the place where the power cord can be unplugged easily from the AC inlet of the product in emergency.
- Do not install the product in the narrow space such as a locker or a cabinet.
- Do not place anything on the product.
- Cover the product to avoid dust when storing.

• The C-HGFIE HG Precentered Fiber Illuminator generates intense heat. Therefore, place it at least 50 cm from the product and arrange a layout where the exhaust heat from the illuminator does not affect the product.

3. Notes on handling optical parts

Scratches or fouling such as fingerprints on the optical parts (such as lenses or filters) will adversely affect the microscope image.

Be careful not to scratch or touch the optical parts. If the optical part gets fouling on it, clean it according to Chapter 5, "Care and Maintenance."

4. Control PC

The product comes with a control PC. Do not use any other PC to operate the product. Do not modify the control PC. Do not install any software other than Nikon recommends.

5. Contents in the Package

- Microscope
- Culture chamber (BS-IM-C chamber)
- 4-quadrant dish positioning adapter (for the BS-IM-C chamber)
- Humidifier water tank, humidifier water tank cover, silicon sheet
- Silicon tubes for the BS-IM-C chamber (two types: wet mixed gas tube, exhaust tube)
- CO₂ mixed gas tube
- Evaporating dish
- Cover of the filter cube port
- Ergonomic controller
- USB cables: one black cable and one blue cable
- RS232C cable
- Sterilizing filter and two adapters for the sterilizing filter
- Hexagonal screwdrivers (three types: 2 mm, 2.5 mm, 3 mm)
- Instruction manuals

Options

- Culture chamber (BS-IM-MC MOT chamber, lid stand)
- Silicon tubes for the BS-IM-MC MOT chamber (two types: wet mixed gas tube, exhaust tube)



1.1 System Configuration

The BioStation IMQ is composed of the following devices.

For handling the control PC, HG precentered fiber illuminator, CO_2 mixer, and CO_2 cylinder, refer to the instruction manuals provided with the devices.



1.2 Microscope



1.2.1 Rear View



Figure 1.2-2 Rear view of the microscope

1.2.2 LED Indicator

The LED indicator is on the top of the microscope.



Figure 1.2-3 LED indicator

 Table 1.2-1
 LED indicator functions

Lamp	Meaning	Emission color	Detail
POWER	Status of the power	Green	This lamp lights up when the power is turned on.
DOOR OPEN	Status of the door	Yellow	This lamp lights up when the slide door or the filter cube port is opened. When both of them are closed, the lamp goes out. When either of them is opened during the time-lapse experiment, the lamp blinks.
NO BOTTLE	Status of the humidifier water tank	Yellow	This lamp blinks when the humidifier bottle is not equipped.
STABLE	Status of the temperature control	Green	This lamp lights up when the temperature of the culture chamber, the humidifier, and so on reach to the setting values and become stable.
TIME-LAPSE	Status of the time-lapse	Green	This lamp lights up during time-lapse experiment.

1.2.3 View When the Front Door Opened

Figure below shows the BS-IM-C chamber.



Figure 1.2-4 Surroundings of the humidifier water tank

1.2.4 View When the Slide Door Opened

BS-IM-C chamber



Figure 1.2-5 Surroundings of the BS-IM-C chamber

BS-IM-MC MOT chamber



Figure 1.2-6 Surroundings of the BS-IM-MC MOT chamber

1.2.5 Locations of Warning Label and Symbols

Figure below shows the BS-IM-C chamber.



1.3 Ergonomic Controller



Figure 1.3-1 Ergonomic controller

Table 1.3-1	Ergonomic controller functions
-------------	--------------------------------

Part name	Function		
Observation method switches	PH Phase contrast is selected and fluorescence filter cubes are automatically removed. FL1 Epifluorescence cube position 1 is selected. FL2 Epifluorescence cube position 2 is selected.		
Shutter open/close switch	The excitation light shutter built in the	microscope is opened or closed.	
Single image capture switch	The currently observed image is captu	ıred.	
Magnification adjustment/illumination intensity adjustment selector switch	It toggles between objective magnification selection and illumination intensity adjustment.		
Memory switch	X, Y, and Z coordinates recorded by time-lapse experiment are recorded.		
UP switch	For magnification adjustment	Magnification is increased.	
	For illumination intensity adjustment	Illumination intensity is increased.	
DOWN switch	For magnification adjustment	Magnification is reduced.	
	For illumination intensity adjustment	Illumination intensity is reduced.	
Indicator A	The LED lamp of the selected observation method lights up.		
Indicator B	The LED lamp is lit when the shutter for excitation light is opened.		
Indicator C	The LED lamp of the selected function lights up.		
Focus knobs	Adjust the focus by rotating the focus You can adjust the focus with either for above, the left focus knob is the main	knobs. ocus knob. In the figure shown knob.	
X stage knob	Observation point is moved in the X direction (right and left).		
Y stage knob	Observation point is moved in the Y direction (back and forth).		

1.4 C-HGFIE HG Precentered Fiber Illuminator (Motorized Operation Type)



This is used for fluorescent observation on the BioStation IM series. The light is controllable from the control PC.

Figure 1.4-1 HG precentered fiber illuminator

1.5 C-HGFIF15 HG Fiber

This leads the light from the HG precentered fiber illuminator into the microscope.



Preparations of a Time-lapse Experiment —

This chapter describes the following procedures: starting up the product, setting a specimen, and operating the ergonomic controller.

2.1 Start-up

1. Turn on the microscope.

Turn the power switch on. Then, the "POWER" lamp of the LED indicator on the top of the microscope lights up.

As soon as the microscope is turned on, the temperature adjustment function starts. The temperature set at the previous operation is the target value. Since there is no set value of previous operation when the microscope is turned on for the first time, the default setting is the target value.

Default temperature setting Temperature of the culture chamber: 37°C Temperature of the humidifier water tank: 37°C

If room temperature is below 18°C or above 28°C the "POWER" lamp of the LED indicator blinks rapidly. Adjust the room temperature properly so that the performance of microscope becomes stable.



Figure 2.1-1 Turning on the microscope



Figure 2.1-2 LED indicator (POWER)

2. Turn on the HG precentered fiber illuminator and the control PC.

For details, refer to the instruction manual for each device.

3. Start up the application software for the BioStation IM and set the temperature.

The standard setting value for the culture chamber is 37°C, but it can be set in the range shown below. For details on setting the temperature, see Section 1.2.2, "Status Display and Settings of the Microscope" in the separate manual, "BioStation IMQ Instructions <Application Software>."

	Standard temperature setting	Temperature range	Necessary condition
Culture chamber	37°C	32 to 38°C	9°C or more higher than the room temperature
Humidifier water tank	The same temperature as that of the culture chamber	32 to 40°C	Equal to or higher than the set value for the culture chamber and equal to or lower than the value 2°C higher than the actual temperature of the culture chamber

Tahlo 2 1-1	Tomnoraturo sottings	for the culture	chamber and the	humidifior	wator tank
	remperature settings			5 munnung	water tarr

Notes on opening the doors on the product

The front door, slide door, and filter cube port must be shut while the product is warmed up or used as a microscope. If you need to open any door, shut it as soon as possible.

4. Warm up the microscope.

It takes approximately three hours to fully warm up the product and stabilize the performance before observation becomes available.

When observation becomes possible, the "STABLE" lamp of the LED indicator on the top of the microscope lights up.

Additionally, the screen display changes from "Unstable" (red) to "Stable" (blue).



Figure 2.1-4 Screen display of the software

5. Turn on the CO_2 mixer.

For details, refer to the instruction manual for the CO₂ mixer.

When using the BS-IM-MC MOT chamber, do not turn on the CO_2 mixer until the microscope is fully warmed up. Check that the "STABLE" lamp of the LED indicator is lit before turning on the CO_2 mixer. Otherwise, condensation may occur within the chamber.

Setting the CO₂ mixer

CO₂ concentration : 5% Amount of flow : 150 ml

The above settings are standard. However, it is recommended to change the values depending on the color of phenol red solution added to the culture medium.

6. Register AF position of the reference mark (only for the BS-IM-MC MOT chamber.)

When using the BS-IM-MC MOT chamber with the AF mode enabled, register the AF position of the reference mark as described below. Check that the "STABLE" lamp of the LED indicator is lit before registering AF position of the reference mark.

Skip this step if the BS-IM-C chamber is used or the calibration is not performed.

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

If the reference mark is not within the screen, follow the instructions in Section 7.4, "Setting up the BS-IM-MC MOT chamber."

If it is in the screen but not placed within the blue frame at the center of the screen, move the reference mark into the blue frame using the jog dial or the ergonomic controller.

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

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



Figure 2.1-5 Reference mark register screen

2.2 Replacing the Filter Cube

The filter for the fluorescence microscopy can be replaced for each specimen.

1. Remove the cover of the filter cube port.

Remove the cover of the filter cube port on the right of the microscope.

When the cover is removed, the "DOOR OPEN" lamp of the LED indicator on the top of the microscope blinks.

When the filter magazine is moved to the far side, it automatically moves to the near side when the cover is removed.



Figure 2.2-1 Removing the cover

2. Remove the filter magazine.

Remove the filter magazine from the sliding bracket as shown.



Figure 2.2-2 Removing the filter magazine

3. Replace the filter cube.

To replace the filter cube, slide it apart from the carrier bracket.

The filter cube shown as "^m" on the display is located at the far side. The filter cube, "^m", is located at the near side.



Figure 2.2-3 Replacing the filter cube

4. Attach the filter magazine.

Insert the filter magazine into the original position and attach the cover to the filter cube port.

