Simple manual 2023.10.26

Keyence all in one fluorescence microscope
How to use BZ-X700

＜Start-up＞

1. Turn on the PC.
2. Turn on the microscope. (Can be used when the blue LED is lit.)
3. Launch BZ-X Viewer on the desktop.
4. Set the sample holder on the microscope.
   If lens exchange and filter exchange are necessary, exchange here.
5. Click [Capture still image].
6. Select sample holder and click [OK].

BZ-X Viewer is capture software.
BZ-X Analyzer is image viewer.

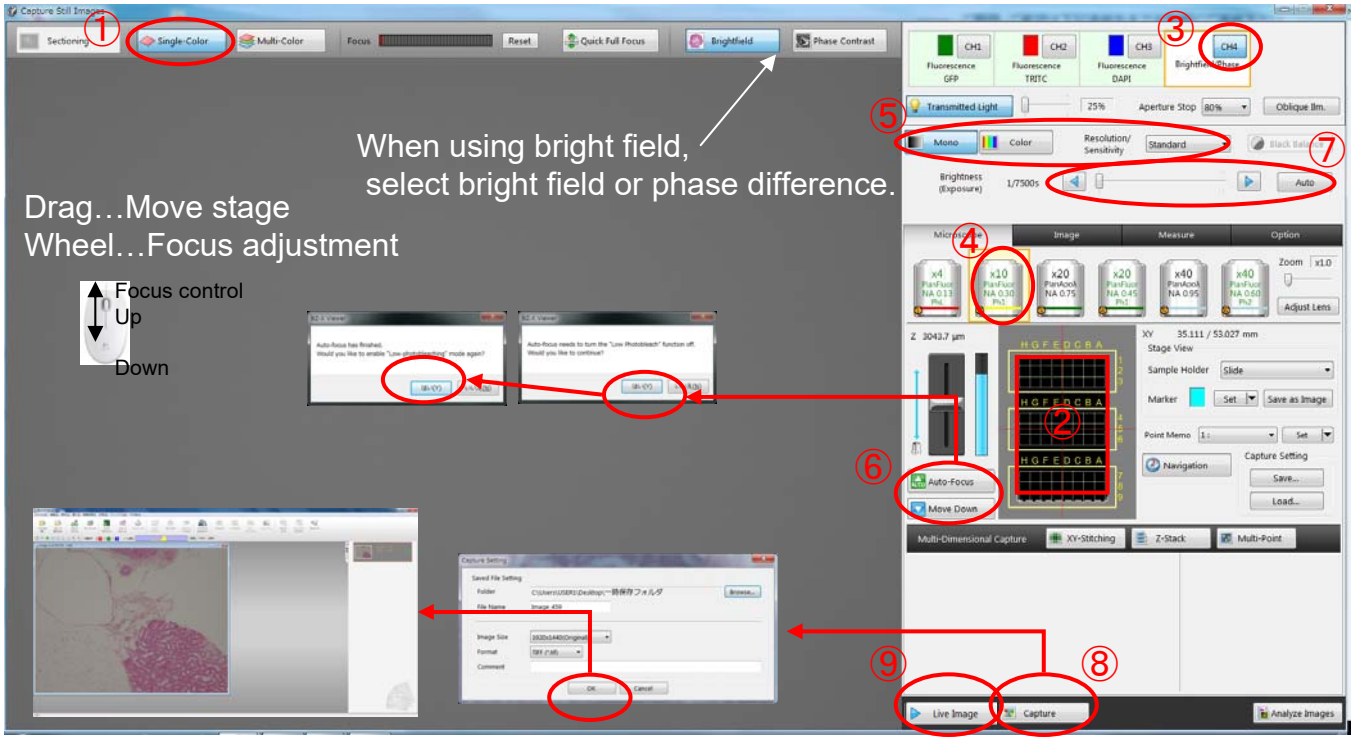
Important!!
You can not take picture of the whole well on outside wells of the micro-plate.

<Basic operation>(Single color capture)

ALL…x4,x10ph
Slide glass…x20,x40
Plastic dish…x20ph,x40ph
If you change the value of the correction ring, you can also use the slide glass.

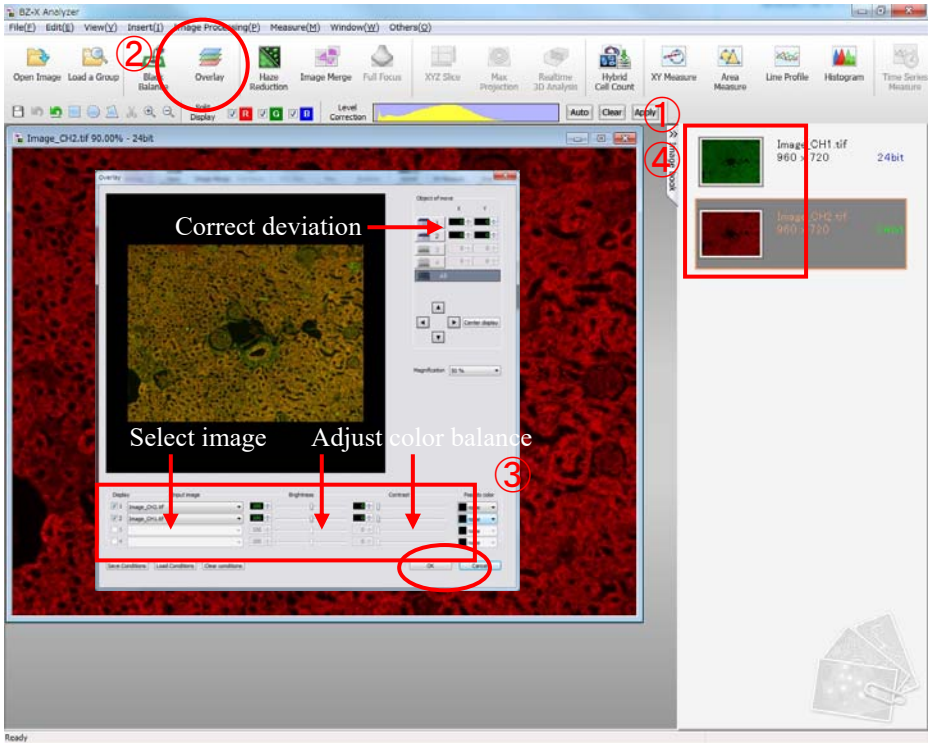
Put on the sample.

1. Click the [Single-Color] button.
2. Click the image of the sample holder to move roughly the stage.
   Drag the preview to move finely the stage.
3. Select the channel you want to capture.
4. Select the objective lens you want to use.
   The phase difference lens checks the value of the correction ring. (cover glass…0.17 , plastic bottom…1.2)
5. To do autofocus, click the button in the order of [Move Down] and [Autofocus].
   When fluorescence is used, a message will be displayed before and after execution, so select [はい](Yes).
   To fine adjustment the focus, turn the wheel of the mouse on the preview screen.
6. Select camera mode. (If necessary, take white balance / black balance where there is nothing.)
   Mono…Mainly for fluorescence and phase difference (High sensitivity , pseudo color display)
   Color…Mainly for bright field (True color , It can also be used for fluorescence)
7. To adjust the brightness, click [Auto] from the brightness item. Fine adjustment with left / right arrow.
8. Click the [Capture] button . Select the save folder, name, image format and click [OK].
   Images are displayed on the BZ-X Analyzer.
9. Click [Live Image] on the BZ-X Viewer and repeat the operation.



<merge the image>

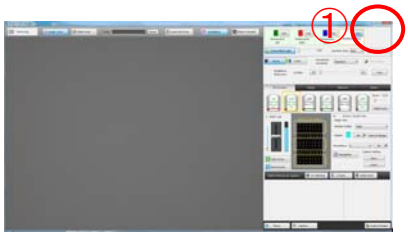
1. Load all the images necessary for merge into “BZ-X Analyzer”.
   (Select “File” - “Open”.)
2. Click the [Overlay].
3. Pull-down the “Input image” item and select all the images necessary for merging.
   Adjust brightness and contrast with “Brightnes” and “Contrast” items.
   Correct the deviation of the position with the “Object of move” item .
   When you are all done, click [OK].
4. Since a merge image is newly created, it is saved with a name.
   (Select “File” - “Save as”.)

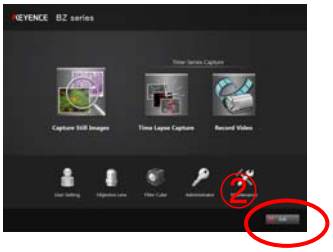


<Shutdown>

1. Close the “BZ-X Viewer”.
2. If you changed the objective lens, filter, holder, restore the setting.
   Click “Exit” to quit the program.
3. Turn off the microscope.
4. After copying the data, exit Windows.
5. Write the record.

The data after a certain period of time is automatically deleted.





④ shutdown

③ Power
(Confirm that the LED is off)

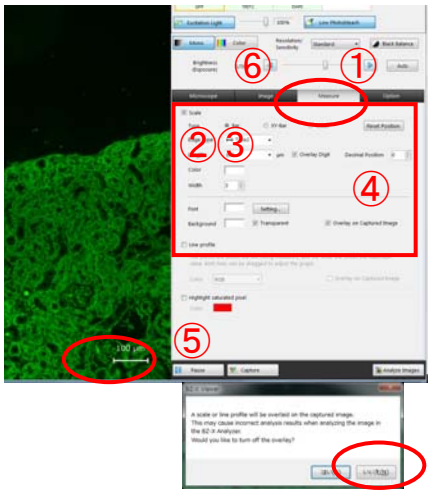
Initial setting of this laboratory
Holder…(outside) multi-plate + (inside) slide
Objective lens
No.1=x4 , No.2=x10Ph , No.3=x20 , No.4=x20Ph , No.5=none , No.6=x40Ph
(Adjust the compensation ring of phase difference lens to 1.2)
Fluorescence filter
Ch1=fluorescence (GFP) , Ch2=fluorescence (TRITC) , Ch3=fluorescence (DAPI) , Ch4=Bright field / phase Cont

<How to insert scale bar>

(How to write before capture)

<u>You can not erase the scale bar after capture.</u>

1. Click “Measure” tab.
2. Check on “scale”. (Scale is displayed.)
3. Select the format of the scale bar.
4. Check on “Overlay on Captured Image”.
5. Move the scale bar in image.
6. Return [Microscope] tab and capture image.



