KEYENCE

96M12292

All-in-one Fluorescence Microscope

BZ-9000 Series

Simple Operation Guide: Capturing Images

- Bright field observation procedure
- Phase contrast observation procedure
- Fluorescence observation procedure
- Maintenance

This guide is a summary of points for capturing clear images with basic functions. Refer to the "BZ-9000 User's Manual" for hardware details, and the "BZ-II Analyzer Reference Manual" for software analysis package details.

Introduction

Thank you for using the BZ-9000 (BIOREVO) all-in-one fluorescence microscope. This product allows even first time users to easily capture beautiful images. In addition, the BZ-9000 can quickly and easily quantify images using the analysis software package.
This guide is a summary for capturing clear images using basic functions. Be sure to read Section I, Preparation before Capturing Images, prior to use.

Safety Precautions

Symbols

The following symbols alert you to important messages. Be sure to read these messages carefully.

⚠WARNING

Failure to follow these instructions may lead to injury (electric shock, burn, etc.).

⚠CAUTION

Failure to follow these instructions may lead to a product damage.

Provides information on operations that can be easily mistaken.

Provides advanced and useful information about the operation.

Provides reference information about the operation.

General cautions

- At startup and during operation, be sure to monitor the functions and performance of this device.
- We recommend that you take substantial safety measures to avoid any damage in the event of a problem.
- Functions and performance are not guaranteed if the device is modified or used in any way other than described in the specifications.
- When the device is used in combination with other devices, functions and performance may be degraded, depending on the operating conditions and surrounding environment. The information and contents described herein are subject to change without prior notice.

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Tips on Correct Use

Be sure to follow the precautions below in order to use the device correctly and to prevent any malfunction.

Handling

Cautions regarding usage

⚠WARNING

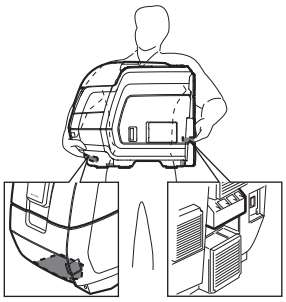
- Turn off the main power of the device before connecting cables or performing maintenance procedures to prevent electric shock.
- Do not bend the power cable or put heavy objects on it. The cable may become damaged and lead to a fire or electric shock. Do not use a damaged cable.
- The power supply code set is not provided with BZ-9000 series. Use the code set that complies with the regulations and standards in the country or region in which the BZ-9000 series are used.
- Do not allow any foreign material to enter the device. Doing so may cause a fire, fault or electric shock.
- Do not remove the case cover. Touching the inside of the device may result in an electric shock.
- Looking directly into the condenser lens of the transparent illumination unit or the transmitted light of the mercury lamp may cause eye damage. Be sure to turn off the main power when mounting or dismounting the lens.
- Replace the mercury lamp or halogen lamp only after the main power has been off for 30 minutes or longer. The lamp is extremely hot and may cause burns.
- Do not touch the motorized stage while it is moving to prevent a hand or finger from being caught.
- This device is Class I equipment. When installing this device, connect the protective grounding terminal of the power supply cord set to the protective grounding conductor of the installation place. Otherwise, it may cause electric shock or product damage.

⚠CAUTION

- Apply the correct power voltage to prevent any malfunction.
- Do not wipe the device with thinner or organic solvent. Doing so may damage the device.
- If the BZ-9000 series becomes dirty, wipe with a dry cloth.
- Do not sit or stand on the device. Doing so may damage the device.
- Do not put things on the device. Doing so may damage the device.
- Be sure to turn off the main power when connecting or disconnecting the power cable. Doing so may damage the device.
- The mercury lamp may burst once its lifetime has expired. The lamp is completely encased in protective glass, so as to prevent any damage in the event that it bursts. Be sure to replace the lamp when it reaches the end of its life to prevent bursting or hearing a cracking sound.
- The device uses precise optical parts. Avoid any vibration or impact. Doing so may damage the device.
- Do not remove or modify the case cover of the device.
- Do not place any object that weighs 300 g or more on the stage. Doing so may damage the device.
- Do not disassemble or modify the device. Doing so may cause a fire or electric shock.

⚠ CAUTION

- When carrying the device, close the panel and disconnect all the cables, and then carry it by the recessed parts (handles) on the base and the rear panel. Carrying the device by other parts may cause damage.



- Do not turn off the main power switch or disconnect the power cable immediately after turning off the POWER switch. The cooling fan for the super-high-compression mercury lamp will not be able to function, which not only shortens the lamp life, but also causes excessive inner heat and leads to a malfunction. The fan stops automatically in three minutes (while the fan is spinning, STABILITY (red) on the status indicator LED flashes). Confirm that the super-high-compression mercury lamp cooling fan has stopped (STABILITY on the status indicator LED should be off) before turning off the main power switch or disconnecting the power cable.
- When turning on the power, be sure to do so after three minutes or more has elapsed since the power was turned off. Otherwise the life of the super-high-compression mercury lamp will be shortened.
- Due to certain characteristics of the mercury lamp, when the power is turned on immediately after being turned off, the lamp may not light up even though the power is turned on. Immediately turn off the power in such a case, and turn it on again after three minutes or more has elapsed.
- Do not unplug the IEEE1394 cable when the device and the control PC are in communication (when the BZ-II Viewer is running). Doing so may damage the device.

Important

The device uses the hard disk drive (HDD) of the control PC to save image data. If the HDD malfunctions, the data stored in the HDD may be lost. Be sure to periodically back up the data stored in the HDD. It is recommended to back up important data immediately. KEYENCE does not guarantee the data stored in the hard disk drive (HDD) of the control PC at the time of repair. When repairing the control PC, be sure to back up the data.

Precautions at the time of a malfunction

⚠ CAUTION

Turn off the power immediately in the following cases. Using the device in abnormal conditions could cause a fire, electric shock, or fault. Contact your nearest KEYENCE office for repair.

- When foreign material or water enters the device.
- When the device is dropped or the case is damaged.
- When the device emits smoke or a strange odor.

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Preparation before Capturing Images

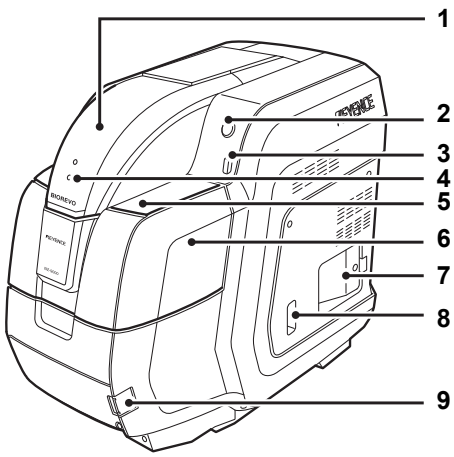
Bright Field Observation

Phase Contrast Observation

I Preparation before Capturing Images

Part names

■ Main unit



1 Transparent illumination unit

The halogen illumination is built-in with the condenser lens and used when observing bright field and phase contrast. Raise it up when mounting a specimen.
When it is raised, the illumination automatically turns off.

2 POWER button

Turn on the power (the main power switch on the rear must already be turned on).
Pressing it once again turns the power off.
"2-8 Startup and Shutdown" (page 2-14) in "BZ-9000 User's Manual"

3 Panel open button

Press this button to open the panel.

4 Status indicator LED

- FLUORESCENCE (white): Illuminates when the mercury lamp is turned on.
- STABILITY (red): Illuminates/flashes depending on the status of the BZ-9000E.

5 Light shielding plate

Can be removed when mounting a specimen. Reinstall it before starting observation.

6 Panel

Open this when mounting a specimen.

7 Mercury lamp house

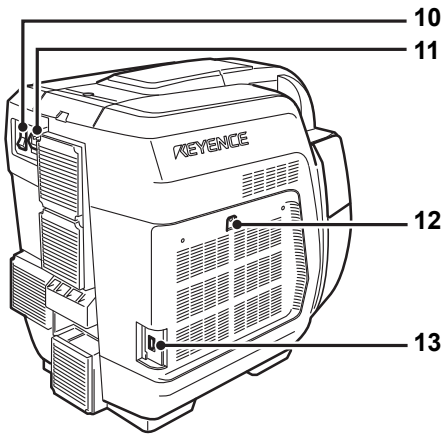
The mercury lamp unit can be replaced from here.
"12-2 Replacing the Mercury Lamp Unit" (page 12-4) in "BZ-9000 User's Manual"

8 Filter slot

Install the diffuse/neutral density filter here.
"2-9 Settings and Installation - Installing the diffuse/neutral density filter" (page 2-25) in "BZ-9000 User's Manual"

9 Filter turret cover button

Press this button to open the filter turret cover.



10 Main power switch

The main power of the BZ-9000E.

"2-8 Startup and Shutdown" (page 2-14) in "BZ-9000 User's Manual"

11 AC 100 to 240 V Inlet appliance

Connect to the power supply with AC power cable.

"2-2 Installing the cable" (page 2-3) in "BZ-9000 User's Manual"

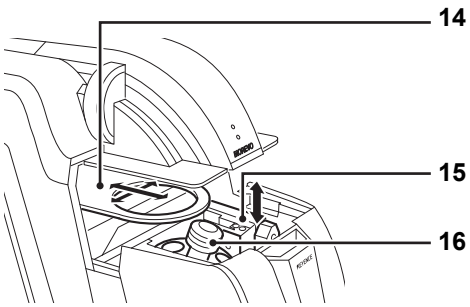
12 Light shielding plate hook

The light shielding plate can be hung here when not in use.

13 IEEE1394 connector (6-pin type)

Connect to the control PC with the provided IEEE1394 cable.

"2-2 Installing the cable" (page 2-3) in "BZ-9000 User's Manual"



14 Motorized XY stage

Moves a specimen in the X and Y-axis directions. Use this to move the observing position of the specimen.

15 Motorized Z stage

Moves the lens in the Z-axis direction. Use this to adjust the focus.

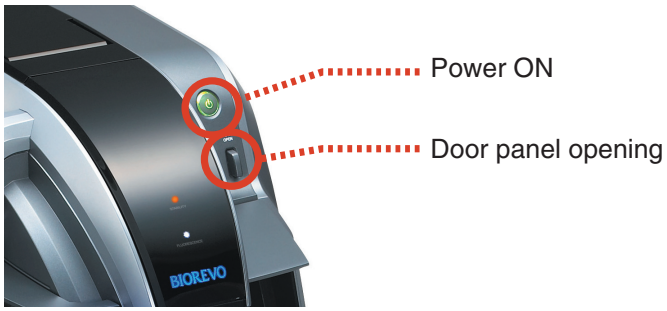
16 Electric revolver

Mount the objective lenses here.

