FV4000-IX83
Operation Manual

EVIDENT

Ver.2

(cellSens-FV ver3.1.1)

Table of contents

Image Acquisition----------

ZDC----------

Table of contents

—Advanced—

—Spectral Imaging—

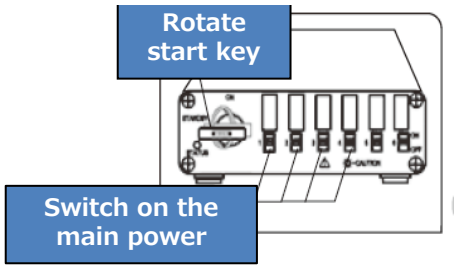
—Appendix—

Image Acquisition

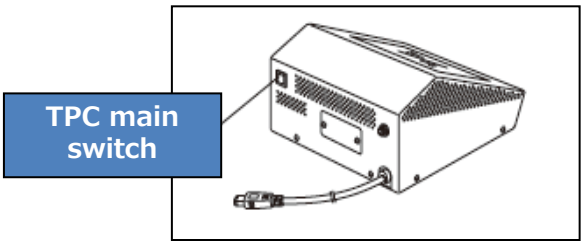
EVIDENT

Starting the system

④Laser Combiner



⑤Touch Panel Controller（TPC）



Turing the PC on

① Turn on the PC.

② Log on Windows with your own user ID. Wait until desktop appears.

【Factory Default ID & password】
Factory User ID : evident
Password : evident

③ Switch on the central power.

Laser controller

④ Rotate the start key and Switch on the main power of each laser.

Touch Panel Controller（TPC）

⑤ Press the main switch of the touch panel controller.

Starting software

⑥ Double-click the [cellSens FV] icon on the desktop.

※Start the software after “Start operation” is displayed on TPC.

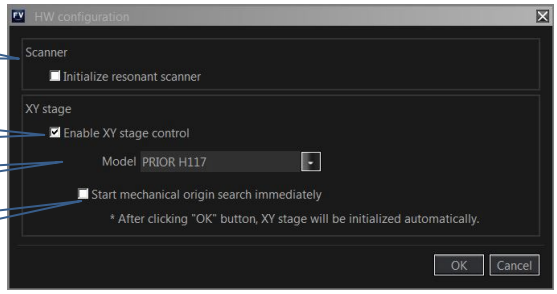
⑦ The dialog box appears. Set each checkbox and click [OK].

Selection whether or not to initialize the resonant scanner

Selection whether or not to control the XY stage by software.

XY stage type

Selection whether or not to move to XY stage origin.



Controller／Objective lens

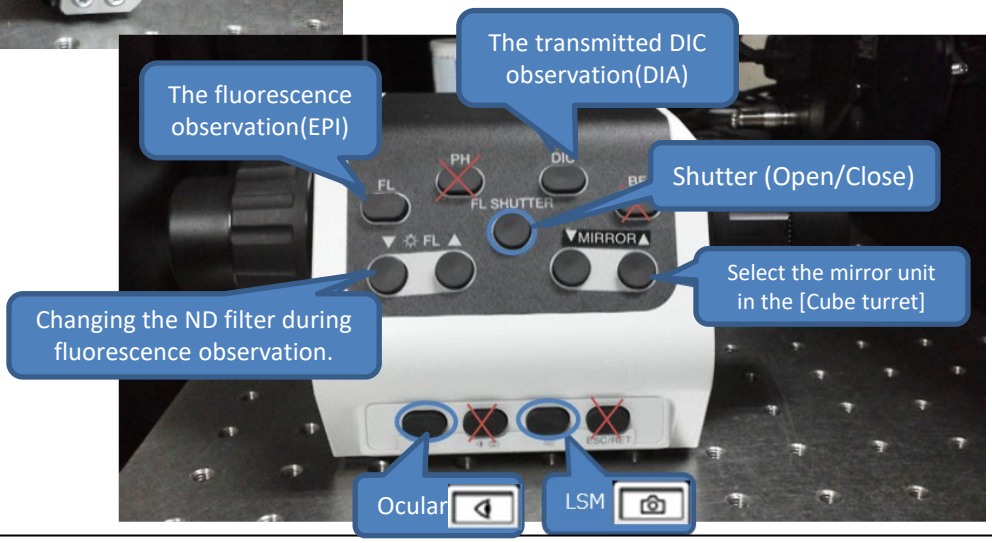
※TPC: Controller

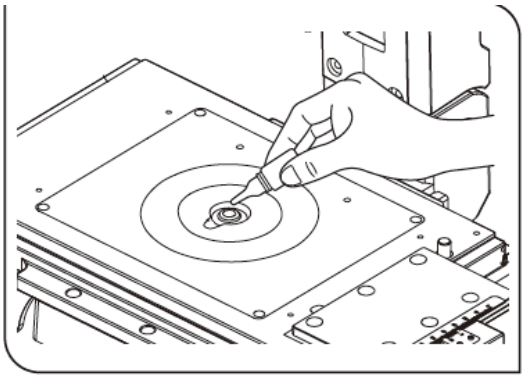
Software: Ocular

All the same meaning.

Use something that is easy to use.

(See the page 7 of this operation manual uses TPC.)

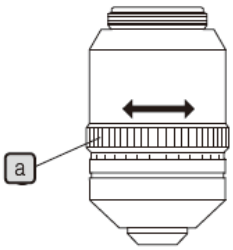




How to use the immersion objective lens

① Set the immersion objective lens in the optical path.
② As shown in the figure on the left, Drop immersion oil on the objective lens.
（1 or 2 drops）
③ Set the specimen and adjust the focus with the focus handle.

※Please note that images will be difficult to see if bubbles enter the oil.
※Please use the immersion oil suitable for the objective lens.
※Clean the objective lens and specimen after use.



[a]Adjustment of Objective Correction collar

① Rotate the correction collar (a) very slightly and re-focus the objective to determine if the image has improved or degrade.
② Repeat the previous step.

Observation via eyepieces

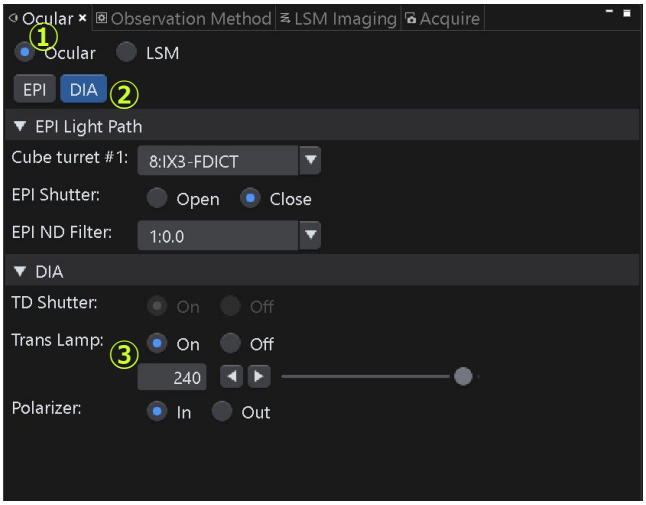
★DIC (Transmitted light)

＊Select the objective lens on the TPC.

① Click “Ocular” in [Ocular] Tool window.

② Click “DIA”.

③ Adjust the brightness via slider in Trans Lamp.



★Fluorescence

＊ Select the objective lens on the TPC.

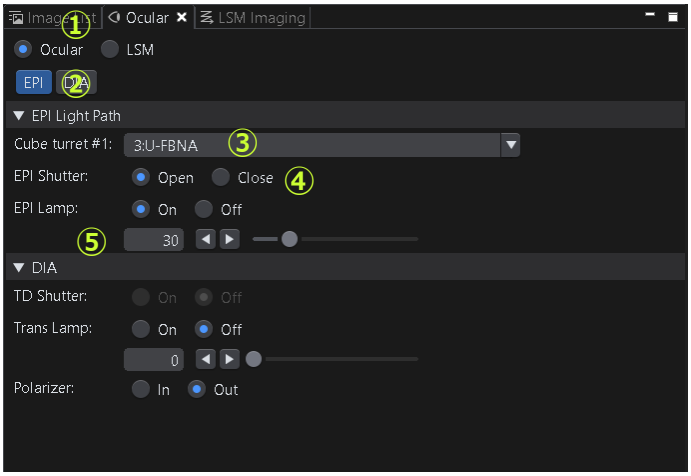
① Click “Ocular” in [Ocular] Tool window.

② Click “EPI”.

③ Select the cube.

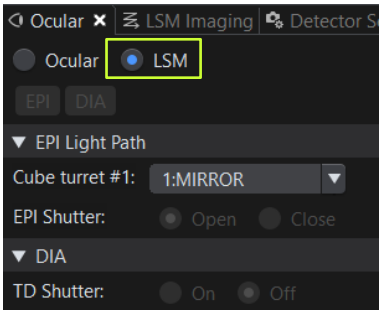
④ Click “Open” in EPI Shutter.
After observation, click “Close”

⑤ Excitation light power can be adjusted by ”EPI Lamp”.

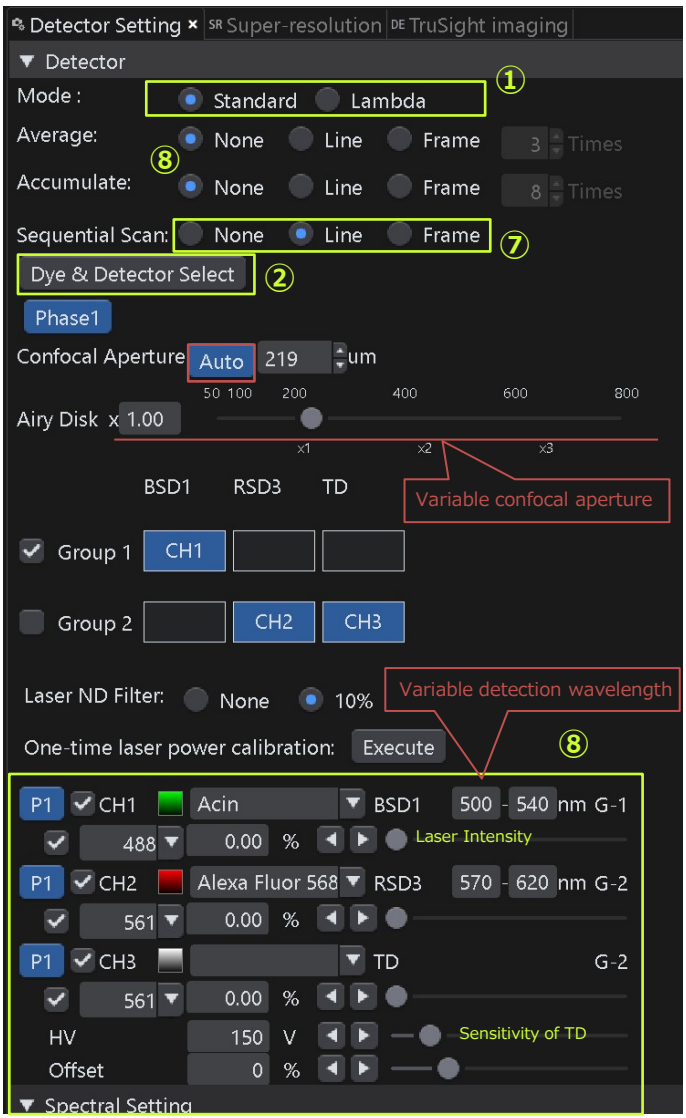


※Display may differ depending on the configuration.

★After observation via eyes, click “LSM”



XY Image Acquisition(1)



① Select “Standard” in [Detector Setting] tool window.

Assign the FL probe and Ch

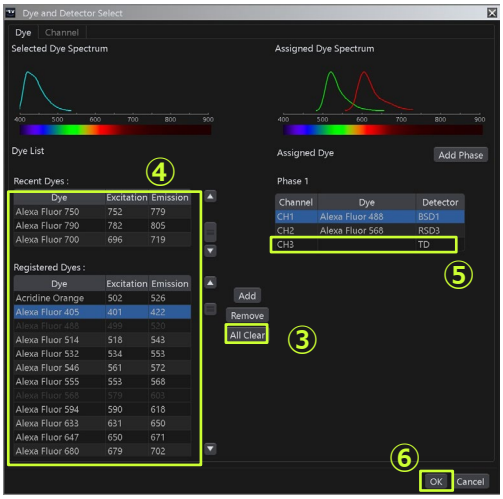
② Click Dye & Detector Select

③ Click All Clear

④ Double click the FL probes to observe。

⑤ When registering the FL probes, TD channel is registered automatically. If you don’t need the channel, double click TD to remove.

⑥ Click OK after selecting all channels.



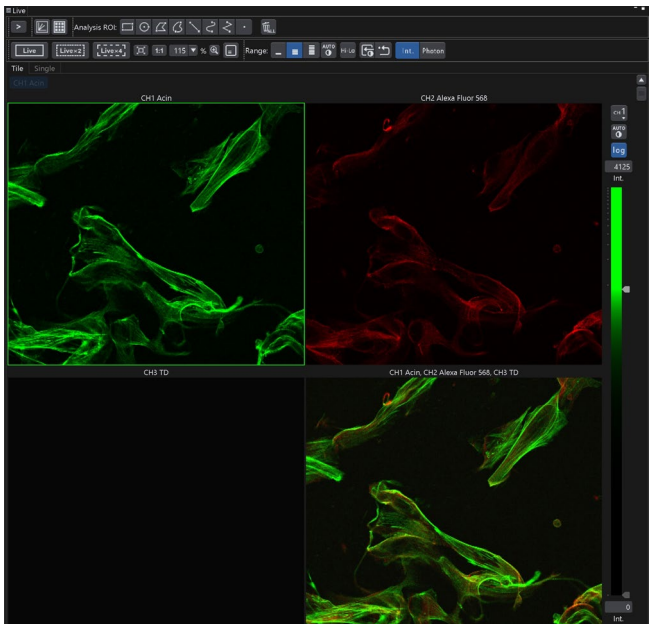
Adjusting the live image

⑦ When you acquire multiple channel image, selecting “Line” in sequential scan is recommended. 。

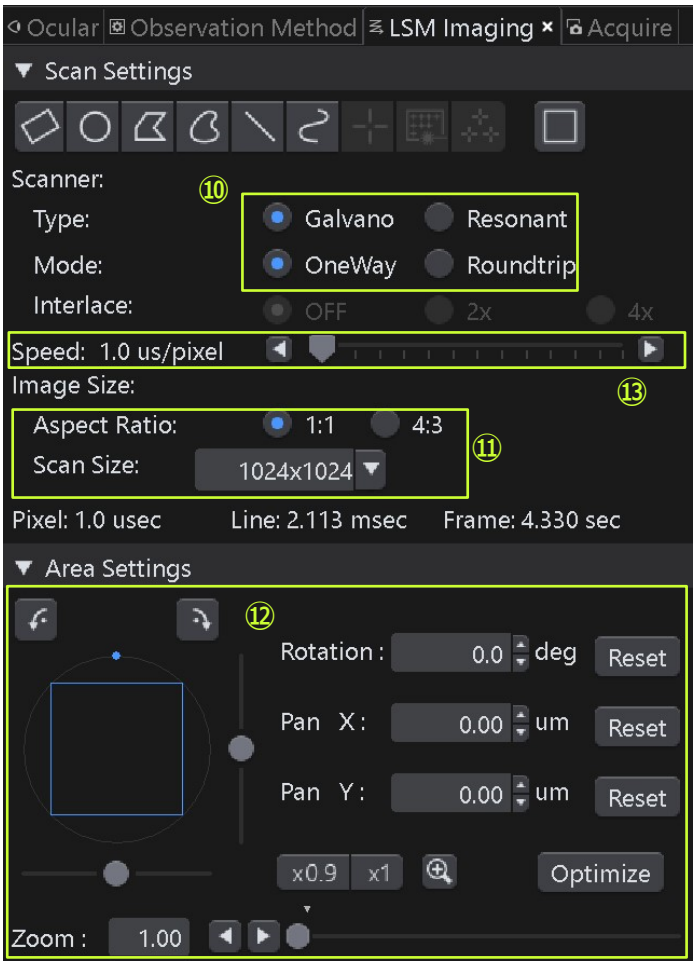
⑧ Click any one of following Live Live×2 Live×4. Adjust focus and Laser Intensity. (HV and Offset on TD.)

⑨ Use “Average” or “Accumulate” to get better S/N.

⑩ Adjust LUT via Range: or vertical bar on the right.



XY Image Acquisition（2）



Setting the scanner in [LSM Imaging]

⑩ Select the type of scanner and mode.

⑪ Set “Scan Size” and “Aspect Ratio”.

⑫ Set “Zoom” and “Rotation”.
Clicking “Optimize”, Zoom changes to make pixel size to ½ of optical resolution.

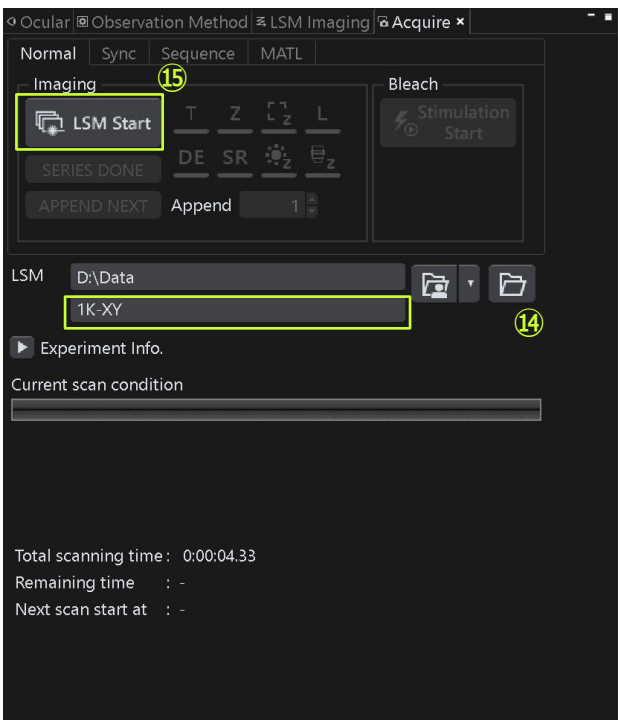
⑬ Set “Speed”. S/N will be better with slower speed.
＊ 1us/pixel will be available only when scan size is lager than 1024x1024.

Starting Acquisition

⑭Select [Normal] tab in [Acquire] Tool Window.
Press the button to open the dialog.

*The acquired images are saved automatically. Series number is added at the end of file name like “×××_0001” and “×××_0002”.

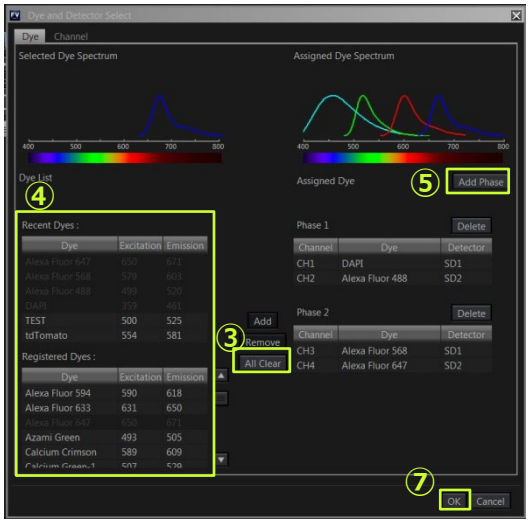
Final check
- ✓ DO NOT use “%” to the file name.
- ✓ Setting such as Timelapse, Z stack, etc. are recognized via icons.

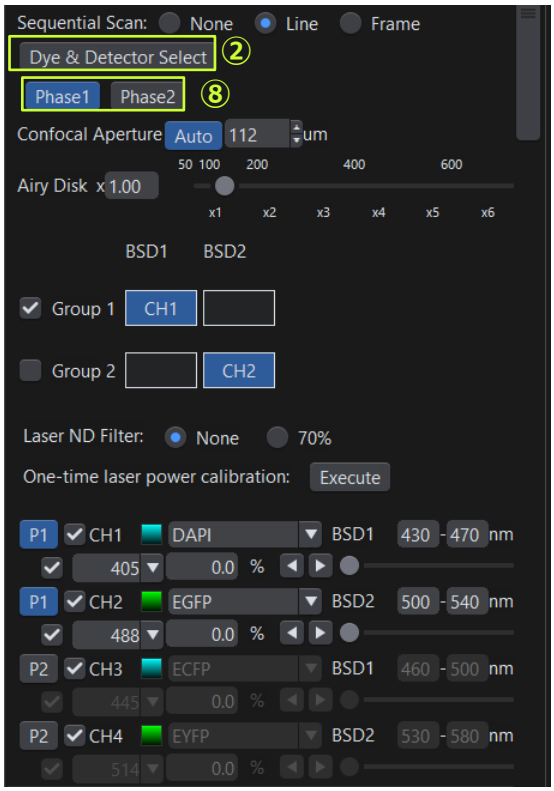


⑮ Press the LSM Start button to start acquiring the image.

Virtual Channel Scan

acquiring multiple CH image whose channel number is lager than the number of detector





① Select “Standard” in [Detector Setting] tool window.

Assigning the detector to channel

② Press the Dye & Detector Select button on [Detector Setting] Tool Window.

③ Press the All Clear button to reset the Assigned Dye.

④ Double-click the name of fluorescence dye to observe.

⑤ Press the Add Phase button to add the phase.

⑥ Drag & drop the “Dye” to observation channel list of the phase you want to add.

⑦ After setting all channels, press the [OK] button .

Adjusting the live image

⑧ Press Phase1 Phase2 or P1 P2 to switch phase.

⑨ Adjust the acquisition setting. Refer the previous pages.