When the cover is attached, the "DOOR OPEN" lamp of the LED indicator on the top of the microscope goes out.



Figure 2.2-4 Inserting the filter magazine

2.3 Checking Water Amount and Supplying Water for the Humidifier Water Tank

Be sure to check the amount of water in the humidifier water tank before operating the product, for time-lapse experiment may last longer than a single day.

Keep the amount of water between the "min" and "max" lines on the humidifier water tank. If the amount of water is not correct, the humidifier may not work correctly.

After refilling the humidifier water tank, warm up the product until the water temperature reaches to the set value and the "STABLE" lamp of the LED indicator lights up.

Checking the amount of water in the humidifier water tank

The "NO BOTTLE" lamp of the LED indicator on the top of the microscope only indicates whether or not the humidifier water tank is set. It does not indicate the amount of water in the tank.

1. Open the front door.

3.

Open the front door by turning the front door open/close knob.



Figure 2.3-1 Opening the front door

2. Detach the quick-release joints of the CO₂ mixed gas tube and the wet mixed gas tube.

Remove the temperature sensor from the

humidifier water tank.



Figure 2.3-2 Removing the quick-release joints



Figure 2.3-3 Removing the temperature sensor

4. Loosen the clamp screw for the humidifier water tank and retract it to remove the humidifier water tank.

When the humidifier water tank is removed, the "NO BOTTLE" lamp of the LED indicator on the top of the microscope blinks.

When it is removed, the humidifier heater is automatically turned off.



Figure 2.3-4 Loosening the clamp screw



Figure 2.3-5 LED indicator (NO BOTTLE)

5. Pour distilled water into the humidifier water tank.

Pour distilled water into the humidifier water tank until it reaches little below the "max" line. Use only distilled water.

6. Follow the removal procedure in reverse order to attach the humidifier water tank to the microscope.

When the humidifier water tank is attached, the "NO BOTTLE" lamp of the LED indicator on the top of the microscope goes out.









Figure 2.3-7 LED indicator (NO BOTTLE)

7. Shut the front door.

Shut the front door properly by turning the front door open/close knob.

2.4 Setting a Specimen

Notes on setting a specimen

When a dish is taken out from an incubator, etc., condensation occurs on the cover of the dish caused by the temperature differences between the incubator and the room. The condensation fades gradually once the dish is set in the culture chamber. Perform the microscopy without the condensation, since the image in the phase contrast microscopy is degraded if the condensation remains.

If the contact surface between the bottom of the dish and the culture chamber is wet, the specimen may move during the time-lapse experiment or go out of focus. Check that the contact surface is free from water and culture medium.

If there is too much specimen in the glass bottom 4-well dish, the specimen may contact the upper lid, resulting in abnormal observation images. When filling the dish with a specimen, only fill up to two-thirds of the height of the dish.

1. Open the slide door.

Open the slide door on the top of the microscope by sliding it backward.

When the slide door is opened, the "DOOR OPEN" lamp of the LED indicator on the top of the microscope lights up.

The figure illustrated at the right shows the case for the BS-IM-C chamber. In this manual, the figure for the BS-IM-C chamber will be illustrated if the operating instruction of the MS-IM-MC MOT chamber is the same as that of the BS-IM-C chamber.



Figure 2.4-1 Opening the slide door

2. Retreat the diascopic illumination unit.

Move the diascopic illumination unit backward by rotating it.



Figure 2.4-2 Retreating the diascopic illumination unit

3. Set specimen in the culture chamber.

BS-IM-C chamber

Open the rotatable cover of the BS-IM-C chamber by rotating it backward, then set the specimen.

- * The 35-mm dish for the CELL-S2 is different from that for the CELL-S2-P.
 - CELL-S2: Glass bottom dish Film bottom 4-quadrant dish

CELL-S2-P: Plastic bottom dish



Figure 2.4-3 Setting specimen

Adjusting the correction ring

In the CELL-S2-P, a correction ring is attached to the objective. Adjustment of the correction ring corrects the spherical aberration caused by the difference in bottom thickness of the plastic bottom dish, and makes images clear.

Set the index of the bottom thickness of the dish in use rotating the knobs near the top of the objective.

For the bottom thickness, contact its manufacturer.



Figure 2.4-4 Correction ring on the objective

When the film bottom 4-quadrant dish is in use

When the film bottom 4-quadrant dish is set, use the 4-quadrant dish positioning adapter.

Place the adapter in the culture chamber, and then set the film bottom 4-quadrant dish, adjusting the adapter notch with the dish partition.

When the 4-quadrant dish is used for the first time, check the positional relation between the cross line displayed on the Wide field screen and the disk partitions for the quadrants.

For details, see Section 7.3, "Notes on Using the Film Bottom 4-Quadrant Dish" in this manual.

After setting the specimen, close the rotatable cover.



Figure 2.4-5 Setting the film bottom 4-quadrant dish

BS-IM-MC MOT chamber

(1) Remove the lid of BS-IM-MC MOT chamber and place it on the lid stand.

Always place the lid on the lid stand located inside of the microscope main unit. If the lid is placed outside of the microscope, it may get cold, causing a longer time re-warming up till the "STABLE" lamp of the LED indicator lights up.



Figure 2.4-6 Setting the glass bottom 4-well dish (1)

(2) Water will accumulate in the water barrier within Water barrier

Figure 2.4-7 Setting the glass bottom 4-well dish (2)

// / / ///

the BS-IM-MC MOT chamber. Use a dropper or other means to remove the water before or after observation.

- (3) Loosen the screws and remove the dish fixing plate.
- (4) Set the glass bottom 4-well dish (with the specimen) into the chamber, place the dish fixing plate on the dish, and secure the plate tightening the screws.
- (5) Seal the BS-IM-MC MOT chamber with the lid.



Figure 2.4-8 Setting the glass bottom 4-well dish (3)

4. Close the slide door.

Return the diascopic illumination unit to the original position and close the slide door by sliding it forward by hands.

When the slide door is closed, the "DOOR OPEN" lamp of the LED indicator on the top of the microscope is turned off.



Figure 2.4-9 Closing the slide door

Set the dish as immediately as possible.

When the slide door is opened, the "STABLE" lamp goes off. When the slide door is shut and the temperature condition is stabilized, the "STABLE" lamp lights up again. The time required to light up the "STABLE" lamp again differs depending on the period of time the slide door was open and the environmental temperature condition. It takes at least ten minutes. Be sure to register time-lapse points after the "STABLE" lamp is lit.

2.5 End

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

This software closes, and the Windows desktop appears.

Perform this operation when you detach the chamber. For the BS-IM-MC MOT chamber, this procedure must be carried out for safe detachment of the chamber. (This procedure triggers the dish mounting part take the position in readiness.)

Temp Chamber	37.0 37.0	Water	37.8 38.0 🛨
Outside	29.3	(unit:deg C)	Stable



2. Turn off the peripheral devices.

Turn off the HG precentered fiber illuminator, CO_2 mixer, and control PC. For details, refer to the instruction manual for each device.

3. Turn off the microscope.

Turn the power switch off. Then, the "POWER" lamp of the LED indicator on the top of the microscope goes out.



Figure 2.5-2 Turning off the microscope



Figure 2.5-3 LED indicator (POWER)



To perform time-lapse experiment, set the observation point, observation conditions, and capturing interval time for specimen.

Close all other running application software on the control PC before setting observation conditions for the time-lapse experiment.

Operation flowchart





3.1 Examining the Specimen

On the Live observation screen, observe the condition and reaction of the specimen when fluorescent reagent is applied. Check whether the specimen is appropriate subject to perform the time-lapse experiment.

1. Display the Live observation screen.

Click the Live observation button.

For details on the Live observation screen, see Chapter 2, "Live Observation Screen," in the separate manual, "BioStation IMQ Instructions <Application Software>."

BioStation IM (Version:2.20 Build:140)			
Live observation	New time-lapse setting		
Figure 3.1-1	Live observation button		

2. Select an observation point.

2-1. When using the BS-IM-MC MOT chamber, click the well switch button to select the well (1 to 4) to be used for observation.

The Well switch button is not shown for the BS-IM-C chamber, as there is no need to switch wells. Proceed to step 2-2.



Figure 3.1-2 Live observation screen (BS-IM-MC MOT chamber)

2-2. Select an observation point by moving the stage.

There are four methods for moving the stage as follows:

- (1) Clicking a point in the Observation point display to move the position to the center of the view
- (2) Clicking a point in the Live observation image display area to move the position to the center of the view
- (3) Operating the jog dial
- (4) Operating the X, Y stage knobs of the ergonomic controller (See Section 1.3, "Ergonomic Controller.")

The screen illustrated below shows the case for using the BS-IM-C chamber. In this manual, the screen for the BS-IM-C chamber will be illustrated if the operating instruction of the MS-IM-MC MOT chamber is the same as that of the BS-IM-C chamber.



Figure 3.1-3 Live observation screen (BS-IM-C chamber)



Figure 3.1-4 Ergonomic controller (stage movement)

3. Select filters and magnifications.

Click the buttons to select filters and magnifications for the observation.

This operation can be performed with the ergonomic controller too. (See Section 1.3, "Ergonomic Controller.")



Figure 3.1-5 Filter buttons and magnification buttons



Figure 3.1-6 Ergonomic controller (filter (observation method) and magnification selection)

4. Focus on the specimen.

Focus on the specimen using the focus buttons or the focus slider.

This operation can be performed with the ergonomic controller too. (See Section 1.3, "Ergonomic Controller.")