"2-9 Settings and Installation - Mounting the objective lens" (page 2-18) in "BZ-9000 User's Manual"

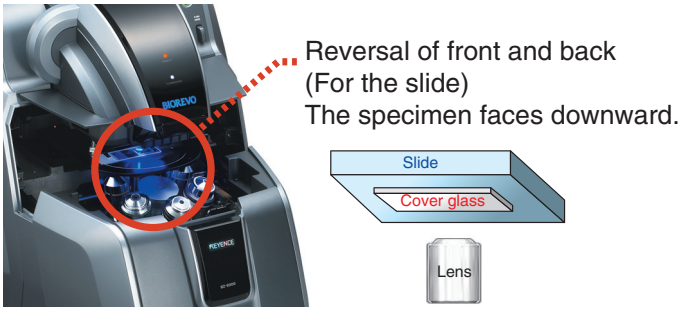
Turning on the power to open the door panel

Turn on the main power switch to the rear of the main unit and then press the power button on the front of the main unit.



Notes on mounting the specimen

For a slide, mount the specimen facing downward.



Cleaning a lens

If a lens is dusty or dirty, the quality of images will deteriorate. Keep lenses covered when not in use to cut down on dust and debris.

<Cleaning method>

Use a lint-free lens cloth or lens tissue to lightly wipe the lens surface in a circular motion. Move from the center of the lens to the outer edge to remove dust and debris.

If the lens is still dirty, sparingly wet a lens cloth or tissue with absolute alcohol and repeat wiping. Then, use a dry cloth or tissue to remove any alcohol residue from the lens.

Never spray or wet the lens directly, always moisten a lens-specific cloth or tissue first.

Selecting a lens

Always use the appropriate lens based on the observation container.

<Selection criteria>

Select a lens according to the thickness of the transparent observation part of a container.

"Optimum lens based on observation container"

Lens name	Slide (cover glass facing downward)	Slide (cover glass facing upward)	Glass bottom dish	Plastic petri dish	Well plate	Phase contrast observation
PlanApo2x	○	△	○	○	○	×
PlanApo4x	○	△	○	○	○	×
PlanApo10x	○	△	○	○	○	×
PlanApo20x	○	×	○	×	×	×
PlanApo40x	○	×	○	×	×	×
PlanApo60x (immersion)	○	×	○	×	×	×
PlanApo100x (immersion)	○	×	○	×	×	×
PlanFluor20x (phase contrast)	△	△	△	○	○	○
PlanFluor40x (phase contrast)	△	△	△	○	○	○

The performance of a lens marked with △ may not be sufficient for observation. Select those lenses marked by ○ as much as possible.

Tips

- A lens is selected according to the thickness of the transparent body on the undersurface of the observation container.
- If the thickness of the transparent body is thin, high-resolution capturing is possible. (The glass bottom dish is better than the plastic petri dish.)
- Inverting a slide and observing through fixed cover glass allows for high resolution imaging. If the cover glass cannot be fixed, imaging can still be done through the slide glass, but resolution will decrease.

Setting a correction ring lens

The correction ring on some lenses must be set to accommodate for container thickness.

"Correction value based on the observation container"

(Correction ring lens)	Slide (observed from the cover glass surface)	Slide (observed from the glass surface)	Glass bottom dish	Plastic petri dish	Well plate
PlanApo40x	0.17 (mm)	×	0.17 (mm)	×	×
PlanFluor20x (phase contrast)	0.17 (mm)	1.2 (mm)	0.17 (mm)	1.2 (mm)	1.2 (mm)
PlanFluor40x (phase contrast)	0.17 (mm)	1.2 (mm)	0.17 (mm)	1.2 (mm)	1.2 (mm)

The above values are reference values. Check the thickness of the observation container and adjust the correction ring accordingly.

Tips

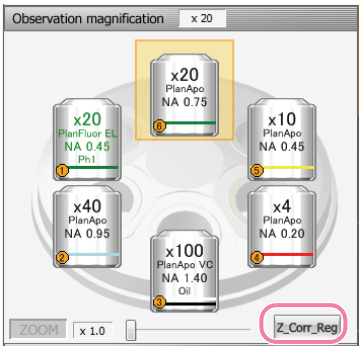
- Adjust the scale of the correction ring lens to the thickness of the viewing area of the specimen container. (The refraction index is corrected according to differences in the container thickness.)
- If the correction ring is set to an inappropriate value, the image will be out of focus.

Checking lens correction registration

The lenses must be registered so that the image will remain in focus when switching between lenses.

<Check method>

1 Click the "Z_Corr_Reg" button in the upper right part of the Viewer screen.



2 A correction registration window appears, as well as red cross-hairs on the image.

3 If "Registered" is not displayed for each lens installed in the revolver, operability deteriorates. Perform correction registration by referring to 🕮 "BZ-9000 User's Manual" P3-5.

Tips

"Correction registration" of the lens is a function to bring the lens into focus and to focus the lens on the screen center position when the lens is switched to a different lens. Be sure to adjust lens correction registration because workability is drastically improved.

⚠ CAUTION

When the following operation is performed, settings of the lens "Correction registration" will be cleared.

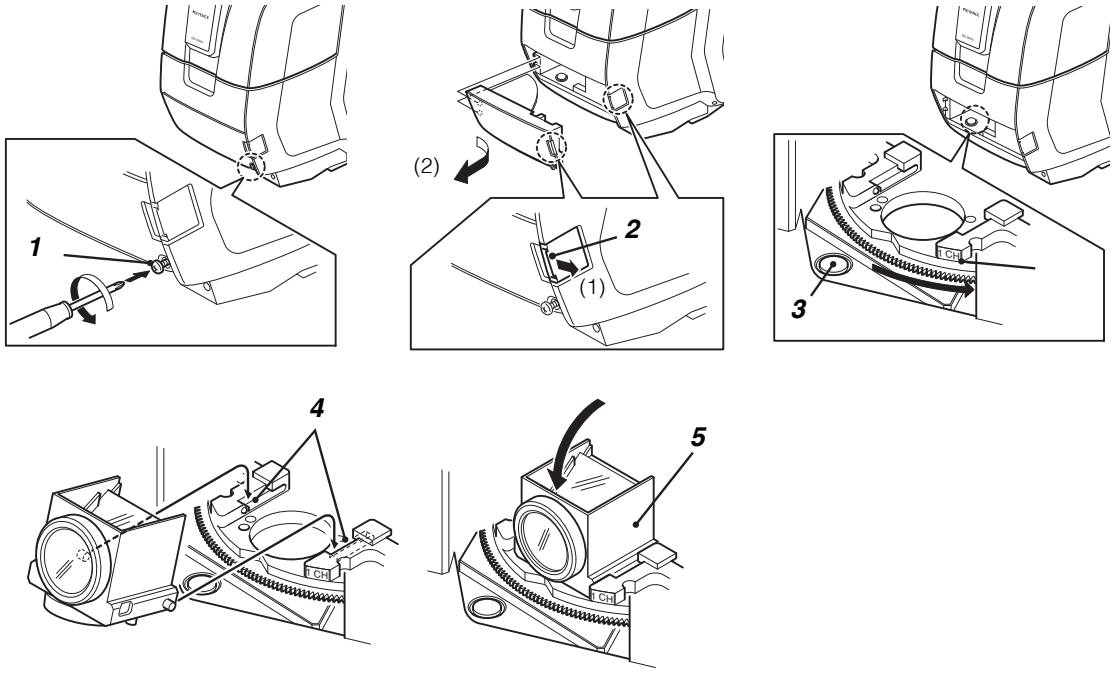
- The "OK" button is clicked on the "Objective lens setting" screen of the initial menu. (Be sure to click the "Cancel" button.)

Checking the fluorescence filter

Make sure the fluorescence filter in the microscope is appropriate for the fluorescence reagent being observed.

<Fluorescence filter check method>

1. Check whether the fluorescence wavelength of the reagent to be observed and the fluorescence filter wavelength set in the main body of the microscope are appropriate. (* If the fluorescence wavelength of the reagent and the fluorescence filter wavelength are inappropriate, fluorescence observation cannot be performed. In such a case, fluorescence observation can be performed with a commercially available filter. If it is not clear whether the wavelengths are appropriate, contact us.)
2. Check the fluorescence filter set in the microscope. Press the "Filter turret cover" button on the lower right of the front of the main body of the microscope to remove the cover. (If the cover cannot be removed, loosen the Phillips head screw below it.)
3. When the cover is opened, you will see the mounted filter cube. Check the name. (CH1: [?] CH2 [?] CH3 [?])
   * When you press the white button in front of you, the filter turret will rotate.



"Standard fluorescence filter wavelengths"

Filter cube name	Excitation wavelength (nm)	Dichroic mirror wavelength (nm)	Absorption wavelength (nm)
DAPI-BP	360±20	400 or longer	460±25
GFP	480±15	505 or longer	510 or longer
DAPI-BP	470±20	495 or longer	535±25
TRITC	540±12.5	565 or longer	605±27.5
TexasRed	560±20	595 or longer	630±30
Cy5	620±30	660 or longer	700±37.5

* Other wavelengths can be captured with commercially available fluorescence filters. Consult with us in order to customize a filter using commercially available glass.

Setting filter channels

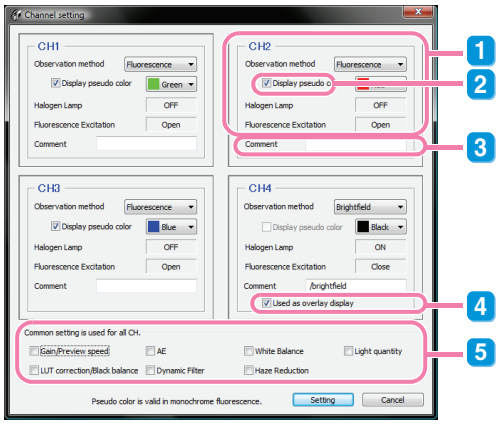
Make sure the filters cubes match the channel settings of the microscope.

<Filter channel check method>

1 Check the name of the fluorescence filter cube mounted on each channel.

2 Click "Channel setting" in the upper right of the Viewer screen and check whether the fluorescence filter mounted in the main body of the microscope is set correctly.

Channel setting button



1 **Selecting an observation method**
Select "OFF", "Bright Field", "Phase Contrast", or "Fluorescence" as the observation method.