⑩ Set “XYZ” and “XYT” setting. Refer the following pages.

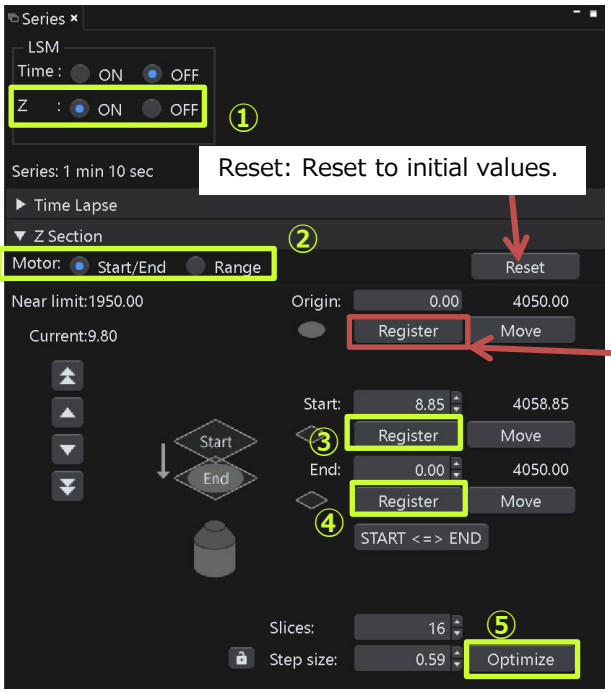
Setting Acquisition

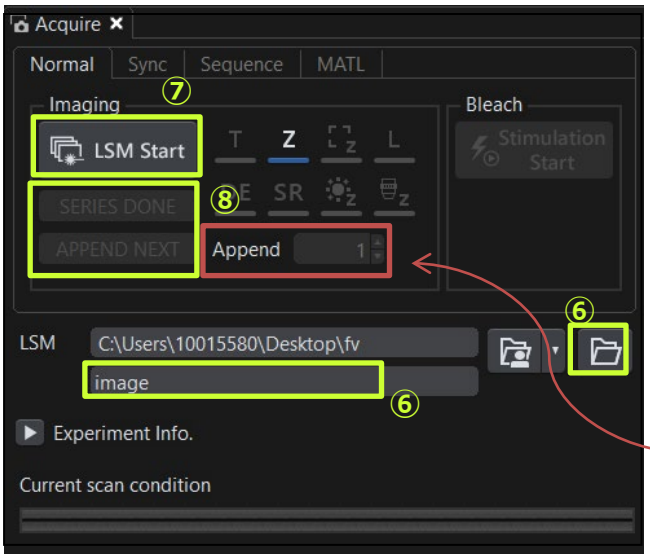
⑪ Select [Normal] tab in [Acquire] Tool Window.
Press the button to open the dialog.

*The acquired images are saved automatically. Series number is added at the end of file name like “×××_0001” and “×××_0002”.

⑮ Press the LSM Start button to start acquiring the image

XYZ Image Acquisition(Start/End)





＊Before starting the following procedure, adjust for XY imaging.

Setting Z series

① Select “ON” in [Z] on [Series] Tool Window.

② Select “Start/End” in [Motor] on [Z section].

Press the Register button, current position is set as 0.00.

Register Start/End position

③ Change the Z position by the focusing knob. Press the Register button in [Start] at the Z position to start acquiring the image .

④ Change the Z position and press the Register button in [End] at the Z position to end acquiring the image.

⑤ Enter a value [Slices] or [Step Size]. Setting one will set other automatically. Pressing the Optimize button, both numerical values “Slice” and “Step Size” are optimized.

Setting Acquisition

⑥ Select [Normal] tab in [Acquire] Tool Window. Press to display the dialog box, then select the folder to save the image.

⑦ Press the LSM Start button to start acquiring the image .

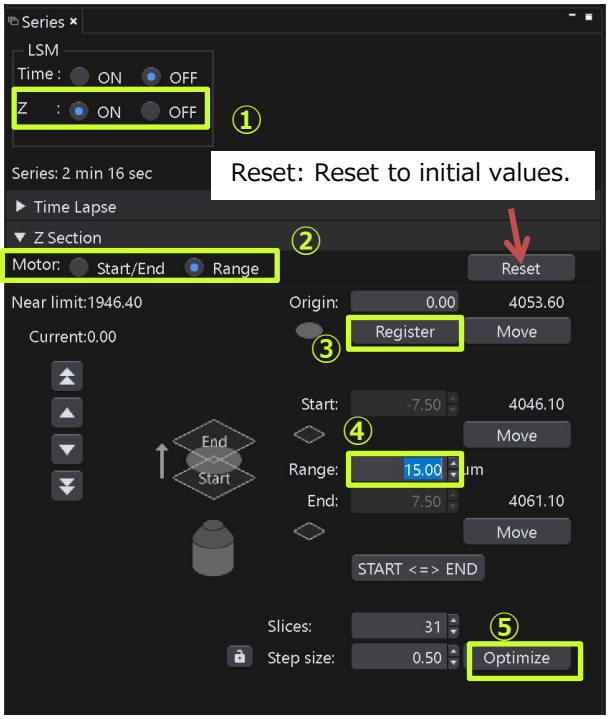
Finishing Acquisition

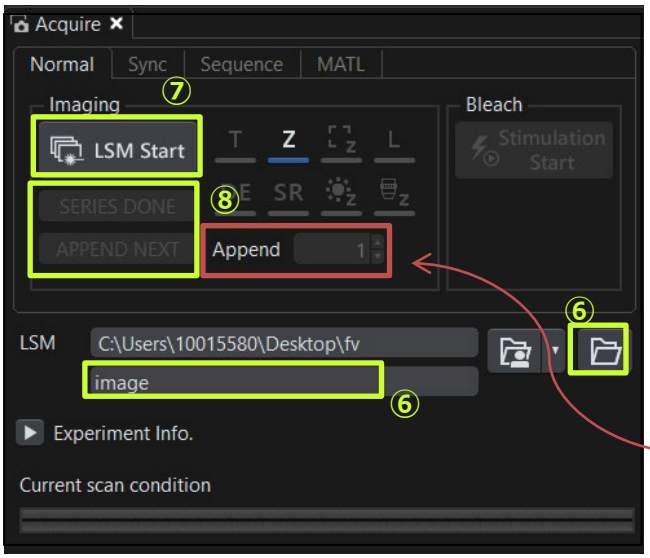
⑧ Finishing acquisition, APPEND NEXT SERIES DONE buttons blink.

Press the SERIES DONE to finalize the image acquisition. If you want additional images from end position, enter the number of additional acquisition and press the APPEND NEXT .

After acquiring, press the SERIES DONE .

XYZ Image Acquisition(Range)





＊Before starting the following procedure, adjust for XY imaging.

Setting the Z-series

① Select “ON” in [Z] in [Series]tool window.

② Select [Range] in [Motor] on [▼Z Section]

Register the Range

③ Change the focus and click Register at the center of the specimen.

④ Enter the Range.

Slices and Step size setting

⑤ Click Optimize to enter the optimize value of Slices and Step size.

Setting Acquisition

⑥ Select [Normal] tab in [Acquire] Tool Window. Press 📁 to display the dialog box, then select the folder to save the image.

⑦ Press the LSM Start button to start acquiring the image .

Finishing Acquisition

⑧ Finishing acquisition, SERIES DONE APPEND NEXT buttons blink .

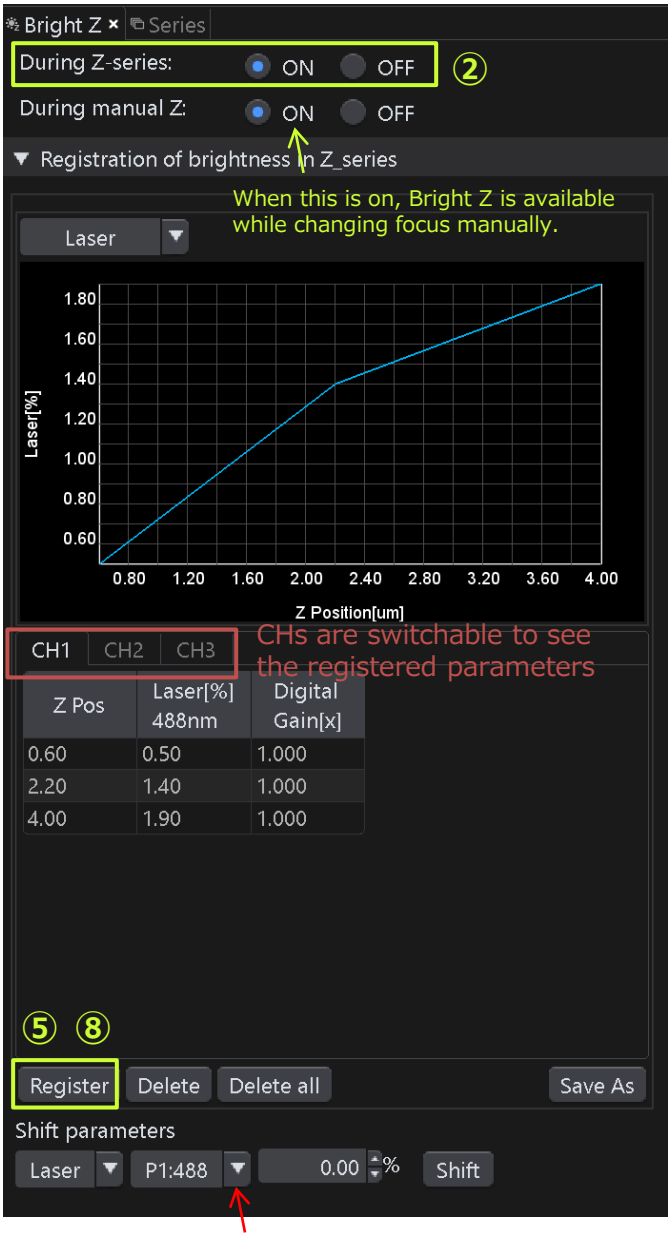
＊ Press the SERIES DONE to finalize the image acquisition. If you want additional images from end position, enter the number of additional acquisition and press the APPEND NEXT .

After acquiring, press the SERIES DONE

Bright Z

scanning while correcting the brightness against the Z position

This function cannot be used with Virtual Z



Intensity of selected wavelength can be shifted。
e.g.
Registered as 0.1%、1.5%、5% at 488nm.
Enter 5% then click “Shift” , re-registered as 5.1%、6.5%、10%.

＊Before setting the following, adjust XY image setting and Z stack condition.

① Select [Tool Window]＞[BrightZ] .

② Select “ON” on ”During Z-series” in [BrightZ] tool window.
(Select “ON” on ”During manual Z” to activate Bright Z during changing focus manually.)

Registering value.

③ Move to start Z position and show Live image.

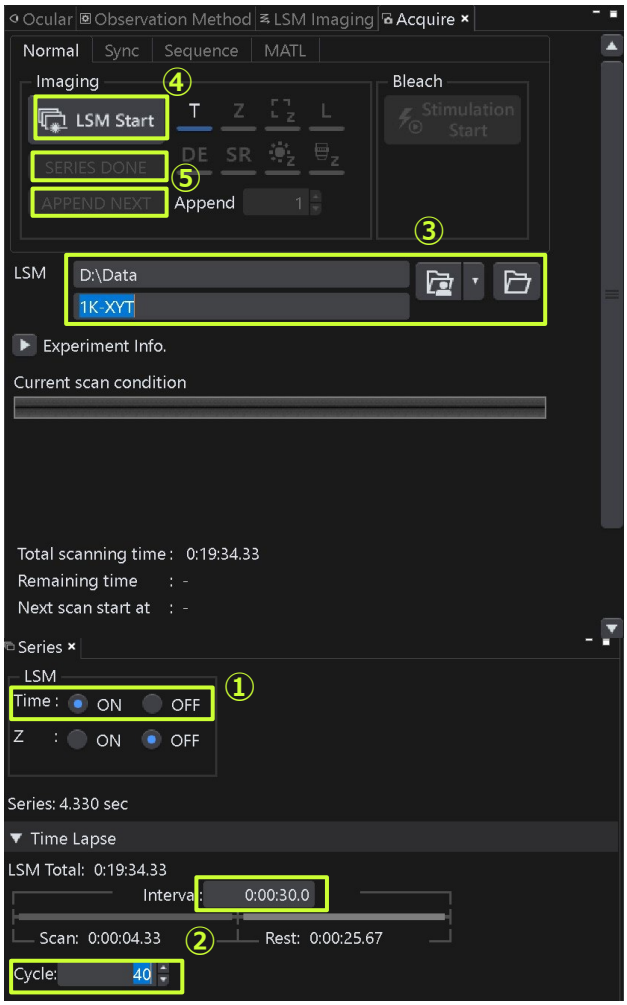
④ Click Register in [BrightZ] tool window to register the parameter.

⑤ Change the focus and change the laser intensity along the depth, click Register each time change the parameters.

⑥ Repeat ⑤ until the end Z position.

⑦ Acquire as same way of XYZ.

XYT Image Acquisition



＊Before starting the following procedure, make adjustments for XY imaging.(refer to page 8-9)

Setting Time Series

① Select ”ON” in [Time] on [Series] Tool Window.

② Set the interval to acquire the image in [Interval] and [Cycle] on [Time Lapse].

If you attempt to set the shorter than the time displayed in [Scan] in [Interval], “FreeRun” appears. In this case, the interval to acquire the image is the time displayed in [scan].

Starting Acquisition

③ Select [Normal] tab in [Acquire] Tool Window. Press the button to display the dialog box, and select the folder to save images.
*The acquire images are saved automatically. Series number is added at the end of file name like “***_001”and “***_002”.

④ Press the LSM Start button to start acquiring the image.

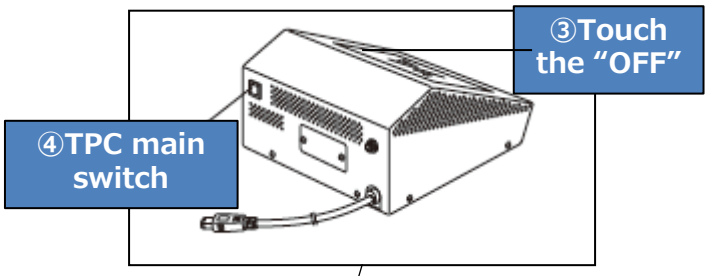
Finishing Acquisition

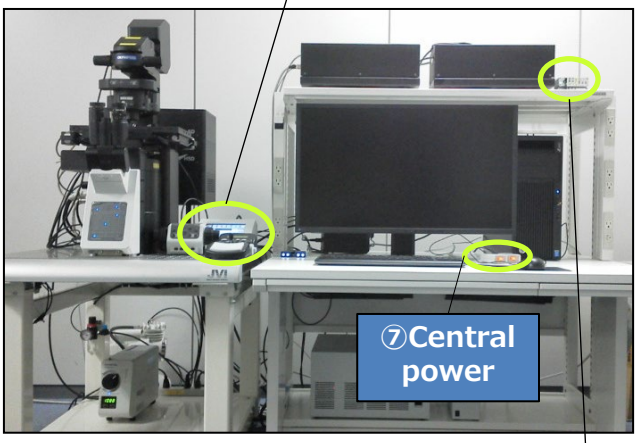
⑤ Finishing acquisition, SERIES DONE / APPEND NEXT button blink.

＊Press the SERIES DONE button to complete the image acquisition. If you want to Additional images under the same condition, enter the number of additional acquisition and press the APPEND NEXT button. After the image is acquired, press the SERIES DONE button.

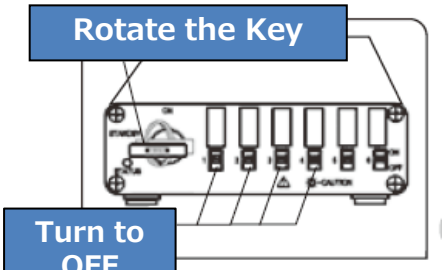
Exiting the system

Touch Panel Controller（TPC）





⑤Laser unit



Exiting the software and PC

① Close the software.
② Shut down the Windows.

Turning OFF the power

Touch Panel Controller（TPC）

③ Tap the “OFF” on display of TPC.
④ Then press the TPC main switch .
※Do not long-press the main switch.

Laser controller

⑤ Turn the laser combiner to OFF.
※Rotate start key of the power.
Supply and set the switch to OFF.

Central power

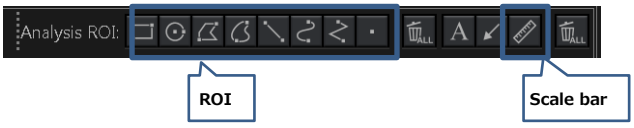
⑥ Turn off the central power.

＊When using immersion oil , clean the objective lens.

2 D view and operation

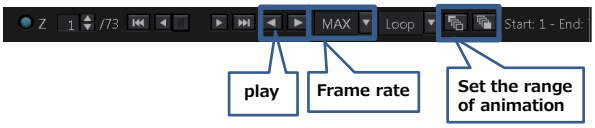
Drawing scale bar and ROI

- A straight line can be drawn by pressing Shift key when left-clicking.



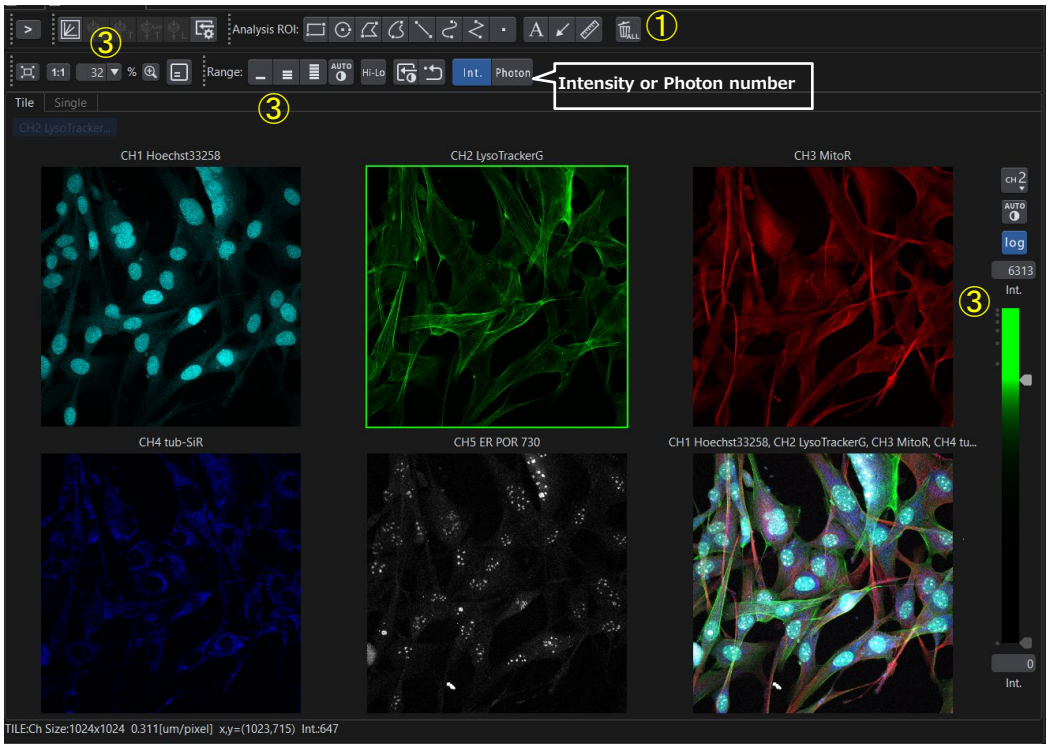
Playing animation of the series images

- These button are used to play animation of the series images.



LUT (Look Up Table)

- Click  and adjust LUT or color , etc.
- Adjust LUT by using vertical bar or 



3D view and operation(1)

Opening the file and displaying 3D image

Open and activate the Z series image.

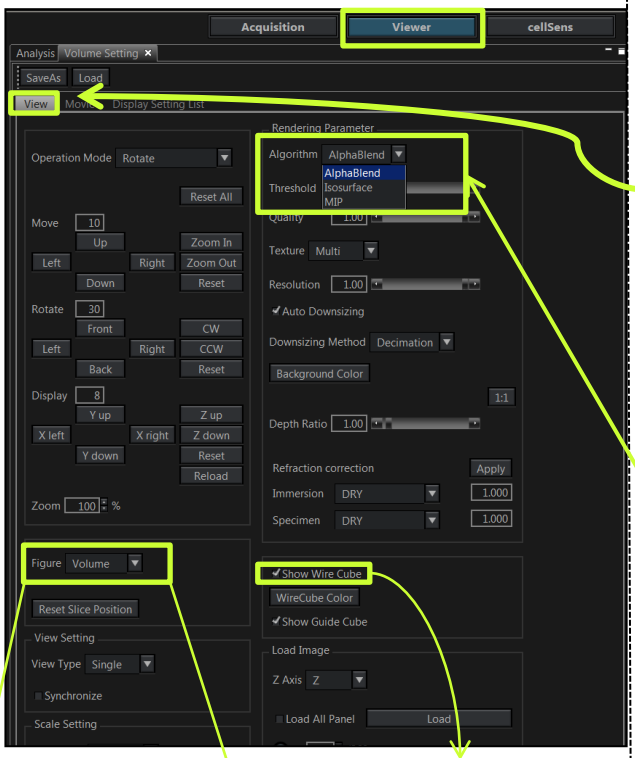
Select [Volume] tab to display 3D image.

Drag the mouse on the 3D image in the direction you want to rotate.

■Zooming 3D image

Mouse wheel **:**Zooms toward the center of the 3D image displayed.

Shift key＋mouse wheel **:** Zooms in the display area



■3D image setting

Select the Viewer button and select [Volume setting] in [Tools window] menu.