After the focus adjustment, the focusing position can be registered with the Fix button at the right of the Z Position.

For details, see Section 2.2, "Setting Observation Conditions (Filter, Magnification, Mode, Z position, and Save)" in the separate manual, "BioStation IMQ Instructions <Application Software>."



Figure 3.1-7 Operation for focusing



Figure 3.1-8 Ergonomic controller (Operation for focusing)

5. Select the observation condition setup mode.

Two modes are available for setting up the observation conditions: the Simple mode and the Manual mode.

Click the mode button to be used.



Figure 3.1-9 Observation condition setup mode

6. Set up the observation conditions.

This section describes the settings on the Manual mode.

For the settings on the Simple mode, see Section 2.2, "Setting Observation Conditions (Filter, Magnification, Mode, Z position, and Save)," in the separate manual, "BioStation IMQ Instructions <Application Software>."

Items can be set up manually. Besides, an existing file can be loaded with the Load settings button to restore the conditions. And the settings of the observation conditions can be saved to an observation condition file. For details on saving observation conditions, see Section 2.2.1, "Saving Observation Condition," in the separate manual, "BioStation IMQ Instructions <Application Software>."

Simple Manual AE(focus) AE			
Load settings			
EPI Lamp 🏾 🍈	100% ৰ — ►		
Exposure time	300ms 🗨 🗧		
Gain	1.00 \triangleleft 📕 🕨 🕨		
Resolution	640 x 480 Binning 💌		
Z Position	150.00 um 🛛 🕞		
	_		

Figure 3.1-10 Setting observation condition on the Manual mode

(fluorescent microscopy)

Simple Manu	AE(focus) AE
Load settings	
DIA Lamp 🛛 🌼	71 <
Exposure time	1499ms
Gain	16.00 4
Resolution	640 x 480 Binning 📃
Z Position	350.95 um

Figure 3.1-11 Setting observation condition on the Manual mode (phase contrast microscopy)

- · AE (focus) button: automatic exposure adjustment with focus priority
- AE button: automatic exposure adjustment (one time)
- EPI Lamp: light intensity adjustment for episcopic illumination (only when FL filter is selected)
- DIA Lamp: light intensity adjustment for diascopic illumination (only when Ph filter is selected)
- Exposure time: exposure time setting
- Gain: gain compensation value setting
- Resolution: image resolution setting

For details on items of the Manual mode, see Section 2.2, "Setting Observation Conditions (Filter, Magnification, Mode, Z position, and Save)," and Section 2.3, "Setting Observation Conditions (Focus Mode, Automatic Exposure, Condition File Loading, Light Intensity, Exposure Time, Gain, and Resolution)," in the separate manual, "BioStation IMQ Instructions <Application Software>."

7. For the BS-IM-MC MOT chamber, repeat the procedure from Steps 2 to 6 to set the observation point for the other wells.

3.2 Registering Time-lapse Observation Points

The Live screen and the Wide field screen are provided to set observation points and conditions of the time-lapse experiment.

This chapter describes the operation methods with the Live screen in principle. The Live screen sets observation points and conditions with an observing live image and the Wide field screen sets them with a captured tiled image.

3.2.1 Observation Point Registration on the Live Screen

1. Display the New time-lapse setting screen.

Click the New time-Lapse setting button.

For details on the New time-lapse setting screen, see Chapter 3, "New Time-lapse Setting Screen," in the separate manual, "BioStation IMQ Instructions <Application Software>."

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

Figure 3.2-1 Displaying the New time-Lapse setting screen

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

Figure 3.2-2 Displaying the Live screen

Click the Live button.

Display the Live screen.

2.

3. Select an observation point.

Select an observation point by moving the stage.

Operations are the same as that on the Live observation screen. (See Step 2 in Section 3.1, "Examining the Specimen.")

4. Select filters and magnifications.

Select check boxes of filters and magnifications for use of observation (multiple filters and magnifications are selectable). Note that at least one filter and one magnification must be selected.

The Ph, Fl1, and Fl2 filters and only the 20x magnification are selected in the figure shown at right. Additionally, the image of 20x for Fl2 filter is currently displayed on the Live screen.

New point Ph Fi1 F12 20× 40× 80× 414x311um 207x156um 104x78um Simple Manual AE(focus)

Figure 3.2-3 Filter buttons and magnification buttons

5. Focus on the specimen.

Focus on the specimen using the focus buttons or the focus slider.

Operations are the same as that on the Live observation screen. (See Step 4 in Section 3.1, "Examining the Specimen.")



Figure 3.2-4 Operation for focusing

6. Select the observation condition setup mode.

Two modes are available for setting up the observation conditions: the Simple mode and the Manual mode.

Click the mode button to be used.



Figure 3.2-5 Observation condition setup mode

7. Set up the observation conditions.

Set the observation conditions for each combination of the filters and the magnifications specified in Step 4.

For the case in Step 4, set the observation conditions for the following combinations:

Ph (phase contrast microscopy) + 20x, Fl1 (fluorescence filter 1) + 20x, and Fl2 (fluorescence filter 2) + 20x

Observation condition setting methods are the same as that on the Live observation screen. (See Step 6 in Section 3.1, "Examining the Specimen.")

8. Register observation points and conditions.

Click the Time-lapse point registration button to register the observation point and the observation conditions.

Repeat the steps for setting up observation conditions and registering the conditions for each observation point to register multiple observation points into the time-lapse experiment scheme.

Start time-lapse			
Points Time Cell name etc Zstack			
 ✓ 1 (Ph 1/180s) (Fi1 1/4s) (Fi2 1/4s) 20x ✓ 2 (Ph 1/180s) (Fi1 1/4s) (Fi2 1/4s) 20x ✓ Delete 			
Time-lapse point registration button			
Load Clear Save			

Figure 3.2-6 Registering observation points

Numbering for the registered observation points All registered observation points will be numbered in sequence of registration. For the BS-IM-MC MOT chamber, the registration number is prefixed with the well number. Time Cell name etc. Points Time Cell name etc (Ph 1/180s) (B-1E 1/4s) (UV2A 1/3s) 20x... Delete 1-1 (Ph 1/180s) (FI1 1/4s) (FI2 1/4s) 20x Delete (Ph 1/180s) (B-1E 1/4s) (UV2A 1/3s) 20x... 1-2 Delete Ph 1/180s) (Fl1 1/4s) (Fl2 1/4s) 20x Delete ✓ 2-1 (Ph 1/180s) (B-1E 1/4s) (UV2A 1/3s) 20x... (Delete) Numbering for registered observation Figure 3.2-7 Figure 3.2-8 Numbering for registered observation points (BS-IM-C chamber) points (BS-IM-MC MOT chamber)

When the Time-lapse point registration button is clicked, the condition is calibrated automatically and the image of the registered position appears.

This is a necessary sequence for a long time-lapse experiment to achieve a stable position repeatability, but sometimes the image may be shifted slightly from the previous one.

If the appeared image needs to be corrected, highlight the observation point to be corrected and adjust its XYZ positions. Then click the Time-lapse point registration button again.

Registering multiple observation points in the Z direction

To register multiple observation points in the Z direction at the same X-Y coordinates, use the Z-stack function.

Specify the travel amount in the Z direction (Step: μ m) and the travel count (Steps). Multiple observation points are set up automatically.

For details on the Z-stack function, see Section 3.1.6, "Time-lapse Experiment Scheme (Zstack Tab)" in the separate manual, "BioStation IMQ Instructions <Application Software>."

Points Time Cell name etc Zstack						
Step: 0.05 Down: 2	🕂 um	Up: 2	ident steps steps			
From: -0.10	um	To: +0.10	um			
Load			Save			

Figure 3.2-9 Zstack tab

3.3 Changing Observation Conditions and Deleting Observation Points

To change an observation condition for each observation point, perform the following procedure.

3.3.1 Changing Observation Conditions

1. Display the Live screen of the New time-lapse setting screen.

Click the Live button. The Live screen of the New time-lapse setting screen appears.

2. Select an observation point to change its setting.

There are two methods for selecting an observation point.

- Click the down arrow button at the right of the New point of the observation condition settings and select an observation point in the list. The Point information dialog box appears.
- On the Points tab, click the observation point to be changed.

The Point information dialog box appears.

To change displayed observation condition settings to new settings, click the Go button. To delete the selected observation point, click the Delete button.

To cancel the changes, click the Close button. The Point information dialog box closes without applying the changes.







Figure 3.3-2 Selecting an observation point



Figure 3.3-3 Selecting an observation point

oint information	i en	
Ph	20× 414x311 um	
DIA Lamp	150	_
Exposure time	5ms	-
Gain	1.00	_
Resolution	640 × 480 Binning	-
Dish	1	-
х	3079.51 um	-
Y	3079.51 um	-
z	150.00 um	-
Comment	test	~
Go	Delete	~

Figure 3.3-4 Point information dialog box
3.3.2 Deleting Observation Points

1. Display the New time-lapse setting screen.

Click the New time-lapse setting button.



Figure 3.3-5 Displaying the New time-lapse setting screen

2. Delete observation point.

On the Points tab, click the Delete button of the observation point to be deleted.

The delete confirmation dialog box appears.



Figure 3.3-6 Deleting the observation point

To delete the observation point, click the OK button.

To cancel the operation, click the Cancel button. The confirmation dialog box closes.



Figure 3.3-7 Delete confirmation dialog box

3.4 Registering the Time-lapse Experiment Time

To perform a time-lapse experiment, specify an interval time and a total observation time.

Registering multiple time-lapse experiment times is available.