2 **Display pseudo color**
If the check box of this item is selected, the observation screen is displayed in the set pseudo color.

3 **Comment**

4 **[Used as overlay display] check box**
When this box is selected, CH4 will be used as an overlay display of the other 3 channels during MultiColor viewing mode.

5 **Check boxes to enable/disable common registration of observation conditions**
Select whether to set a function to common per channel.

<Setting procedure>: For CH1 [GFP-BP] CH2 [TRITC] CH3 [DAPI-BP]
"Example of channel setting 1"

	CH1	CH2	CH3	CH4
Observation method	Fluorescence	Fluorescence	Fluorescence	Bright field
Display pseudo color	レ	レ	レ	
Color	Green	Red	Blue	Black
Comment	GFP-BP	TRITC	DAPI-BP	
Overlay display				レ

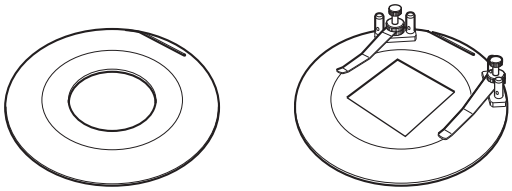
<Setting procedure>: For CH1 [GFP-BP] CH2 [Phase contrast] CH3 [None]

"Example of channel setting 2"

	CH1	CH2	CH3	CH4
Observation method	Fluorescence	Phase contrast	None	Bright field
Display pseudo color	レ			
Color	Green	Black		Black
Comment	GFP-BP	Phase contrast		
Overlay display				レ

Selecting a stage plate

Select a stage plate based on the observation container.



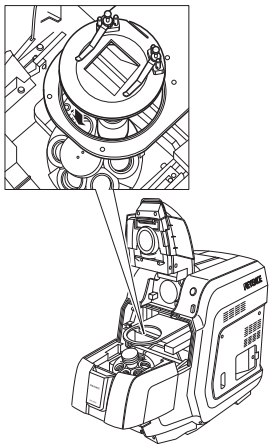
"Stage plate based on observation container"

Type of stage plate	Slide	35-mm dish	100-mm dish	Well plate
Circular plate	○	○	△	△
Square plate (with stage clips)	○	×	×	×
Square plate (without stage clips)	○	×	○	○

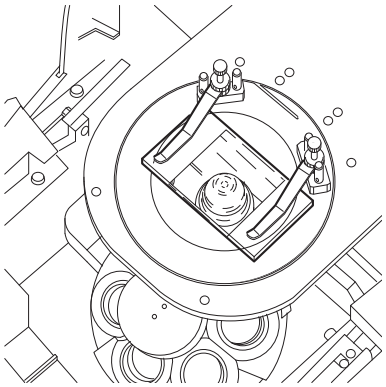
* Operability of the plates marked by △ deteriorates because the view range narrows.

* "Square plate (without stage clips)" means that the two stage clips fixed by screws are removed.

Install the stage plate with the cut facing to the back so that it produces a symmetric appearance from the center of the stage.



As shown in the figure below, the stage clips are used to fix a specimen such as a slide.



Setting shooting conditions

Set an image size, a file extension, and a file storage destination.
(Register the following items by switching the rightmost tabs of the Viewer.)

"Shooting registration conditions by functional tab of the Viewer"

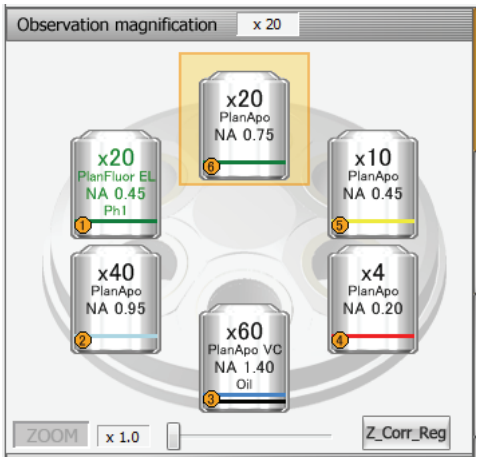
	Camera setting tab	Option tab
Shooting conditions (selection of full size pixel count)	680x512 to 4080x3072	
Setting for well plate observation (removal of lens crash check flag)		レ (cleared)
Format for saving		Select a format for saving from TIFF BMP JPEG
Setting the file storage location		Select a path from the "Browse" button

* When observing the well plate, you must remove the above "lens crash check flag".
* If the "lens crash check flag" is removed, the lenses set on both sides of the observation lens may crash with the observation stage. To avoid this crash, alternately mount a tall lens and a short lens on the lens revolver.

II Bright Field Observation

Selecting a lens

Of the set lenses, click a lens with the lowest magnification to start observation.
The lens enclosed by yellow is the lens currently being used.
If you click other lenses, the lens turret will rotate to the selected lens.



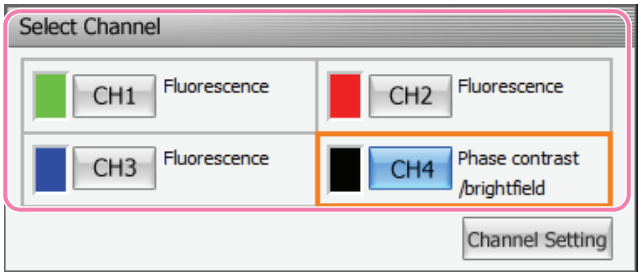
Selecting the "Color" tab

Click the "Color" tab of the Viewer to switch to the color camera.

* For a cultivated cell, etc., you can also select "monochrome" mode. Select color mode or monochrome mode tailored to the purpose of observation.

Selecting a channel

Click the "Brightfield" channel from the channels set in "examples of channel setting by observation example" on P15.



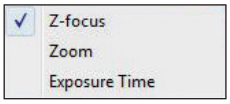
Selecting "Auto" for exposure time

Click "Auto" to the right of Exposure time.

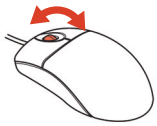
Using the mouse and keyboard for focusing

After right-clicking on the observation screen,

select "Z focus".



Adjust the focus by scrolling the mouse wheel.



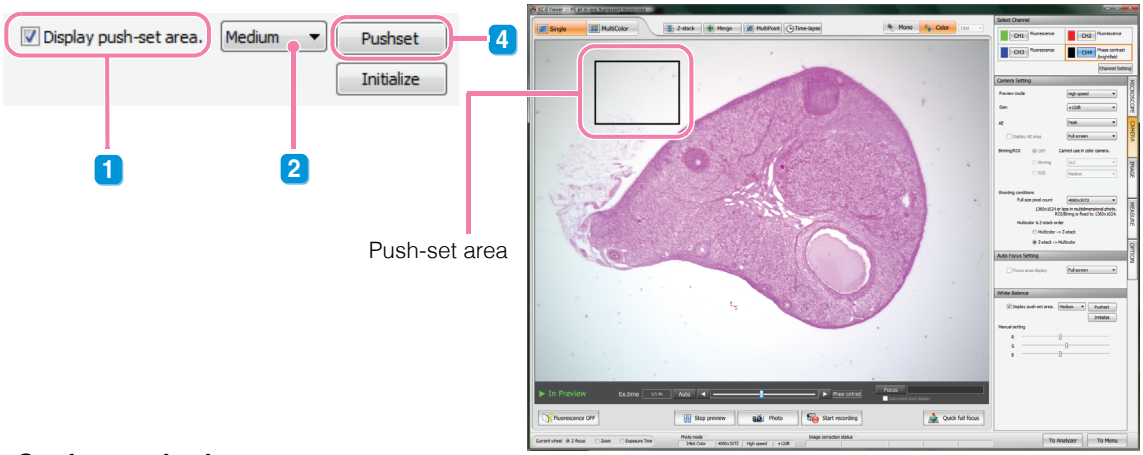
Performing focusing with not only the mouse wheel but also the "Shift" and "Ctrl" keys improves operability.

	Very course adjustment	Course adjustment	Normal adjustment	Fine adjustment
Shift+Ctrl+mouse wheel	○			
Ctrl+mouse wheel		○		
Mouse wheel			○	
Shift+mouse wheel				○

* Be sure to memorize and use the above keyboard operations.

White balance

Adjust the white balance in the Camera tab on the right side of the Viewer screen.



<Setting method>

1. Select the [Display push-set area.] check box.
2. Click ▼ of the [Display push-set area.] list box and select [Large], [Medium], [Small], or [Full screen].
3. Drag the push-set area to the position where you want to adjust the white balance.
4. Click the [Pushset] button.

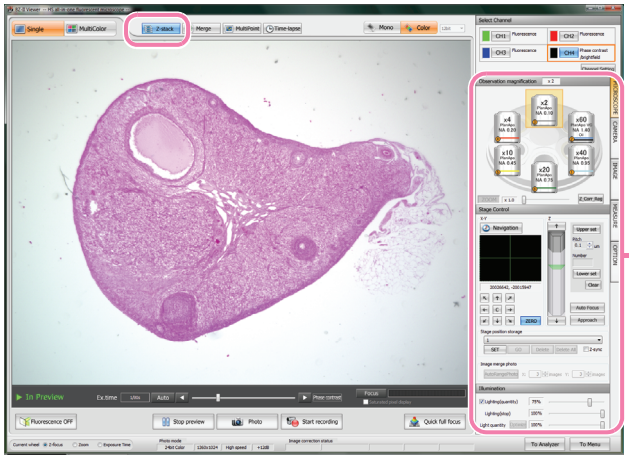
"Full focus" function

Use the "full focus" (or "Z-stack") function for thick or uneven samples.

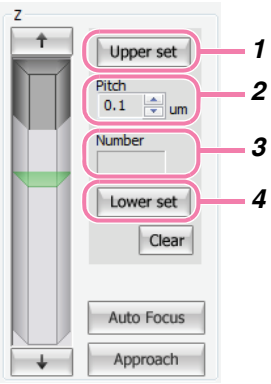
- Thick or uneven samples can be particularly difficult to image and accurately analyze, especially when only part of the image is in focus.
- Refer to 🕮 "BZ-9000 User's Manual" P9-1 "Capturing Thick Specimens".

<Full focus method>

1 Click the "Z-stack" tab of the Viewer screen.



2 Adjust the focus until the uppermost portion of the sample is in focus. Pause here and click "Upper set."



1	**Upper set button**	: Registers the upper limit in the Z-axis direction.
2	**Pitch**	: Adjusts the photo interval.
3	**Number**	: Displays the number of images to be captured.
4	**Lower set button**	: Registers the lower limit in the Z-axis direction.