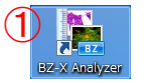
Click [いいえ](No) if this message displayed.

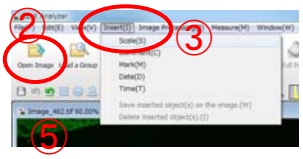
(How to write after capture)

<u>No same format as the method of writing when capture.</u>

<u>Can not fix the scale position.</u>

1. Launch the BZ-X Analyzer.
2. Select [Open image].
3. Select the “Insert” → “Scale” from menu.
4. Don’t change calibration value.
   Select the format of the scale bar and click [OK].
   If you want to change the font size, write it as a comment.
5. Move the scale bar in image.
6. Save as image.
   (There is confirmation of scale bar writing. Can not erase the scale bar after writing.)





<How to save capture settings>

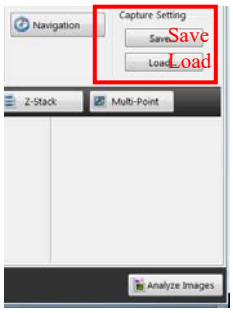
You can save the capture settings in the setting file.

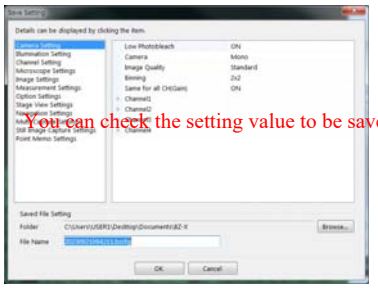
<Save the setting>

1. Make the state you want to save. (Also includes the position of multi position)
2. Click [Save] from the “Capture setting” item.
3. Specify save destination and file name and click [OK].

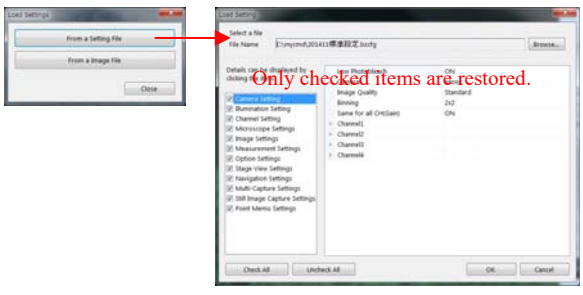
<Load the setting>

1. Click [Load] from the “Capture setting” item.
2. Select the “from setting file”.
3. Check on the item you want to load.





You can check the setting value to be saved.

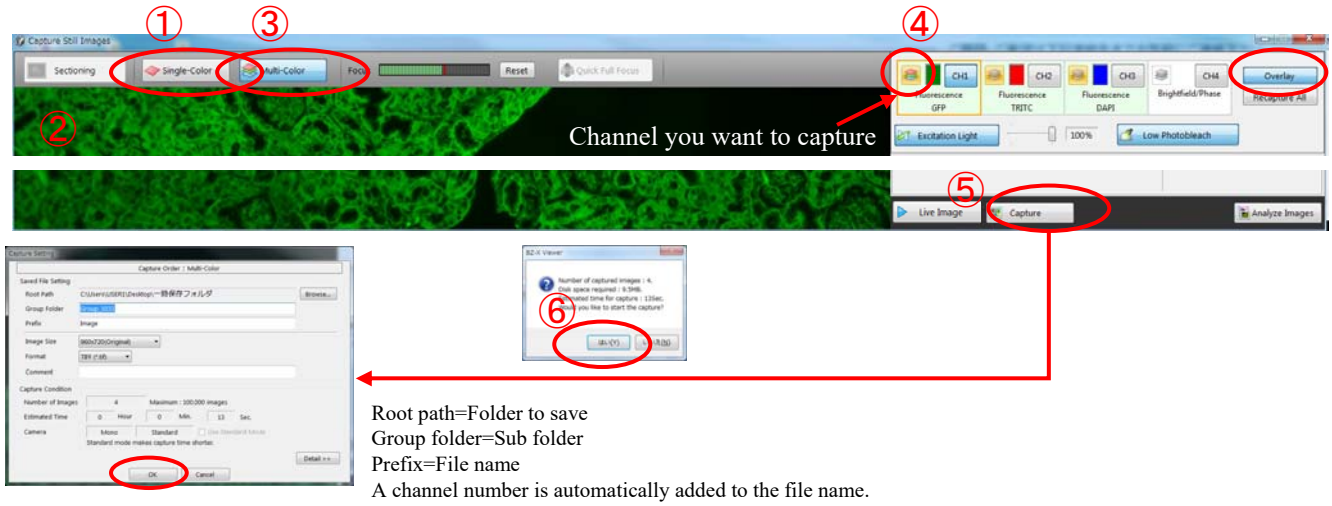


Only checked items are restored.

<Multi color capture>

Sequential shoot and get overlay image.
For basic operation, refer Single color capture.

1. Click [single-color] button.
2. Adjust the focus and adjust the brightness of all colors to capture in multicolor capture.
3. Click [Multi-color] button .
4. Check on all the channels you want to capture.（Icon turns orange）
5. Click the [Capture] button. Select folder, name, format to save and click [OK].
6. As confirmation comes out, click [はい](Yes).
7. The shoot image is displayed on the BZ-X Analyzer.
8. Click [Live image] on the BZ - X Viewer and repeat the operation.



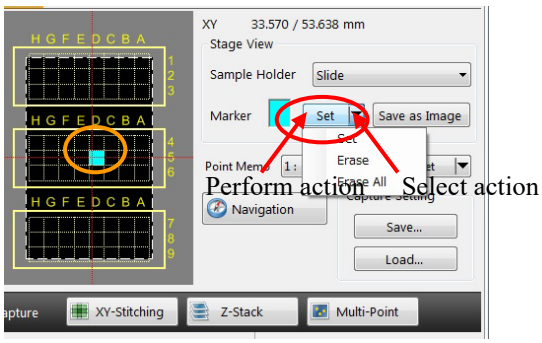
Root path=Folder to save
Group folder=Sub folder
Prefix=File name
A channel number is automatically added to the file name.

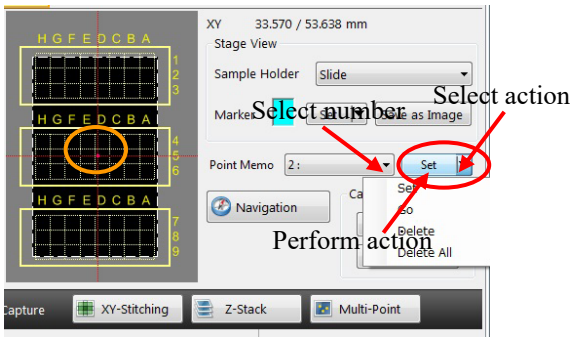
<Marker >

Approximate location record. (This is not XYZ coordinates)
Select [Set] from pull down menu and click [Set] button. (The color of current location will change.)
In case of unnecessary, select [Erase] from pull-down menu and click [Erase] button.

<Point memo> useful function!

Record accurate XYZ coordinates. (Max 30 points)
Select the number you like from the pull down menu.
Select [Set] from pull down menu and click [Set] button. Coordinates are registered in the selected number.
If you execute the [set] with the registered number, the setting will be overwritten.
Select a number and click [Go] to move to the registered location.
In case of unnecessary, select number and [Delete] from pull-down menu and click [Delete] button.





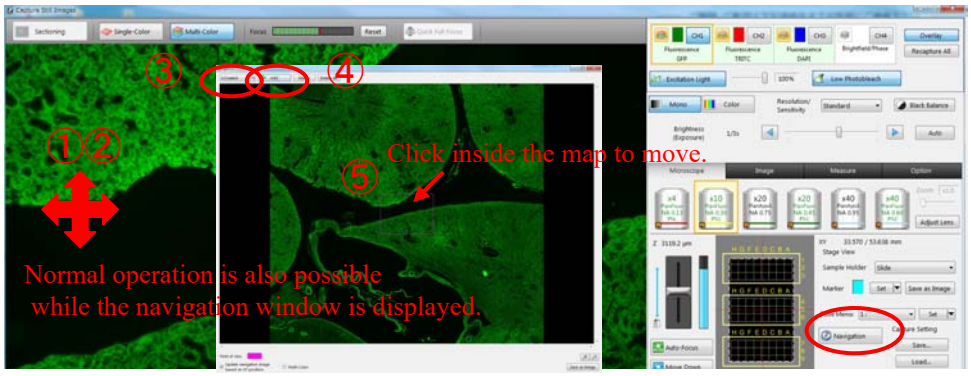
<Navigation>

Useful to create a map with low magnification and use it for movement at high magnification.

Create a simple reduced map.

1. Move the stage to the center of the map.
2. Adjust the focus and brightness.
3. Select unused registration number from the navigation pull down menu.
4. Click [Add] button. Create a map while the stage moves.
   If you want to stop halfway, click [stop].
5. Click in the map you created and move to that place.
   Navigation map can be used even by changing the objective lens.

In case of unnecessary, select number and click [Delete] button to delete.



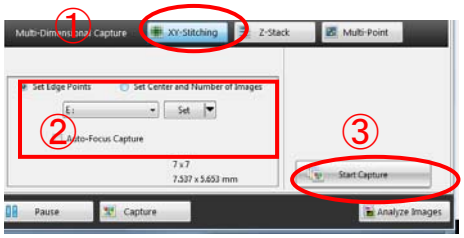
<Image stitching>

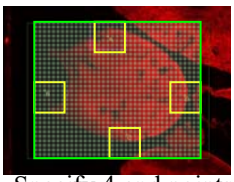
Stitch the images and create a wide range of images.