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

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

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

	Acquisition cycle	Total time R	ounds
	0h 20m 00s	10h 00m 00s	31 Deleto
	0h 00m 10s	0h 10m 00s	61 Deloto
	0h 10m 00s	20h 00m 00s	121 Delete
N	ew		Clear

3.4.1 Registering the Time-lapse Experiment Time for Image Capturing

1. Change the time-lapse experiment scheme view to "Time."

Click the Time tab of the New time-lapse setting screen.

Points Time Cell name etc Z	stack
✓ 1 (Ph 1/180s) (Fl1 1/4s) (Fl2 1/4s) 20x ✓ 2 (Ph 1/180s) (Fl1 1/4s) (Fl2 1/4s) 20x	Delete Delete
-	Clear

Figure 3.4-2 Switching to the Time tab

2. Set time-lapse experiment time.

Click the New button.

The Timelapse dialog box (for new registration) appears.



Figure 3.4-3 New button

 Timelapse

 Acquisition cycle
 1

 Total time
 2

 Rounds
 2

 As soon as possible 0h 00m 34s.

 Add

 Close

Figure 3.4-4 Setting capturing interval time and total observation time

Input capturing interval time in the Acquisition cycle box and total observation time in the Total time box.

The number of rounds (Rounds) is automatically calculated from values of Acquisition cycle and Total time and displayed.

3. Register time-lapse experiment time.

Click the Add button.

The Timelapse dialog box is closed and the time-lapse experiment time set on the Time tab is registered.

To register multiple time-lapse experiment time, repeat this procedure.

Acquisition cycle	10	seconds 💌
Total time	10	minutes 💌
Rounds	60	
	As soon a:	s possible Oh OOm 34s.

Figure 3.4-5 Registering capturing interval time and total observation time

╶┓



Figure 3.4-6 Displaying interval time

Details on setting time-lapse experiment time

If the registered time-lapse experiment time cannot be executed, a warning mark appears in upper right corner of the time-lapse experiment scheme view.

Acquisition cycle	Total time	Rounds
0h 10m 00s	10h 00m 00s	60 Delete
		warning ma

3.4.2 Registering the Time-lapse Experiment Time for Stream Capturing

To perform stream capturing during a time-lapse experiment, configure the stream setting following the procedure shown below.

Stream setting limitations

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

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

1. Change the time-lapse experiment scheme view to "Time."

Click the Time tab on the New time-lapse setting screen.

Points	Time Cell name e	tc (Zstack)
1-1	(Ph 1/180s) 20x	(Delete)
1-2	(B-1E 1/4s)20x	Delete
		Clear

Figure 3.4-8 Selecting the Time tab

2. Set the stream time.

Click the New button.

The Timelapse dialog box (for new registration) appears.

Points Time	Cell name	e etc Zs	tack
Acquisition cycle	Total time	Rounds	
New			Clear

Figure 3.4-9 Clicking the New button

Check the Stream	setting checkbox.
------------------	-------------------

Set the stream capturing time in the Stream time box (setting range: 1 to 3600 seconds.)

The shortest interval time to be set is displayed below the Stream setting box.

Acquisition cycle	1	minutes 💌
Total time	2	minutes 💌
Stream blocks	2	18
Stream setting Stream time	10	sec
	As soon a	ıs possible Oh OOm 19s.

Figure 3.4-10 Setting the stream time

3. Set the time-lapse experiment time.

Input the capturing interval in the Acquisition cycle box. This value must be equal to or longer than the value shown below the Stream setting box.

Input the total observation time in the Total time box.

The number of Stream blocks (number of captures) is automatically calculated from values of Acquisition cycle and Total time.

Total time	La al	24 /12 /2 /2
rotar time	60	minutes 💌
Stream blocks	4	
▼ Stream setting		
Stream time	10	sec

Figure 3.4-11 Setting the Acquisition cycle and Total time

4. Register the time-lapse experiment time.

Click the Add button.

The Timelapse dialog box closes, and the registered time-lapse experiment time is displayed on the time-lapse experiment scheme view.

When Stream setting is enabled, the Time tab is replaced with Stream tab.

Repeat this procedure to register multiple time-lapse experiment times.

- 🔽 Stream setting Stream time	10	sec	
724	As soon a	is possible Oh OOm 19s.	
Add		Close	

Figure 3.4-12 Registering the Stream time

Acquisition cycle	Total time	Blocks	Stream	
0h 15m 00s	0h 45m 00s	4	10sec	(Delete)
New				Clear

Figure 3.4-13 Displaying the Stream setting



3.5 Changing and Deleting a Time-lapse Experiment Time

Change or delete the registered time-lapse experiment time when it cannot to be executed.

3.5.1 Changing the Time-lapse Experiment Time

1. Display the New time-lapse setting screen.

Click the New time-lapse setting button.



Figure 3.5-1 Displaying the New time-lapse setting screen

2. Select the time-lapse experiment time to be changed.

On the Time tab, click the time-lapse experiment time to be changed.

The Timelapse dialog box (for setting change) appears. Change the settings of the time-lapse experiment.

Points Time	Cell name e	c Zstack
Acquisition cycle	Total time	Rounds
0h 10m 00s	20h 00m 00s	121 Delete
New		Clear
Load		Save

Figure 3.5-2 Time

Time-lapse experiment time

To close the Timelapse dialog box with the changes applied, click the Apply button. To delete the selected time-lapse experiment time, click the Delete button.

To close the Timelapse dialog box without applying the changes, click the Close button.

elapse		
Acquisition cycle	10	seconds 💌
Total time	20	minutes 💌
Rounds	121	7
	As soon as po	ssible Oh 20m 34s.
Apply	Delete	Close

Figure 3.5-3 Timelapse dialog box

3.5.2 Deleting the Time-lapse Experiment Time

1. Display the New time-lapse setting screen.

Click the New time-lapse setting button.



Figure 3.5-4 Displaying the New time-lapse setting screen

2. Delete the time-lapse experiment time.

On the Time tab, click the Delete button shown next to the time-lapse experiment time to be deleted.

The delete confirmation dialog box appears.

Cell name e	tc Zstack
Total time	Rounds
20h 00m 00s	121 Delete
	Clear
	Save
	Cell name e Total time 20h 00m 00s

Figure 3.5-5 Deleting the time-lapse experiment time

To delete the selected time-lapse experiment time,
click the OK button.

To close the confirmation dialog box without deleting, click the Cancel button.



Figure 3.5-6 Delete confirmation dialog box

3.6 Performing a Time-lapse Experiment

1. Make sure that the microscope is ready for time-lapse experiment.

Check that the status of the culture chamber and humidifier water tank appears to be "Stable."



Click the Start time-lapse button.

The Confirmation window of time-lapse experiment settings appears.

When settings are appropriate for time-lapse experiment, click the Start time-lapse button. The Windows Save As dialog box appears.

To change the settings, click the Back to setting button.

Water 38.1 38.0 ÷ Temp Chamber 37.0 37.0 🕂 Outside 29.3 (unit:deg C) Stable Figure 3.6-1 Checking condition of the microscope for time-lapse experiment Water 38.1 38.0 ÷ Temp Chamber 37.0 37.0 + Outside 29.3 (unit:deg C) Stable Start time-lapse Points Time Cell name etc... Zstack I (Ph 1/180s) (FI1 1/4s) (FI2 1/4s) 20x Delete 🗹 2 (Ph 1/180s) (Fl1 1/4s) (Fl2 1/4s) 20x Delete Performing time-lapse experiment Figure 3.6-2 Confirmation window Total rounds 3 Total time Oh 01 m 00s Point X(um) Y(um) Z(um) Filter Objective 1919.45 1919.45 1919.45 1919.45 1919.45 1959.52 1959.52 1959.52 1959.52 1959.52 149.00 149.50 150.00 150.50 Ph Ph Ph Ph Ph Ph 20x 20x 20x 20x 20x 20x 20x 20x 34567 151.00 3319.92 4720.39 3600.40 2079.72 150.00 150.00 Start time-lapse Back to setting

Figure 3.6-3 Checking settings for time-lapse experiment

3. Save time-lapse experiment results in a file.

Input a file name for time-lapse experiment results and click the Save button.

The Time-lapse images in process screen appears and time-lapse experiment starts automatically.

Save As	? 🔀
Save in: 🗀 Image	· ← 🗈 📸
ा test.nex ति test_061017.nex	
File name:	Save
Save as type: Experiment files (*.nex)	Cancel

Figure 3.6-4 Saving the time-lapse experiment result file



Figure 3.6-5 Time-lapse images in process screen

3.7 Observing the Process of the Time-lapse Experiment

When time-lapse experiment is started, the Time-lapse images in process screen automatically appears. This screen shows process of time-lapse experiment. This screen cannot be displayed by operating any buttons.

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

For details on the Time-lapse images in process screen, see Chapter 4, "Time-lapse Images in Process Screen," in the separate manual, "BioStation IMQ Instructions <Application Software>."

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

Table 3.7-1	Channels display and Points	display
-------------	-----------------------------	---------

3.7.1 Channels Display



Figure 3.7-1 Time-lapse images in process screen (Channels)

3.7.2 Points Display

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



Figure 3.7-2 Time-lapse images in process screen (Points)

3.8 Changing Observation Conditions during a Time-Lapse Experiment

To change the observation conditions such as the coordinates of the registered observation points and exposure conditions during a time-lapse experiment, pause the time-lapse experiment and after changing the settings, restart the time-lapse experiment.

Note that the clock for time-lapse experiment does not stop even if the time-lapse experiment is paused. If a capture timing comes while the observation conditions are being modified, no image is captured and a black image is recorded.