Tips

When setting a capturing pitch manually, see the following table for appropriate capturing pitch:

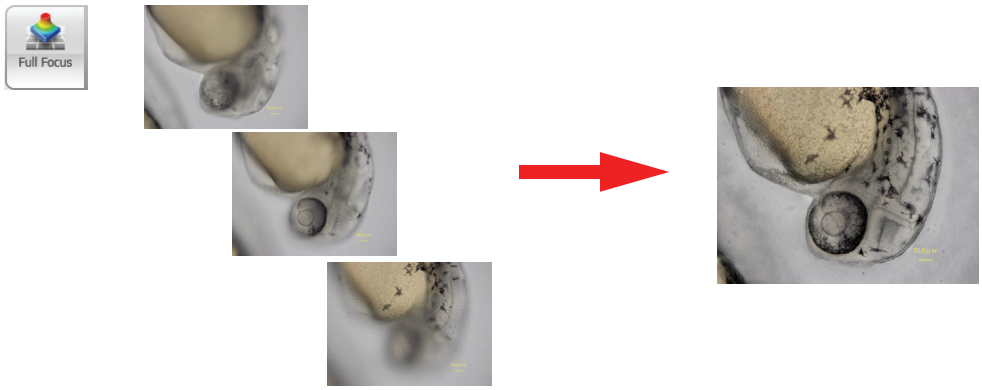
Lens name	Long depth of field (micron)	Appropriate (capturing) pitch
PlanApo20x	1.0	1.0 to 2.0
PlanApo40x	0.6	0.6 to 1.2
PlanApo60x (immersion)	0.4	0.4 to 0.8
PlanApo100x (immersion)	0.4	0.4 to 0.8
PlanFluor20x	2.7	2.7 to 5.4
PlanFluor40x	2.0	2.0 to 4.0

3 Next, pause the lens where the lowest area of the sample is in focus and click "Lower set".

4 Click "Photo" and set a path save destination of a photo file to start capturing.

5 After capturing, select the photo image folder from "Loading as a group" in the Analyzer and click "Load".

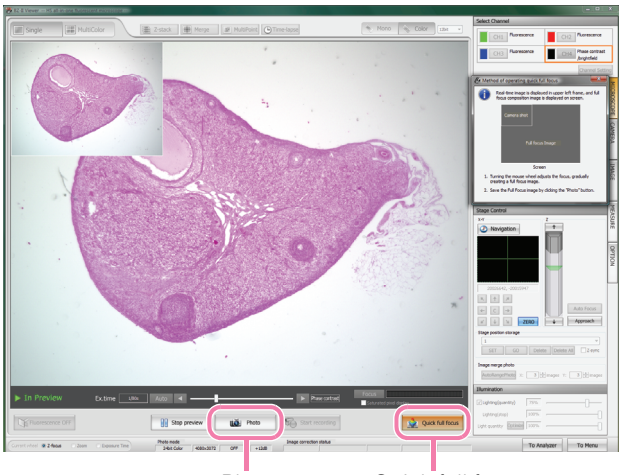
6 Clicking the "Full focus" icon in the Analyzer after loading the grouped image synthesizes a full focus image.



"Quick full focus" function

To create a fully focused image during observation in the Viewer screen, use the "quick full focus" function.

- Quick full focus allows you to quickly overcome depth of field limitations within the observation image.



<Quick full focus method>

1 Focus on the uppermost area of the sample, and then click the "Quick full focus" button on the bottom of the Viewer screen.
The real-time image from the camera is displayed on the top left of the screen and a full-focus composite image is displayed on the full screen.

2 When viewing the camera image, scroll the mouse wheel downward until the bottom of the object comes into focus.

Tips

Scroll the mouse wheel as slowly as possible.
If you scroll the mouse wheel quickly, the full-focus image may be unable to be synthesized.

3 Click the [Photo] button to save the image.

"Navigation" function

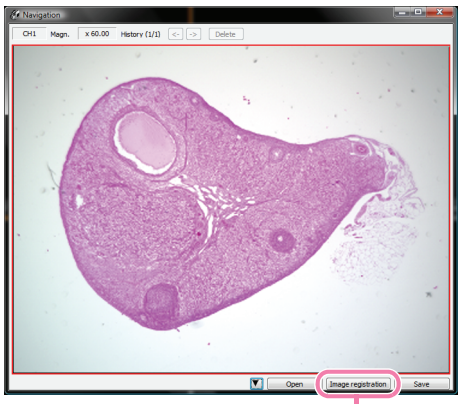
The "Navigation" function improves observation efficiency.

- The majority of observation time is spent aligning a capturing point. The "Navigation" function can drastically reduce capturing time.
- Alignment is also easy with an oil immersion lens. The immersion lens allows wide-range position movement and observation without having to switch to a low magnification lens.
- Refer to 🕮 "BZ-9000 User's Manual" P3-6 "Using the Navigation Function".

<Navigation method>

1. Observe the image with a low magnification lens.
2. Click the "Navigation" button.

3. After clicking the "Image registration" button, change the observation magnification to a higher magnification.



"Stage position storage" function

The "Stage position storage" function allows you to set and recall points of interest within a sample.

- Never again get lost within your sample while using high magnification.
- Up to 30 coordinates can be registered, including the focus.
- Refer to 🕮 "BZ-9000 User's Manual" P34 Operation Screen "8 Stage position storage".

Stage storage position check box

Select the [Stage storage position] check box. The coordinates stored by the stage position storage are displayed.

"Image merge" function

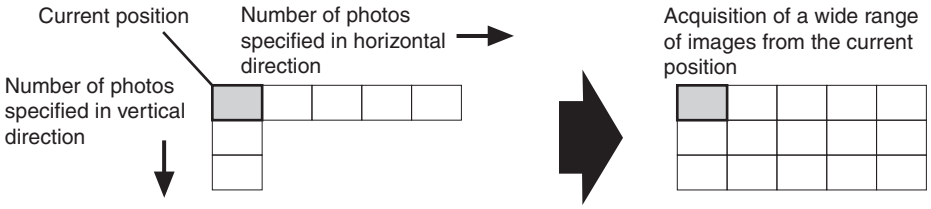
Image merge allows a wide-view, high resolution image to be seamlessly stitched together.

- Obtain a high magnification, high resolution image over a much larger area of your sample.
- Easily view distributions across your entire sample at high resolution.
- Refer to 🕮 "BZ-9000 User's Manual" P3-7 "Image Merge Photo".

<Imager merge method 1 - creating a merge with a set number of photos>

1 Click the "Merge" button.

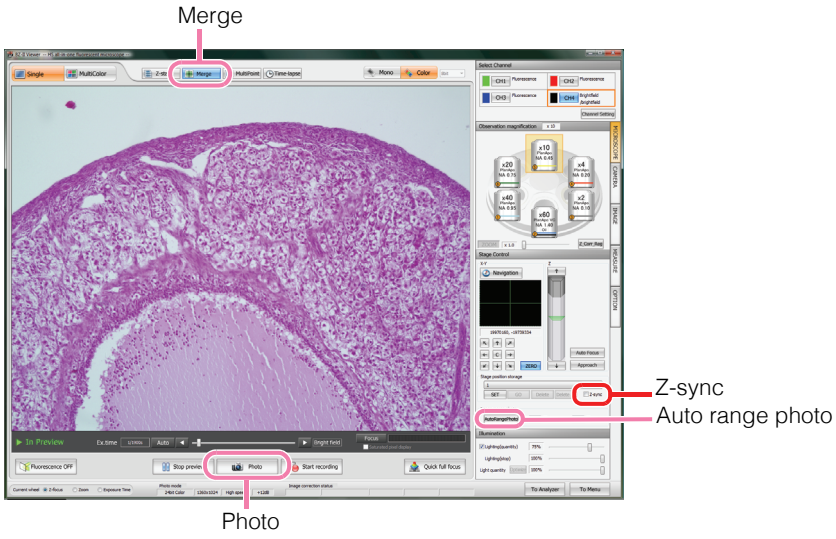
2 Enter the number of photos to be captured in the horizontal and vertical directions in the "Image merge photo" areas.



3 Click the "Photo" button.

<Image merge method 2 - creating a merge with a specific area range>

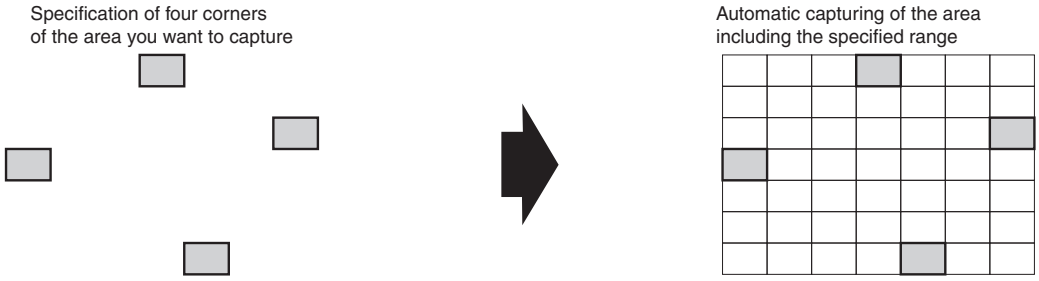
1 Click the "Merge" button.



2 Select the "Z-sync" check box, move the stage to the topmost part of the area you want to capture, and click the "SET" button.

3 Likewise, move the stage to the left, right, and bottom of the area and click the "SET" button.

Register coordinates for four points of the top, bottom, left, and right with the "SET" button.



4 Click the "AutoRangePhoto" button.

The following confirmation dialog box appears.

5 Click the "OK" button.

The number of photos required to capture the area including the specified four points is automatically entered and the stage moves to the top left (bottom right on the coordinates) of the area.

III Phase Contrast Observation

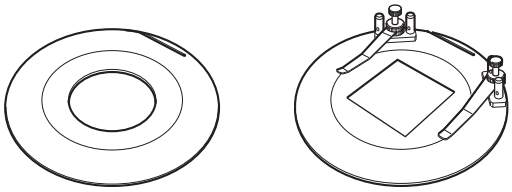
Important setting to use when the observation container is a well plate

Remove " レ " (flag) of "Lens crash check" located in the option tab of the Viewer.

* The normal height of the objective lens will no longer be in focus due to the height of the well plate, so removing this restriction allows you to correctly focus the lens.