For the explanation of basic operation, see the items of “Single color capture” or “Multi color capture”.

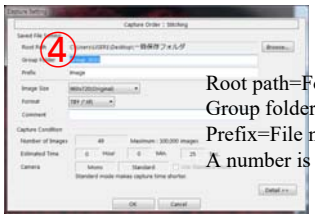
1. Click the [XY-Stitching] button. (Button turns blue)
2. Specify the northernmost, southernmost, westmost, and easternmost points of the range.
   Select “Set Edge Points”.
   Move to the northernmost point and adjust the focus.
   Select “A:” from pull down menu and click [Set] button.
   (Coordinate of the northernmost point is registered as point “A”.)
   In the same way, the remaining 3 places are registered as point “B”, point “C” , point “D”.
   If there are places where the focus position is largely different from 4 points, register that point as well.
   (Max 10points)

   To redo point registration from the beginning,
   select "delete all" from the pull-down menu and click button.
3. Click [Start capture] button.
4. Select folder, name, format to save and click [OK].
   As confirmation comes out, click [はい](Yes).
5. After capturing is complete, click [Open Image Folder] button.
6. Open the file (that name is image) of the microscope icon. (BZ-X Analyzer starts up.)

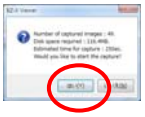


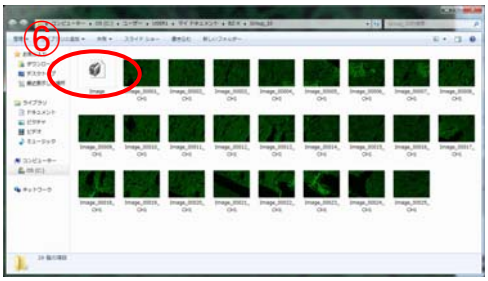


Specify 4 end points. (Green is the specified range)
The range is set so that all 4 points are taken.

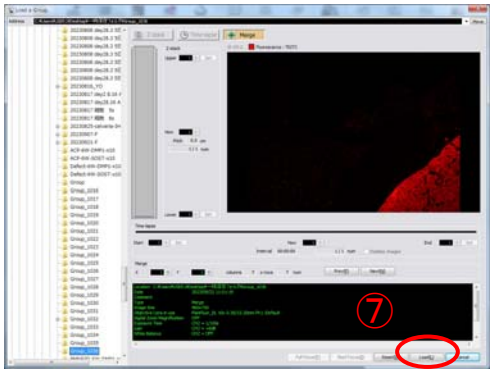


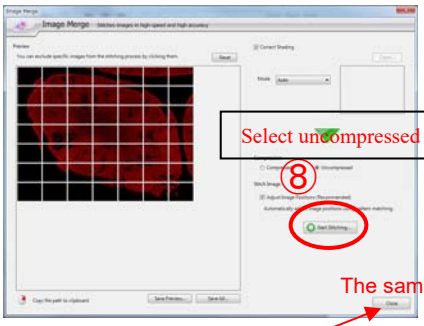
Root path=Folder to save
Group folder=Sub folder
Prefix=File name
A number is automatically added to the file name.



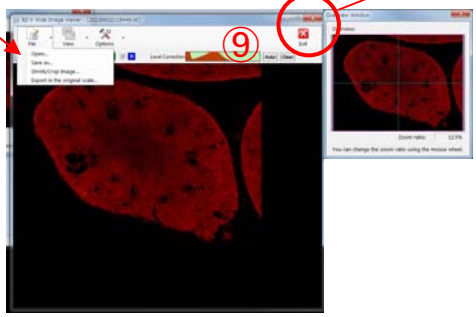


7. Confirm that image folder is selected. click [Load] button.
8. Click [Start stitching] button.
9. After a while stitching image is created. It is the highest image quality, but dedicated format.
   If you want a general format, usually end without saving. (Save it if necessary.)
   Click [Exit] button.
   Click [Close] button in the image Merge window.
   The stitching image is displayed in BZ-X Analyzer.
   Save the stitching image with a name.

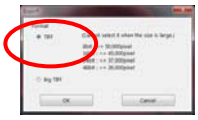


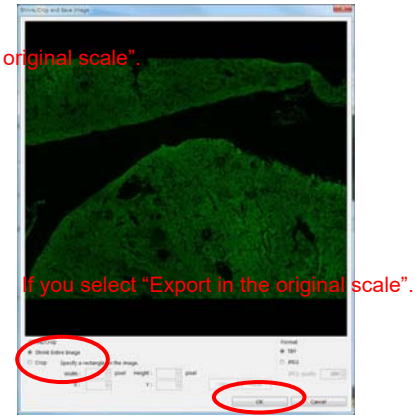


Save as ... Dedicated format (view only)
Shrink / Crop Image … for BZ-X Analyzer and general
Export in the original scale … for general (High quality)



If you select “Export in the original scale”.

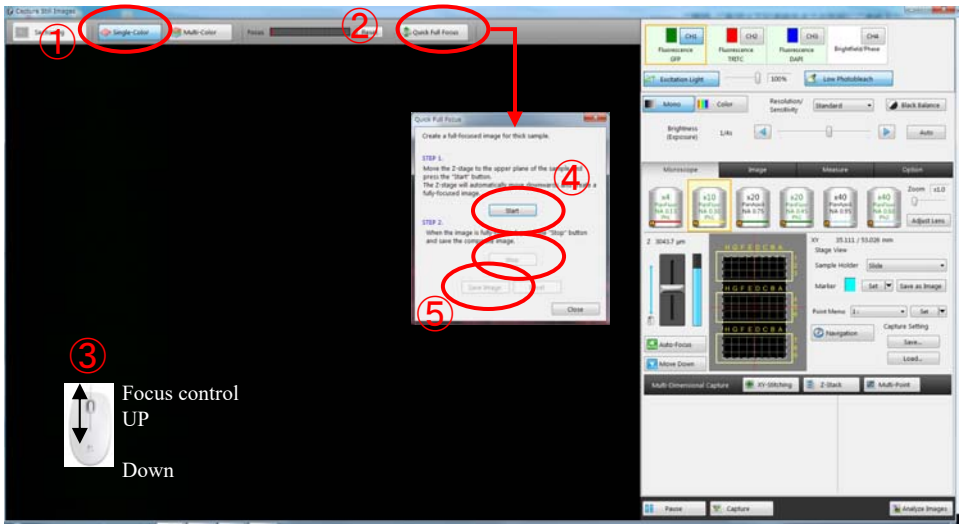




<Quick full focus>

Capture while changing the focus and create a single focused image. (single color capture only)

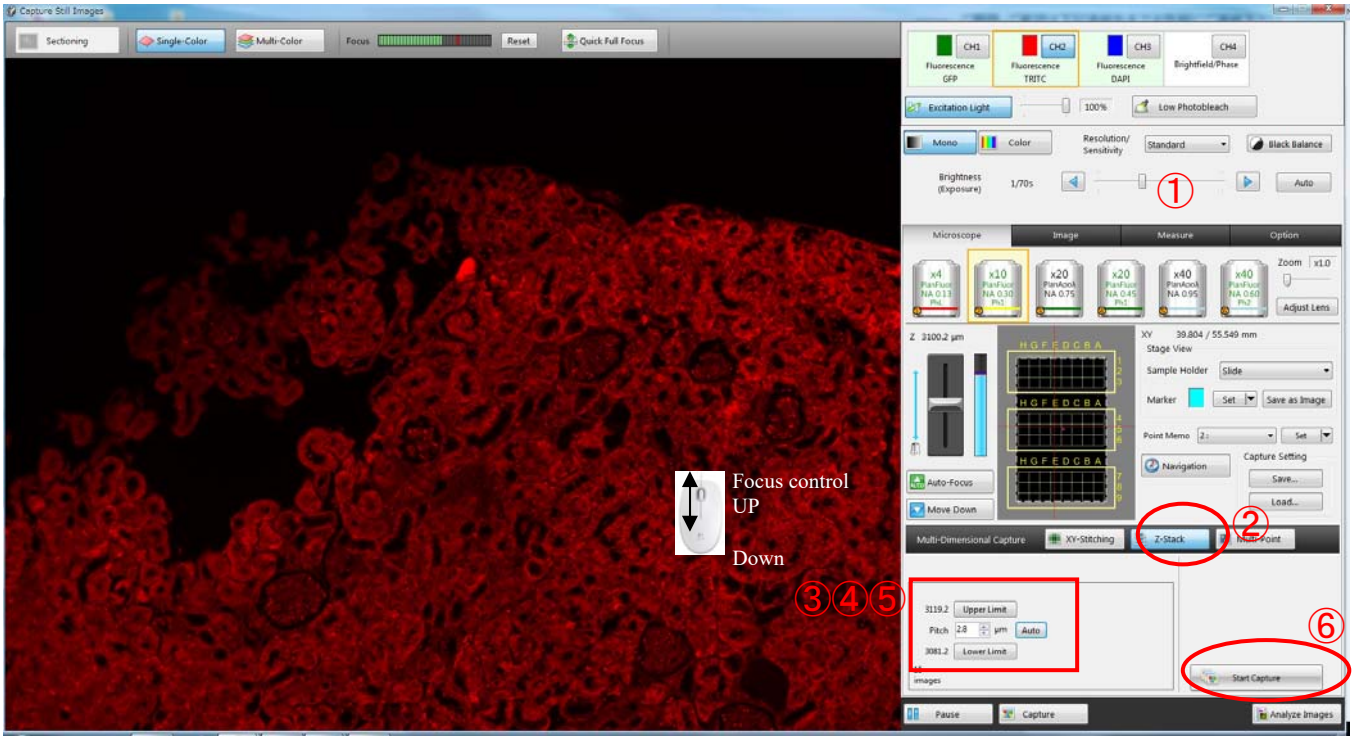
1. Adjust the brightness with a single color mode.
2. Click [Quick Full Focus] button.
3. Move the focus to the top of the sample.
4. Click [Start] button. Focus starts moving downward. The displayed images are synthesized in real time.
   Click [Stop] when the image does not change.
5. Click [Save Image] and save it.