1. Pause the time-lapse experiment temporarily.

On the Time-lapse images in process screen displayed during a time-lapse experiment, click the Pause button. The Pause confirmation dialog box appears.



Figure 3.8-1 Pause a time-lapse experiment

To pause the time-lapse experiment, click the Yes button.

To close the confirmation dialog box without pausing the time-lapse experiment, click the No button.

2. Change the observation conditions for time-lapse.

Click the Modify time-lapse setting button.



Figure 3.8-2 Pause confirmation dialog box



Figure 3.8-3 Changing the time-lapse settings

3. Select an observation point to change its setting.

On the Points tab, click the observation point to be changed.

The Point information dialog box appears.

1	(Ph 1/90s) (FI1 1/4s) (FI2 1/4s) 20x	Delete
2	(Ph 1/90s) (FI1 1/4s) (FI2 1/4s) 20x	Delete
/ 3	(Ph 1/90s) (FI1 1/4s) (FI2 1/4s) 20x	Delete
1	(Ph 1/90s) (FI1 1/4s) (FI2 1/4s) 20x	Delete
15	(Ph 1/90s) (FI1 1/4s) (FI2 1/4s) 20x	Delete

Figure 3.8-4 Selecting an observation point

Click the Go button on this dialog box. The objective is moved to the selected observation point and the live image for the observation point is displayed.

To cancel the changes, click the Close button. The Point information dialog box closes without applying the changes.

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

Figure 3.8-5 Point information dialog box

4. Change the observation conditions for registered observation point.

Operate the ergonomic controller or software to modify the coordinate and/or exposure conditions.

Operations are the same as that on the Live observation screen. (See Step 2-2, 4, and 6 in Section 3.1, "Examining the Specimen.")



Figure 3.8-6 Changing the time-lapse settings

5. Register and update the modified observation point and observation conditions.

Click the Time-lapse point registration button to register and update the observation point and the observation conditions.

	Start time-lapse	
Points Time Cell name etc Zstack		
✓ 1	(Ph 1/90s) (FI1 1/4s) (FI2 1/4s) 20x	(Delete)
2	(Ph 1/90s) (FI1 1/4s) (FI2 1/4s) 20x	Delete
✓ 3	(Ph 1/90s) (FI1 1/4s) (FI2 1/4s) 20x	Delete
✓ 4	(Ph 1/90s) (FI1 1/4s) (FI2 1/4s) 20x	Delete
✓ 5	(Ph 1/90s) (FI1 1/4s) (FI2 1/4s) 20x	Delete
Time-lapse point registration button		
Load Save		

Figure 3.8-7 Registering and updating observation point

6. Restart the time-lapse.

Click the Start time-lapse button to restart the time-lapse.

Point	s Time Cell name etc Z	stack
1	(Ph 1/90s) (FI1 1/4s) (FI2 1/4s) 20x	(Delete)
• 2	(Ph 1/90s) (FI1 1/4s) (FI2 1/4s) 20x	Delete
• 3	(Ph 1/90s) (FI1 1/4s) (FI2 1/4s) 20x	Delete
• 4	(Ph 1/90s) (FI1 1/4s) (FI2 1/4s) 20x	Delete
• 5	(Ph 1/90s) (FI1 1/4s) (FI2 1/4s) 20x	Delete

Figure 3.8-8 Restart the time-lapse

3.9 Checking Images after the Time-lapse Experiment

This screen automatically appears when time-lapse experiment completes. On this screen, loading and playing the saved file of time-lapse experiment results are available. There are the Channels display and the Points display for the Time-lapse images Acquired screen. Various functions are different from those of the Time-lapse images in process screen.

For details on the Time-lapse images Acquired screen, see Chapter 5, "Time-lapse Images Acquired Screen," in the separate manual, "BioStation IMQ Instructions <Application Software>."

3.9.1 Channels Display



Figure 3.9-1 Time-lapse images Acquired screen (Channels)

3.9.2 Points Display

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

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



Figure 3.9-2 Time-lapse images Acquired screen (Points)



Misuse of the product may adversely affect performance, even if the product is not damaged. If any of the following troubles occurs, be sure to check the following table for possible causes before requesting service.

Contact your nearest Nikon representative if the troubles cannot be resolved by taking the following countermeasures.

4.1 Troubleshooting on the Microscope Main Unit

4.1.1 Troubleshooting on Image Viewing

Trouble	Cause	Countermeasure
The field of view is	The light guide fiber is not connected correctly.	Connect it correctly. (See page 61.)
vignetted or uneven in brightness. The image is invisible or	The objective or specimen is contaminated with dirt or dust.	Clean the objective or specimen.
dark.	The specimen is stored in nonstandard case.	Store the specimen in the specified case.
Dirt or dust is seen in the viewfield.	The objective or specimen is contaminated with dirt or dust.	Clean the objective or specimen.
It is impossible to	The dish in use is not the specified dish.	Use the specified dish. (see page 13.)
focus on the target cell.	You are trying to focus on a floating cell.	Focus on the cell that exists on the bottom of the 35-mm dish. This product is designed to observe the specimen that exists on the bottom of the dish.
	The specimen setting part of the culture chamber is wet.	Clean the specimen setting part.
The image goes out of focus during time-lapse experiment.	The temperature in the product installation place changes rapidly.	Use the product in an air-conditioned room. However, do not install the product in the place where the product is directly blown by the wind from the air conditioner, nor the place in the temperature changes rapidly such place as near the door.
	The time-lapse experiment was started before the "STABLE" lamp lights up.	Start the time-lapse experiment after the "STABLE" lamp is lit.
	The time-lapse point was registered before the "STABLE" lamp lights up.	Register time-lapse points after the "STABLE" lamp is lit.
The XY position of the specimen shifts during a time-lapse experiment.	The specimen setting part of the culture chamber is wet.	Clean the specimen setting part.
Images in phase contrast microscopy are dark.	The diascopic illumination unit is moved to the escape position.	Place the diascopic illumination unit into the optical path.
The image is not	The thickness of the glass bottom dish is different from the reference value. (for CELL-S2)	Use the glass bottom dish whose thickness is 0.17 mm.
clear.	The adjustment of the correction ring of the objective is not correct. (for CELL-S2-P)	Turn the correction ring to set the mark to the thickness of the plastic bottom dish.

Trouble	Cause	Countermeasure
Image contrasts are poor in fluorescent observation.	The sliding shade is open and ambient light enters in.	Close the sliding shade.

4.1.2 Troubleshooting on Operation

Trouble	Cause	Countermeasure
	The power cord is not connected.	Connect it correctly. (See page 59.)
The product cannot be turned on.	The local voltage to be used is different from the voltage specified to the product.	Check the local voltage and connect the product to the proper power supply.
The temperature	Warming-up started after turning on the power does not complete yet.	Wait until warming-up completes. (See page 8.)
chamber is unstable. (The "STABLE"	The slide door remains opened, or it is frequently opened and closed.	Minimize opening of the slide door and wait until the temperature stabilizes.
lamp goes out.)	The ambient temperature changes rapidly.	Install the product in the place where the temperature is stable.
	The set temperature is extremely high or low.	Adjust the temperature to be controllable range in the culture chamber.
	The slide door remains open.	Close the slide door.
	The slide door was opened during warming-up.	Minimize opening of the slide door and wait until the temperature stabilizes.
	The cover on the filter cube port is removed.	Attach the cover on the filter cube port. (See page 9.)
The temperature inside the culture	The front door of the microscope remains open.	Close the front door.
chamber does not	The duct is tipped up.	Pull the duct down.
setting value.	Warming-up started after turning on the power does not complete yet.	Wait until warming-up completes. (See page 8.)
	The temperature sensors are not attached.	Attach the temperature sensors for the culture chamber and the humidifier water tank. (See pages 54 to 56.)
	The temperature control function or the temperature sensors is damaged.	Contact your nearest Nikon representative.
	The room temperature is extremely high or low.	Use the product at a temperature between 18 and 28°C.
CO₂ concentration does not increase.	The CO_2 cylinder is not connected, or the valve is shut.	Connect the CO ₂ cylinder correctly and open the valve.
	The tube for the CO ₂ mixer is disconnected.	Connect the tube for the CO_2 mixer correctly. (See page 57.)
	The CO ₂ mixer is turned off.	Turn on the CO ₂ mixer.
	The gas pressure in the CO_2 cylinder decreases.	Replace the CO ₂ cylinder.
	The CO_2 mixer or the CO_2 concentration sensor is damaged.	Contact your nearest Nikon representative.

Trouble Cause		Countermeasure
The ergonomic controller cannot be operated.	The cable is not connected correctly.	Connect it correctly. (See page 59.)
The temperature of the humidifier water does not rise nor reach the setting value.	The temperature sensor is not attached.	Attach the temperature sensor for the humidifier water tank. (See page 56.)
	Water in the humidifier water tank is not enough.	Add distilled water in the humidifier water tank. (See page 10.)
The pH of the culture medium changes rapidly.	The tubes are not connected correctly.	Connect the tubes correctly. (See page 57.)
	The rotatable cover on the culture chamber remains opened.	Close the rotatable cover on the culture chamber. (See page 13.)
	The gas pressure in the CO ₂ cylinder decreases.	Replace the CO ₂ cylinder with new one.
	The CO_2 mixer is damaged.	Refer to the instruction manual for the CO_2 mixer.
Cells die unexpectedly.	The excitation light is too strong.	Weaken the excitation light.
	The cells are subjected to the excitation light for a long time.	Shorten the time of excitation light irradiation.
Unable to perform the fluorescent microscopy.	The fluorescent filter is not in the optical path.	Attach the filter cube. (See page 9.)
	The HG precentered fiber illuminator is not turned on.	Turn on the HG precentered fiber illuminator.
	The light guide fiber is not connected correctly.	Connect the light guide fiber correctly. (See page 61.)
	The fluorescent shutter is closed.	Open the fluorescent shutter.
Condensation forms on the cover of the glass bottom dish for a long time.	The duct is tipped up.	Pull the duct down.