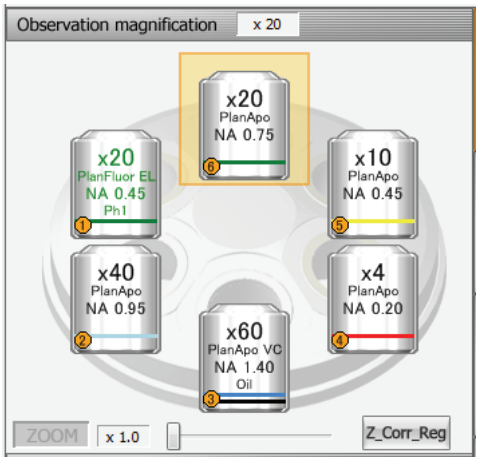
Selecting a stage plate

Select the stage plate corresponding to the container from P15 "Stage plate based on observation container".
(Remove the two stage clips fixed by screws. After using the stage plate, return the stage clips to their original positions.)



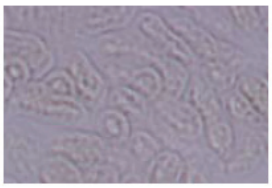
Selecting a lens

Of the set lenses, click a lens with the lowest magnification to start observation.
The lens enclosed by yellow is the lens currently being used.
If you click other lenses, the lens turret will rotate to the selected lens.
Select the optimum lens as the capturing lens from P11 "Optimum lens based on observation container".

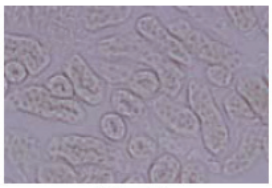


Setting a correction ring lens

If a captured image is out of focus, see P11 "Correction value based on observation container" and adjust the correction ring of the lens to create a high contrast and focused image.



Before correction



After correction

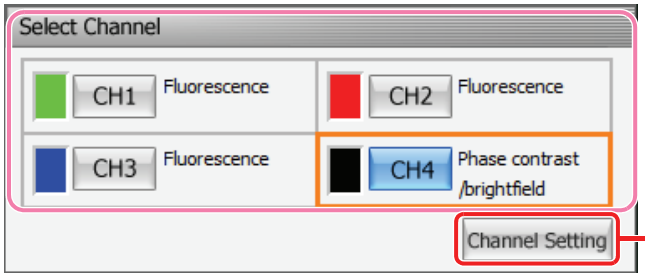
Selecting the "Color" tab

Click the "Color" tab of the Viewer to start the color camera.

* For a cultivated cell, etc., you can also select "monochrome" mode. Select color mode or monochrome mode tailored to the purpose of observation.

Selecting a channel

Select the "Phase contrast" channel.



Channel setting

Tips

If the selected channel is not "Phase contrast", change the channel to "Phase contrast" by clicking the "Channel setting" button.

Selecting "Auto" for exposure time

Click "Auto" to the right of Exposure time.

Using the mouse and keyboard for focusing

Performing focusing with not only the mouse wheel but also the "Shift" and "Ctrl" keys improves operability.

	Very course adjustment	Course adjustment	Normal adjustment	Fine adjustment
Shift+Ctrl+mouse wheel	O			
Ctrl+mouse wheel		O		
Mouse wheel			O	
Shift+mouse wheel				O

* Be sure to memorize and use the above keyboard operations.

"Navigation" function

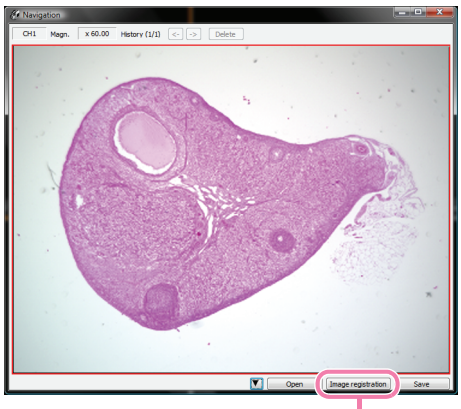
The "Navigation" function improves observation efficiency.

- The majority of observation time is spent aligning a capturing point. The "Navigation" function can drastically reduce capturing time.
- Alignment is also easy with an oil immersion lens. The immersion lens allows wide-range position movement and observation without having to switch to a low magnification lens.
- Refer to 🕮 "BZ-9000 User's Manual" P3-6 "Using the Navigation Function".

<Navigation method>

1. Observe the image with a low magnification lens.
2. Click the "Navigation" button.

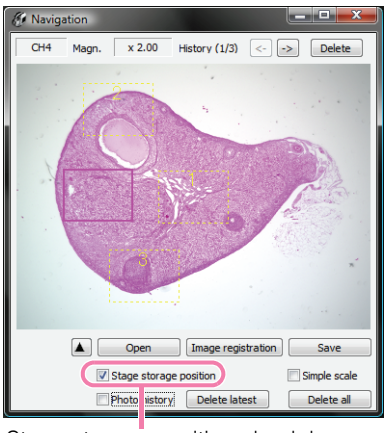
3. After clicking the "Image registration" button, change the observation magnification to a higher magnification.



"Stage position storage" function

The "Stage position storage" function allows you to set and recall points of interest within a sample.

- Never again get lost within your sample while using high magnification.
- Up to 30 coordinates can be registered, including the focus.
- Refer to 🕮 "BZ-9000 User's Manual" P3-4 Operation Screen "8 Stage position storage".



Stage storage position check box

Select the [Stage storage position] check box. The coordinates stored by the stage position storage are displayed.

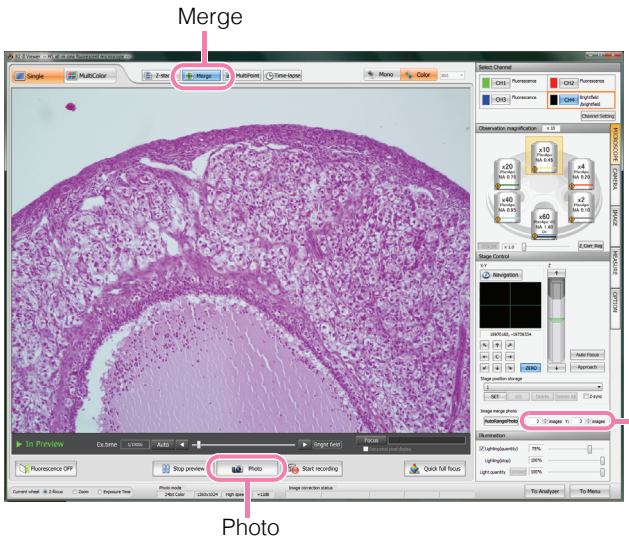
"Image merge" function

Image merge allows a wide-view, high resolution image to be seamlessly stitched together.

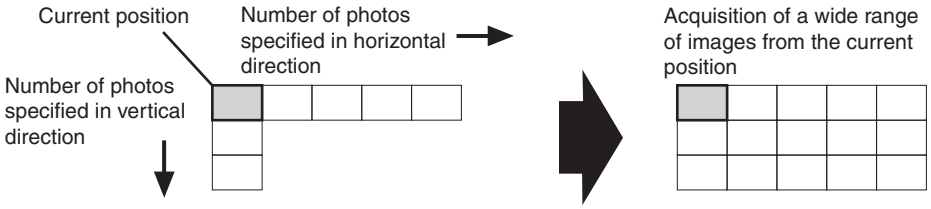
- Obtain a high magnification, high resolution image over a much larger area of your sample.
- Easily view distributions across your entire sample at high resolution.
- Refer to 🕮 "BZ-9000 User's Manual" P3-7 "Image Merge Photo".

<Imager merge method 1 - creating a merge with a set number of photos>

1 Click the "Merge" button.



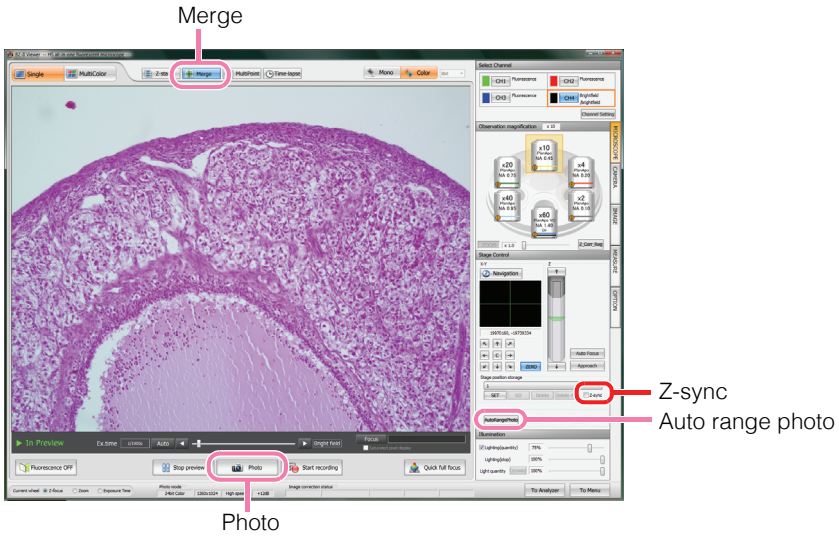
2 Enter the number of photos to be captured in the horizontal and vertical directions in the "Image merge photo" areas.



3 Click the "Photo" button.

<Image merge method 2 - creating a merge with a specific area range>

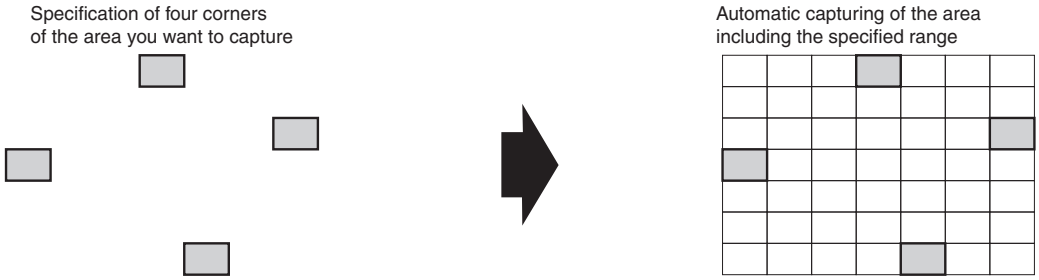
1 Click the "Merge" button.



2 Select the "Z-sync" check box, move the stage to the topmost part of the area you want to capture, and click the "SET" button.

3 Likewise, move the stage to the left, right, and bottom of the area and click the "SET" button.

Register coordinates for four points of the top, bottom, left, and right with the "SET" button.