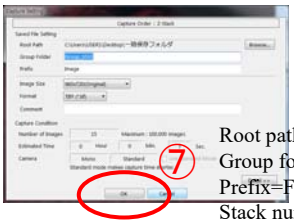


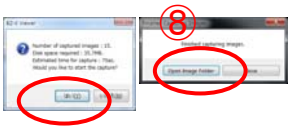
<Full focus>

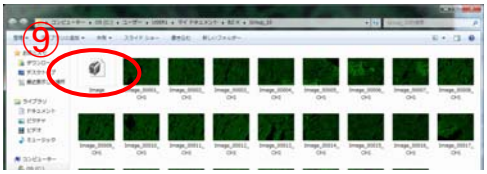
After capture while changing the focus, create one focused image.
Multi color can also be used together.

1. Adjust the location and brightness.
2. Click [Z-Stack] button. (Button turns blue)
3. Focus on the top of the sample. Click [Upper Limit] button.
4. Focus on the bottom of the sample. Click [Lower Limit] button.
5. Click [Auto] button.
6. Click [Start Capture] button.
7. Select folder, name, format to save and click OK.As confirmation comes out, click [はい](Yes).
8. After capturing is complete, click [Open Image Folder] button.
9. Open the file (that name is image) of the microscope icon. (BZ-X Analyzer starts up.)
10. Confirm that image folder is selected. Click [Load] button.
11. After the file opens in BZ-X Analyzer, click [Full Focus] button.
12. Click [Normal images] button.
13. A full focus image is created. Save full focus image with a name.

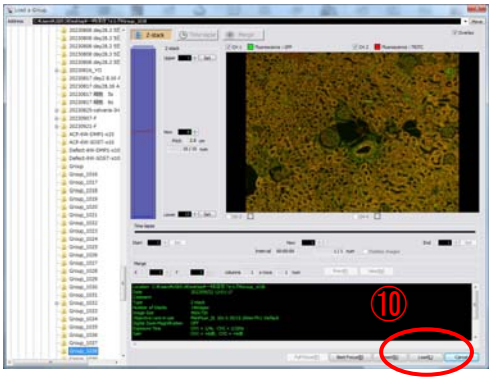


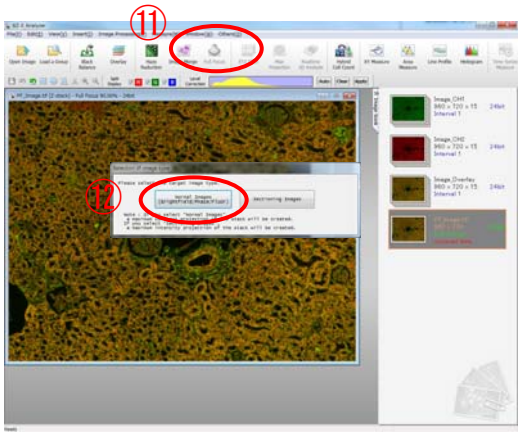






Root path=Folder to save
Group folder=Sub folder
Prefix=File name
Stack number is automatically added to the file name.



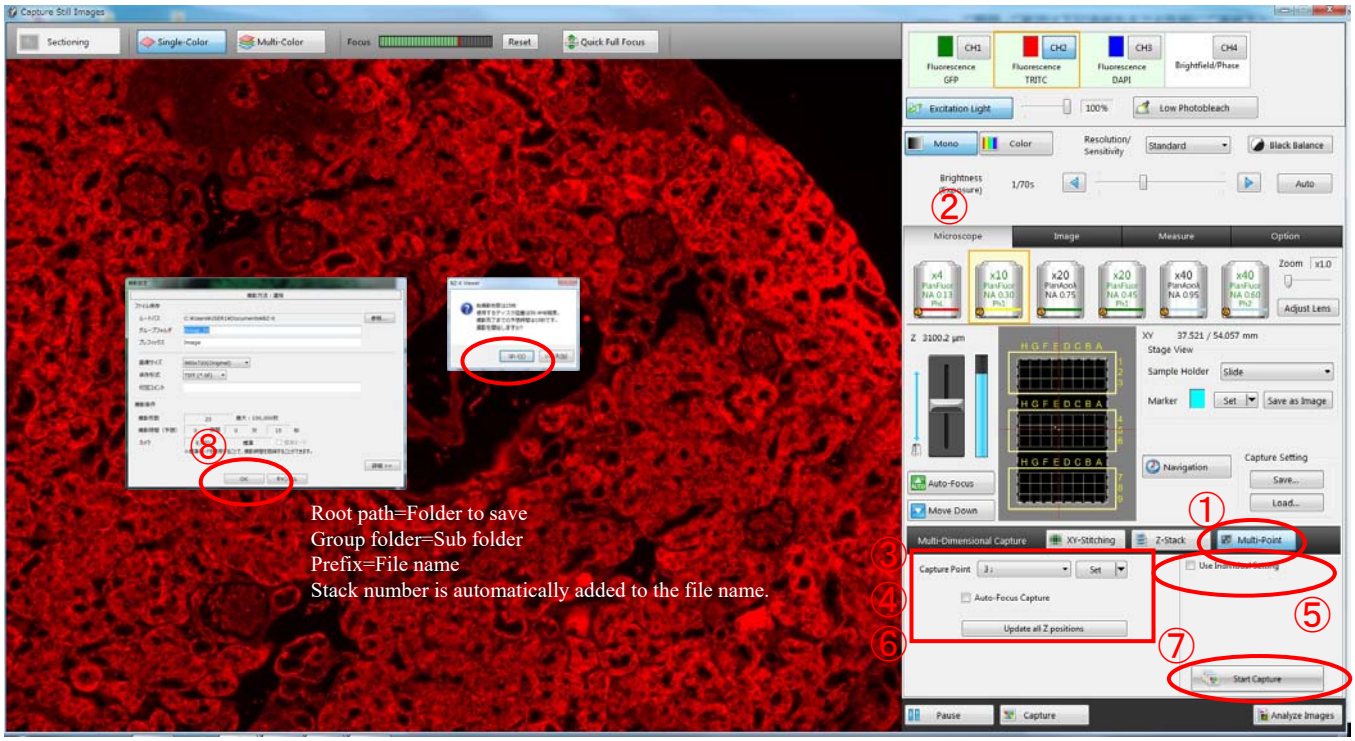


<Multi point>

Capture the specified position automatically.

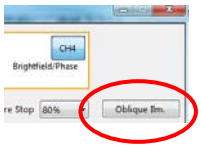
1. Click [Multi-point] button. (Button turns blue)
2. Adjust brightness and focus at the place you want to capture.
3. Set the [Capture point] to 1. Click [Set] button. Change to new number.
4. To autofocus at each point, check on “Auto Focus Capture”.
5. If you want to adjust the brightness for each point, check on “Use Individual setting”.
6. Register other points with the same operation. (point 2,3…) Max 99 points
7. Click [Start capture] button.
8. Select folder, name, format to save and click [OK]. As confirmation comes out, click [はい](Yes).

Individual settings can also change lens

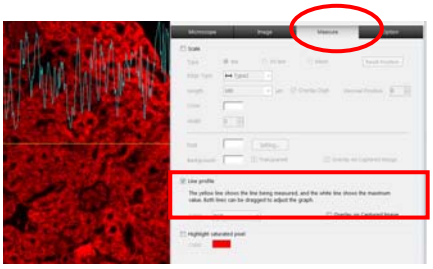


<Other functions>

Oblique Ilm…Change the method of lighting and get the contrast.
Zoom…Use digital zoom. Up to 3 times. (Can only be operated when using a single color.)
Adjust lens…It is used for correction ring operation and apply oil operation.
Line profile…Display a graph of fluorescence intensity.



Functions can be used in combination.
for example…XY stitch + Z stack = Extensive full focus image



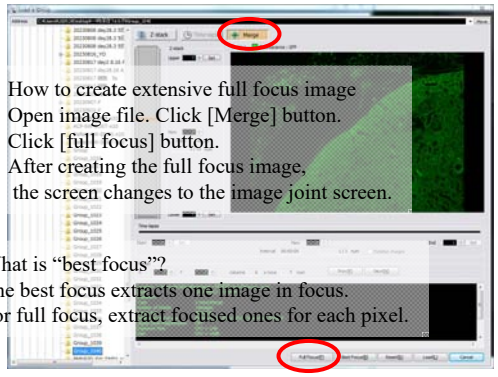
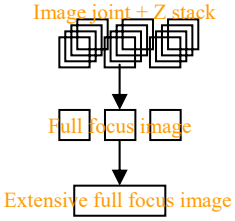
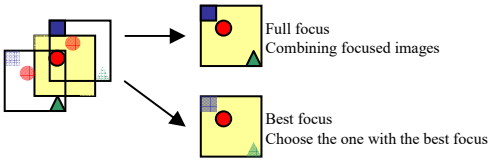


Image of creation procedure

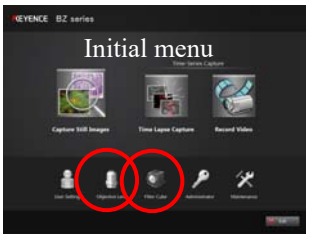


Difference between full focus and best focus



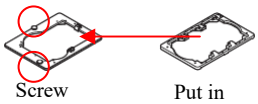
How to change BZ-X device settings

Launch the “BZ-X Viewer”.
(If it is in use, close the capture screen and return to the initial menu.)



<How to change stage holder>

[for multi-plate, for general, for general (large space)]
Combine the two screws and screw them to the stage.



[for slide glass, for 35mm dish]
Attach the multi-plate holder to the stage.
Place the holder for slide glass or 35mm dish into the plate holder.