[Volume setting] Tool Window is displayed.

Select [View] tab in [Volume setting].

■Selecting the algorithm

[Algorithm] : The field is used to express the 3D image from following 3 types.

1） MIP : Maximum Intensity Projection

The MIP method reflects the maximum intensity of the object preferentially on the image.

Therefore ,the context of the object is not taken in account, and the area with the high intensity even in the object can be extracted.

2） Isosurface

The Isosurface method draws the area where the intensity variation volume of the object is large as a top surface and reflects it on the image. Therefore, only the top surface is drawn.

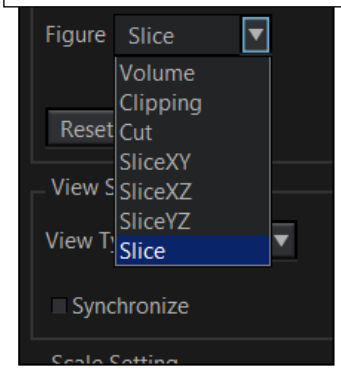
3） AlphaBlend

The α blend method reflects the intensity on the top surface of the object preferentially to the image.

Therefore , the context of the object is displayed properly.

Ticking this checkbox will display Wire Cube in 3D view.

Observe the cross section. (cf. next page)



■Save the 3D image

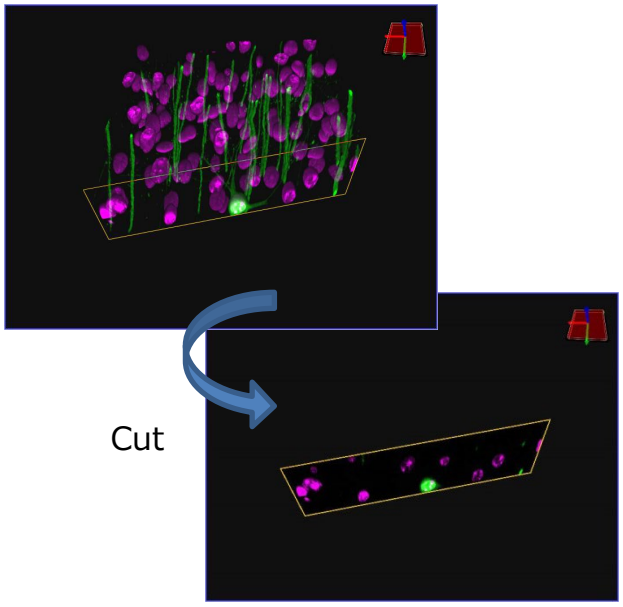
①Right click of the mouse on the 3D image and select “ Save Display”.

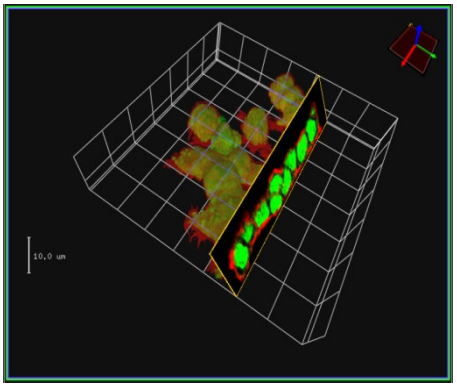
②Input the File Name and Format.

③Click the [OK] button.

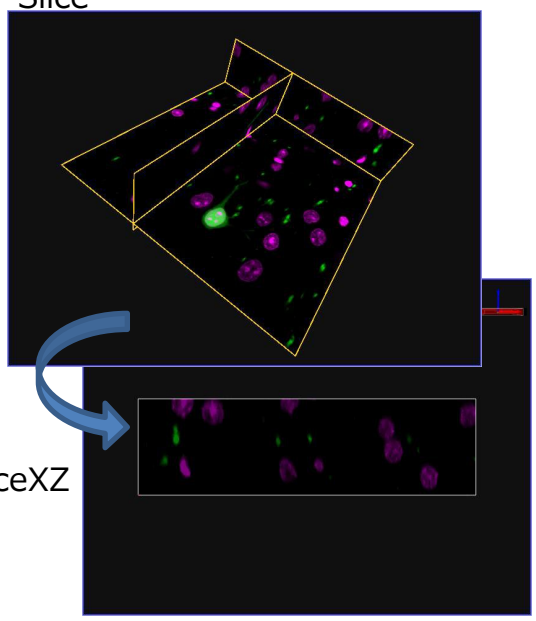
3D view and operation（2）

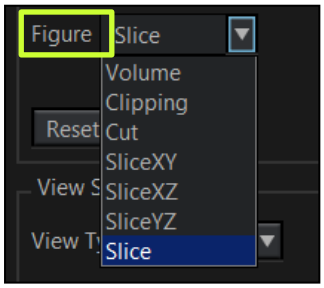
Clipping





Slice





■Clipping/Cut

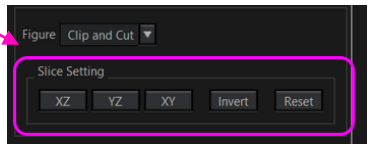
Displays the yellow frame in the image constructed in 3D.

Dragging this frame with the mouse will display only the area visible from the frame.

■Clip and cut

Show “clipping” and “cut” at the same time.

changing the cross collection XY/YZ/XZ by pressing these button.



■Slice

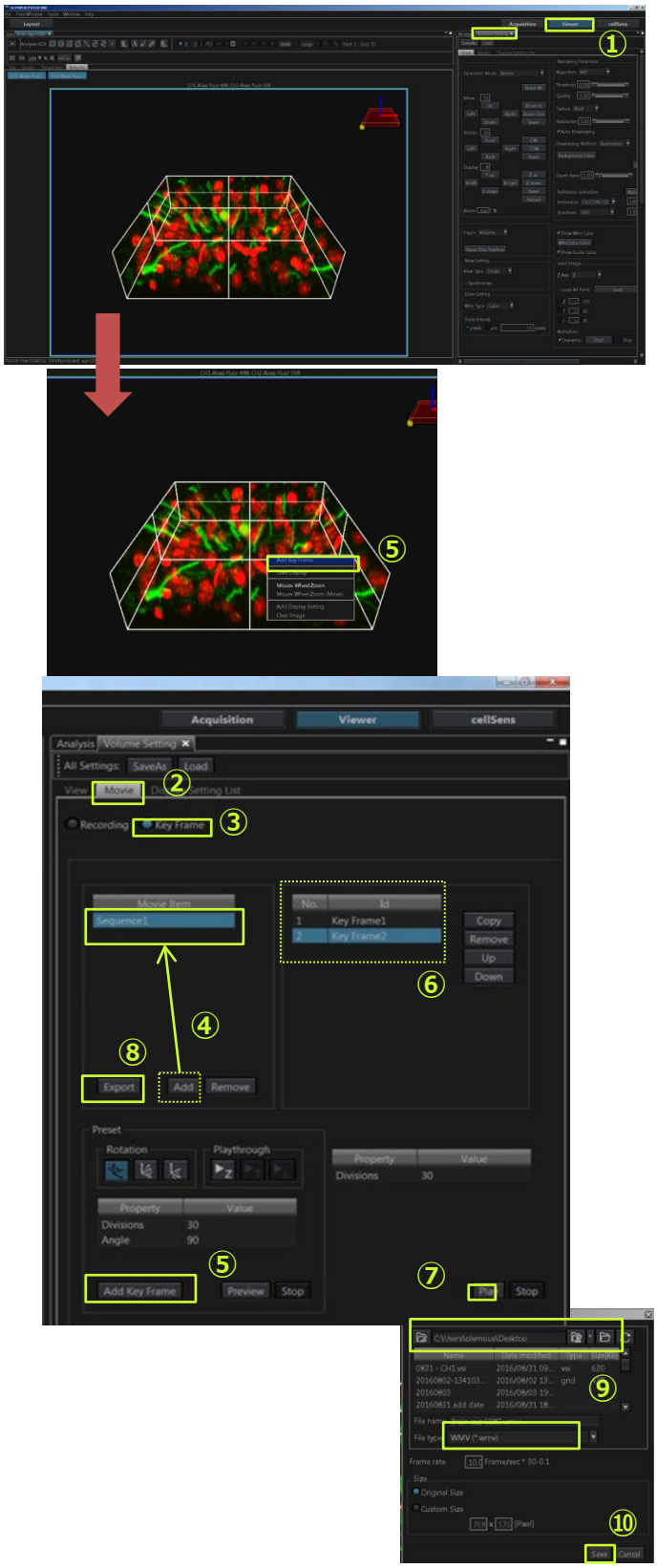
Create the cross-sectional view sliced in XY/XZ/YZ directions in the image constructed in 3D image, and displays the image sliced in each direction.

The cross sections can be moved by dragging the mouse.

＊SliceXZ/YZ/XY

After setting the position and angle eith Slice, switch to “SliceXZ” ,”SliceYZ” or “SliceXY”, the cross section of the position determined in Slice is displayed.

Creating the movie



＊Opening the file and displaying 3D image

① Press the Viewer button and select [Volume setting] in the [Tool Window] menu.

② Select [movie] tab in [Volume setting] tool window.

③ Select “Key Frame” in [Movie Item]

④ Press the Add button.
“Sequence1” is shown below “Key Frame”.
Select “Sequence1”in [Movie Item].

Registering the Key Frame

⑤ Move the 3D image in [Image] Window by dragging and right-click at the desired status.
When you select [Add Key Frame] in the menu display, the display status is registered and “Key Frame X” is displayed in [Id] in [Volume setting] Tool window.

⑥ Repeat ⑤ and register the statuses you want to display as Key Frames.

⑦ When you press the Play button, the image between Key Frame is interpolated automatically to play back the movie.

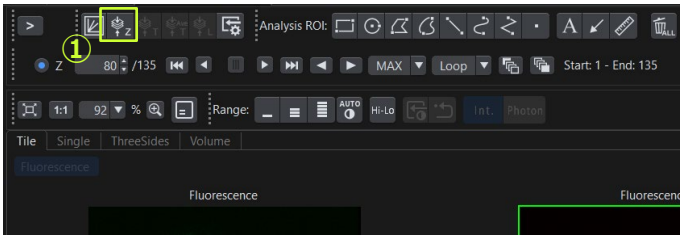
Exporting movie

⑧ Select “Sequence xx” in [Movie Item] and select the movie you want to export.
Press the Export button.

⑨ Press the button to select the folder of the save destination.
Set the [File name] and [Frame rate] by entering them directly.

⑩ Press the Save button.

Projection(1)

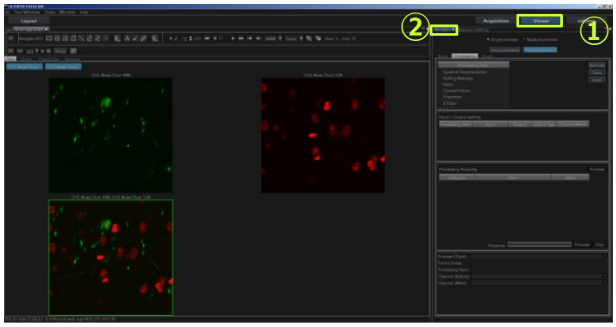


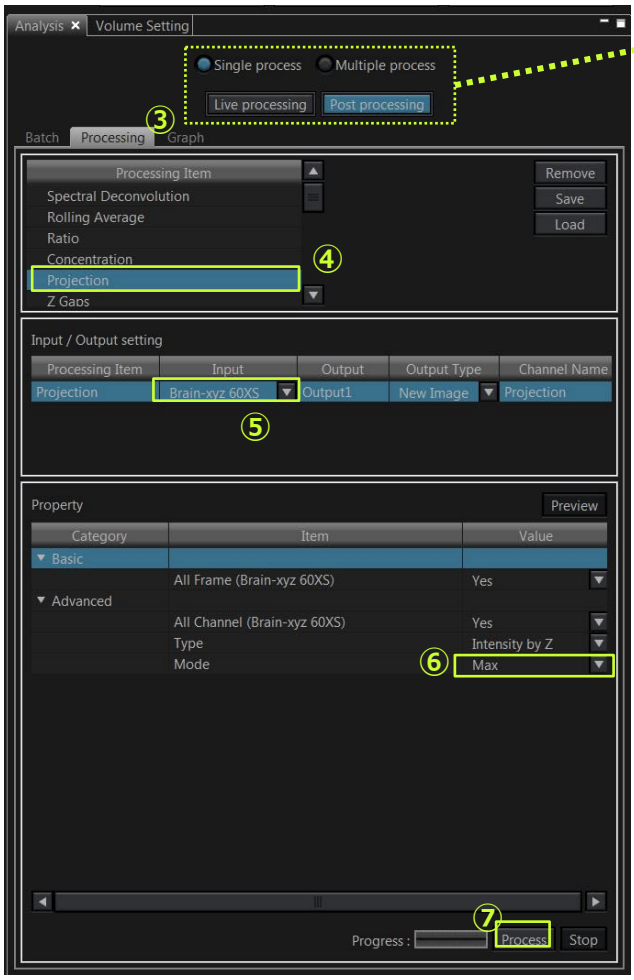
① Click  to show projection image。

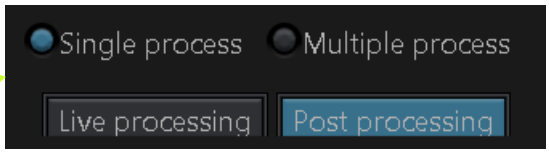
② File > Save As > Projection Image OR right-click on the image > Save As > Projection Image.

③ Save the image (.OIR is the extension only available)

Projection(2)







① Press the Viewer button to switch to “Viewer mode”.

② Select [Analysis] in the [Tool Window] menu.

③ Select “Single process” and “Post processing” as the illustration below.

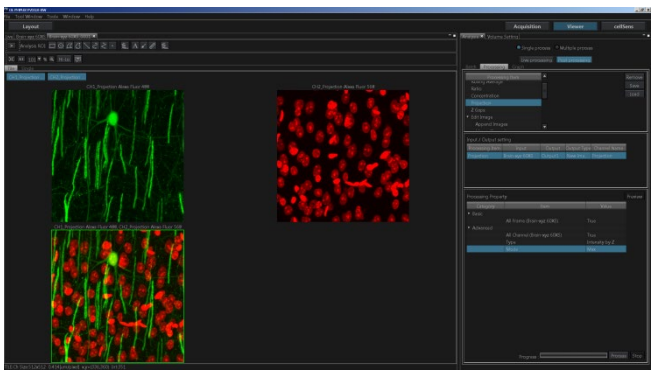
④ Select the [Processing] tab and resister “projection” in [Processing Item].

⑤ Click the [Input], and select the images for image processing.

⑥ Select “MAX” in [Mode] in [Processing Property].

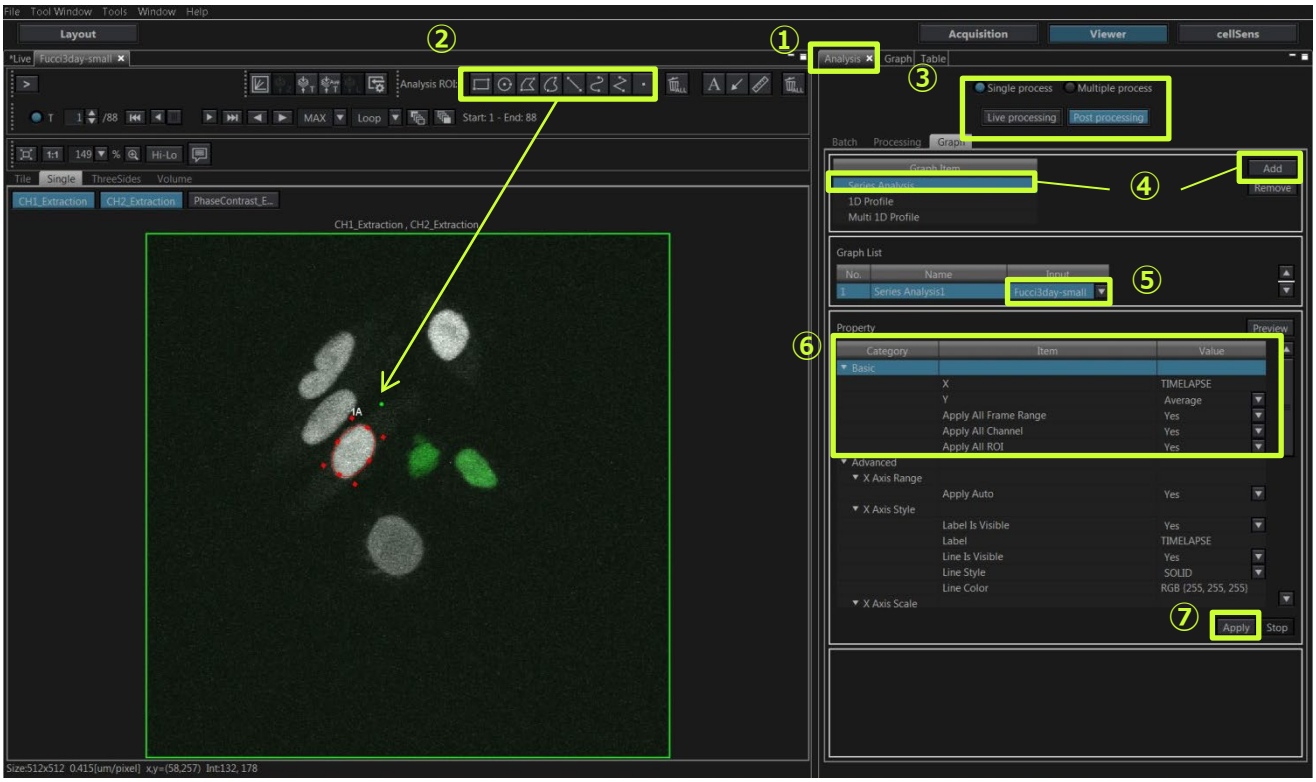
⑦ Press the Process button.

⑧ The image processing results are displayed in [Image] window, and saved to the same location where the original imaged were saved.



Drawing a Intensity Graph : Series Analysis

(Specifying ROI on the series image)



① Select [Analysis] in the Tool window menu.

② Select the ROI tool in [Analysis ROI], and specify the area you want to measure on the item.

③ Select “Single process” and “Post processing”.

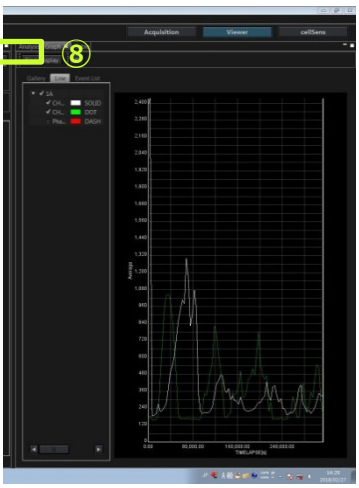
④ Select the [Graph] tab, and Select [Series Analysis] , and press the Add button.

⑤ Click the [Input], and select the images for image processing.

⑥ Set details of items in [▼basic] in [Property].

- Apply All Frame Range→Yes (Measuring for all frames)
  No (Measuring for specified frame)
- Apply All Channel→Yes (Measuring for all channels)
  No (Measuring for selected channels)

⑦ Press the Apply button allows you to draw all graphs registered in [Graph List].



▼[Graph] tab

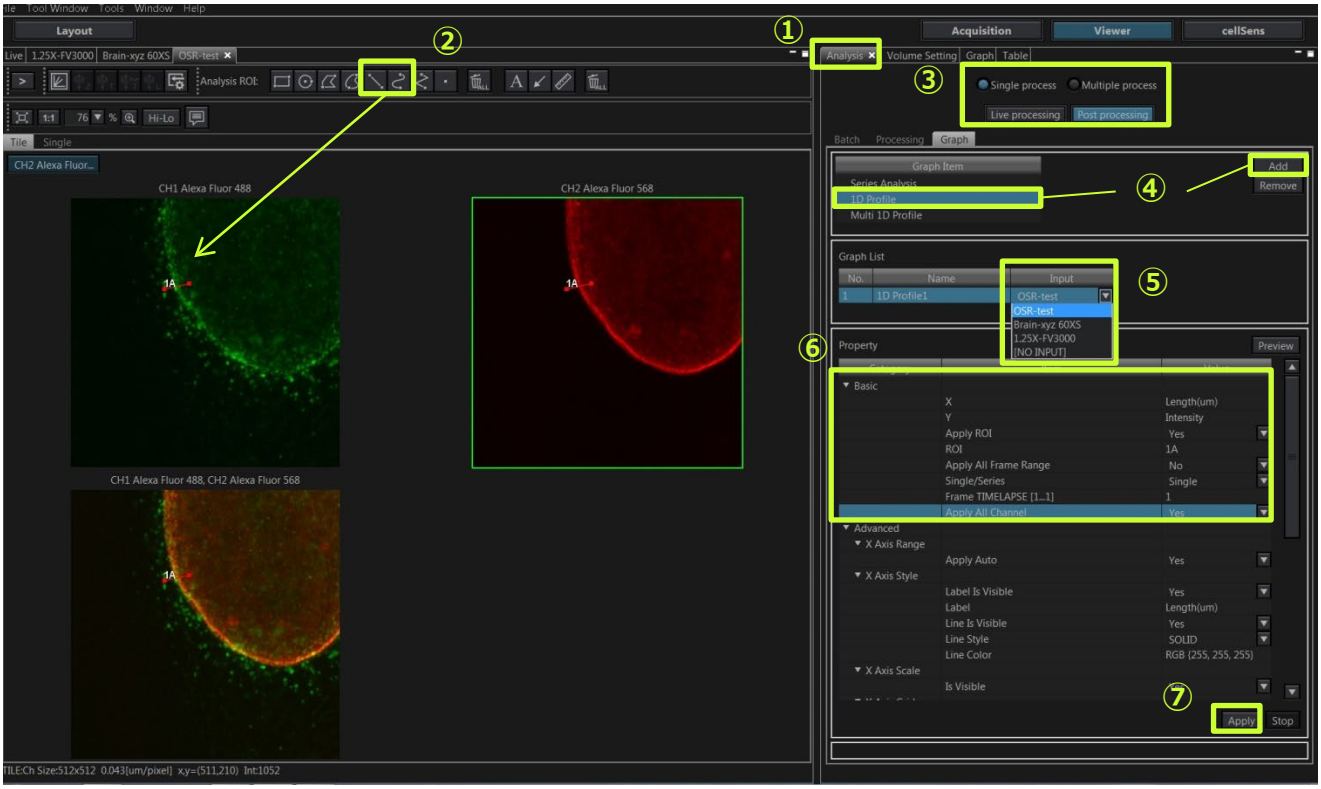
- You can change the color in [Line] tab.
- Press the Save Display button to save the graph.