4 Click the "AutoRangePhoto" button.

The following confirmation dialog box appears.

5 Click the "OK" button.

The number of photos required to capture the area including the specified four points is automatically entered and the stage moves to the top left (bottom right on the coordinates) of the area.

IV Fluorescence Observation/Single Photo

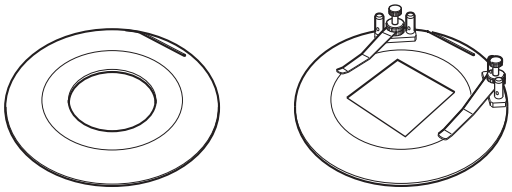
Important setting to use when the observation container is a well plate

Remove " レ" (flag) of "Lens crash check" located in the option tab of Viewer.

* The normal height of the objective lens will no longer be in focus due to the height of the well plate, so removing this restriction allows you to correctly focus the lens.

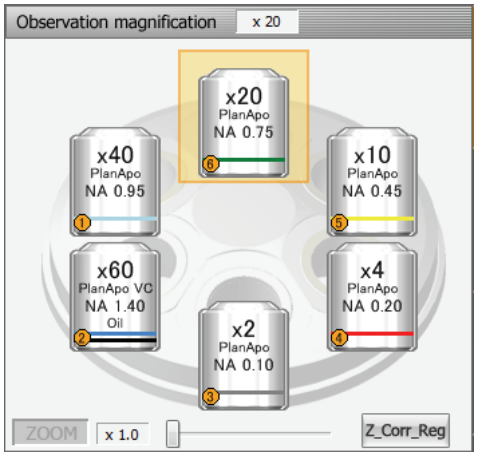
Selecting a stage plate

Select the stage plate corresponding to the container from P15 "Stage plate based on observation container".
(Remove the two stage clips fixed by screws. After using the stage plate, return the stage clips to their original positions.)



Selecting a lens

Of the set lenses, click a lens with the lowest magnification to start observation.
The lens enclosed by yellow is the lens currently being used.
If you click other lenses, the lens turret rotates to the selected lens.
Select the optimum lens as the capturing lens from P11 "Optimum lens based on observation container".

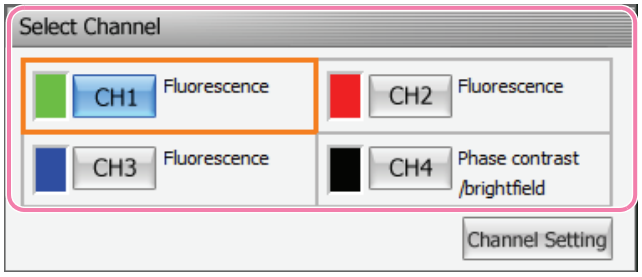


Selecting the "Mono" tab

Perform fluorescence observation in "monochrome". In "monochrome", fluorescence observation can be performed with a sensitivity three times higher than in "color". The monochrome resolution is about two times higher than the color resolution, so it is possible to capture fluorescent images with clear contrast.

Selecting a filter channel

Make sure the fluorescence filter in the microscope matches the fluorescence wavelength of the reagent being observed.
(Refer to P13 "Fluorescence filter wavelengths".)
Make sure the fluorescence filter cube in the microscope match the channel settings.
(Refer to P14 "Filter channel check method".)



Manually adjusting the exposure time with the slide bar

Manually adjust the exposure time of fluorescence observation tailored to the purpose of the observation.

* If exposure time mode is set to "Auto", the observation target may become too bright or a weak signal may become invisible.

Using the mouse and keyboard for focusing

Performing focusing with not only the mouse wheel but also the "Shift" and "Ctrl" keys improves operability.

	Very course adjustment	Course adjustment	Normal adjustment	Fine adjustment
Shift+Ctrl+mouse wheel	○			
Ctrl+mouse wheel		○		
Mouse wheel			○	
Shift+mouse wheel				○

* Be sure to memorize and use the above keyboard operations.

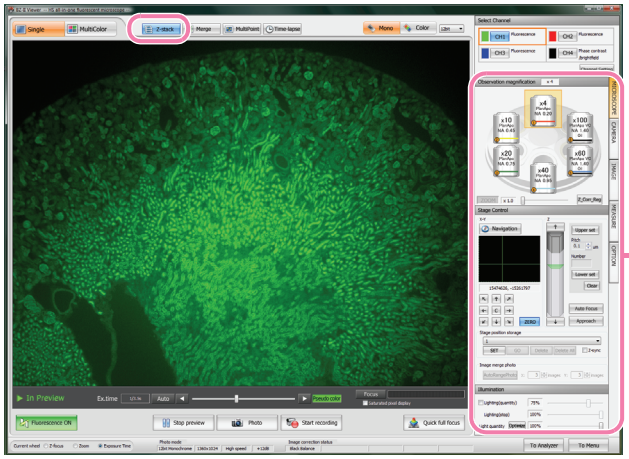
"Full focus" function

Use the "full focus" (or "Z-stack") function for thick or uneven samples.

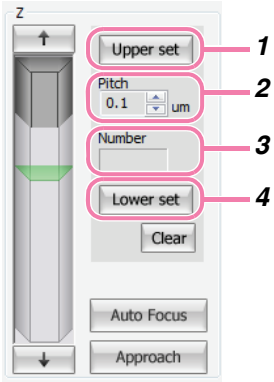
- Thick or uneven samples can be particularly difficult to image and accurately analyze, especially when only part of the image is in focus.
- Refer to 🕮 "BZ-9000 User's Manual" P9-1 "Capturing Thick Specimens".

<Full focus method

1 Click the "Z-stack" tab of the Viewer screen.



2 Adjust the focus until the uppermost portion of the sample is in focus. Pause here and click "Upper set."



1 **Upper set button** : Registers the upper limit in the Z-axis direction.
2 **Pitch** : Adjusts the photo interval.
3 **Number** : Displays the number of images to be captured.
4 **Lower set button** : Registers the lower limit in the Z-axis direction.

Tips

When setting a capturing pitch manually, see the following table for appropriate capturing pitch:

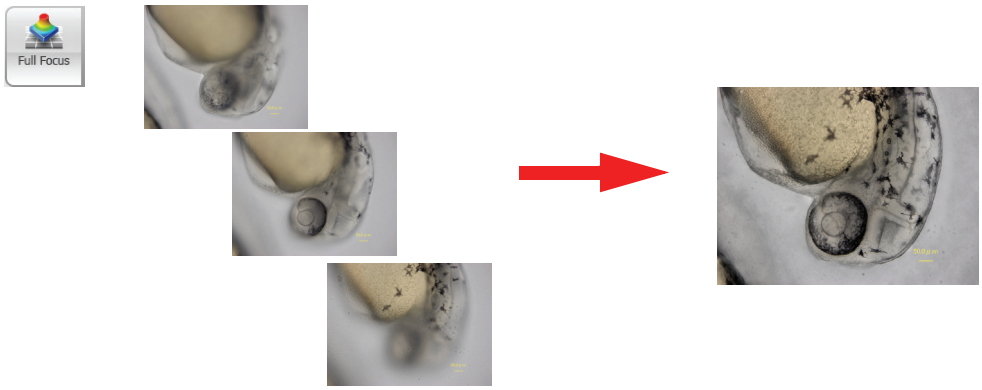
Lens name	Long depth of field (micron)	Appropriate (capturing) pitch
PlanApo20x	1.0	1.0 to 2.0
PlanApo40x	0.6	0.6 to 1.2
PlanApo60x (immersion)	0.4	0.4 to 0.8
PlanApo100x (immersion)	0.4	0.4 to 0.8
PlanFluor20x	2.7	2.7 to 5.4
PlanFluor40x	2.0	2.0 to 4.0

3 Next, pause the lens where the lowest area of the sample is in focus and click "Lower set".

4 Click "Photo" and set a path save destination of a photo file to start capturing.

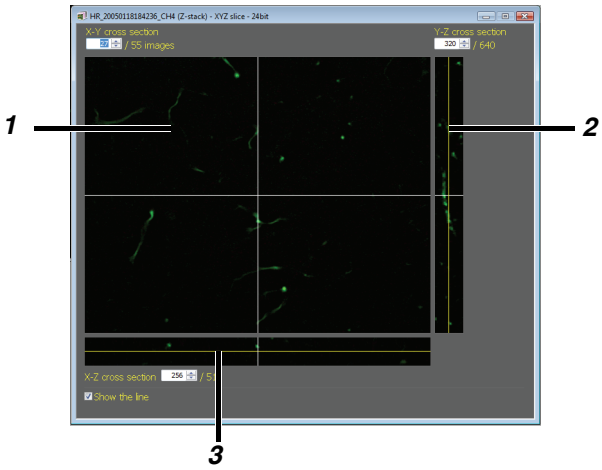
5 After capturing, select the photo image folder from "Loading as a group" in the Analyzer and click "Load".

6 Clicking the "Full focus" icon in the Analyzer after loading the grouped image synthesizes a full focus image.



<When creating an image of a cross-section direction such as XZ and YZ> (XYZ slice)

Clicking "Max Projection" after loading the image allows you to create a full-focus image.



1 The XY cross-section is displayed as a preview.
2 The YZ cross-section is displayed as a preview.
3 The XZ cross-section is displayed as a preview.

To increase (or decrease) the Z-axis scale of the "max projection", double-click "Interval" of the Z-stack image book icon located in the right end of Analyzer and adjust the interval value.

"Navigation" function

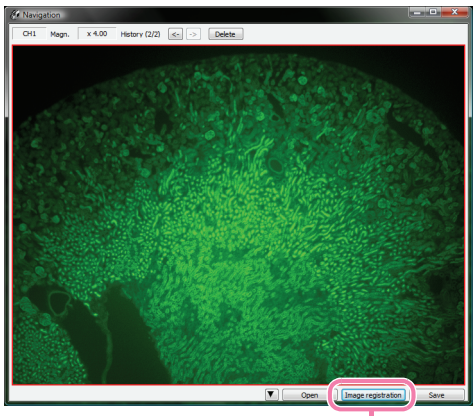
The "Navigation" function improves observation efficiency.