Be sure to restore defaults after changing.
Default setting…for multi-plate + slide glass holder

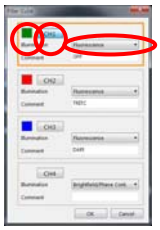
<How to change the objective lens>

Click the [Objective lens] button from initial menu.
Click the icon of the objective lens you want to replace.
As the lens moves to the frontmost position, replace with the one you want to use.
Select the name of the objective lens you want to use from the list.
Click the green upward arrow under the objective lens icon.
(The icon changes to the objective lens you want to use.)
Click [OK] button to close the setting screen.

Usable objective lens
PlanApo_λ 2x 0.10/8.5mm
PlanApo_λ 40x0.95/0.25-0.16mm
PlanApo_λ 100xH 1.45/0.13mm Oil

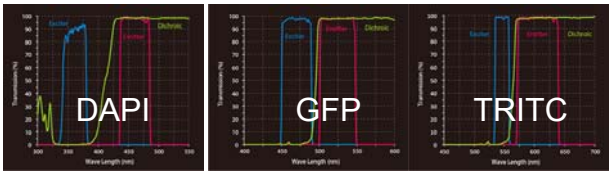
Be sure to restore defaults after changing.
Default setting
①PlanFluor_DL 4x0.13/16.50mm PhL　②PlanFluor_DL 10x0.30/15.20mm Ph1
③PlanApo_λ 20x0.75/1.00mm　④S_PlanFluor_ELWD_ADM 20xC 0.45/8.20-6.90mm Ph1
⑤None　⑥S_PlanFluor_ELWD_ADM 40xC 0.60/3.60-2.80mm Ph2
(Adjust the correction ring of the S_Plan objective lens to 1.2.)

<How to change the fluorescent filter>



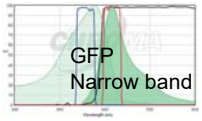
Click the [Filter cube] button.
Click the place (channel button) where you want to replace the filter.
(The filter moves to the frontmost position.)
Open front panel and replace filter cube.
（Pull up the front side of the cube and pull out the cube.
To set up, first insert protrusions on both sides and next lower the front side.)

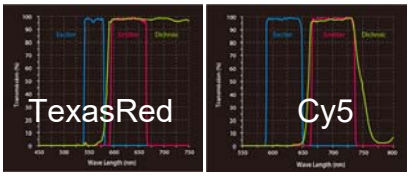
Select “Fluorescence”, “Bright Field/Phase Cont”, “OFF” from the pull-down menu.
In the case of fluorescence, click the color and select the false color.
Click [OK] button to close the setting screen.

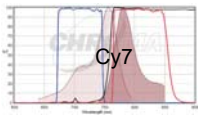


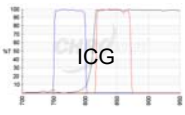
Be sure to restore defaults after changing.
Default setting
Ch1=FL (GFP) , Ch2=FL (TRITC) , Ch3=FL (DAPI) , Ch4=BF/Ph

Name	Excitation	Dichroic	Emission
DAPI	360/40	400	460/50
GFP	470/40	495	525/50
GFP narrow	480/20	495	510/20
TRITC	545/25	565	605/70
TexasRed	560/40	585	630/75
Cy5	620/60	660	700/75
Cy7	710/75	760	810/90
ICG	775/50	810	845/55









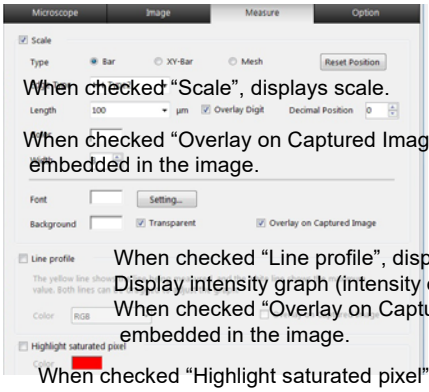
BZ-X option settings

Image tab…Perform image quality adjustment at the time of preview.

Adjust the contrast using the lookup table.

Improve image quality using image filter and haze reduction.

Measure tab…Display scale, line profile, saturated pixels.

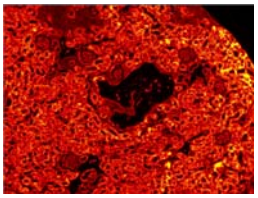


When checked “Scale”, displays scale.

When checked “Overlay on Captured Image”, embedded in the image.

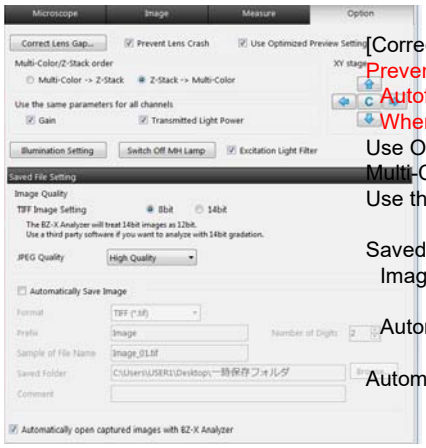
When checked “Line profile”, displays line profile.
Display intensity graph (intensity on yellow line).
When checked “Overlay on Captured Image”, embedded in the image.

When checked “Highlight saturated pixel”, displays saturated pixels.



The yellow area is saturation.

Option tab…Change the device settings.



[Correct Lens Gap]…Correct focus position and center location for each lens.
Prevent Lens Crash…When this check is off, the limit is canceled.
Autofocus works only to the limit position. When you cancel the limit, be careful not to hit the lens.
When unchecked, PlanApo x20, x40 lens should not be used.
Use Optimized Preview Setting…Improve the quality of the preview.
Multi-Color/A-Stack order…Change priority order.
Use the same parameters for all channels…Illumination and detector settings are common.(Defaults are all ON.)

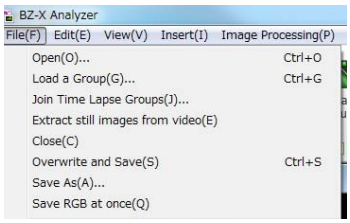
Saved File setting
Image Quality…Setting of gradation of TIFF file and image quality of JPEG file.

Automatically Save Image…When checked, it will be automatically saved with the specified settings.

Automatically open captured images with BZ-X Analyzer…Open the image with BZ-X Analyzer after capture.

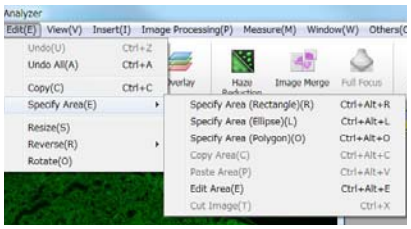
How to use BZ-X Analyzer

Red letters are frequently used functions.



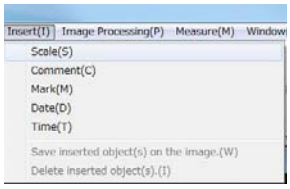
File

Open…Open the single image. (Multichannel image is a single image.)
Load a group…Open the stack image.
Select a folder and press [Load] button. (Used for multi function images.)
Save RGB at once…Separate the color image into RGB.



Edit

Copy…Duplicate image.
Specify Area …Make a frame of the specified shape.
Specify Area (Rectangle , Ellipse , Polygon)
You can perform the following operations after specifying the area.
Copy Area…Copy the shape.
Paste Area…Paste the shape.
Edit Area…Adjustment of frame.
Cut Image…Cut out specified area into new file.
Resize…Reduce the image in 1% increments.
Reverse , Rotate…Mirror image , Rotate image. There is a limit on the image size.



Insert

Scale…Insert scale bar of the specified length. Do not touch the calibration value.
Comment , Mark , Date , Time …Insert specified character.
Save inserted object on the image…Write object in image.
(If write has not been executed, ask when closing.)
Delete inserted object…Delete all inserts before writing.

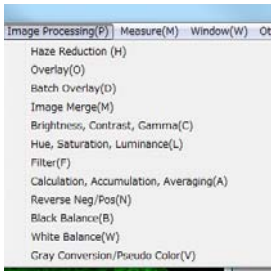
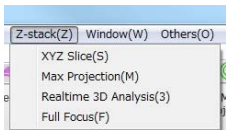


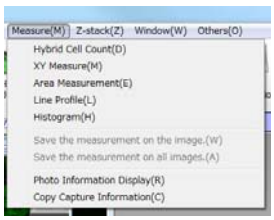
Image processing

Haze reduction…Remove blur.(Change the parameters while watching the preview.)
Images to be used for intensity calculation should not be processed.
Overlay…Merge the image. (Select the image to be merged in the pull-down menu.)
Image marge…Stitch of stack images. (Just read the file and press [start stitching].)
Brightness etc…Image adjustment.
Filter…Averaging, edge emphasis, etc.
Calculation , Accumulation , Averaging…Calculate image.
Black / White balance…Correct background. (Just pick the background.)
Gray conversion / pseudo color…Convert color image.



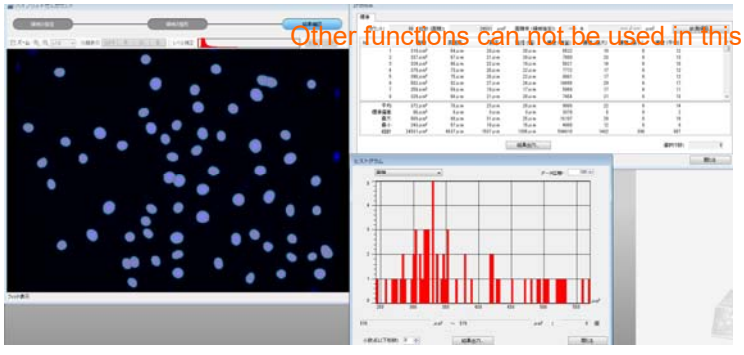
Z stack (Menu is displayed only for Z stack image.)

Full focus…Create one focused image. (Press in the order of full focus, normal image.)
For other 3D-related functions, please refer to the attached document.