▼[Table] tab

- Press the SaveAs button to save the results as CSV file.

Drawing average intensity profile : 1D profile

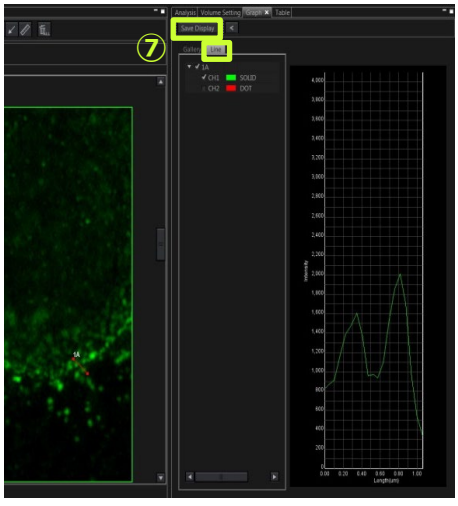
(the arbitrary line viewing the series image)



① Select [Analysis] in the Tool window menu.
② Select the ROI tool in [Analysis ROI], and specify the area you want to measure on the item.
③ Select “Single process” and “1D profile”.
④ Select the [Graph] tab, and Select [Series Analysis] , and press the Add button.
⑤ Click the [Input], and select the images for image processing.
⑥ Set details of items in [▼basic] in [Property].

・Apply All Frame Range→Yes （Measuring for all frames）
No （Measuring for specified frame）
・Apply All Channel→Yes （Measuring for all channels）
No （Measuring for selected channels）

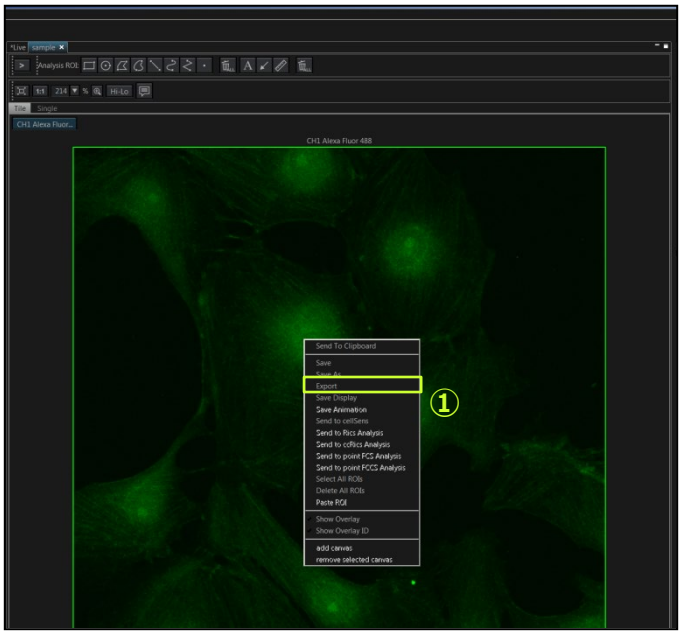
⑦ Press the Apply button, [Graph] tab and [Table] tab appears.

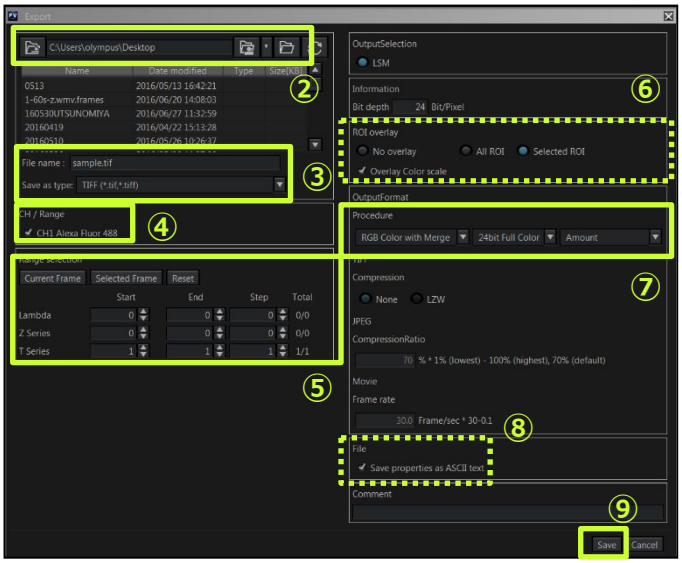


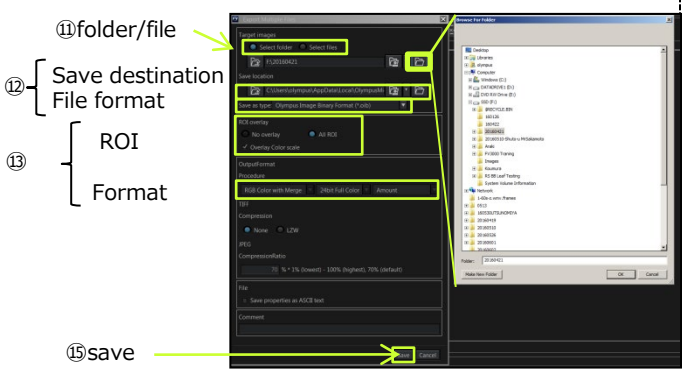
▼[Graph] tab
・You can change the color in [Line] tab.
・Press the Save Display button to save the graph.

Exporting the image

*the image can be exported in the file format which can be used by other software.







A. Exporting a single image

① Right-click on the image to be exported. Select [Export] in the menu displayed. The [Export] dialog box appears.

② The save destination of the image to be displayed in ②.
If necessary, press the button to select the folder of the save destination.

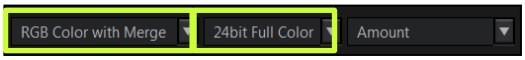
③ Set the file name and select the file type in [Save as type].

④ Select the channel to be exported in [CH/Range].

⑤ Set the range to be exported and the number of steps in ⑤.

⑥ Set whether or not to overlay the ROI over the image to be exported.

⑦ When general purpose format is selected in [Save as type], select the method to export channels and the bit color.



⑧ Ticking this checkbox will output the properties in the text.

⑨ Press the Save button.
The image will be exported.

B. Exporting Multiple images

⑩ In the [File] menu on the software screen, select [export multiple files].
The [Export multiple files] dialog box appears.

⑪ Select the method to export images.

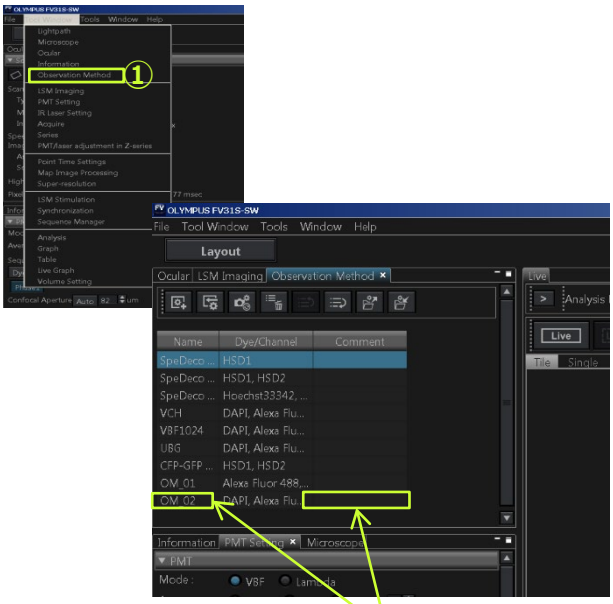
⑫ Press the button to select the folder or the file to be exported.

⑬ Select the save destination and the file format to be exported in [Save as Type].

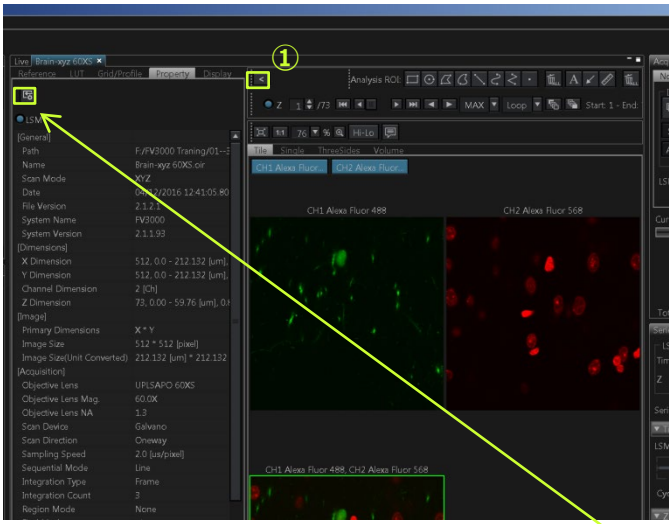
⑭ Same as 4~8 of A.

⑮ Press the Save button. The image will be exported.

Reloading and saving Observation Method



Direct input by double-click



Save/ load the observation method

① Select the [Observation Method] in [Tool Window] menu.

＊Each function

 :to load the observation condition selected in the list

 :to save the current condition and add the list

 :to update the current conditions

 :to delete the condition from the list

 :to sort the list

 :to import/export the methods

Click the acquiring condition

① Press the  button and open sub pane.

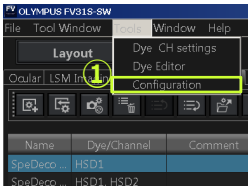
② Select [Property] tab and check the acquiring conditions.

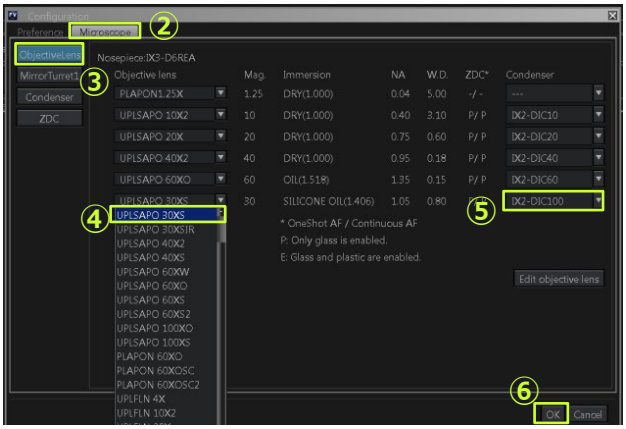
Load acquisition parameters from acquired image

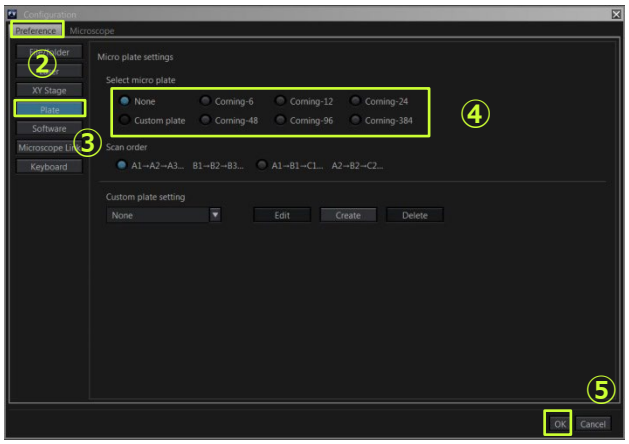
*Read actual acquisition condition from the image and reflect them in the software setting

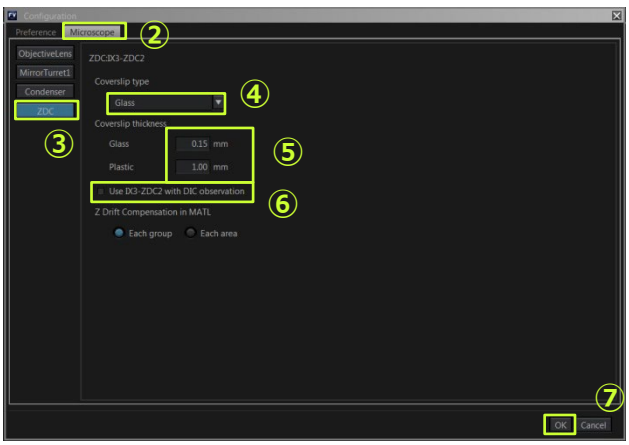
・In [Property] in sub pane, press the  button.

Configuration









■Changing the objective lens

＊Exchange the objective lens to be used.

① Select [Configuration] in [Tool] menu. The [Configuration] dialog box appears.
② Select [Microscope] tab.
③ Select [Objective Lens].
④ Select the name of the mounted objective lens.
⑤ Specify the optical elements to be switched by interlocking during the switchover of the objective lens.

＊Select the DIC which is same number as the objective lens.
example) 30X→IX2-DIC30
100X→IX2-DIC100

⑥ Press the OK button.

■Specifying the micro plate

① Select [Configuration] in [Tools] menu. The [Configuration] dialog box appears.
② Select [Preference] tab.
③ Select [Plate].
④ Select the micro plate to be used.
⑤ Press the OK button.

■For Z drift compensation

① Select [Configuration] in [Tools] menu. The [Configuration] dialog box appears.
② Select [Microscope] tab.
③ Select [ZDC] tab.
④ Specify the coverslip type to be used.
⑤ Enter directly the thickness of the coverslip to be used.
⑥ If you use the DIC, tick the check box ⑥.
⑦ Press the OK button.

ZDC

※Option

EVIDENT

Maintain Focus with the Z-Drift Compensation(ZDC) system

■ZDC（Z Drift Compensator ）

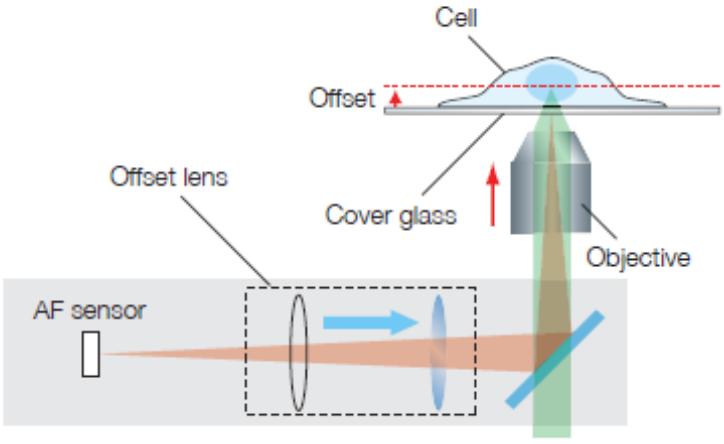
The ZDC uses minimally-phototoxic infrared light to identify the location of the sample plane.

ZDC makes it easy to reproduce any preset focus position.

It avoid focus drift due to temperature changes or additional of reagents.

The ZDC is also compatible with silicone objective and plastic bottom vessels.

IX3-ZDC2 Z-Drift Compensator Optical Path Diagram



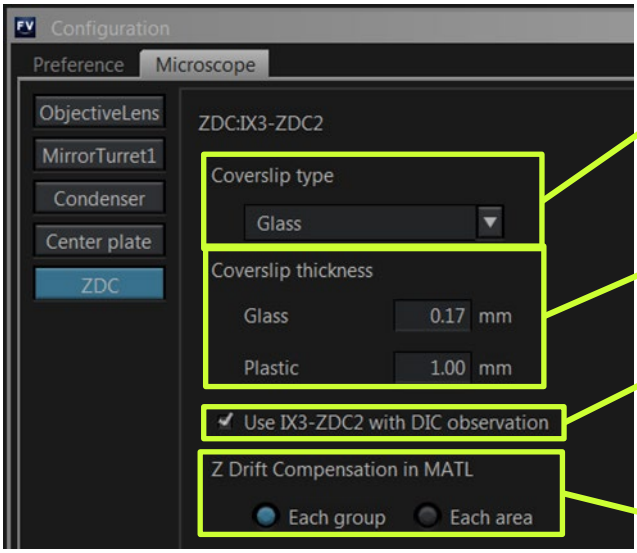
■Objective lens for ZDC2

Objective lens		Glass bottom dish （t=0.17mm）		Plastic dish （t=1.0mm）	
		Continuous	One shot	Continuous	One shot
Dry	UPLXAPO4X	×	×	–	–
	UPLXAPO10X	○	○	–	–
	UPLXAPO20X	○	○	–	–
	UPLXAPO40X	○	○	–	–
Long working distance	UCPLFLN20X	○	○	○	○
	LUCPLFLN20X	○	○	○	○
	LUCPLFLN40X	○	○	×	○
	LUCPLFLN60X	○	○	×	○
silicone	UPSAPO30XS	○	○	–	–
	UPLSAPO40XS	○	○	–	–
	UPLSAPO60XS	○	○	–	–
	UPLSAPO100XS	○	○	–	–
oil	UPLXAPO60XO	○	○	–	–
	UPLXAPO100XO	○	○	–	–
	PLAPON60XOSC	○	○	–	–
water	UPLSAPO60XW	○	○	–	–

Configuration :ZDC

① Select [Configuration] in [Tools] menu. The [Configuration] dialog box appears.
② Select [Microscope] tab.
③ Select [ZDC] tab.

If there setting are not made correctly, the autofocus may not work properly.



Coverslip type（Glass / Plastic)

This field is used to set the material of coverslip you will use.

Coverslip thickness

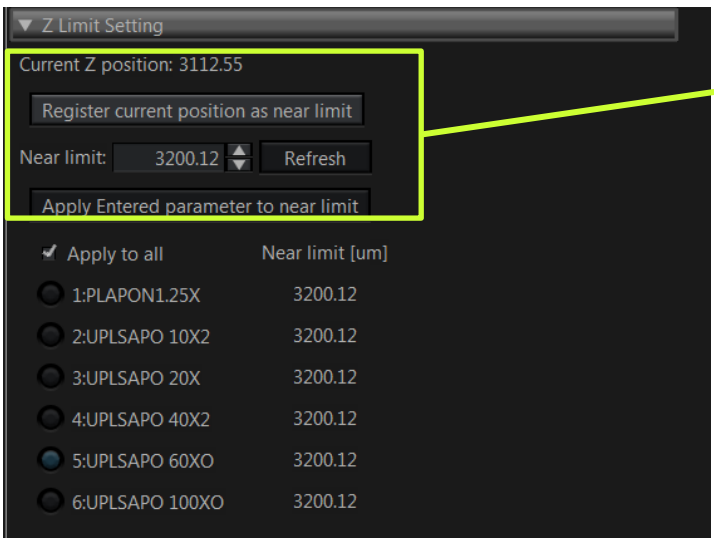
Use DIC observation
（Objective lens:100X）
Tick→The DIC prism is placed in the light path
Uncheck→without the DIC prism in the light path

ZDC in MATL
This field is used to select the timing to execute the ZDC when acquiring the image by MATL

④ Press the [OK] button.

■Near Limit

① Select [Microscope] in [Tool Window].
② In [▼Z limit Setting], set the Near limit to all objective lenses.



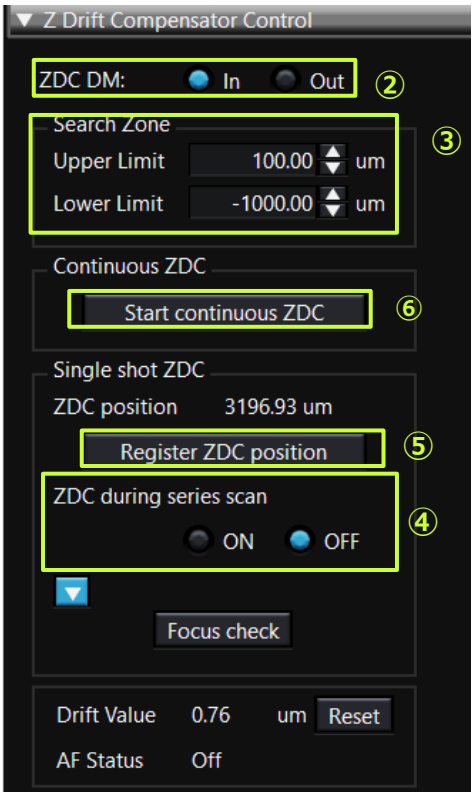
If the Near limit is not set properly, the autofocus may be failed.
Recommended value
: focus position＋500μm

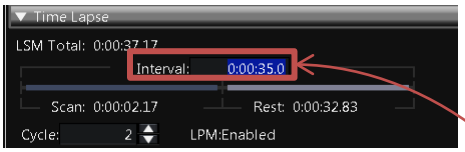
Register current position as near limit
:set the current Z coordinate as a Near limit

Apply Entered parameter to near limit
:register the adjusted position as a Near limit

XYT Image Acquisition (with the Z drift compensation)

◆ZDC◆





【Attention】

If [Rest] is shorter than 31 seconds, Z drift correction works only first scanning. For every time working, set the [Interval] so that the [Rest] to be 31sec or longer.