4.2 Troubleshooting on the BS-IM-MC MOT chamber

Trouble	Cause	Countermeasure
Dirt or dust is seen in the view field.The reference glass is contaminated with dirt or dust.Clean the reference glass		Clean the reference glass.
It is impossible to focus on the target cell.	The dish in use is not the specified dish.	Use the specified dish.
The image goes out of focus during time-lapse experiment.	The reference glass is contaminated with dirt or dust.	Clean the reference glass.
	Condensation occurs on the reference glass.	Adjust the temperature settings for the humidifier water tank and the amount of flow of the CO_2 mixer.
	The 4-well dish is not secured.	Place the dish fixing plate, and secure it tightening the screws.
	Too much culture medium is put in each well of the dish.	Put an appropriate amount of culture medium. (See page 12.)
Image contrasts are poor in phase contrast microscopy.	mage contrasts are poor in phase contrast microscopy.Observing point is close to the partition wall of the well.Since each partition wall is relation in the Y direction, observe the located near the center of the w (Y=2000 to 4000.)	

4.2.1 Troubleshooting on Image Viewing

4.2.2 Troubleshooting on Operation

Travela	0	0
Irouble	Cause	Countermeasure
It takes a long time to stabilize the temperature inside the culture chamber.	The lid of the culture chamber lost its heat for being left outside of the microscope.	Place the lid on the lid stand located inside of the microscope.
The pH of the culture medium changes significantly. Cells die unexpectedly.	The lid of the culture chamber is not placed properly.	Place the lid properly. (See page 14.)
Condensation occurs in the culture chamber.The CO2 mixer is turned on while microscope is warmed up.		Turn the CO_2 mixer on after the temperature of the microscope is stabilized.
The Well switch button is not displayed or well switching is not performed.	The power cable for BS-IM-MC MOT chamber is not attached.	Attach the power cable for BS-IM-MC MOT chamber, and then reboot the microscope and the application software.



5.1 Cleaning Lenses

Do not let dust, fingerprint, etc. get on the lenses. Dirt on the lenses, filters, etc. will adversely affect the view of image. If any of the lenses gets dirty, clean them as described below.

- Remove dust with a soft brush or lightly wipe off with a gauze.
- Only if there are fingerprints or grease on a lens, dampen lightly a piece of soft, clean cotton cloth, lens tissue, or gauze with absolute alcohol (ethyl or methyl) and gently wipe off the dirt.
- Do not use any solvents other than absolute alcohol as they may damage the lens adhesion surfaces. Especially, do not use petroleum benzine for the lenses or filters.
- Absolute alcohol is extremely flammable. Keep this flammable solvents away from fire or sparks emitted when turning on/off the power switch of the illuminator.
- Follow the instructions provided by the manufacturer when using absolute alcohol.

5.2 Cleaning Inside the Culture Chamber

- Use soft clean lens tissue, cotton cloth or gauze moistened with a little absolute alcohol (ethyl or methyl).
- When a specimen is spilled onto the culture chamber, check that the specimen is hazardous or not. If the specimen is hazardous, follow your standard facility procedures.
- The BS-IM-C chamber and the humidifier water tank can be removed and treated with the autoclave sterilization.
- The BS-IM-MC MOT chamber cannot be treated with autoclave sterilization. If the specimen is spilled into the BS-IM-MC MOT chamber and enters moving parts or gaps between the glasses, contact your nearest Nikon representative.

5.3 Cleaning the Exterior of the Product

- For persistent dirt, dampen a piece of gauze with diluted neutral detergent and wipe gently.
- · Using organic solvents may result in discoloration of the plastic parts.

5.4 Disinfection

- Disinfect the product with 70% medical alcohol in ordinary cases.
- When a specimen is spilled onto the microscope, check that the specimen is hazardous or not. If the specimen is hazardous, follow your standard facility procedures.
- Using organic solvents may result in discoloration of the plastic parts.

5.5 Maintenance of the Humidifier

- Check the amount of water in the humidifier water tank before operating and refill it if necessary.
- Water is poured into the evaporating dish as the exhaust air from the culture chamber contents water supplied by the humidifier.

Clean the evaporating dish if necessary.

5.6 Storage

- Store this product in a dry place where mold is not likely to form.
- Put the dust-proof cover over this product to protect it from dust.
- For using an illuminator or etc., turn off the power (flip the power switch to the "O" side) of this product and wait until the lamp house cools down completely before setting the dust-proof cover back.

5.7 Regular Inspections (Charged)

Regular inspection (charged) is recommended to maintain the performance of the product. Contact your nearest Nikon representative for details.



6.1 Operation Principles

Manipulate the Control PC or ergonomic controller to move the observation position or change the magnification and observe or capture an image of a cultured specimen on or in a vessel such as a Petri dish.

6.2 **Performance Properties**

Model		CELL-S2	CELL-S2-P	
Imaging optics	Configuration	A single objective and a second-objective combination Variable magnification by selecting a second-objective		
	Objective	f = 5, NA = 0.8 Plan Fluor 40x DL (specially designed for the culture microscope)	f = 10, NA = 0.5 Plan Fluor 20x DL With a correction ring (correction range: 0.6 to 1.4 mm) (specially designed for the culture microscope)	
	Intermediate magnification (second-objective)	0.5x (f = 100) / 1.0x (f = 200) / 2.0x (f =	= 400)	
	Optical magnification	20x/40x/80x	10x/20x/40x	
Tiled	Phase contrast microscopy	6 minutes	1 minute 30 seconds	
image capture time	Fluorescent microscopy (1 channel)	13 minutes 20 seconds	2 minutes 20 seconds	
(full view) Phase * ¹ fluores micros	Phase contrast, fluorescent-1, fluorescent-2 microscopy (3 channels)	46 minutes 7 minutes 20 seconds		
1 round	One observation point	BS-IM-C chamber: 1 second		
time for a time-lapse experiment	Phase contrast microscopy (1 channel)	BS-IM-MC MOT chamber * ² AF-ON mode: 1 minute 25 seconds AF-OFF mode: 30 seconds		
	One observation point	BS-IM-C chamber: 2 seconds		
	Fluorescent microscopy (1 channel)	BS-IM-MC MOT chamber * ² AF-ON mode: 1 minute 30 seconds AF-OFF mode: 35 seconds		
	Five observation points	BS-IM-C chamber: 25 seconds		
Phase contrast microscop (1 channel)	Phase contrast microscopy (1 channel)	BS-IM-MC MOT chamber * ² AF-ON mode: 2 minutes 50 seconds AF-OFF mode: 2 minutes		
	Five observation points	BS-IM-C chamber: 30 seconds		
	Fluorescent microscopy (1 channel)	BS-IM-MC MOT chamber * ² AF-ON mode: 3 minutes 10 seconds AF-OFF mode: 2 minutes 25 seconds		
	Five observation points	BS-IM-C chamber: 60 seconds		
	Phase contrast, fluorescent-1, fluorescent-2 microscopy (3 channels)	BS-IM-MC MOT chamber * ² AF-ON mode: 5 minutes 5 seconds AF-OFF mode: 4 minutes 20 seconds		

Model		CELL-S2	CELL-S2-P
Dish for BS-IM-C chamber specimens		35-mm glass bottom dish / 35-mm film bottom 4-quadrant dish (Hi-Q4)	35-mm plastic bottom dish
	BS-IM-MC MOT chamber	Glass bottom 4-well dish (model No. 155383, manufactured by Nalge Nunc International K.K.)	Cannot be mounted

*1: The tiled image capture time (magnifications: Full) and the 1 round time for a time-lapse experiment shown above are a guide value for the standard specifications. They vary depending on the observation points and exposure conditions.

*2: The time shown above indicates that the same number of observation points is set for each of the four wells for the BS-IM-MC MOT chamber.