Measurement

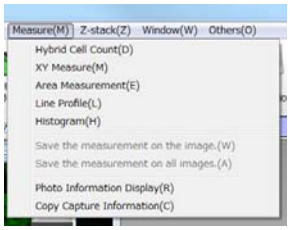
Hybrid cell count…Isolate cells with fluorescence intensity or hue.
Calculate the count, area, brightness of the isolated cells. (See the figure below)
Contact KEYENCE when you can not do it well.
Photo information display…Display of objective lens, exposure etc.
Copy capture information…Copy the capture condition from another file.
（You can open files that can not be opened by BZ-X Analyzer.）



Other functions can not be used in this laboratory. (Paid option)

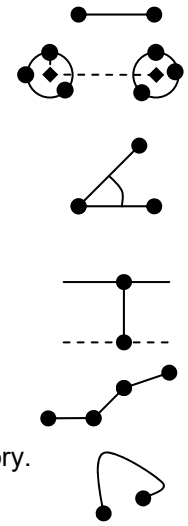
How to use BZ-X Analyzer (extra manual)

These functions can not be used on a PC connected to a microscope.
If you would like to use these function please use the 180 days trial version.

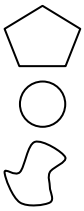



Measurement menu

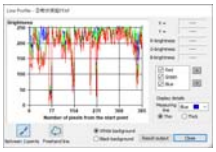
Hybrid cell count…Perform cell count. See the separate manual for usage.
XY measure…Measure distance. Select the drawing mode and draw the object on the screen.
Between 2 points…Measure the distance from the clicked place to the next clicked place.
Radius…Measure radius of circle. Specify three points and draw a circle.
Centers…Measure the distance between the center points of two circles.
Count…The place you clicked is marked. The number of marks is counted and displayed.
Angle1…Measure the angle. Specify three points. The third point is the corner.
Angle2…Measure the cross angle of the two lines.
Click twice to draw a straight line.
When the second line is drawn, the angle of the cross part is measured.
Perpendicular…Measure the length of the perpendicular.
Click twice to draw a baseline. When you draw a perpendicular with the third click,
the length is measured. Double click to end.
Parallel…Same usage as perpendicular.
Segment line…Measure the total distance of a polygonal line.
Click to bend. Double click at the end point.
Freehand line…Draw freehand lines. Click at the start point. Move the mouse and draw a trajectory.
Click again at the end point
Garbage box…Delete the selected object.
Delete all…Delete all objects.
(in the other window)
Result output…Export the list as a text file.



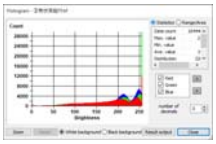
Area measurement…Measure area. Select the drawing mode and draw the object on the screen.
Polygon…Draw a polygon. Same usage as polygonal line.
Circle draw…Draw a circle. Same usage as radius.
Freehand line…Draw a free figure. Same usage as curve of size measurement.
Run…Select the [Run] button and click inside or outside the drawn shape.
The area of the corresponding place is displayed in the list.
(in the other window)
Result output…Export the list as a text file.



Line profile…Display the intensity graph on the line.
You can select “between 2-points” or “Freehand line”.
Drawing method is same as size measurement.
Move the mouse on the graph to display the information and location.
When clicking the [Result output] button,
the coordinates and the intensity value are export as a text file.



Histogram…Display the intensity distribution graph of the image.
The [Result output] button can export histogram data to a text file.

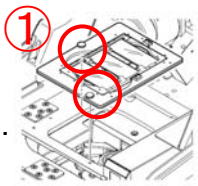


Keyence all in one fluorescence microscope
How to use BZ-X700

Method of stage chamber setup

Installation method

①Remove stage mounter.（Screws are fixed in 2 places.）
Remove the PlanApo20x (black letters) lens.
Adjust the correction ring of 20x, 40x (green letters) objective.



Cover glass=0.17
Plastic bottom=1.2

②Assemble the chamber unit.
If you use a 35mm dish, set 2 dishes.

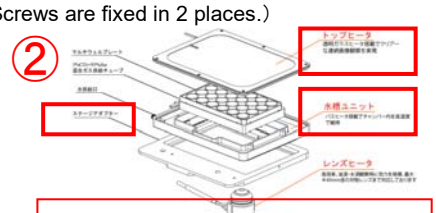
③Attach the chamber unit on the microscope and fix it with screws.（Screws are fixed in 2 places.）

④Inject distilled water slowly from the tube. (About 48ml)

⑤Connect the CO2 gas line.

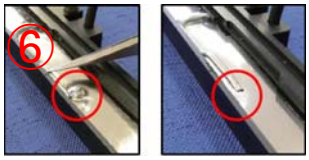
⑥Confirm that the gas has reached the chamber unit.
After confirming the bubbles, make the gas line floating.

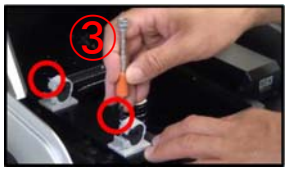
⑦Pull out the cables and tubes from the front panel and close the lid.
Is there a margin for the tube from the front?



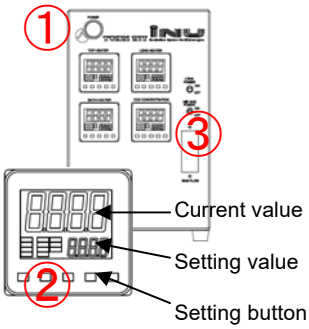
Set in the microscope
in an assembled state without water.

Open the clamp
when injecting water.





How to set up the controller



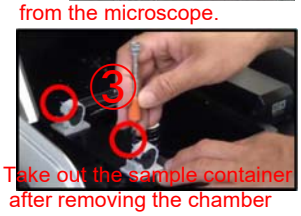
①Turn on the controller's main switch.
②Set temperature and CO2 concentration with setting button.
③Turn on mix gas switch.
(Wait about 30 minutes to stabilize the incubator.)
(To reduce focus deviation,
put the sample in the incubator and wait for about 30 minutes.)

(Default value) Recommended value	Dish	Well
TOP	(49.0) 48.5	(48.5) 48.5
BATH	(39.0) 38.5	(39.0) 38.5

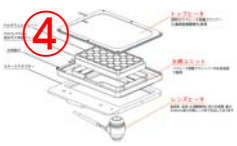
Removal method

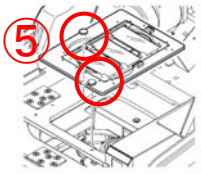
①Disconnect the CO2 gas line.
②Use a syringe to drain the chamber.
③Detach the chamber unit.
④Wipe off the chamber water completely.
Wipe the top heater and chamber unit with 70% ethanol and dry.
⑤Attach the stage mounter. Leave the lid open and let the interior dry.
Install the PlanApo20x lens.
⑥Turn off the controller.

Drain water
before removing the chamber
from the microscope.



Take out the sample container
after removing the chamber
from the microscope.





※Lens heater is unnecessary for phase difference lens.
(mainly oil immersion lens)
If you use a lens heater, pass the cord through the gap of the lid of the filter and wind the heater around the lens.
Turn on the lens heater switch of the controller.
※When using the lens heater, lens change and multipoint shooting are not available.

Keyence all in one fluorescence microscope
How to use BZ-X700

Time lapse (constant interval capture)

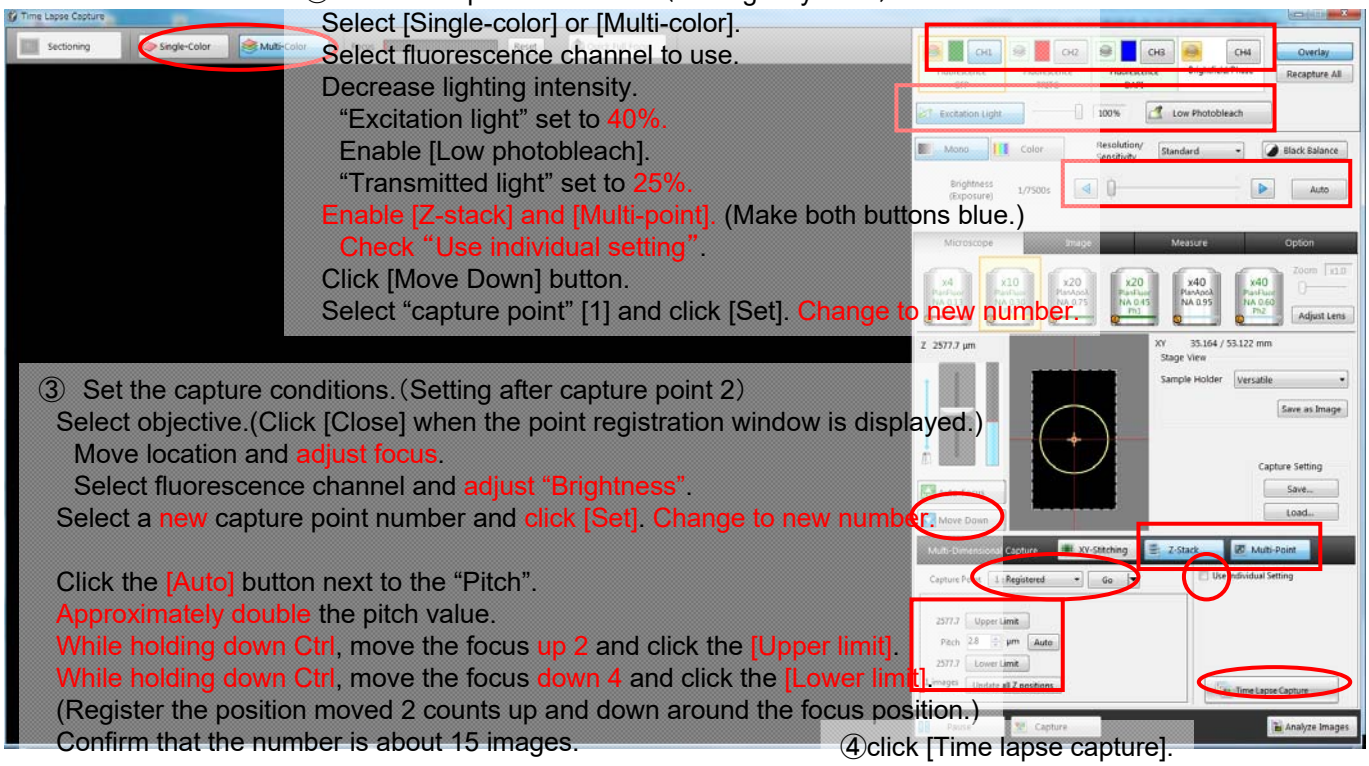
Use only Phase contrast lenses.
Adjust the correction ring of 20x and 40x objective.