＊In advance, prepare to use ZDC.
(See page15 for details.)
＊Set for acquiring the series image.

Setting near limit

① Select [Microscope] in [Tool Window] menu.
Press the Register current position as near limit button to set the Near limit to all objective lenses in [▼Z limit Setting].

ZDC setting

② Set [ZDC DM] in [ZDC Control] on [Microscope] Tool Window to “In”.

③ Specify the search zone of the coverslip position in [Upper Limit] / [Lower Limit] in [Search Zone] .

④ Set [ZDC during series scan] to "ON".

⑤ Adjust focus and press the Register ZDC position button .The coverslip top surface position is acquired.
When the Z drift compensation is successful, the buzzer sounds beeps once only. If the Z drift compensation is not successful, the buzzer sound beeps three times.

⑥ When performing the Z drift compensation continuously during series scan, press the Start continuous ZDC button in [Continuous ZDC] .
As this function performs the drift compensation in real-time during series scan, the focusing can be at a high-speed.

・High-speed scanning→Continuous AF
・If the rest time is longer than 30 seconds
→You had better not use Continuous AF

⑦ Press LSM Start in [Normal] tab in [Acquire] Tool Window to start acquisition.

Mortorized Stage

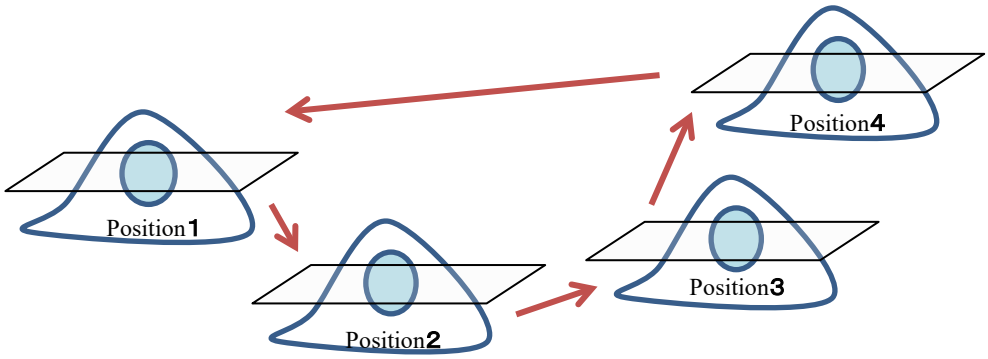
*Option

EVIDENT

◆only for motorized stage ◆

Option : Motorized stage

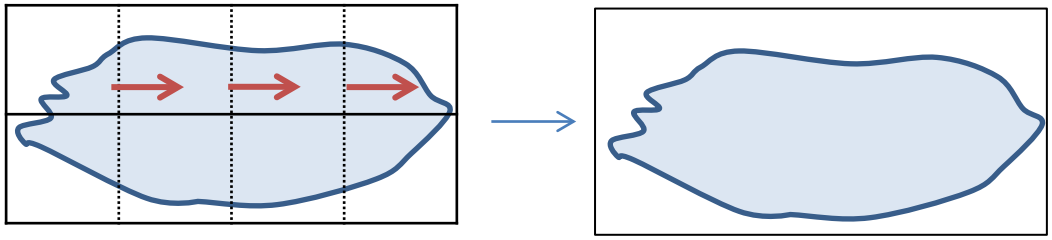
■ Multi Area Time Lapse



- Register multiple areas and repeatedly acquire images .
- It is also possible to set the interval.
- Time lapse date of multiple area can be acquired at once.

■ Acquiring stitched image

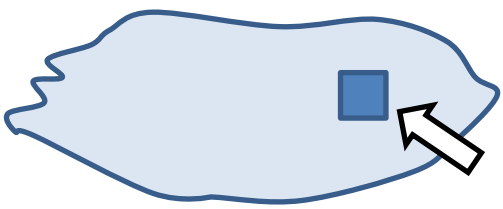
- Acquire the adjacent area in order.
  And stitch the image to create a single wide field of view image.



■ Map image

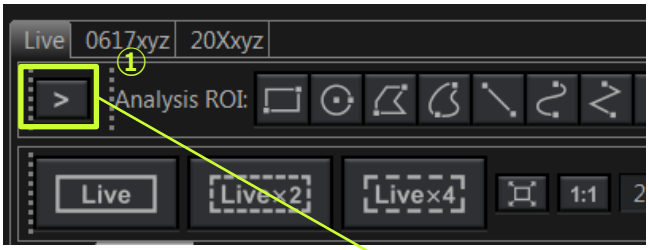
- Display wide field of view image acquire with low magnification objective lens.
- And it can be used as a guide when looking for the field of view.

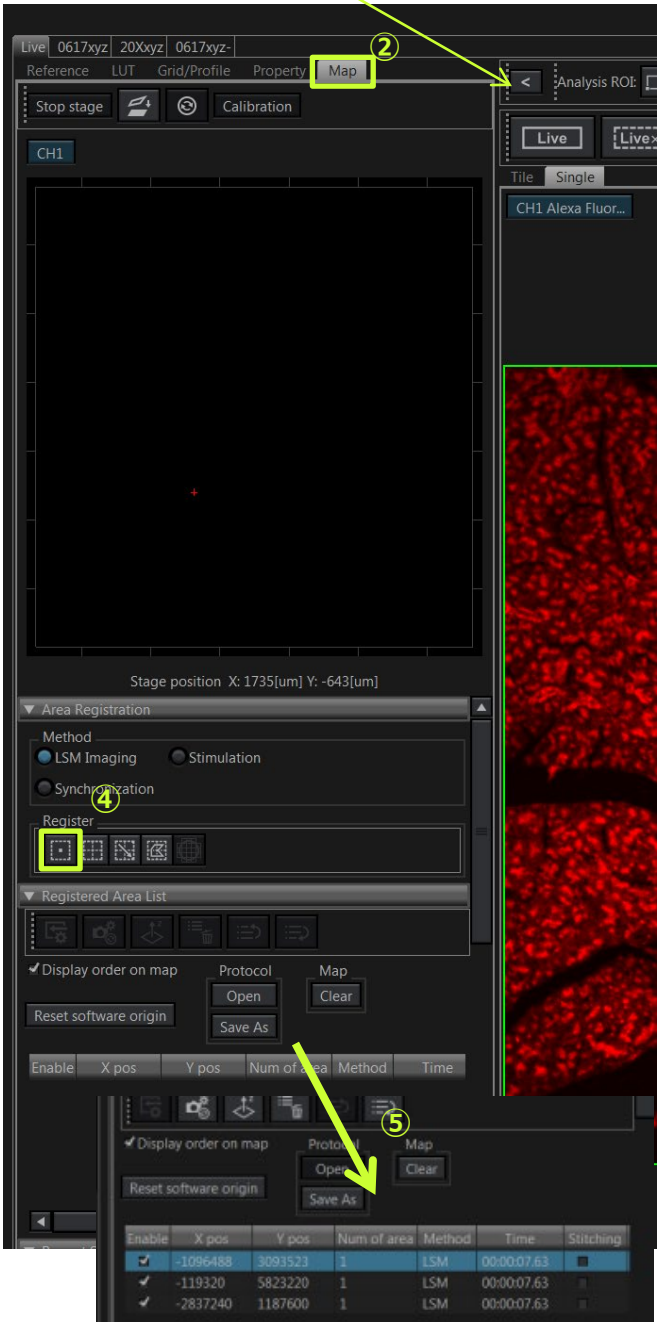
★Double-click on the map image, the motorized stage moves so that it becomes the center of the image.



Multi Area Time Lapse Imaging Acquisition

◆Only for Motorized stage◆





＊Before starting the following procedure, make adjustments for XY imaging.

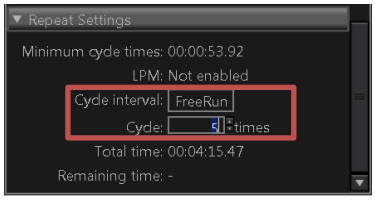
① Press the [>] button in [Live] Window.
② Select the [Map] sub pane.

Registering the group of MATL

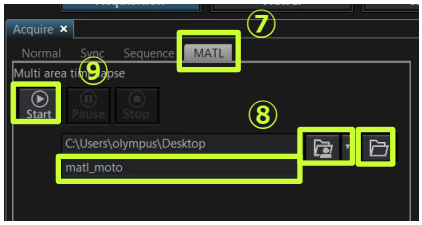
③ Move the specimen to a desired position and adjust the live image. If you want to acquiring the XYZ image, make adjustments for Z series additionally.
④ Press the [ ] button to register the position and its image acquisition condition.

【Attention】
All acquiring conditions(XY position, focus position, laser intensity, sensitivity, series setting, and so on.) are registered when you press the [ ] button. So, Register after you finish adjusting all conditions.

⑤ Repeat the operation of ③ and ④ to register for multi area timelapse.
⑥ Set the interval to acquire the image in [Interval] and [Cycle] on [Repeat Setting].

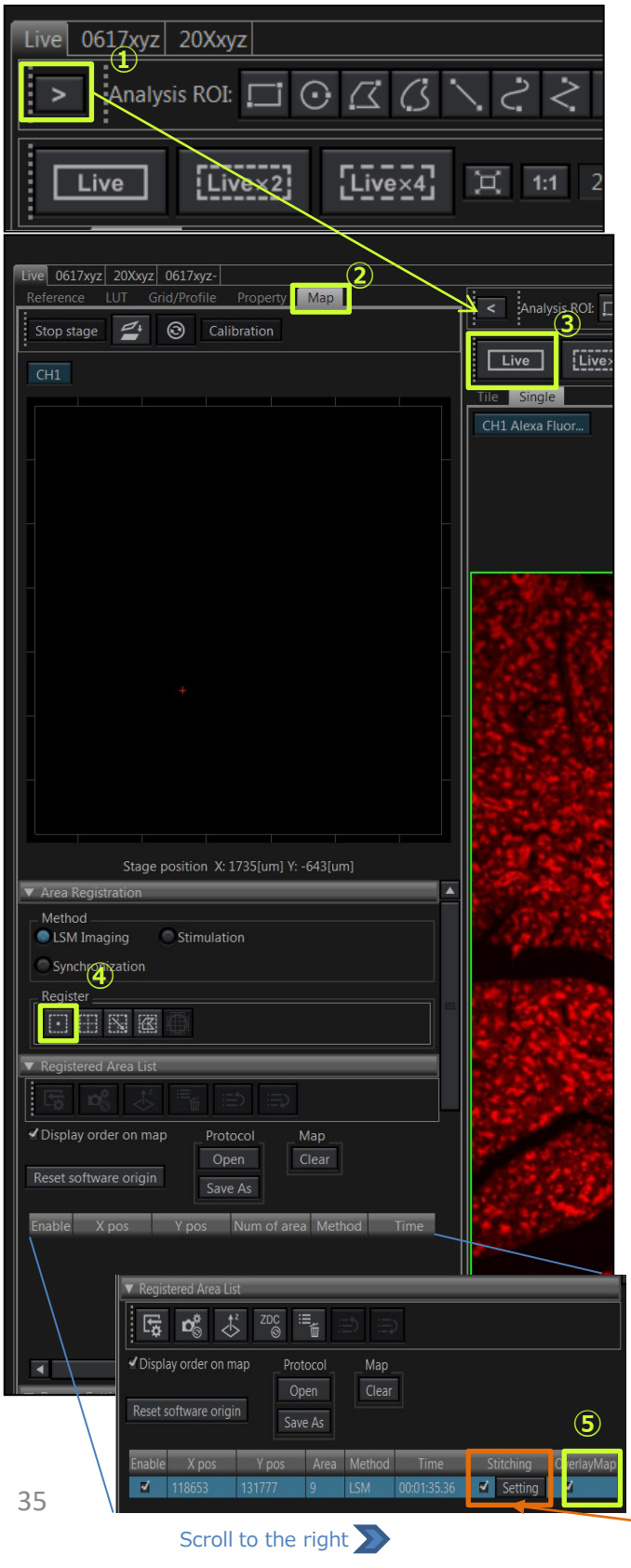


⑦ Select [MATL] tab in [Acquire] Tool Window.
⑧ Press the [ ] button to display the dialog box, and select the folder to save the images.
⑨ Press [Start] button to start acquiring the image.



Multi Area Time lapse using Map Image (1)

◆Only for motorized stage ◆



＊Before starting the following procedure, make adjustments for XY imaging.

① Press the [>] button in [Live Window], the sub pane appears.
② Select [Map] tab.

Create the map

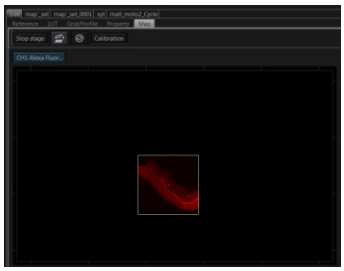
③ Bring the image into focus and adjust acquiring parameters using the low magnification objective lens.
④ Press the [ ] button to register the position and acquiring parameters.

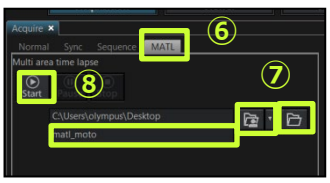
【Attention】
All parameters register by pressing the [ ] button. When acquiring a map image, Check [LSM] in [Series] Tool Window whether it is selected “OFF” [Z] and [Time] .

⑤ Scroll to the right in registered are List and Ticking [Overlay Map].

Map image acquisition

⑥ Select [Acquire] Tool Window in [Tool Window] menu and select [MATL] tab.
⑦ Press the [ ] button to display the dialog box, and select the folder to save the images.
⑧ Press [Start] button to start acquiring the image. Finishing the acquisition, map image is displayed in [Map] tab.



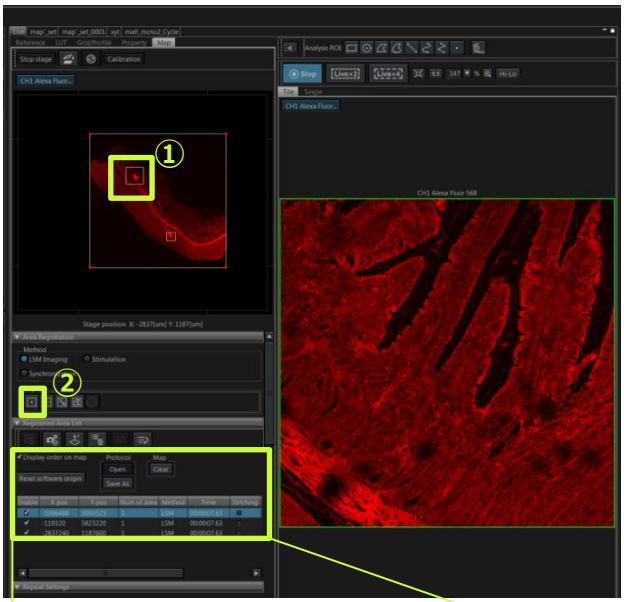


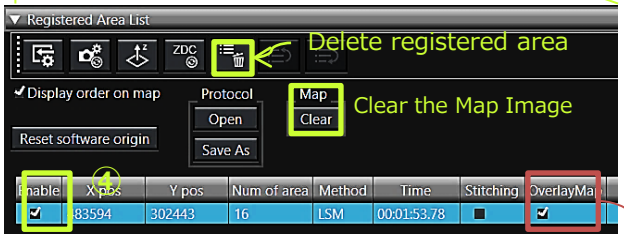
Scroll to the right

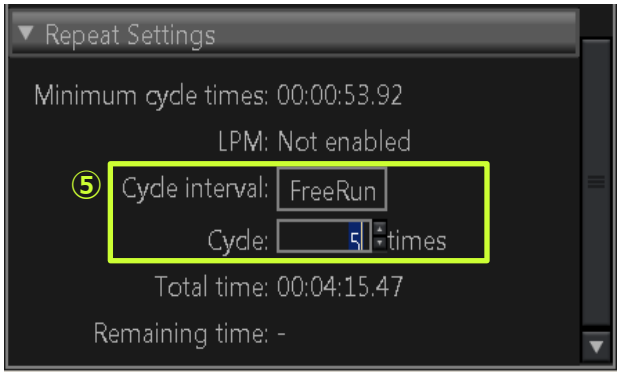
Check when automatic processing
→ Setting : Select matching reference frame

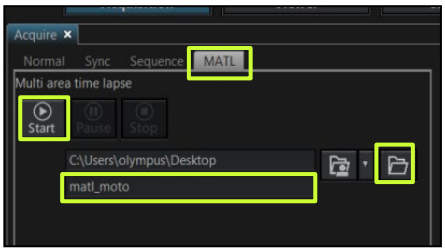
Multi Area Time lapse using Map Image(2)

◆Only for motorized stage◆









Registering the multiple area

① Set the higher magnification objective lens. double-clicking on the map image, the stage is moved to at the center of the map.

② Press the  to register the position and acquiring parameters.

③ Repeat ①, ② register the multi areas that you want to acquire images.

④ Check the registered area List whether it is ticked to ”Enable” at the area which you want acquire images.

【Attention】
All acquiring conditions(XY position, focus position, laser intensity, sensitivity, series setting, and so on.) are registered when you press the  button. So, Register after you finish adjusting all conditions.

⑤ If necessary, set [Cycle interval] and [Cycle].

If you want to see the image on map in real-time, tick the “OverlayMap”.

⑥ Select [MATL] tab in [Acquire] Tool Window.

⑦ Press the  button to display the dialog box, and select the folder to save the images.

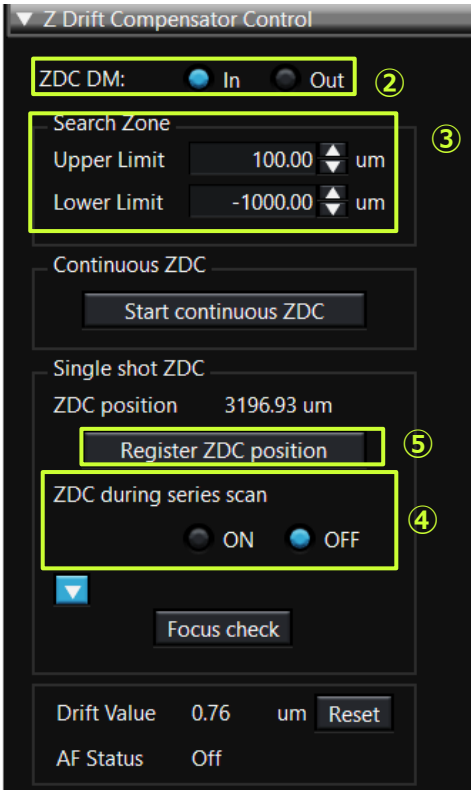
⑧ Press  button to start acquiring the image.

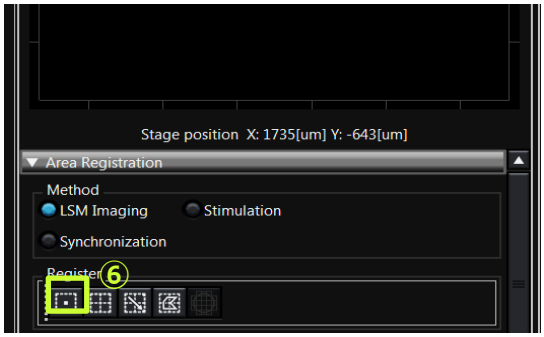
⑨ Finishing the acquisition, map image is displayed in [Map] tab.

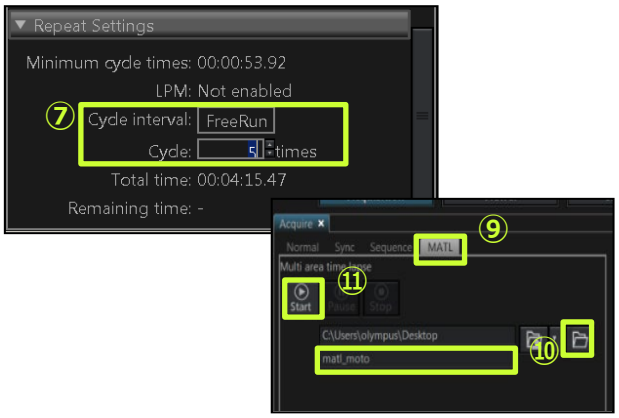
Multi Area Time Lapse (with the Z drift compensation)

◆only for mechanical stage ◆

◆ZDC◆







＊Before starting the following procedure, make adjustments for XY imaging.

ZDC setting (See page 24 for details.)

① In [▼Z Limit Setting], press the Register current position as near limit button to set the Near limit to all objective lenses.

② Set [ZDC DM] to “In” and place the dichroic mirror of ZDC in the light path.

③ Specify the search zone of the coverslip position in [Upper Limit] / [Lower Limit] in [Search Zone] using the Origin coordinate of Z as a base point.

④ Set [ZDC during series scan] to "ON".

⑤ Adjust focus and Press the Register ZDC position button of [Coverslip position]. The coverslip top surface position is acquired.

*When the Z drift compensation is successful, the buzzer sounds beeps once only. If the Z drift compensation is not successful, the buzzer sound beeps three times.

⑥ Press the button to register areas.

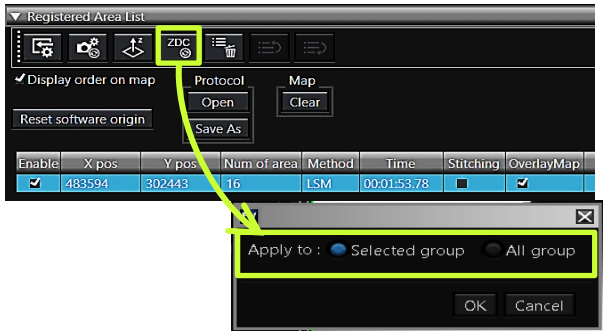
⑦ Set [Cycle interval] and [Cycle].