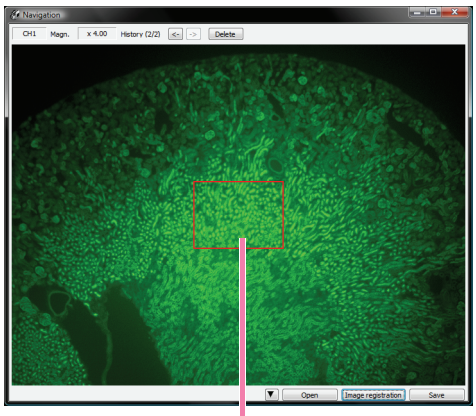
- The majority of observation time is spent aligning a capturing point. The "Navigation" function can drastically reduce capturing time.
- Alignment is also easy with an oil immersion lens. The immersion lens allows wide-range position movement and observation without having to switch to a low magnification lens.
- Refer to 🕮 "BZ-9000 User's Manual" P3-6 "Using the Navigation Function".

<Navigation method>

1. Observe the image with a low magnification lens.
2. Click the "Navigation" button.

3. After clicking the "Image registration" button, change the observation magnification to a higher magnification.





"Stage position storage" function

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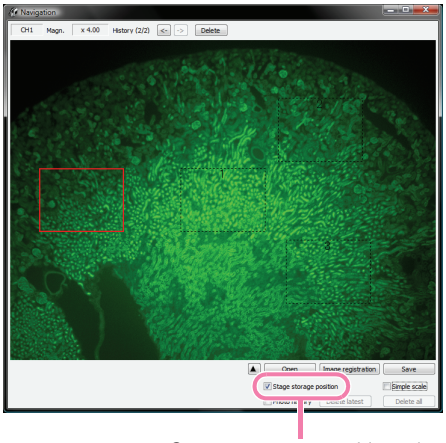
- Never again get lost within your sample while using high magnification.
- Up to 30 coordinates can be registered, including the focus.
- Refer to 🕮 "BZ-9000 User's Manual" P3-4 Operation Screen "8 Stage position storage".



Select the [Stage storage position] check box.
The coordinates stored by the stage position storage are displayed.

Stage storage position check box

"Image merge" function

Image merge allows a wide-view, high resolution image to be seamlessly stitched together.

- Obtain a high magnification, high resolution image over a much larger area of your sample.
- Easily view distributions across your entire sample at high resolution.
- If the image joint function is used in the fluorescent image, merge quality when "black balance" is not applied becomes higher than merge quality when "black balance" is applied.
- Refer to 🕮 "BZ-9000 User's Manual" P3-7 "Image Merge Photo".

<Imager merge method 1 - creating a merge with a set number of photos>

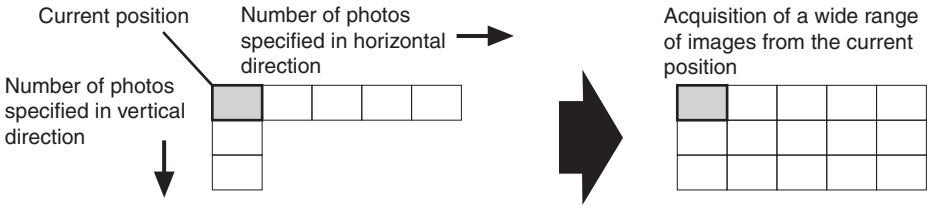
1 Click the "Merge" button.

Merge

Image merge photo

Photo

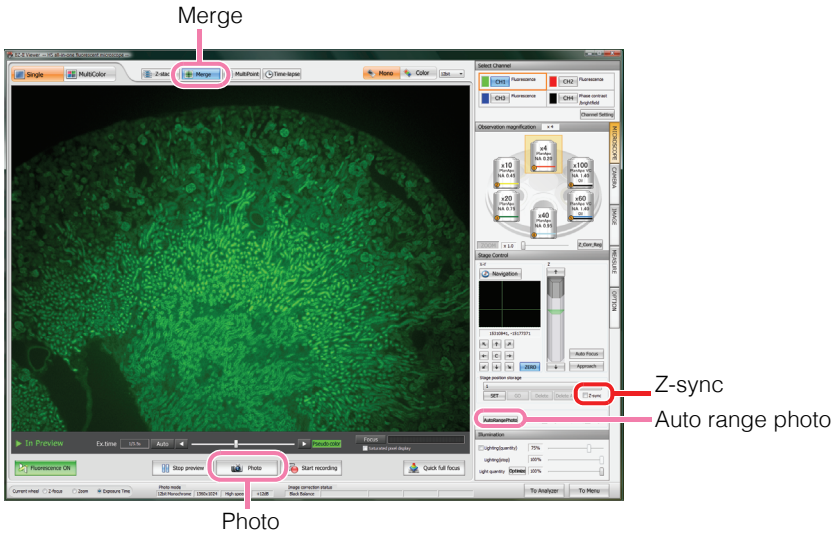
2 Enter the number of photos to be captured in the horizontal and vertical directions in the "Image merge photo" areas.



3 Click the "Photo" button.

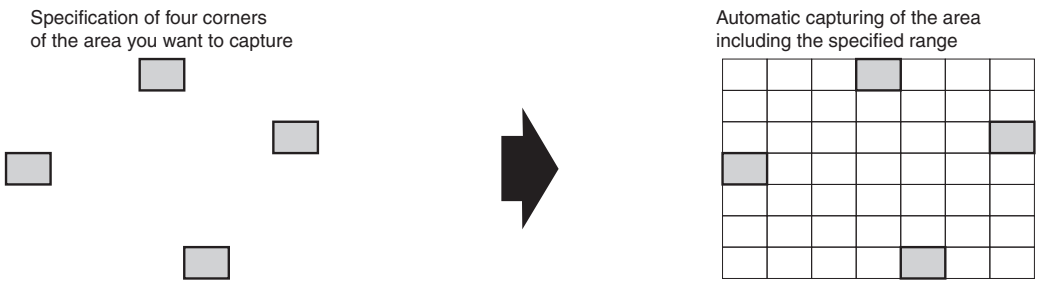
<Image merge method 2 - creating a merge with a specific area range>

1 Click the "Merge" button.



2 Select the "Z-sync" check box, move the stage to the topmost part of the area you want to capture, and click the "SET" button.

3 Likewise, move the stage to the left, right, and bottom of the area and click the "SET" button.
Register coordinates for four points of the top, bottom, left, and right with the "SET" button.



4 Click the "AutoRangePhoto" button.
The following confirmation dialog box appears.

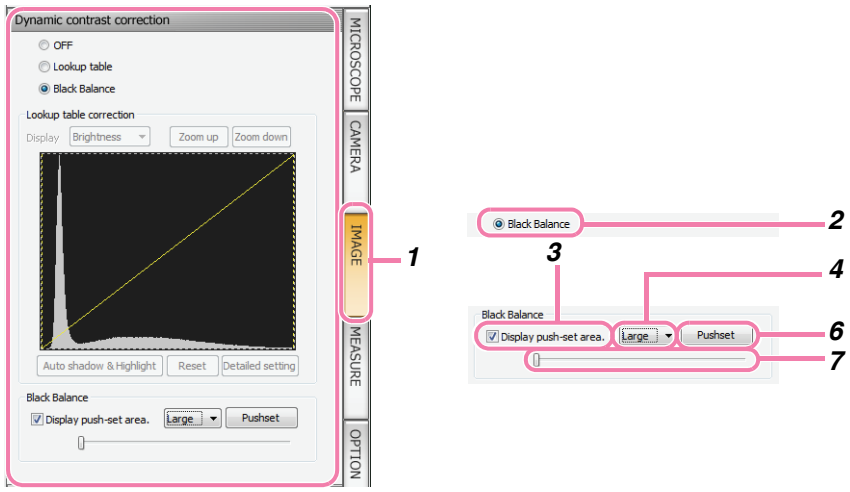
5 Click the "OK" button.
The number of photos required to capture the area including the specified four points is automatically entered and the stage moves to the top left (bottom right on the coordinates) of the area.

"Black balance" function

Removes background fluorescence on the preview screen. (Black balance function)

- Black balance reduces fluorescence background noise to enable more accurate observation and analysis.

<Black balance method>



1. Click the "Image" tab of the Viewer screen.
2. Select the "Black Balance" check box of "Dynamic contrast correction".
3. Select the "Display push-set area." check box of "Black Balance".
4. From "Display push-set area.", select "Large", "Middle", or "Small".
5. When a rectangular frame appears on the screen, drag it to where the background fluorescence is the strongest.
6. Click "Pushset" of "Black Balance" to end black balance.
7. Fine-tune black balance with the slider bar required.

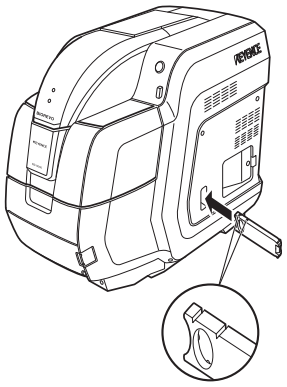
"Diffuse filter" function

The diffuse filter is useful when there is contrast between the center and periphery of the viewing screen.

- Sometimes contrast remains because light in the center of the screen is strong and thus the periphery of the screen becomes dark.

<Setting method>

1. Insert the "Diffuse filter (ND)" into the "Filter slot" on the right side facing the main body of the microscope.
2. The light intensity in the center of the screen is reduced, so the lighting becomes constant across the screen.



"Light quantity adjustment" function

When you want to reduce toxicity as much as possible and capture an image.
(Light quantity adjustment function)

- Use this function to reduce phototoxicity and photobleaching of delicate samples.
- Weakening the light quantity lengthens the excitation time (exposure time) but the sample lifetime can be lengthened because the damage to the specimen is reduced.

<Setting method>

From the microscope operation screen, select the light quantity of the mercury lamp from 5%, 10%, 20%, 40%, and 100%.

"Camera high-speed preview" function

When the focus cannot be adjusted because the signal is weak and the exposure time is too long. (Camera high-speed preview function)

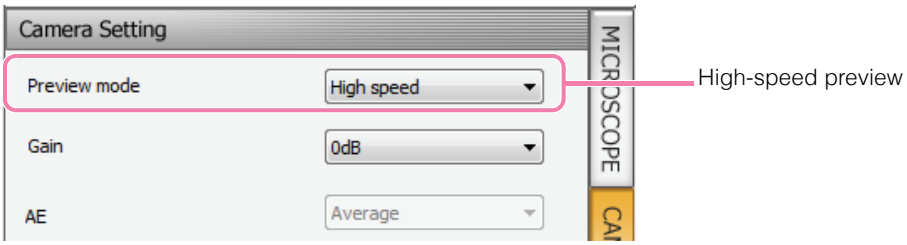
- This function enables you to adjust focus, even when the frame rate is long due to a long exposure time.
- This function increases the camera sensitivity to make the frame rate of the screen higher and enable focusing.