①Select [Time lapse capture] from the main menu.
Select the sample holder to use.

Method of time lapse (fixed range)

For time-lapse capture,
Z stack and multi-point capture are used together.
(In order to follow the focus position)
In order to prevent the temperature drop caused by the objective,
lower the objective at capture point 1.
After putting the sample,
it takes about 30 minutes for the focus position to stabilize.

② Set the capture conditions.(Setting only once)
Select [Single-color] or [Multi-color].
Select fluorescence channel to use.
Decrease lighting intensity.
"Excitation light" set to 40%.
Enable [Low photobleach].
"Transmitted light" set to 25%.
Enable [Z-stack] and [Multi-point]. (Make both buttons blue.)
Check "Use individual setting".
Click [Move Down] button.
Select "capture point" [1] and click [Set]. Change to new number.



③ Set the capture conditions.(Setting after capture point 2)
Select objective.(Click [Close] when the point registration window is displayed.)
Move location and adjust focus.
Select fluorescence channel and adjust "Brightness".
Select a new capture point number and click [Set]. Change to new number.

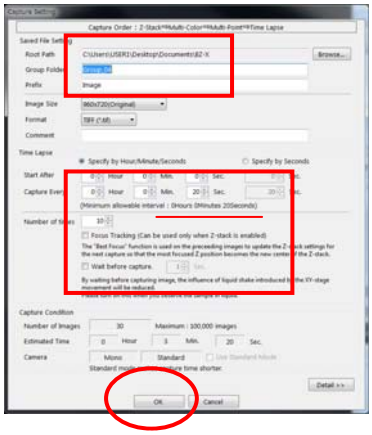
Click the [Auto] button next to the "Pitch".
Approximately double the pitch value.
While holding down Ctrl, move the focus up 2 and click the [Upper limit].
While holding down Ctrl, move the focus down 4 and click the [Lower limit].
(Register the position moved 2 counts up and down around the focus position.)
Confirm that the number is about 15 images.

④click [Time lapse capture].

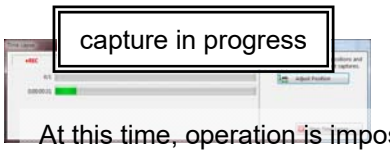
Repeat step 3 if there is a next capture point.
After registering all capture points, check the focus again.
Select a capture point number and click [Go]. Check the focus.
If the focus is out of focus, re-set the upper and lower limits.

Focus up

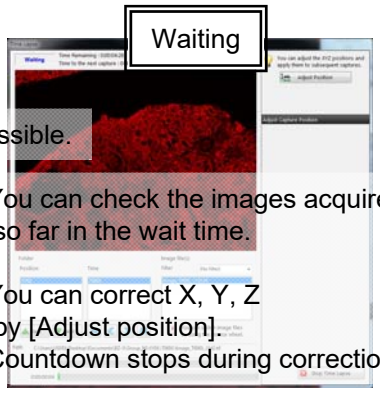
Focus down



⑤Confirm the folder and name to save.
Enter "Capture Every" at least "Minimum allowable interval".
Enter the "Number of times".
Check "Wait before capture".
(Waiting time is about 5s) (not use focus tracking)
Click [OK] to start capture.
The capturing screen and waiting screen are displayed alternately
until the capture ends.

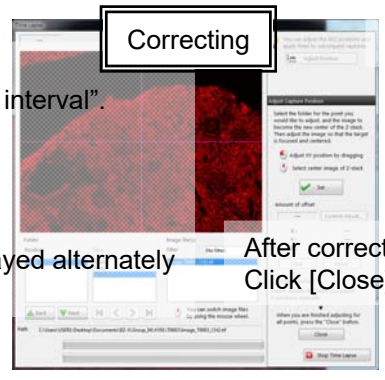


At this time, operation is impossible.



You can check the images acquired so far in the wait time.

You can correct X, Y, Z by [Adjust position].
Countdown stops during correction.



After correcting the position, click [Set].
Click [Close] to return to the waiting screen.

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Time lapse (constant interval capture)

Use only Phase difference lenses.
Adjust the correction ring of 20x and 40x objective.

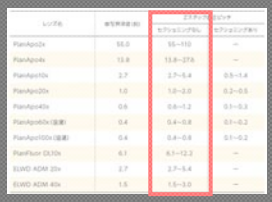
①Select [Time lapse capture] from the main menu.
Select the sample holder to use.

Method of time lapse (focus tracking)

For time-lapse capture,
Z stack and multi-point capture are used together.
(In order to follow the focus position)
In order to prevent the temperature drop caused by the objective,
lower the objective at capture point 1.
After putting the sample,
it takes about 30 minutes for the focus position to stabilize.

② Set the capture conditions.(Setting only once)
Select [Single-color] or [Multi-color].
Select fluorescence channel to use.
Decrease lighting intensity.
"Excitation light" set to 5% to 20%.
(The exposure time should be 3 seconds or less.)
"Transmitted light" set to 25%.
Enable [Z-stack] and [Multi-point]. (Make both buttons blue.)
Check "Use individual setting".
Click [Move Down] button.
Select "capture point" [1] and click [Set]. Change to new number.

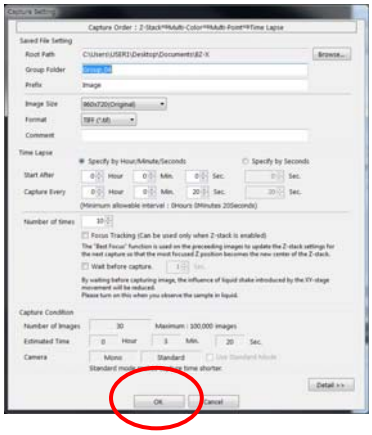
③ Set the capture conditions.(Setting after capture point 2)
Select objective. (Click [Close] when the point registration window is displayed.)
Move location and adjust focus.
Select fluorescence channel and adjust "Brightness".
Select a new capture point number and click [Set].
Change to new number.



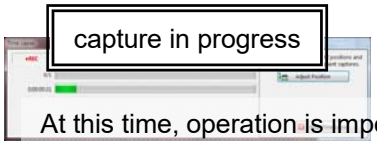
Set the pitch to the value in the table.
Click the [Upper limit] at the focus position.
Shift the focus down and click the [Lower limit].
Set the lower limit so that the number is about 10 images.

Repeat step 3 if there is a next capture point.
After registering all capture points, check the focus again.
Select a capture point number and click [Go]. Check the focus.
If the focus is out of focus, re-set the upper and lower limits.

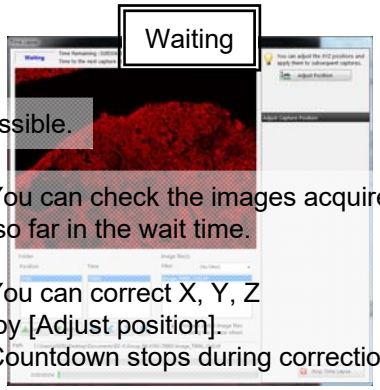
④Click [Time lapse capture].



⑤Confirm the folder and name to save.
Enter "Capture Every" at least "Minimum allowable interval".
Enter the "Number of times".
Check "Focus tracking" and "Wait before capture".
(Waiting time is about 5s)
Click [OK] to start capture.
The capturing screen and waiting screen are displayed alternately until the capture ends.

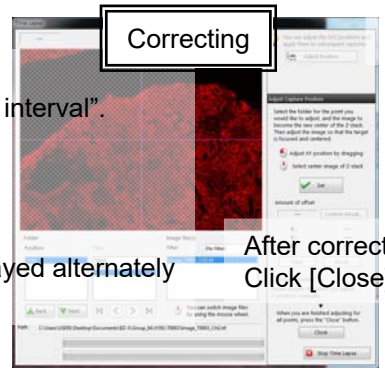


At this time, operation is impossible.



You can check the images acquired so far in the wait time.

You can correct X, Y, Z by [Adjust position].
Countdown stops during correction.



After correcting the position, click [Set].
Click [Close] to return to the waiting screen.

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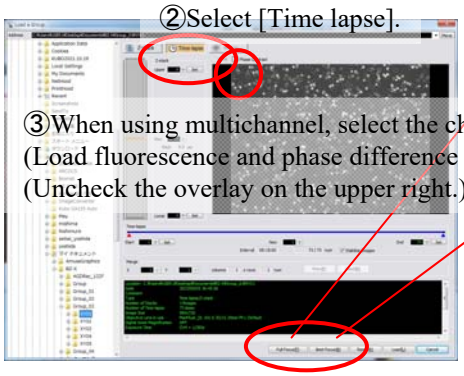
Time lapse data processing method

①Open Image file in data folder.

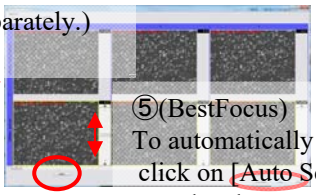
②Select [Time lapse].



③When using multichannel, select the channel to load.
(Load fluorescence and phase difference separately.)
(Uncheck the overlay on the upper right.)