⑧ Select [MATL] tab in [Acquire] Tool Window.

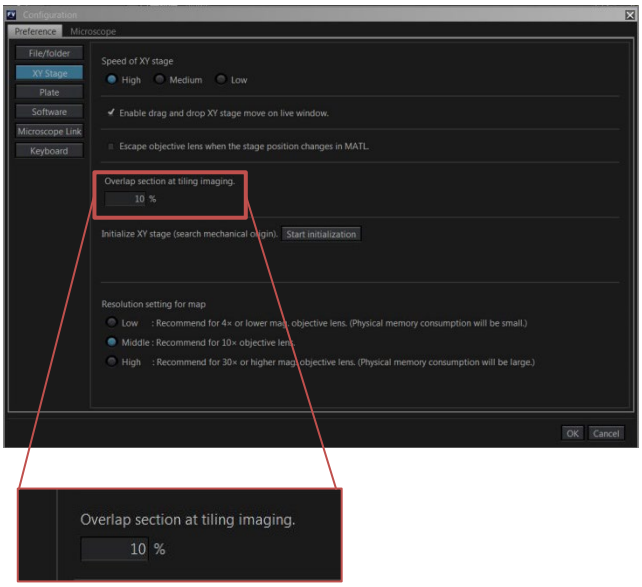
⑨ Press the button to display the dialog box, and select the folder to save the images.

⑪ Press Start button to start acquiring the image.

*Update the setting of ZDC position



Overlap section at tiling imaging



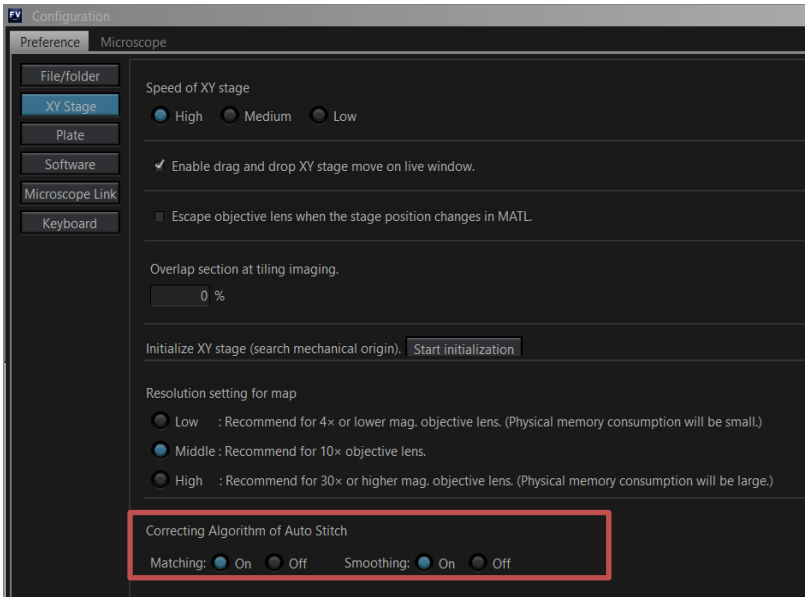
① Select [configuration] in [Tools] to open the dialog on the left.

② Select [XY stage] in the [Preference] tub.

③ Enter the number directly in “Overlap section at tiling imaging”
＊example↓
Objective lens 10X or less:20%～30%
10X or more:15%～20%

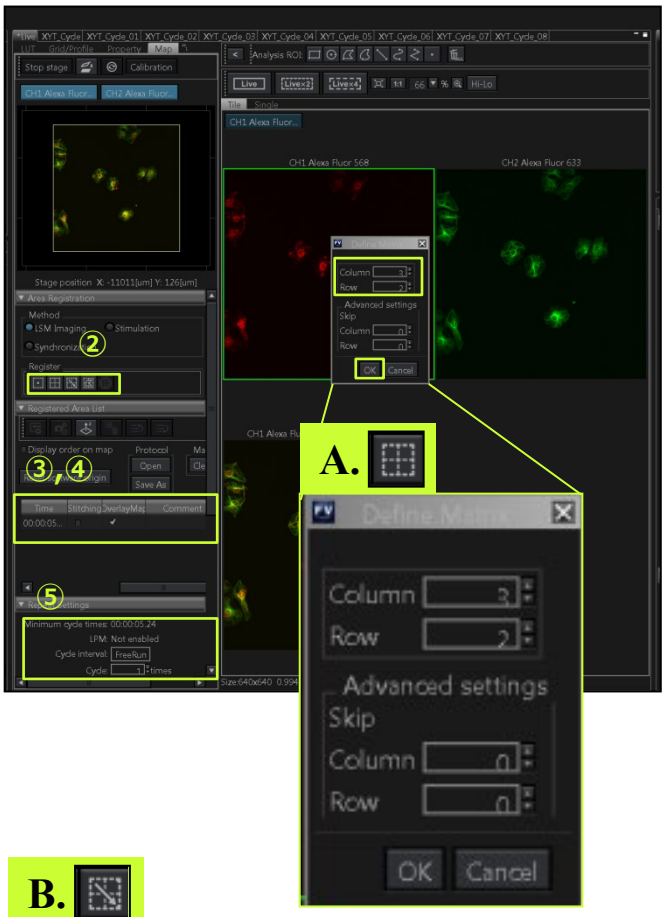
④ Press the [OK] button.

～Setting of automatic stitching process～

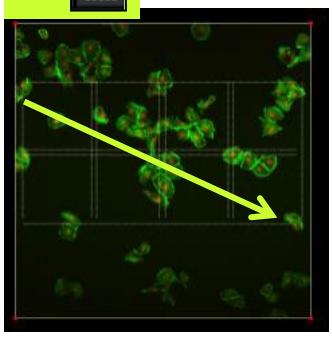


Matching/Smoothing : On
→→ Enables matching and smoothing processing even during automatic stitching.
(* V2.5.1 ~)

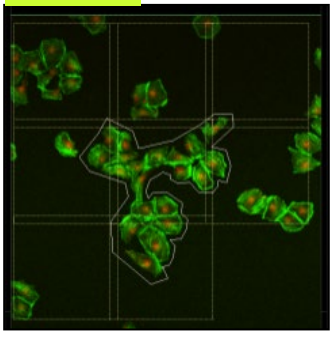
Acquiring the stitched image using Map Image(1)



B.



C.



① Before starting the following procedure, make adjustment for XY imaging.

② Setting the acquiring area.

＊3 types

A. Register the Column×Row

1. Press the ⬚ in area registration. The [Define Matrix] dialog box is appears.
2. Enter the number of Column and Row.
3. Press the [OK].
   ※This button registers an area with the center being the stage position at the time the button is pressed.

B. Drawing a rectangular ROI in the Map

1. Refer to page 19-20, create a map image.
2. Press the ▨ button.
3. Drawing a rectangular ROI in the Map image display area, and then registered on the list.

C. Drawing a polygonal ROI in the Map

1. Refer to page 19-20, create a map image.
2. Press the ▨ button.
3. Drawing a polygonal ROI in the Map image display area.
4. Right click of the mouse to complete the ROI, and then registered on the list.

Acquiring the stitched image using Map Image(2)

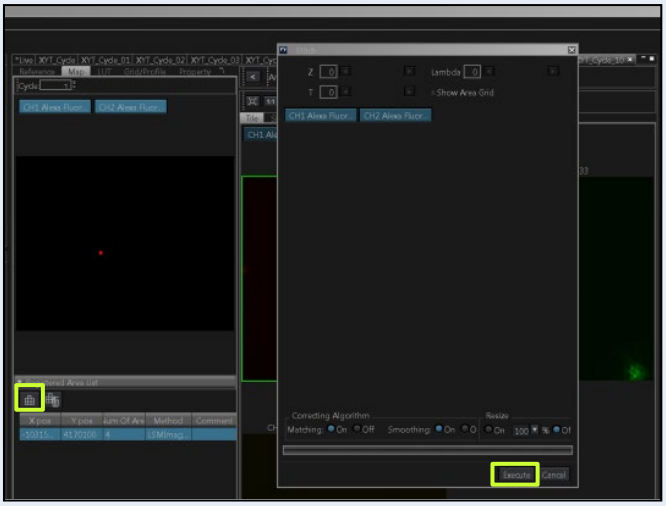
Processing to stitch after acquiring the image.

① Open the image.

② Press the  button.

The [Stitch Dialog box] appears.

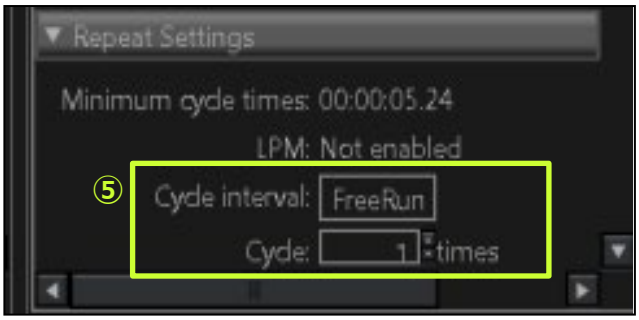
③ After the image is displayed in the dialog box, press the Execute button.



③ Check the registered area List whether it is ticked to “Enable” at the area which you want acquire images.

④ If you want to see the image on map in real-time, tick the “OverlayMap”.

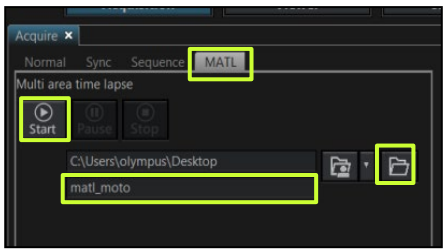
⑤ If necessary, set [Cycle interval] and [Cycle].



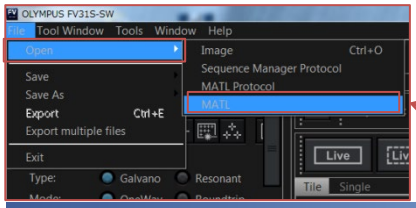
⑥ Select [MATL] tab in [Acquire] Tool Window.

⑦ Press the  button to display the dialog box, and select the folder to save the images.

⑧ Press the  button to start acquiring the image.
Finishing the acquisition, map image is displayed in [Map] tab.

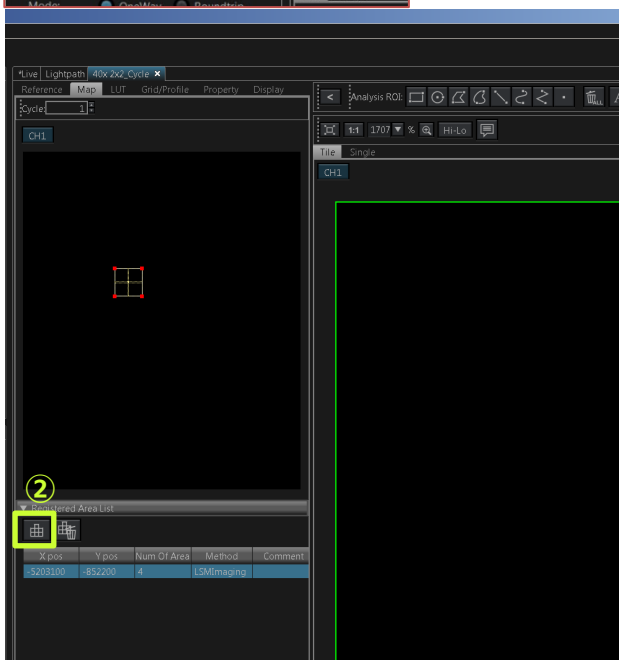


Stitching



① Open the date you acquired.

How to open the image after closing the image.
1)Select File>Open>MATL
2)Select the folder. And open「matl.omp2info]
3)After open the file, subpanel is opened. And select the Map tub automatically.

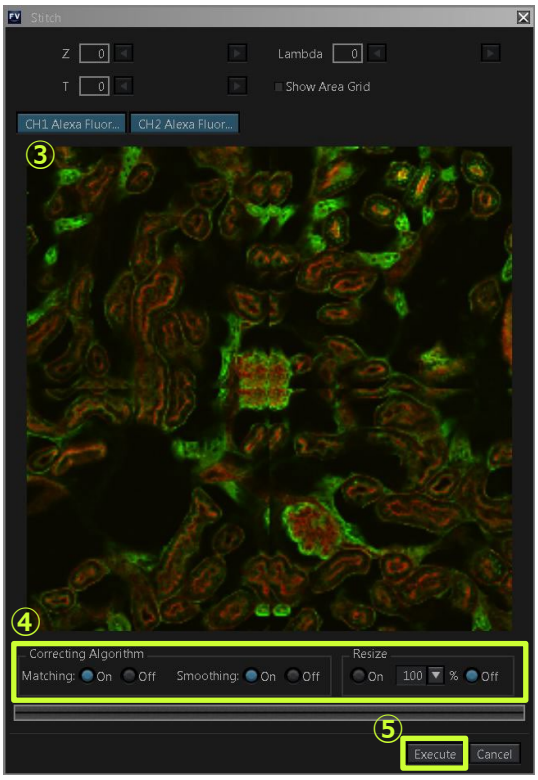


② Click on the top left of the image in the Map tub to select all image. And then click the  button.

③ Stich window is displayed. In a few seconds, preview image is displayed.
※Too large date takes time to display the preview image.
※Since the images are arranged side by side without consideration of margin, they are displayed in a duplicate state as shown in the figure.

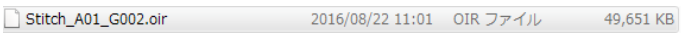
④ Select the processing content.

*Matching→Correct overlapping parts
*Smoothing→Make the boundary smoother, making it less noticeable.
*Resize→Reduce the image.



⑤ Press the Execute button to start stitching.

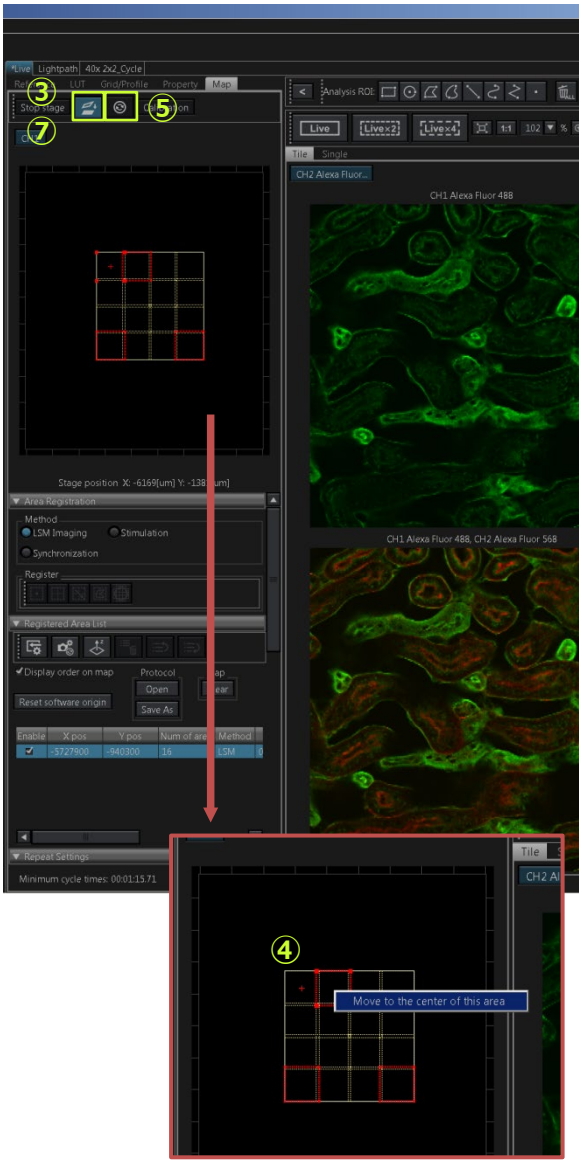
⑥ The name of the date after stitching is 「Stitch_***」.



★In case of the large date

・If it exceeds 1GB, the file is divided.
File name：「Original file name_00001（serial number）」
＊please note that images can not be opened when there are no divided file.
・there are restrictions on export. Please use “Resize”.

Adjusting the tilt in Z direction ～Focus Map～



① Before starting the following procedure, make adjustment for XY imaging.

② Setting the acquiring area (more than 2X2). (p.39)

③ Click the button .
The Red focus point is displayed at 3 positions in MATL ROI.
※If necessary, drag&drop the focus point on the map image display area to change the XY position of the focus point.
※Moving the XY stage position of the focus point moves the XY stage moves in conjunction with it.

④ Click the Red frame to turn it Green.
Right-click on the green frame to perform [Move to the center of this area].

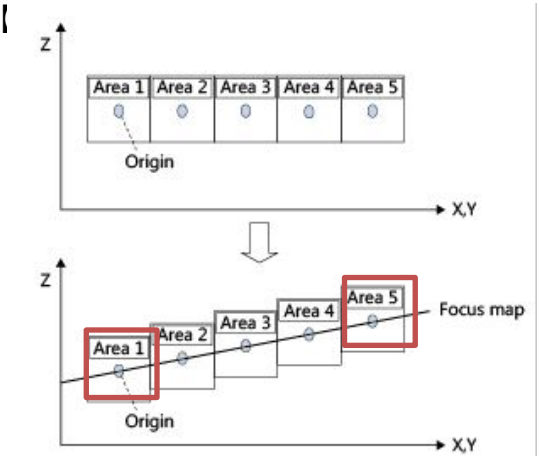
⑤ Adjust the Z position, and click the button in the state of the green frame.
The Z position of the focus point is updated.

⑥ Repeat ④,⑤ at 3 positions.

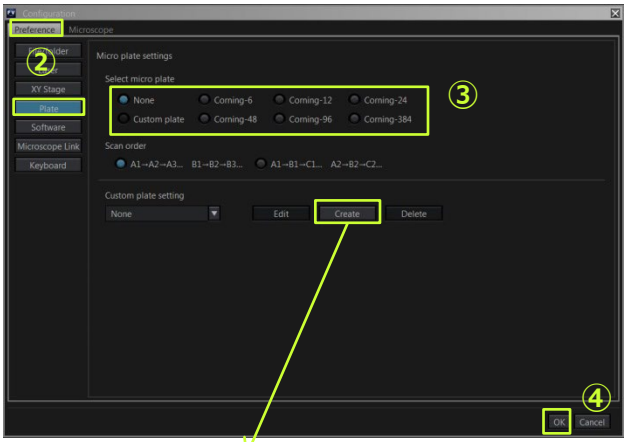
⑦ Click the button again.
Then the focus point is disappeared.

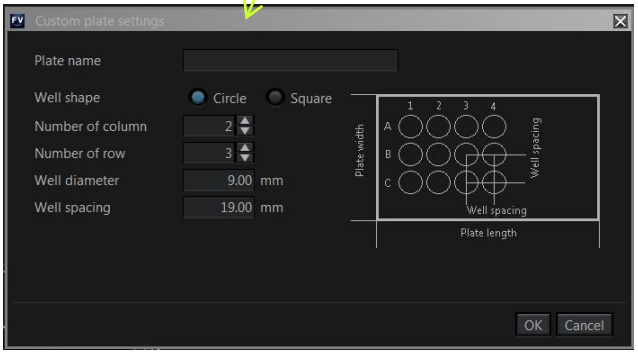
⑧ Start acquiring MATL.

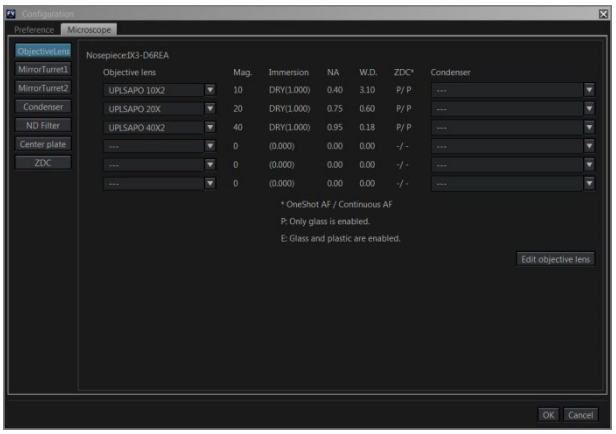
Offset the origin position in each field using the surface (focus map) calculated from 3 focus points.⇒



Setting of the Well Plate







■Setting

① Select [Tools]>[Configuration].
② Select the [Preference] tub .
③ Select the [Plate] on the left list.
③ Select the appropriate well.
※Corning is preset.
④ Press the OK button.

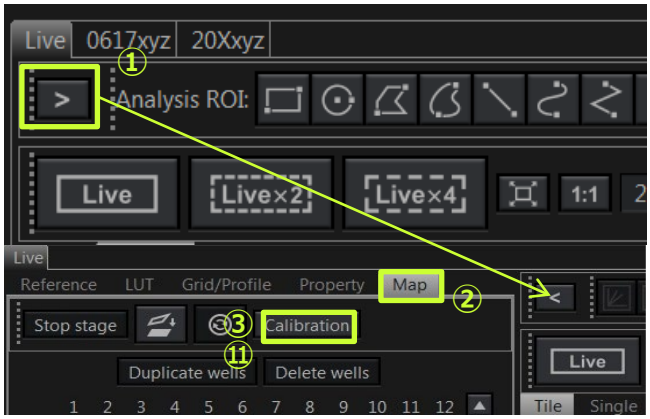
Register plate in Custom plate setting

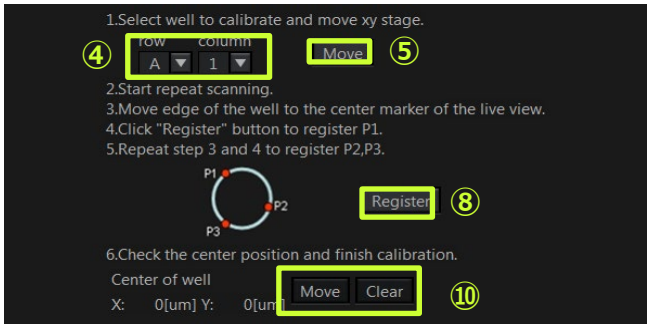
(ex : circle well)
Plate name
Well shape : circle or square
Number of column : Lateral well(line)
Number of row : Vertical well(column)
Well diameter
Well spacing : distance between the center of the well

■Stage : IX3-SSU

→Center plate must be set.
① Select [Tools]>[Configuration].
② Select the [Microscope]tub in the window.
③ Select [center plate] on the left list.
④ Select ”IX3-HOW” (well plate holder).
⑤ Press the OK button.