Tips

- The exposure time is intentionally changed to "7.5 frames/second", so point adjustment and field search can be easily performed.
- By increasing the camera sensitivity, the noise of the image may stand out but the image can be used for focusing.
- When "Photo" is clicked during this mode, the captured image will have the original exposure time.

<Setting method>

1 Click the "Camera Setting" tab of the Viewer.



2 Select High speed from the pull down menu.

"Binning" function

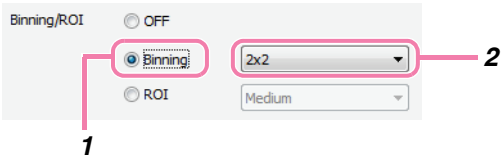
Use binning when the image is hard to confirm on the screen because the fluorescence signal is very weak.

- Sometimes a sample requires a very long exposure time because the fluorescence signals are very week.
- High-speed capturing is enabled by virtually widening the light-receiving area by bundling several adjacent light-receiving elements and amplifying the signal (binning function).

<Binning method>

1 Select the "Binning" check box of the "Camera Setting" tab on the Viewer screen.

2 Select "Binning" from 2x2, 4x4, and 8x8.



Tips

- The sensitivity increases and the frame rate is also improved.
- The sensitivity is inversely proportional to the resolution.

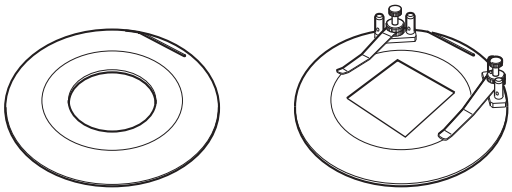
"Listing of sensitivity, number of frames/second, and resolutions by binning setting"

(Binning setting)	2×2	4×4	8×8
Sensitivity (standard time rate)	4 times	16 times	64 times
Number of frames/second	30 frames	60 frames	100 frames
Resolution (standard time rate)	1/4	1/16	1/64

V Fluorescence Observation/Multi-Stained Photo

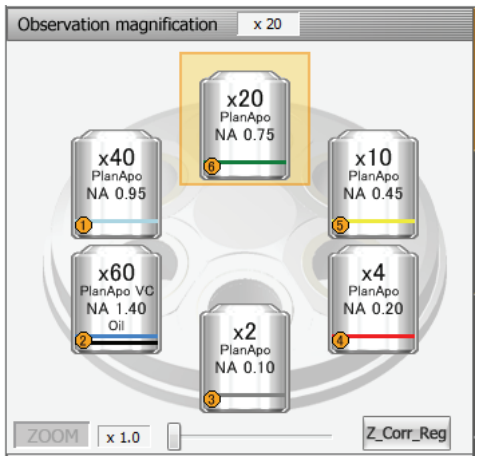
Selecting a stage plate

Select the stage plate corresponding to the container from P15 "Stage plate based on observation container".
(Remove the two stage clips fixed by screws. After using the stage plate, return the stage clips to their original positions.)



Selecting a lens

Of the set lenses, click a lens with the lowest magnification to start observation.
The lens enclosed by yellow is the lens currently being used.
If you click other lenses, the lens turret rotates to the selected lens.
Select the optimum lens as the capturing lens from P11 "Optimum lens based on observation container".

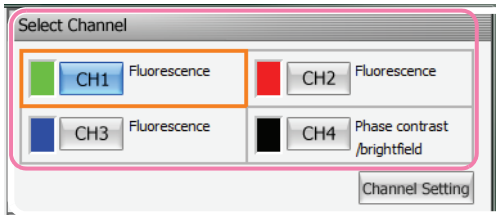


Selecting the "Mono" tab

Perform fluorescence observation in "monochrome". In "monochrome", fluorescence observation can be performed with a sensitivity three times higher than in "color". The monochrome resolution is about two times higher than the color resolution, so it is possible to capture fluorescent images with clear contrast.

Selecting a filter channel

Make sure the fluorescence filter in the microscope matches the fluorescence wavelength of the reagent being observed.
(Refer to P13 "Fluorescence filter wavelengths".)
Make sure the fluorescence filter cube in the microscope match the channel settings.
(Refer to P14 "Filter channel check method".)



Manually adjusting the exposure time with the slide bar

Manually adjust the exposure time of fluorescence observation tailored to the purpose of the observation.

* If exposure time mode is set to "Auto", the observation target may become too bright or a weak signal may become invisible.

Using the mouse and keyboard for focusing

Performing focusing with not only the mouse wheel but also the "Shift" and "Ctrl" keys improves operability.

	Very course adjustment	Course adjustment	Normal adjustment	Fine adjustment
Shift+Ctrl+mouse wheel	○			
Ctrl+mouse wheel		○		
Mouse wheel			○	
Shift+mouse wheel				○

* Be sure to memorize and use the above keyboard operations.

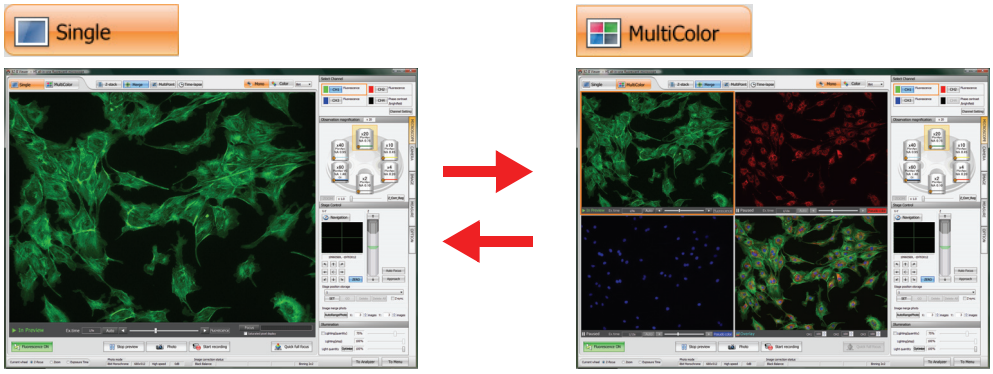
Tips

- Determine where to perform multiple observation by referring to IV Fluorescence Observation/ Single Photo.
- For clear multiple observation, also refer to IV Fluorescence Observation/Single Photo on P31-P39.

Selecting the "MultiColor" tab

<Setting method>

1. Click the "MultiColor" tab located in the top left of the Viewer screen.
   Four divided screens are linked to the channel setting positions in the top right of the Viewer screen.
   The screen enclosed by an orange frame border is "active". Adjust the focus and the exposure time.
2. Click another divided area (screen) and likewise adjust the focus and the exposure time.
3. Finally clicking the screen in the bottom right of the four divided screens enables creation of a multi-stained overlay image.



Tips

- The key point for clean overlay creation is to shorten the exposure time of each channel as much as possible and to weaken the fluorescence background.
- Applying black balance to each channel as required is also efficient. (*For "Black balance" setting method, see P36.)

VI Maintenance

Replacing the mercury lamp unit

- For instructions on how to replace the halogen lamp and fuse, refer to 🕮 "BZ-9000 User's Manual" P12-1.

The average life of the super-high-compression mercury lamp for illumination is about 2,000 hours (at room temperature). If the operating hours have exceeded 2,000 hours, or an error message urging you to replace the lamp is displayed, replace the lamp with the following steps.

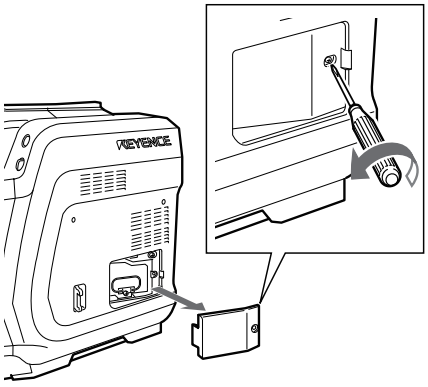
⚠WARNING

High voltage
Turn off the power before replacing the lamp.
As high voltages are generated in the lamp and the cables while power is being supplied, replacing the lamp may cause an electric shock.
High temperature
Replace the lamp only after the power has been turned off for 30 minutes or more. The lamp is extremely hot and may cause burns.

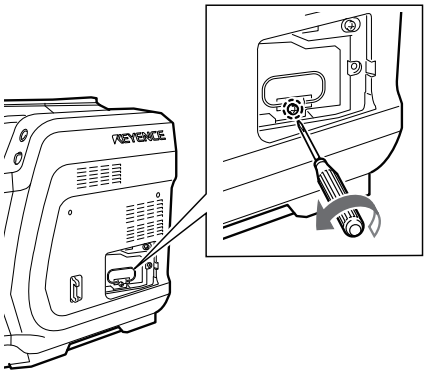
⚠CAUTION

The mercury lamp may burst once its lifetime has expired. The lamp is completely encased in protective glass, so as to prevent any damage in the event that it bursts. Be sure to replace the lamp when it reaches the end of its life to prevent bursting or hearing a cracking sound.

1 Loosen the lamp cover screw and remove the lamp cover.



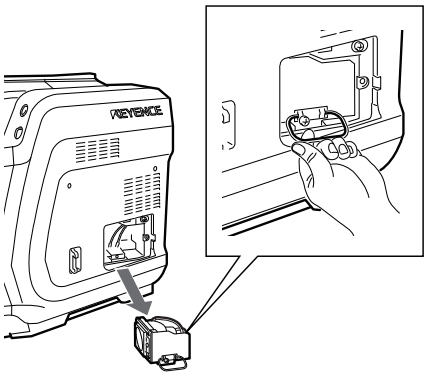
2 Loosen one screw of the lamp unit.



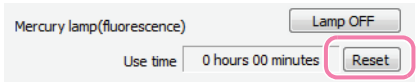
3 Hold the handle of the lamp unit and draw out the lamp unit.

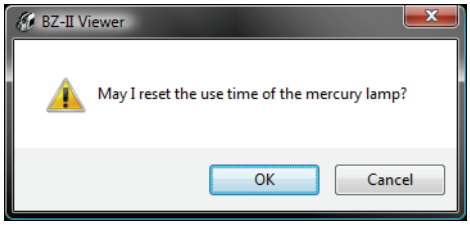
Important

Use an extra-high pressure mercury lamp for OP-85674 BZ2 as the replacement lamp.



4 After replacing the lamp, reset [Use time] of the mercury lamp in the [Option] tab.
When you click the [Reset] button, the following dialog box is displayed:
When you click the [OK] button, the displayed [Use time] returns to zero.





Specifications are subject to change without notice.

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