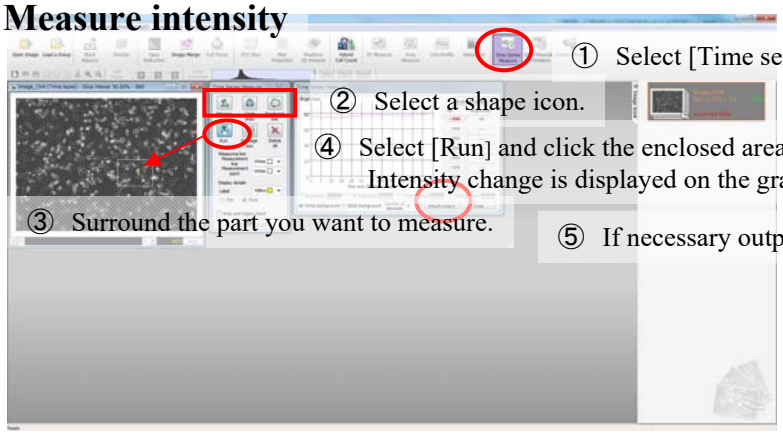
④Choose [Full focus] or [Best focus].
(If you are not using the Z-stack, Click [Load].)

⑤(FullFocus)
Select [Normal image].



⑤(BestFocus)
To automatically select the optimum focus position, click on [Auto Select].
To select by yourself, use the slide bar to display the image at the optimum focus position from each Z stack image and click the [Select].
Finally click [Load].

Measure intensity



① Select [Time series measure] of menu.

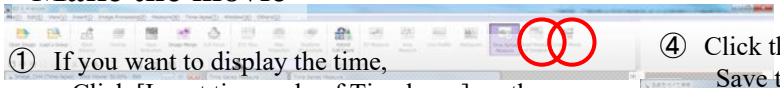
② Select a shape icon.

③ Surround the part you want to measure.

④ Select [Run] and click the enclosed area.
Intensity change is displayed on the graph.

⑤ If necessary output it to the text file with the [Result output] button.

Make the movie

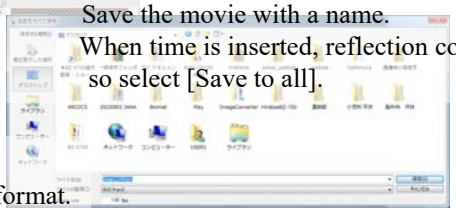


① If you want to display the time,
Click [Insert timescale of Timelapse] on the menu.
If you do not need the Time lapse time, go to ④.

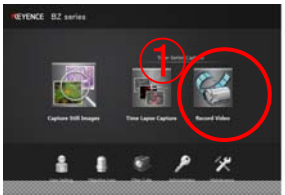
② Set the format and click [OK].

③ Move the inserted time.
Double-click the inserted time to edit the format.

④ Click the [Create movie] on menu.
Save the movie with a name.
When time is inserted, reflection confirmation is displayed, so select [Save to all].



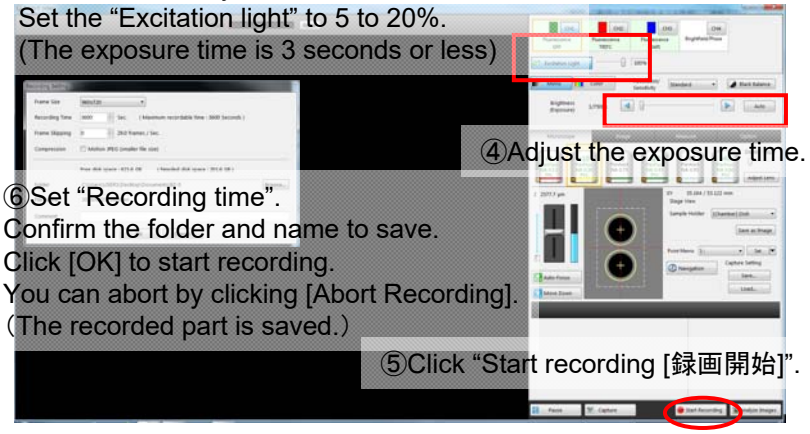
Recording video



①Select [Record Video] from the main menu.

②Move location and Choice the lens.
Adjust the focus.

③Set the intensity of “Transmitted Ilm” to 25%.
Set the “Excitation light” to 5 to 20%.
(The exposure time is 3 seconds or less)



④Adjust the exposure time.

⑥Set “Recording time”.
Confirm the folder and name to save.
Click [OK] to start recording.
You can abort by clicking [Abort Recording].
(The recorded part is saved.)

⑤Click “Start recording [録画開始]”.

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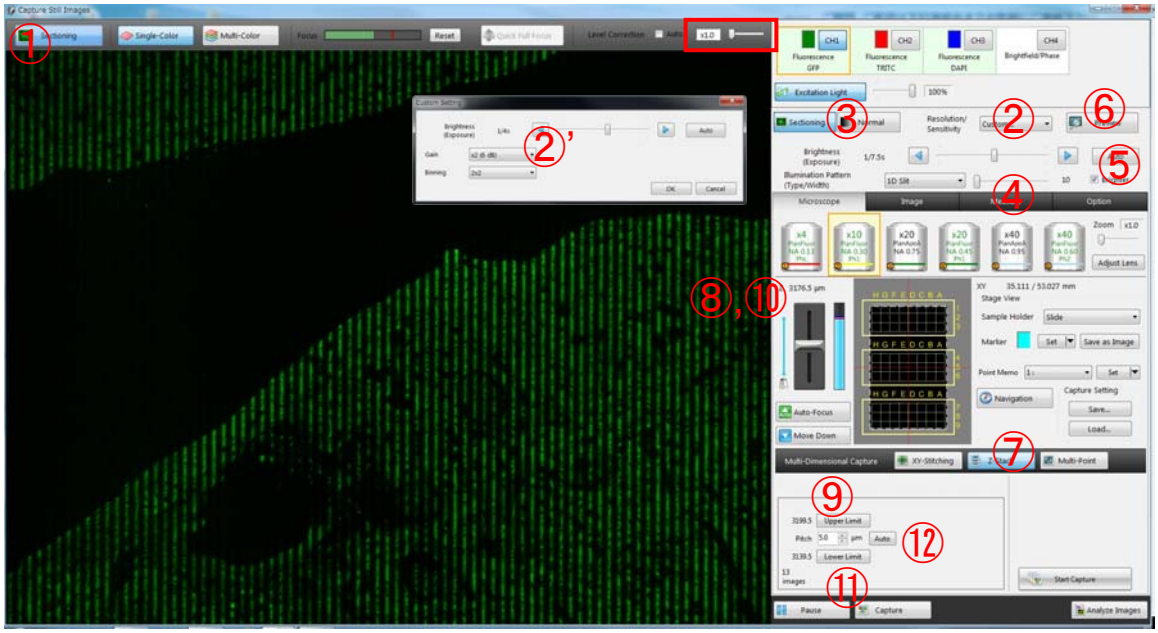
Method of sectioning



1. Click the [Sectioning] button to switch to sectioning mode. (button turns blue)
2. Select [Custom] in “Resolution / Sensitivity” menu. Set “Gain” to x8.
3. Click [Normal] button to switch to sectioning mode. Adjust focus.
   Click [Sectioning] button to switch to sectioning mode.
4. Select slit type and width in Illumination pattern menu.
   If the stripes do not appear, the image cannot be capture.
   Choose numbers that make the stripes look nice.
   Basically, the smaller the number, the higher the resolution.
   The higher the number, the lower the resolution, but you can capture deeper images.
5. Click [Auto] button on “Brightness”. (Make fine adjustments to avoid saturation.)
6. Click [Preview] button to check image.
   Set “Level correction” to x1.
   Click [Capture] to capture the sectioning image.
   (Multi-colors are also possible.)

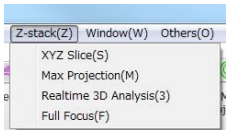
If you want to use Z stack, continue to set up Z stack.

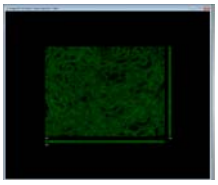
Z-Stack setting

7. Click [Z-Stack] button to switch to Z-Stack mode. (button turns blue)
8. Check the focus number. Move the focus towards the larger number.
   Stop when you can no longer see the stripes.
9. Click [Preview] and confirm that no displayed. Click [Upper limit] button.
10. Move the focus towards the smaller number. Stop when you can no longer see the stripes.
11. Click [Preview] and confirm that no displayed. Click [Lower limit] button.
12. Click [Auto] button on pitch. Double the pitch number.
    Click [Start capture] to start Z-stack.

How to use BZ-X Analyzer (extra manual)

This function can be used only sectioning data.



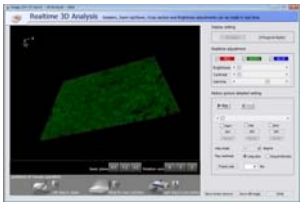


Z stack (Menu is displayed only for Z stack images)

XYZ slice…Display plan and cross section images.
Move the line and change the section position.
You can save section images.

Max projection…Image synthesis is performed with the maximum intensity value on the same axis.
Only fluorescence images function normally.
You can save maximum projection image.

Realtime 3D analysis…You can create a stereoscopic image and move freely.
You can save still images and rotating movie.
Dragging a stereoscopic image will change the angle.
Scale with the mouse wheel.
When you right-drag the cross section, the cross section position changes.
(Effective when you want to show the inside.)



If you select [Orthogonal display], the XYZ slice will be displayed.
(When you right-drag the cross section, the cross section position changes.)
Adjust brightness and contrast with real time adjustment.

Motion picture detailed setting…Items necessary for creating animation.
Change the angle to the starting position and click the [Set] button under “Start”.
Change the angle to the end position and click the [Set] button under the “End”.
(If you set “Mid”, it will become an animation that changes in angle)
Select the “play methods” and angle step.
Set the frame rate between 5 and 50.
Click the [Frame rate] button.
You can check the animation with the [Play] button.
When you click the [Save still image] button,
displayed image saves in TIFF or JPEG image.
When you click the [Save motion picture] button, movie saves in avi file.

Full focus…Make full focus image.
Select a normal image or sectioning images.