Calibration of the well plate





Please confirm !
Visually check the position of objective lens if the well you moved in ⑤ matches the well specified with ④ (ex:A1).

When they do not match, move the motorized stage to the specified well using a joystick.

＊Before starting the following procedure, make adjustment for XY imaging.

Set the Map mode

① Select [Live] window. And select the [>] button to display the tool on the left side of the LIVE image.

② Double click the [Map] tub.

Performing the calibration

③ Press the [Calibration] button ,and then the Position adjustment mode is displayed in the Map Image display area.

④ Select the well to be adjusted in the [row] and [column]
(ex)A01 well : ([row]=A, [column]=1)

⑤ Press the [Move] button to move the stage for the center of the well to be adjusted.

⑥ Press the [Live] button to scan the Image with the marker displayed.

⑦ Moving the stage so that the edge of the well coincide with the cross marker.

⑧ Press the [Register] button at matching point.(P1)

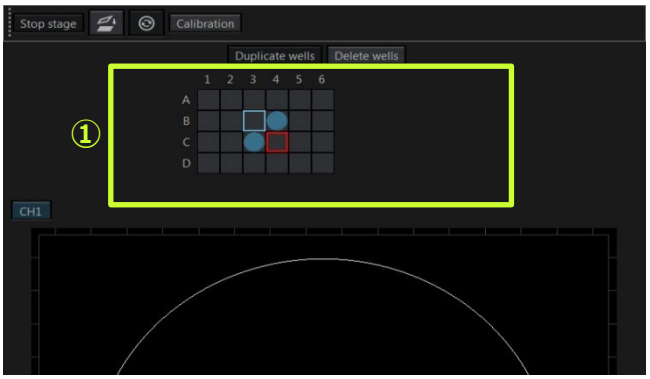
⑨ Repeat ⑦ and ⑧ to register P2 and P3 point.

Attention !
As a result of registering P1, P2, P3, if you can not calculate a circle from the 3 points (eg 2 points out of 3 points or all 3 points are collinear), an error message will be displayed .
In that case, please try again from P1 registration

⑩ Press the [Move] button to move the center of the well .
Make sure that the well center is set correctly. If it is not set correctly, clear the registration of P1,P2,P3 by pressing [Clear] button and register again.

⑪ Press the [Calibration] button again to release the Position adjustment mode.

Map Image display area for well plate(1)



When selecting plate type in [Plate], it is displayed for microplate.

Pattern diagram for well plate

① Line is alphabet , column is number.

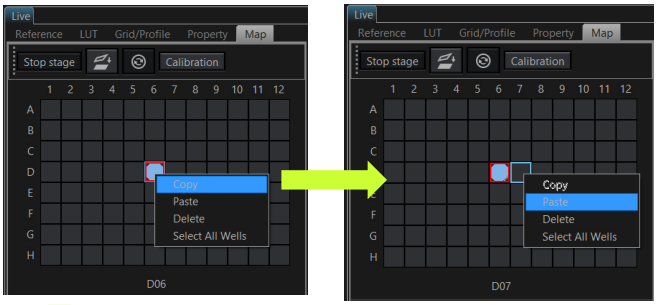
: well in the stage operation range
click →selected
double click →move to center of the well

: well where the stage located

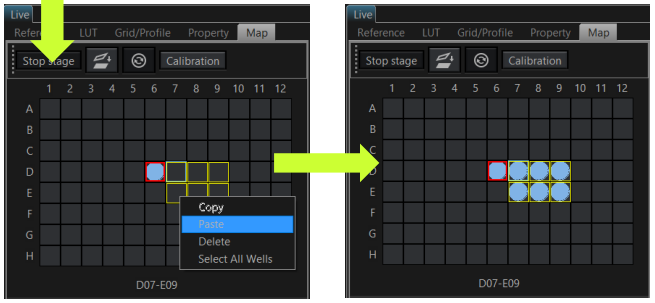
: well selected

: Well set as an image selection area and further setting that area to “Enable”.

①Copy ②Paste



②-※Paste（Multiple wrell）



Duplicate the well set as and image selection area

① Right click on the well to open the menu.
- Copy acquisition settings of the well
- Paste acquisition settings of the well
- Delete acquisition settings of the well
- Select All Wells in the plate

② Select “Paste” on the target well

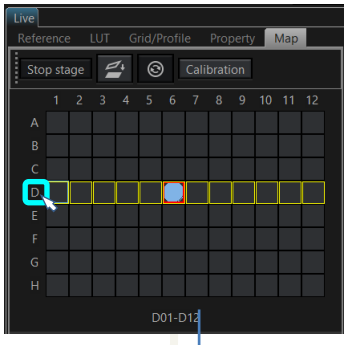
※Dragging on the wells to select the Multiple wells.
Yellow frame means selected wells.
It is possible to paste it there.

Map Image display area for well plate(2)

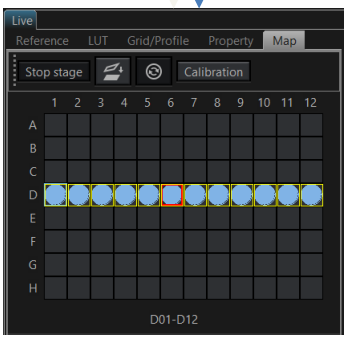
Copy and Paste of the scan setting of the one well

- Target row and column can se selected by clicking on row/column nuber.
- All the wells can be selected by context short cut key.

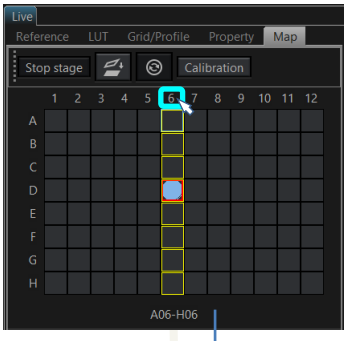
1. Select one row



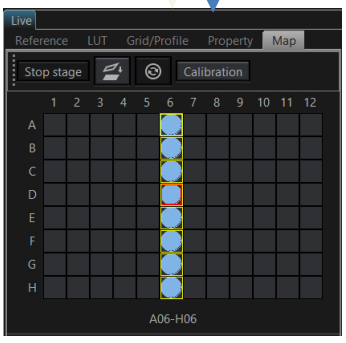
2. Paste



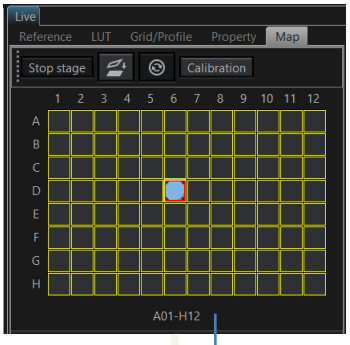
1. Select one column



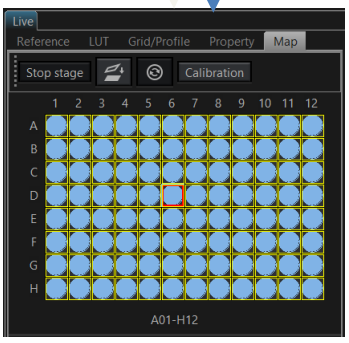
2. Paste



1. Select all wells



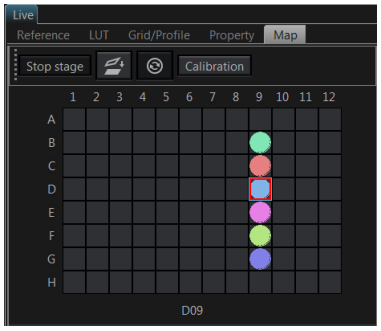
2. Paste



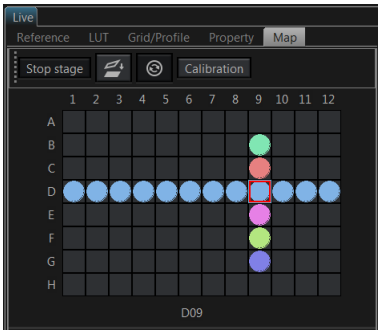
Color labeling of well

- Newly registered (not copied from other well) wells are labeled with different color.
- If one of these well is copied to other wells, they have same color label.

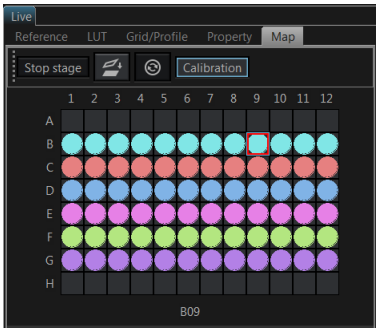
Different setting in eack well



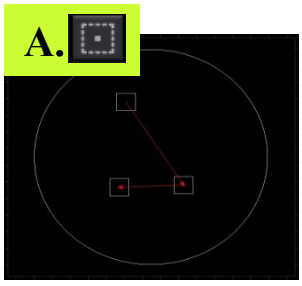
Setting of D9 was paste to all wells in rowD

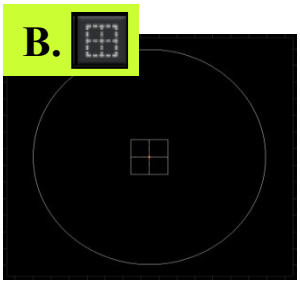


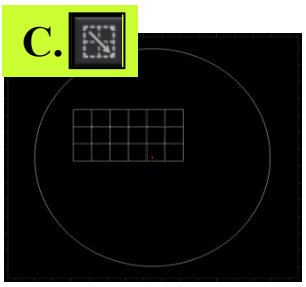
Same setting in each row

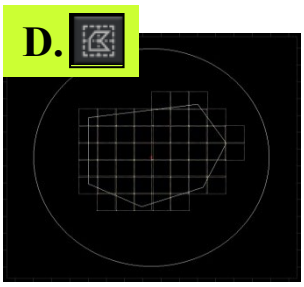


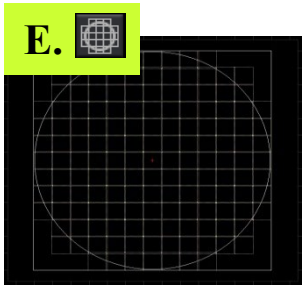
Registration of Image Acquisition area











① Select the [LSM Imaging] in [Area Registration] on [Map] tab.

② Register the Image acquisition area.

A.Specify any area

1. Press the button to resister an area with the stage position at the time the button is pressed.

B.Resister the ColumnXRow

1. Press the in area registration. The [Define Matrix] dialog box is appears.
2. Enter the number of Column and Row.
3. Press the [OK].
   ※This button registers an area with the center being the stage position at the time the button is pressed.

C. Drawing a rectangular ROI in the Map

1. Press the button.
2. Drawing a rectangular ROI in the Map image display area, and then registered on the list.

D.Drawing a polygonal ROI in the Map

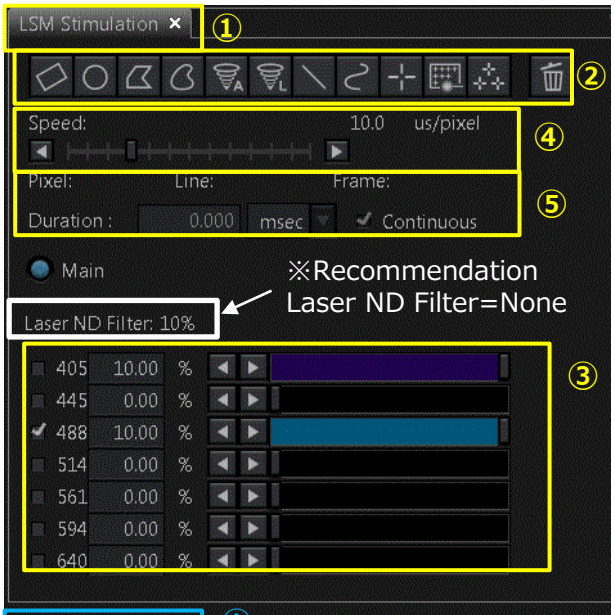
1. Press the button.
2. Drawing a polygonal ROI in the Map image display area.
3. Right click of the mouse to complete the ROI, and then registered on the list.

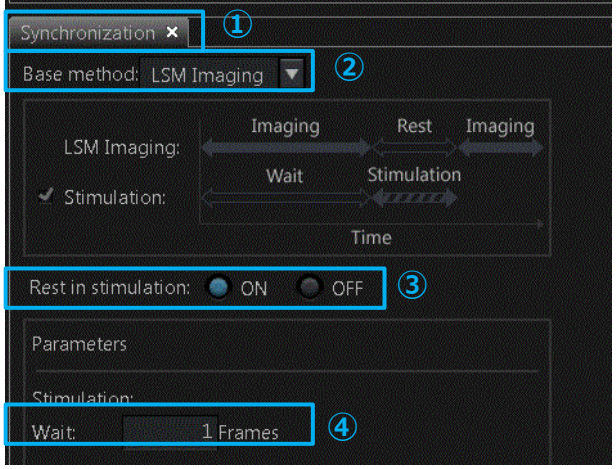
E.Register all areas of the well

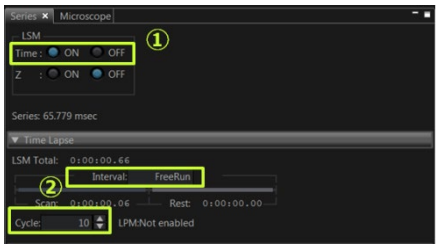
1. Press the button.

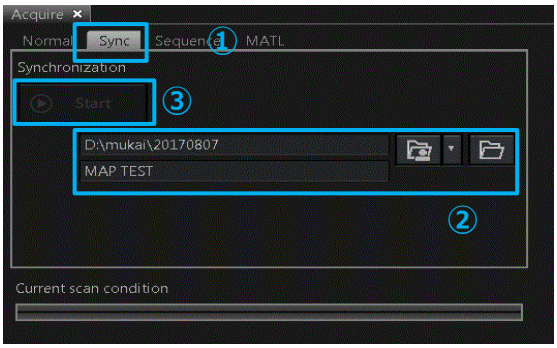
Advanced

Stimulation









Condition setting

① Select the [LSM Stimulation] in [Tool Window] menu.

② Select the ROI and resister the stimulation area on the Image.

Tornado scan
A:trnado scan at a constant angular velocity with a circle.
L:trnado scan at a constant linear velocity with a circle.

※When changing the Mode of scanner, ROI is reset.
To specify the ROI, display the LIVE image once,

③ select the laser wavelength for stimulation and adjust the intensity.

④ Set the speed (us/pixel).

⑤ Remove the check of the [Continuous] and enter the time of duration and unit of time.
※Continuous : in case of setting the Start/Stop of stimulation manually.

Creating a Time Line

① Select the [Synchronization].

② Select the “LSM Imaging” in [Base Method].
（=Start TimeLine simultaneously with image capture.）

③ Select “OFF” in [Rest in stimulation].
※ON:Image capture continues even during light stimulation.

④ Enter the wait Frame: from Image acquisition to start stimulation.

Set the T series(Total number of the frame)

① Select “ON” in [Time] on [Series] tool window.

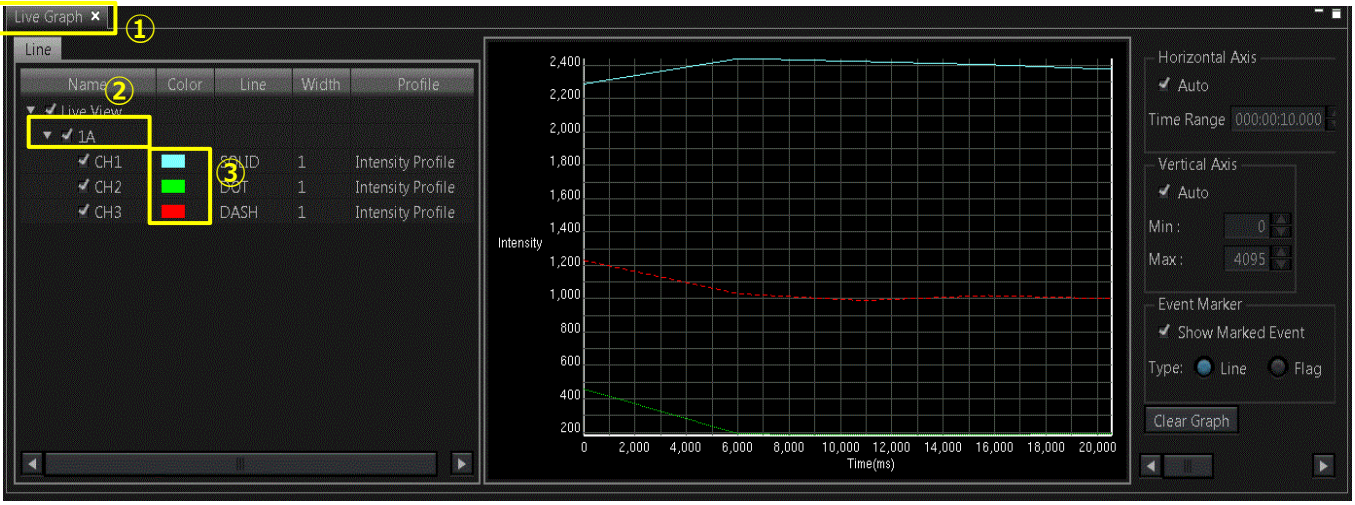
② Set the interval to acquire the image in [Interval] and [Cycle] on [▼Time Lapse].

Execution of TimeLine

① Select [Sync] tab in [Acquire] window.
Press the button to display the dialog box, and select the folder to save the images.

② Press the Start button to start acquiring the image.

Stimulation



Drawing the graph of brightness with LIVE image

① Select the[Live Graph] in the [Tool window] menu.

② Specifying the place checked the intensity by drawing the ROI in [Analysis ROI].

③ Ticking these checkboxes displays the intensity profile.

④ To change the color, double-click the displayed color. When the ... button appears, click it to display a dialog box.

⑤ change the color in the box.

⑥ Press the Start button to start imaging and drawing the intensity graph.

⑦ Right click on the graph and select the [SaveDisplay] to save the image of the graph.

When drawing and analyzing a luminance graph **after image acquisition**
→ refer to p.22 “Creating a luminance graph: Series Analysis”

※OSR license

Super Resolution:FV-OSR

The resolution can be improved to around 120 nm by using FV-OSR.

① Adjusting focus and set Laser Intensity(%), Sensitivity(HV), Gain and Offset on [PMT setting] Tool Window.

② Select the [Super-resolution] in the [Tool Window].

③ When "Super-resolution imaging" is turned on, the following items are set automatically.

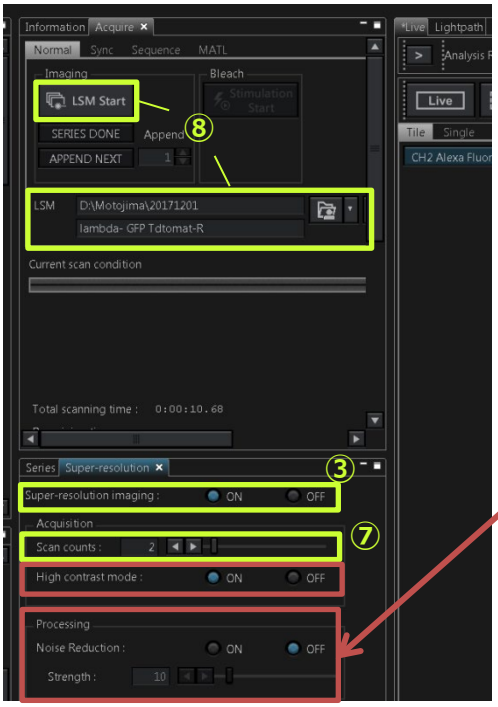
■Following parameters are set automatically
- ・Scanner : Galvano / Oneway
- ・Pinhole size
  →The image will be darker because it is squeezed smaller.
- ・Zoom
  →Lager scan size leads lager field of view.

④ Remove the DIC slider from the light path and set ZDC DM to “Out” in the “Microscope” window.
(It affect the image quality.)

⑤ Adjust the focus and brightness again.
※ Because the high magnification zoom is applied, please be careful about fading.

⑥ It is also possible to combine Z stack .