Camera Environmental condition to be controlled	DS-Qi1Mc CCD device: Monochrome 2/3 inch, 1280 x 960 pixel Flame rate 10 fps, progressive scan Cooling temperature: Ambient = -5° C Quantization: 12 bits Culture chamber The temperature, humidity and CO ₂ concentration are controlled. (For CO ₂ , the CO ₂ mixer controls the concentration.) Temperature setting range for the culture chamber: 32 to 38°C Standard environmental conditions: Temperature 37°C, humidity 95% or more, CO ₂ 5%		
Observation means	With a PC monitor or so on (No eyepiece is equipped.)		
Operation method	Center control with a PC Ergonomic controller		
Місгоѕсору	Epi-fl microscopy and diascopic phase contrast microscopy		
Light source	Fluorescent light source: HG Precentered fiber illuminator (separate-typed) Diascopic light source: Built-in high intensity red LED (wavelength: 625 nm)		
Observable range in the X and Y directions	 BS-IM-C chamber (standard) 6 mm x 6 mm (The specimen position is fixed but the objective position moves horizontally.) BS-IM-MC MOT chamber (optional) Objective movement: 6 mm x 6 mm Well switching function: 36.6 mm (12.2 mm x 3) in the Y direction 		
Stroke in the Z direction	1.25 mm (The objective position moves vertically.)		
Interface	 Microscope USB 2.0 device x 2 (for CCD camera x 1 and for microscope x 1) RS232C x 1 (for HG precentered fiber illuminator) Special interface for the ergonomic controller Special interface for an external device 		
Input rating	100 VAC to 240 VAC, ±10%, 50/60 Hz, 3 A maximum		
Power cord	 For countries where the supply voltage is 100 V to 120 V (excluding Japan): UL Listed detachable cord set (3 conductor grounding Type SVT, No. 18 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 set (3 conductor grounding Type HO5VV-F, 3 m long maximum, rated at 250 VAC minimum For Japan: Power cord set conforming with the Electrical Appliance and Material Safety Law (with PSE mark) (3 conductor grounding Type VCTF3 x 0.75 mm² 3 m long maximum rated at 125 VAC minimum) 		

Software	OS: Windows XP professional SP2 E Windows Vista Business 32 bits SP1 E Windows 7 professional 32 bits E	
Operating condition	Temperature: Humidity: Altitude: Pollution degree: Installation category: Electric shock protection class: Indoor use only	18 to 28°C 85% RH maximum 2000 m maximum Degree 2 Category II Class I
Storage condition	Temperature: Humidity: Altitude: Pollution degree: Installation category: Electric shock protection class: Indoor use only	0 to 40°C 85% RH maximum 2000 m maximum Degree 2 Category II Class I

6.3 Physical Properties

Transportation condition (when the product is packed)	Temperature: Humidity:	-20 to +50°C 90% RH maximum (no condensation)
External dimensions and weight	External dimensions: Weight:	220 mm (width) x 400 mm (height) x 620 mm (depth) (excluding protrusions) Approximately 30 kg
Safety standard compliance	 FCC 15B Class A satisfied. This equipment has been to digital device, pursuant Par provide reasonable protect operated in a commercial e This equipment generates, installed and used in accom- interference to radio comm area is likely to cause harm to correct the interference of This class A digital apparat numérique de la classe A e This product complies with CE Marking In vitro diagnostic medical of Compliance with which EN EN1658 and EN60825-1. Low voltage directive satisf EMC directive 	ested and found to comply with the limits for a Class A t 15 of the FCC Rules. These limits are designed ion against harmful interference when the equipment is environment. and can radiated radio frequency energy and, if not dance with the instruction manual, may cause harmful unications. Operation of this equipment in residential iful interference in which case the user will be required own expense. us complies with Canadian ICES-003.Cet appareil est conforme à la norme NMB-003 du Canada. Australian EMI (AS/NZS CISPR11).



7.1 Assembly and Connection

Be sure to read the "Safety Precautions" and follow the all instructions given there before assembling the product.

7.1.1 System Configuration

The BioStation IMQ is composed of the following parts.



Figure 7.1-1 Configuration of the BioStation IMo

Necessary tool

The following tools are required to assemble the product.

Hexagonal screwdrivers (accessory)

7.1.2 Removing the Fixture

Caution in removing the fixture

When removing the fixture, be sure not to drop fixture securing bolts into the product. If the product is operated with the dropped bolts left inside, it may result in a malfunction.

Loosening the bolt securing the diascopic illumination unit

Open the slide door of the microscope and remove the fixture.

The diascopic illumination unit is secured with a hexagonal socket head bolt (1 piece). Fully loosen the bolt with the hexagonal screwdriver (2 mm).

And then, retract the diascopic illumination unit.



Figure 7.1-2 Loosening the bolt securing the diascopic illumination unit

Removing the fixture for the Z moving part of the objective

The fixture for this position is a screw-in type. Remove the fixture by rotating counterclockwise by hands.



Figure 7.1-3 Fixture of the Z moving part of the objective (Detached)



Figure 7.1-4 Removing the fixture for the Z moving part of the objective

Removing the fixture for the XY moving part of the objective

The fixture for this position is a screw-in type. Remove the fixture by rotating counterclockwise by hands.



Figure 7.1-5 Fixture for the XY moving part of the objective (Detached)

Removing the fixture for the floating unit

The fixture for the floating unit is secured with seven hexagonal socket head bolts (M3: three pieces, M4: four pieces). Remove the bolts with the hexagonal screwdriver (2.5 mm and 3 mm).



Figure 7.1-7 Fixture for the floating unit (Detached)



Figure 7.1-6 Removing the fixture of the XY moving part of the objective



Figure 7.1-8 Removing the fixture for the floating unit

Removing the bar clamping the magnification selector dial and the filter magazine

Remove the cover of the filter cube port and check that the clamp bar is attached.

The clamp bar is secured with three M4 hexagonal socket head bolts. Loosen the bolts with the hexagonal screwdriver (3 mm).

* Loosen but not remove those bolts.

Hexagonal socket head bolt (three pieces)



Clamp bar

Figure 7.1-9 Removing the bar clamping the magnification selector dial (1)

Rotate the clamp bar counterclockwise by 90 degrees around the hexagonal socket head bolt located at the center as shown in the figure at right.

Secure the clamp bar, tightening the hexagonal socket head bolt located at the center.

Retighten the other two bolts securely so as not to fall.



Hexagonal socket head bolt (three pieces)

Figure 7.1-10 Removing the bar clamping the magnification selector dial (2)

7.1.3 Attaching Accessories

Attaching the culture chamber

1. Open the slide door.



Figure 7.1-11 Opening the slide door

Diascopic illumination unit

Figure 7.1-12 Retreating the diascopic illumination unit



Figure 7.1-13 Attaching the culture chamber

2. Retreat the diascopic illumination unit. Additionally, tip up the duct.

3. Attach the culture chamber. BS-IM-C chamber

Set the BS-IM-C chamber and secure it with three hexagonal socket head bolts using the hexagonal screwdriver (2.5 mm.)

BS-IM-MC MOT chamber

Set the BS-IM-MC MOT chamber and secure it with three knurled screws.

4. Insert the temperature sensor into the culture chamber.

The temperature sensor port on the culture chamber is magnetic.

When the temperature sensor reaches the mounting position, clicking noise occurs and it is secured with the magnetic mechanism.

Be sure to attach the temperature sensor.

If no temperature sensor is attached, the temperature in the culture chamber cannot be controlled properly.



Figure 7.1-14 Attaching the temperature sensor

Attaching the humidifier water tank

- Pour distilled water into the humidifier water tank.
 Pour distilled water into the humidifier water tank until it reaches little below the "max" line.
- 2. Open the front door of the microscope. Place a silicon sheet on the humidifier heater.



Figure 7.1-15 Opening the front door



Figure 7.1-16 Retracting the clamp screw for the humidifier water tank

3. Loosen the clamp screw for the humidifier water tank and retract it.

4. Set the humidifier water tank on the humidifier heater.

Make sure that the lid gasket seals the top of the tank completely.



Figure 7.1-17 Setting the humidifier water tank

5. Insert the clamp screw for the humidifier water tank into the original position and tighten it.

Caution in tightening the clamp screw for the humidifier water tank

When tightening the clamp screw for the humidifier water tank by hand, do not tighten it excessively. It might cause the humidifier water tank crack.

6. Insert the temperature sensor into the humidifier water tank.



Be sure to attach the temperature sensor.

If no temperature sensor is attached, the humidification cannot be controlled properly.

7. Place the evaporating dish to the specified position.



Figure 7.1-18 Securing the humidifier water tank



Figure 7.1-19 Setting the temperature sensor



Figure 7.1-20 Setting the evaporating dish

7.1.4 Connecting the Tubes

Connect the culture chamber, humidifier water tank, CO₂ mixer, regulator, and CO₂ cylinder with the tubes.

For CO_2 mixer, regulator, and CO_2 cylinder handling, follow the instructions in the manual supplied with the unit or from the manufacturer.

The piping from the CO_2 cylinder to the CO_2 mixer has a section with high CO_2 concentration (100%) and high pressure.

Leakage from this section may lead to a high possibility of suffocation, and handle it with extra care. Check periodically if any cracks or looseness of the tubes and connected areas exist.

Use the CO₂ mixer whose specifications match below.

CO₂ mixed gas concentration: 5% (adjustment desirable)

CO₂ mixed gas flow: 150 ml (adjustment desirable)

CO₂ mixed gas pressure: approx. 0.02 MPa

When a 5% CO_2 cylinder is connected, the CO_2 mixer is not necessary; however, a flow meter is required instead.

The regulator with a flow meter is also available in this case.

Follow the above specifications for the flow and the pressure of CO_2 mixed gas concentration if the regulator with a flow meter is used.

The CO₂ concentration may have to be adjusted when relatively weak cells such as primary cells are used.

It is recommended to use the 100% CO₂ cylinder and the CO₂ mixer together.



Figure 7.1-21 Piping diagram (BS-IM-C chamber)



When connecting tubes to the BS-IM-MC MOT chamber, be sure to use the tubes supplied with the chamber. Connect the wet mixed gas tube to the IN port of the chamber, and the exhaust tube to the OUT port. The positions of the IN/OUT ports are reversed from the ones for the BS-IM-C chamber.



Figure 7.1-23 Surroundings of the humidifier water tank



Figure 7.1-24 Surroundings of the culture chamber

7.1.5 Connecting the Cables

Connect the microscope, control PC, HG precentered fiber illuminator, CO_2 mixer, and ergonomic controller with the cables.



USB cable connection

Connect the microscope and the control PC with two USB cables.

1. Connect the camera USB cable (blue).

Use the blue USB cable to connect the upper USB connector (Camera USB) on the back of the microscope to the upper left USB connector on the back of the control PC.

2. Connect the microscope USB cable (black).

Use the black USB cable to connect the lower USB connector (Microscope USB) on the back of the microscope to the lower left USB connector on the back of the control PC.

USB cable connection on the back of the control PC shown at right below is an example of the back panel of the XW-6400 manufactured by Hewlett-Packard Co.



Figure 7.1-26 USB cable connection on the back of the microscope



Figure 7.1-27 USB cable connection on the back of the control PC

3. Connect the USB cables of devices such as a mouse and a keyboard.

For the USB cables of devices such as a mouse and a keyboard, connect them to vacant USB connectors other than the two connectors on control PC described above.

Caution for the USB cable connections

If you change the connector for the USB cable after the setup, the OS of the control PC may request you to reinstall three device drivers for this product. To avoid this, connect the USB cables to the locations specified in this section and do not change the connections. If the OS of the control PC requests you to reinstall the device drivers, check that the USB cables are connected to the specified connectors.

If a USB cable is connected to an incorrect connector, reconnect it to the specified connector.

Additionally, be sure to use the provided USB cables for operational stability.
Attaching the light guide fiber

1. Insert the light guide fiber.

Insert the light guide fiber into the light guide fiber port on the rear of the microscope as far as it goes.

The light guide fiber is inserted by approximately 17 cm into the system.

2. Remove the cover for the light guide fiber clamping point.

Secure the light guide fiber.

the light guide fiber clamping point.

screwdriver (2.5 mm).

First make sure that the clamp screw is loose enough to permit the fiber to be fully inserted until it stops and then tighten the clamp screw with the hexagonal

After securing the light guide fiber, attach the cover for

And then, pull the light guide fiber to make sure that it



Figure 7.1-28 Inserting the light guide fiber



Figure 7.1-29 Removing the cover for the light guide fiber clamping point



Figure 7.1-30 Attaching the light guide fiber

is secured.

3.

Caution for the light guide fiber attachment

Before attaching the light guide fiber, loosen the clamped floating unit. When the floating unit is clamped, the guide hole on the housing is not aligned with the attaching fit hole on the floating unit, and the light guide cannot be fully inserted to the end.

7.1.6 Attaching the Fixture

Before carrying or shipping the microscope, attach the fixture to the microscope.

Setting the movable parts to clamping positions

Before the fixture can be attached, all moving parts must be in the proper position.

- 1. Turn off the power switch of the microscope. The cover of the filter cube port must be attached. If not, the filter cube switching mechanism does not move to the clamping position.
- 2. While pressing any two buttons on the front panel of the ergonomic controller, turn on the microscope.

You can release the two buttons once the floating unit starts to move.

3. Each movable part moves to the clamping position automatically after initialization. When all movable parts move to their clamping positions, all LED indicator on the upper surface of the

product blink for approximately three seconds.

Turn off the power switch of the microscope. 4.







Figure 7.1-32 Ergonomic controller

Attaching the fixture

The microscope has five clamp positions as described below. Follow the removal procedure in reverse order to attach the fixture to the microscope.

- * For clamping procedure, refer to "Removing the Fixture" in this chapter.
- Clamping the magnification selector dial and the filter magazine
- Attaching the fixture for the floating unit
- Attaching the fixture for the XY moving part of the objective
- Attaching the fixture for the Z moving part of the objective
- Clamping the diascopic illumination unit

CAUTION

Caution for the floating unit clamping

Before clamping the floating unit, remove the light guide fiber.

If the floating unit is clamped while the fiber is attached, excessive force is applied to the edge of the fiber, and the fiber may be unable to be removed or even may be damaged.

7.1.7 Parts Treatable in Autoclave Sterilization

The parts shown below can be treated in autoclave sterilization.

However, after treating each part with an autoclave, dry it completely with an apparatus such as dryer (60° C).



Figure 7.1-33 Parts treatable in autoclave sterilization

These parts have not been sterilized when arrived. Sterilize them as necessary. Note that the sterilizing filter is also not sterilized.

The sterilizing filter and tubes are consumables. They must be replaced when deteriorated or degraded.

Do not treat BS-IM-MC MOT chamber in autoclave sterilization.

7.2 Operating the Ergonomic Controller

7.2.1 Changing the Focus Knob Position

Default setting of the ergonomic controller is as follows: the right knob moves the stage in the X and Y directions and the left knob controls focusing.

If the right and left knobs are switched, the knob on the left side can move the stage in the X and Y directions and another knob on the right side can control focusing.

This chapter indicates the procedure for switching these knobs.



Figure 7.2-1 Default setting of the ergonomic controller

The focus knob is attached by a magnet and can be

Remove the focus knob on the left.

removed by hand.

1.



Figure 7.2-2 After switching the knobs on the ergonomic controller



Figure 7.2-3 Removing the focus knob on the left



Figure 7.2-4 Loosening the clamp screw fixing the handle arm

2. Use the hexagonal screwdriver (2 mm) to loosen the clamp screw fixing the bottom of the handle arm.

3. Remove the focus knob on the right. Retract the handle arm to the other side and gently remove the focus knob on the right by hand.

4. Attach a focus knob to the right and left sides. Switch the right and left knobs and attach each of them. To attach the focus knob, align the locating pin on the focus knob with the locating pin hole on the ergonomic controller and then attach the focus knob.



Figure 7.2-5 Removing the focus knob on the right



Figure 7.2-6 Attaching the focus knob



Figure 7.2-7 Focus knob

5. Tighten the clamp screw fixing the bottom of the handle arm.

Rotate the handle arm back to the other side and tighten the clamp screw.



Figure 7.2-8 Securing the handle arm

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7.2.2 Adjusting the X Stage Knob and the Y Stage Knob

Adjusting the height of the knobs

The height of the X stage knob and the Y stage knob can be changed. Hold the knobs and move them vertically to your convenient height.



Adjusting the torque of the knobs

There are torque adjustment wheels between the X stage knob and the Y stage knob. Move down the X stage knob and move up the Y stage knob to their limits. To increase the torque of a knob, rotate the wheel so that it moves closer to the knob. (To increase the torque, rotate the Y stage knob torque adjustment wheel counterclockwise viewed from the top, and rotate the X stage knob torque adjustment wheel clockwise viewed from the top.)





7.3 Notes on Using the Film Bottom 4-Quadrant Dish

Setting Hi-Q4 (film bottom 4-quadrant dish) in the Preference menu displays a cross line on the observation point indication (pink crosshair), indicating the registered time-lapse observation point belongs to which quadrant (I to IV).

For information, see Section 3.2.1, "Observation Point Verification Display" in the separate manual, "BioStation IMQ Instructions <Application Software>."

When the 4-quadrant dish is used for the first time, capture a tiled image on the Wide field screen, and then check the positional relation between the cross line displayed there and the disk partitions for four quadrants.

Usually, the cross line is in the partition thickness, but if the position where the culture chamber is secured to the main unit slips out of place, the partitions may be off from the cross line. If so, loosen the clamp bolt for the culture chamber, adjust it, and then secure it again.

For details on capturing a tiled image, see Section 3.2, "Wide Field Screen" in the separate manual, "BioStation IMQ Instructions <Application Software>."

If the partitions of the 4-quadrant dish do not align with the cross line, the quadrants may be incorrectly identified when a place close to a partition is registered as a time-lapse observation point.



Figure 7.3-1 New time-lapse setting screen (Wide field screen) (When Hi-Q4 is set)

7.4 Setting up the BS-IM-MC MOT chamber

When attaching the BS-IM-MC MOT chamber to the microscope for the first time or after re-attaching the chamber, register the position of the reference mark as described below. Check that the "STABLE" lamp of the LED indicator is lit before performing this procedure.

1. Click the Marker seek button on the reference mark register screen (displayed upon software startup).

The area around the reference mark is captured (three images each in the X and Y directions), and displayed on the screen in a tiled pattern.

Three images are captured for the Z direction also (reference position/positive side/negative side).



Figure 7.4-1 Reference mark register screen

- 2. Move the Z-axis scroll bar to display the screen with the clearest focus on the reference mark.
- **3.** Double-click the tile with the reference mark. The clicked image is enlarged.



Figure 7.4-2 Tiled display of the area around the reference mark

4. Move the reference mark into the blue frame at the center of the screen using the jog dial or the ergonomic controller.



Figure 7.4-3 Positioning the Reference mark



Figure 7.4-4 Ergonomic controller (stage movement)



Figure 7.4-5 Registering the AF position of the Reference mark

5. Click the Mark registration button.

The AF positions of the reference marks for wells 1 to 4 are registered in order.

This will take about four minutes.

When registration is complete, the Live observation screen appears.

Automatic focus (AF)

For position calibration, AF is performed on the reference marks written on the reference glass that is attached at the bottom of the dish mounting part. Single reference mark is aligned with each well of the dish.

Calibration is not carried out when the well is switched on the Live observation screen.

Calibration is carried out to make the observation point registration available each time the well is switched on the New time-lapse setting screen.

Calibration OFF mode (About the New time-lapse setting screen)

By default, the calibration using the reference marks is carried out each time the well is switched. However, the mode not to perform the calibration (Calibration OFF mode) is available for a quick setting. Do not select the Calibration OFF mode for promoting the position accuracy.

Calibration ON mode:	Well switching takes about 15 seconds due for completion of AF.
	1 round for a time-lapse experiment takes about 1 minute (for four calibrations.)
Calibration OFF mode:	Well switching takes only about 1 second. 1 round for a time-lapse experiment

takes about 5 seconds.

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