⑦ ”scan counts” is used to set the cumulative number when acquiring the super-resolution image. (Recommendation : 2-8 times)

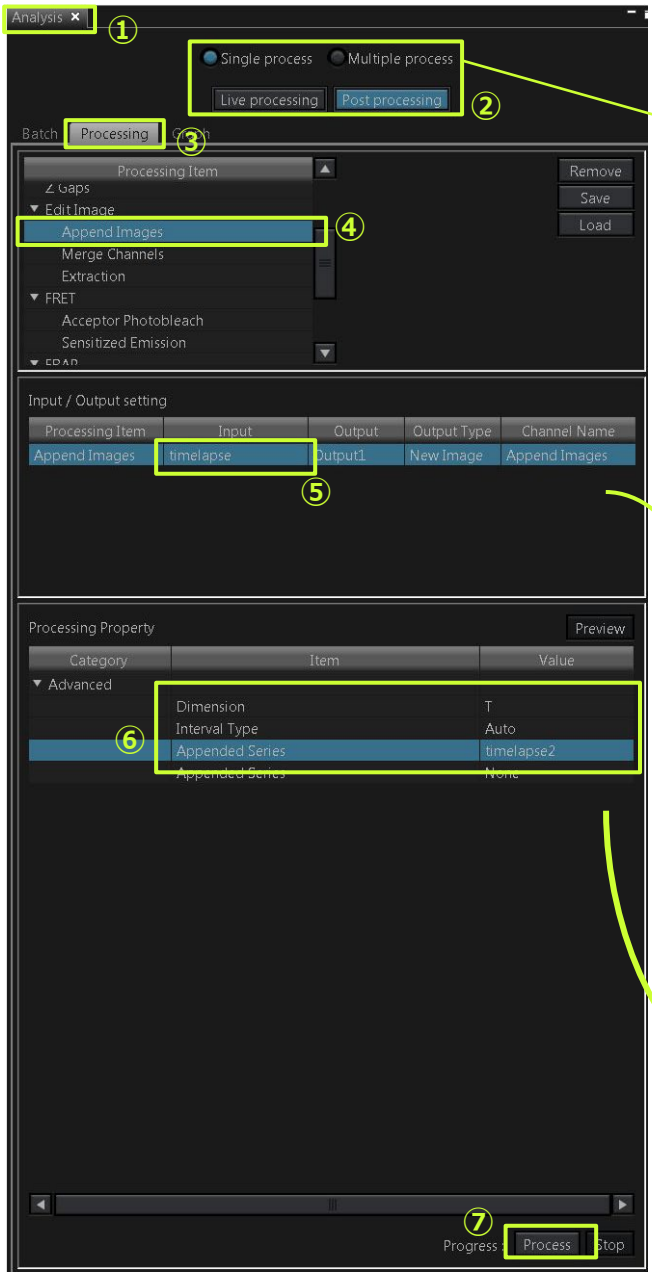


■High contrast mode
→ON: High contrast mode
OFF: Standard mode

■Noise Reduction
→The noise is reduced while acquiring the super-resolution image and it is saved as an image different from the super-resolution image.
(Recommendation : Strength10-40)

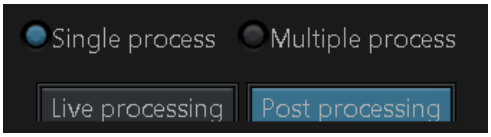
⑧ Select the folder and set the name of the image. Press the LSM Start button.

Append Images



① Select the [Analysis] in the [Tool window] menu.

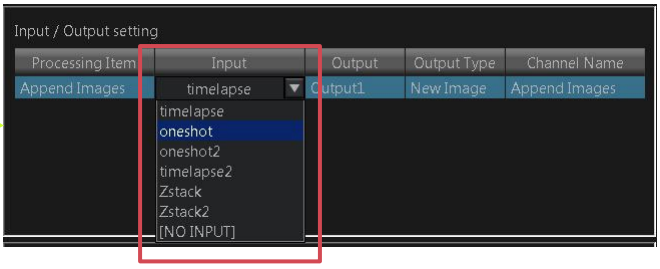
② Select the [Single process] and [Post processing] in the [Analysis] tool window.



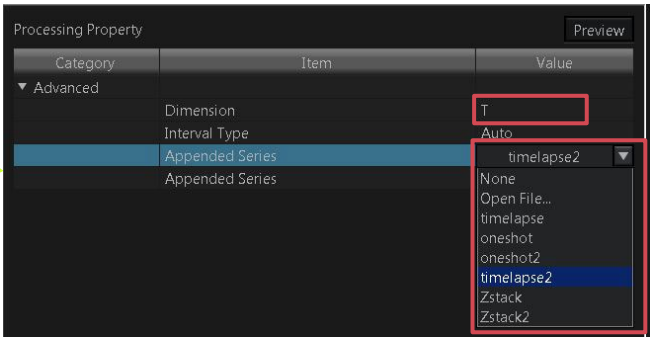
③ Open the [Processing] tab.

④ Select “Append Images” in the [▼Edit Images].

⑤ Click the [Input], and select the image for image processing.

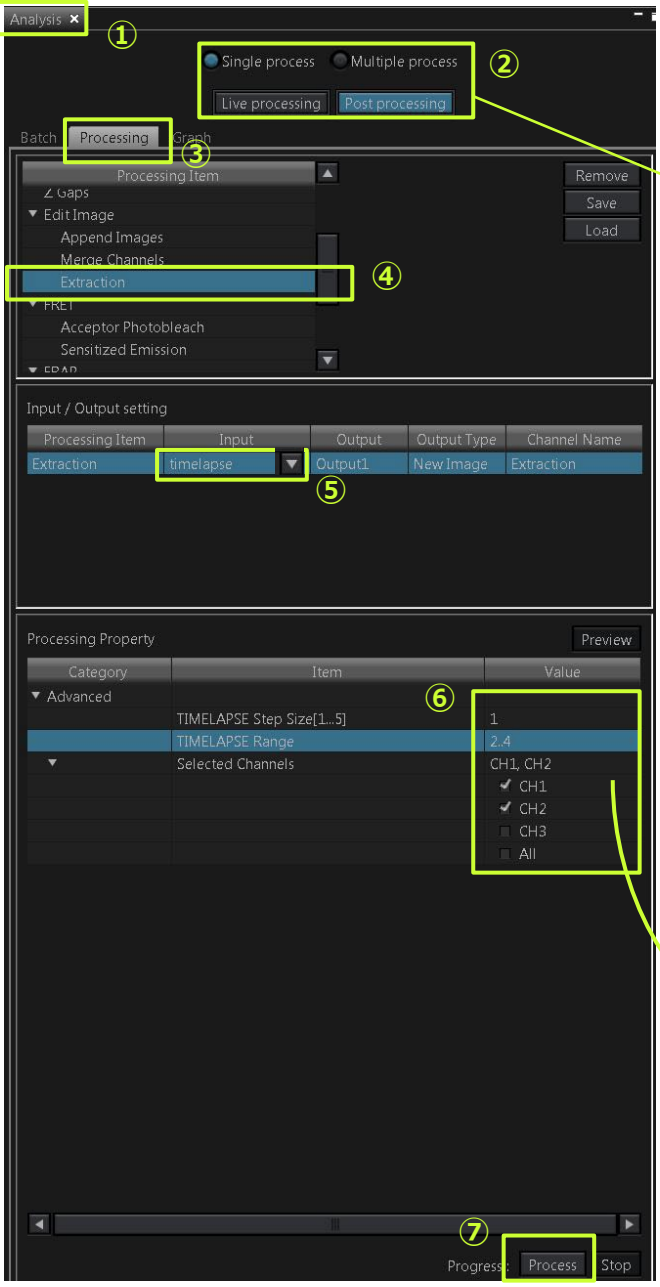


⑥ Select the axis for series to connect images when adding the image in “Dimension” and select the image to be added in “Append Series”.



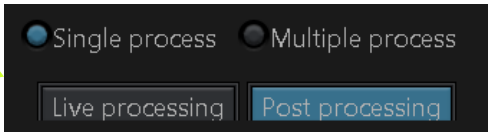
⑦ Press the Process button.

Extraction



① Select the [Analysis] in the [Tool window] menu.

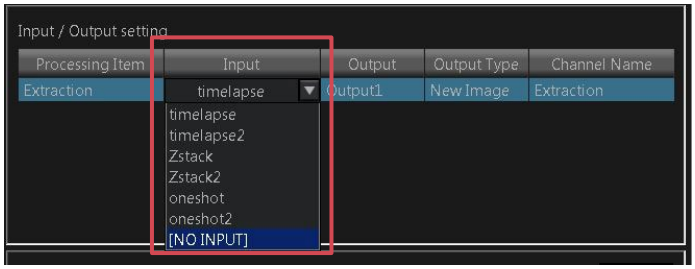
② Select the [Single process] and [Post processing] in the [Analysis] tool window.



③ Open the [Processing] tab.

④ Select “Extraction” in the [▼Edit Images].

⑤ Click the [Input], and select the image for image processing.



⑥ Setting the value of item in the [Processing Property].

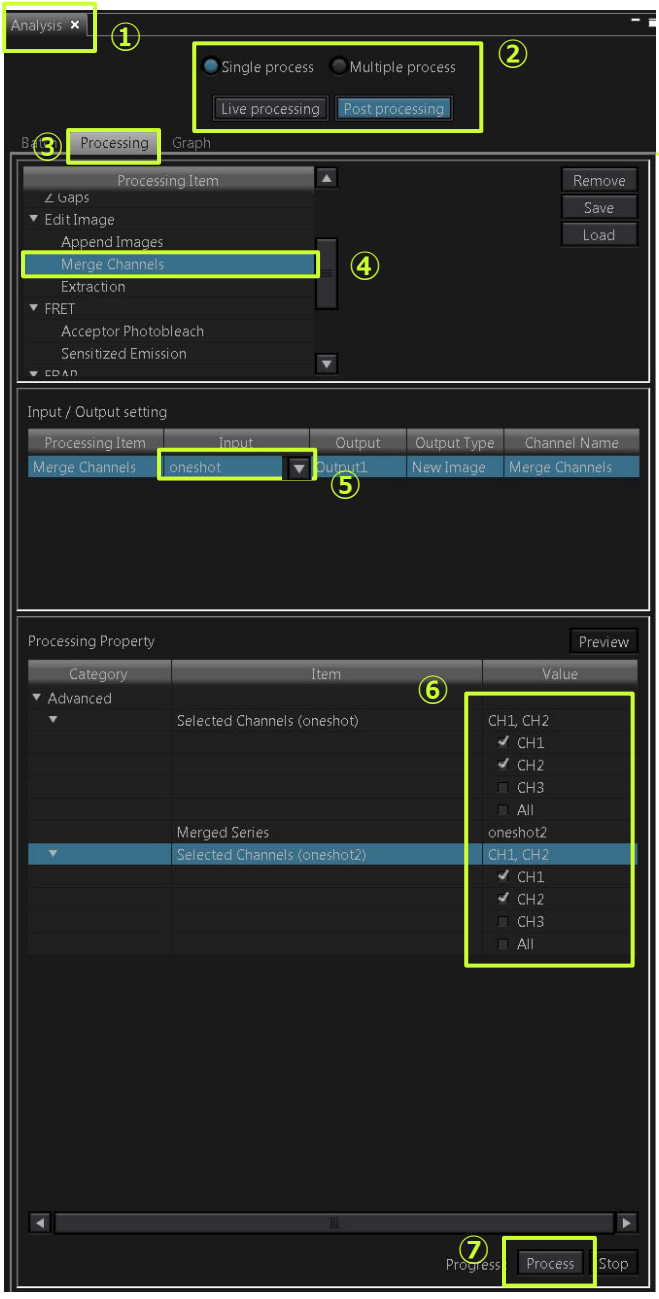
i. Setting the interval among frames (ex: 1)

ii. Setting the section to extract the image from the series image.
(ex:2~4→2…4)
※For「… 」, use the one entered on the software from the beginning.

iii. Select the channel to extract the image.
“All” allows you to extract all channels.

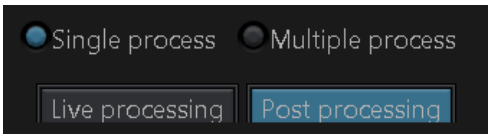
⑦ Press the Process button.

Merge Channels



① Select the [Analysis] in the [Tool window] menu.

② Select the [Single process] and [Post processing] in the [Analysis] tool window.

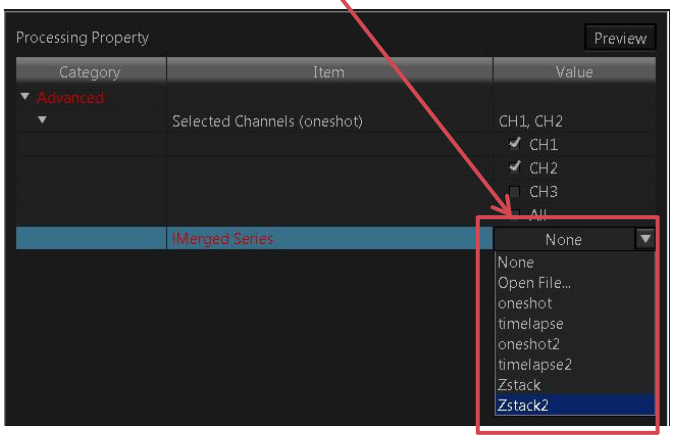


③ Open the [Processing] tab.

④ Select “Merge Channels” in the [▼Edit Images].

⑤ Click the [Input], and select the image for image processing.

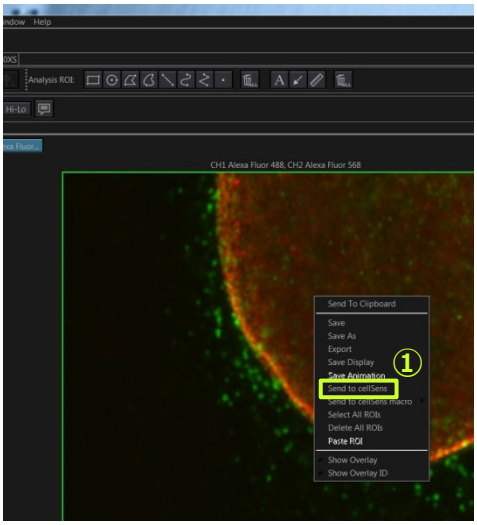
⑥ Select the different image and channel to be composed in the [Processing Property].

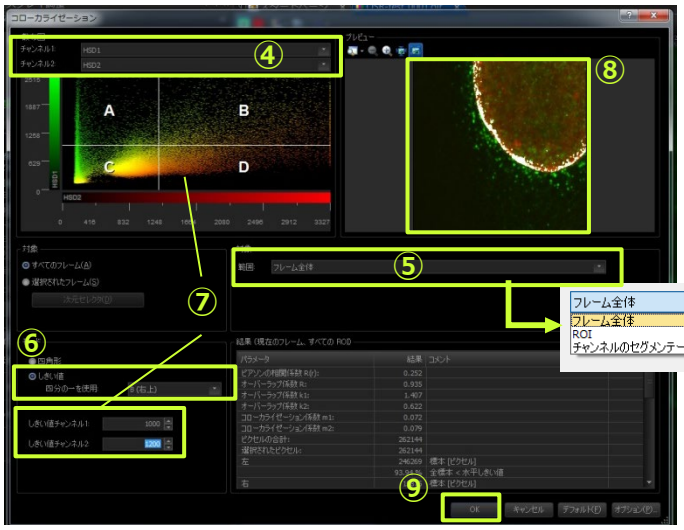


⑦ Press the Process button.

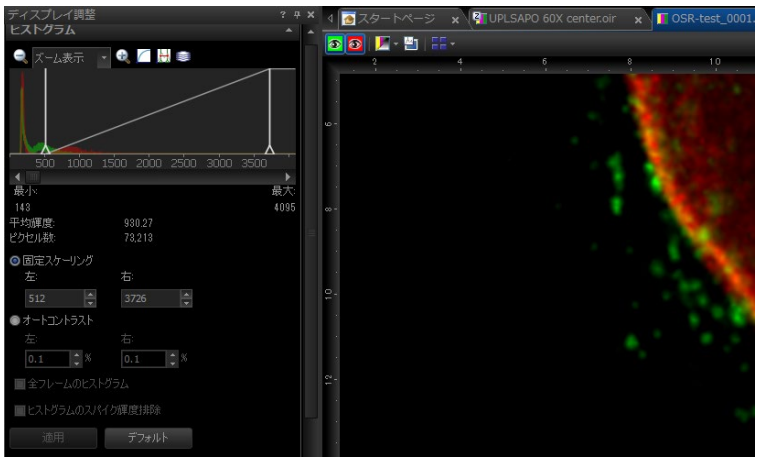
Colocalization

~cellSens~





① Open the Image on FV software and Right-click to select [Send to cellSens].
② cellSens starts up.
③ Select the [Colocalization] .(Measure>Colocalization)
④ Select the two color channel on which you want to perform the measurement colocalization.
⑤ In the target area group, select the target range of the analysis.
⑥ Select the “Threshold” in [Mode] and select the “B(upper right) in the Use quarter.
⑦ In the scatterplot, define the intensity range.
⑧ In the preview, the pixels whose intensity values are within the quandrant that has been selected are shown in white.
⑨ Click the [OK] button to finish the measurement of colocalization.
⑩ Numerical date can be output with the [File>Export to >Excel].



■Result

Peason’s Correlation Coefficient
Overlap Coefficient
Colocalization Coefficient
Total amount of pixels
Selected pixels and % of A~D

■Tips of setting the threshold

①Select the [View]>[ToolWindow]>[Colocalization]
②Adjust the brightness and Backgroung with [Adjust Display] to see the signal.
③the optimized number of threshold is “Right” in Fixed scaling.

※Option：cellSens 3D Deconvolution

3D Deconvolution

~cellSens~

① Open the acquired Image (Z stack) and double click on the image. Select the [Send to CellSens].
② cellSens is displayed.
③ Select the [Process]>[Deconvolution]>[Constrained Iterative…].
④ Choosing images for the deconvolution filter in “Apply on”.(All frame… or Selected frames…)
⑤ Press the Next >> button.
⑥ Choose the “Laser Scanning Confocal” or “FLUOVIEW FV3000” in Modality.
⑦ Set the Algorithm and parameters.

⑧ Press the Finish button to start the process.
(press the Verify button to display a preview of the resulting image.)

■Enough resolution is required for successful the Deconvolution

ex) In case of use the 60xO Objective lens (NA1.3)
- ImageSize：more than 800x800
- Zoom：more than 1.5X
- Z Step size：less than 3um
- Z Slice：the more slices, the better.

TruAI Noise Reduction

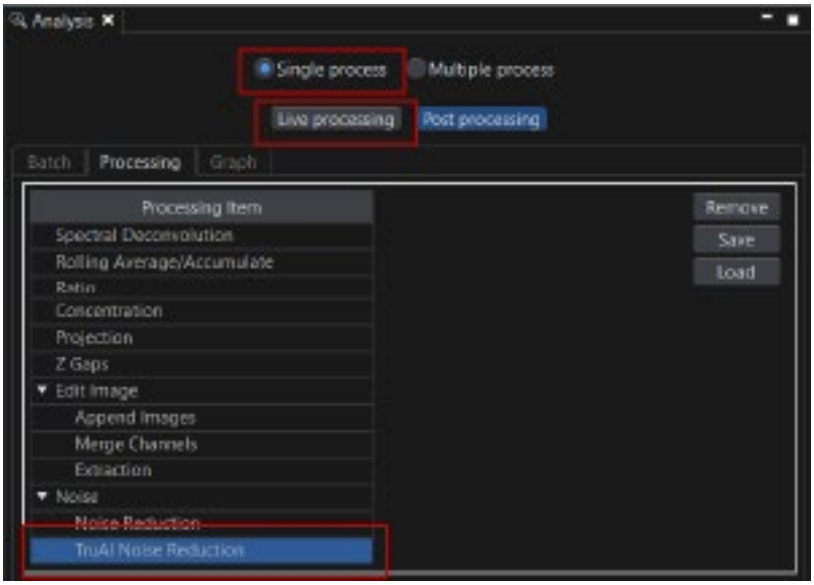
※FV40S-AINR option

High S/N image will be expected by using AI noise reduction.

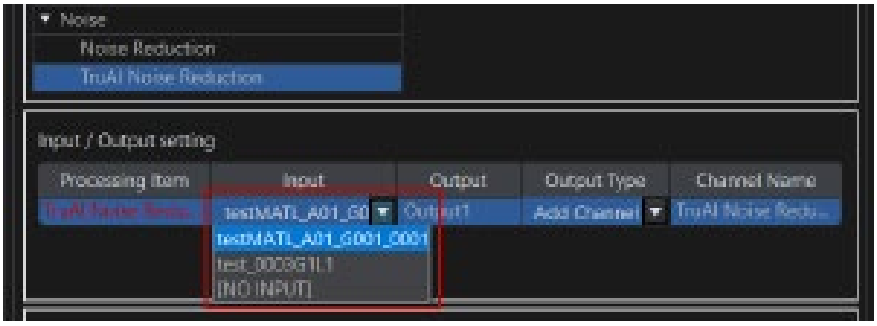
① Open the images.

② Show Analysis tool window by Tool Window > Analysis.

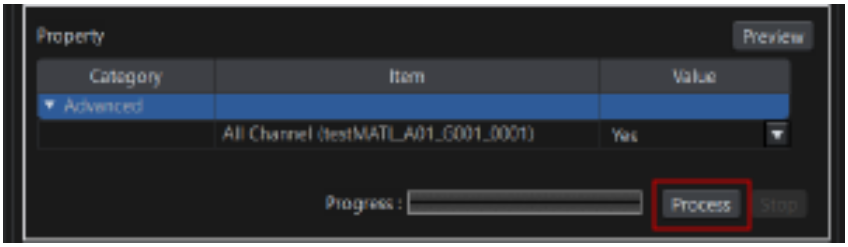
③ Select [Single process] and [Post processing] then select [TruAI Noise Reduction] in Processing Item.



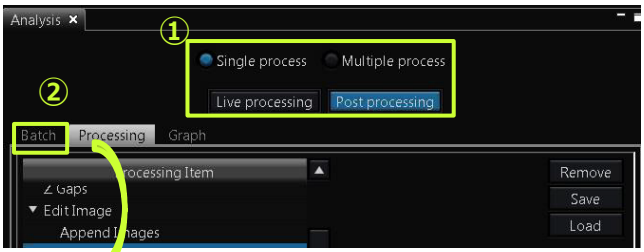
④ Select the image in [Input].
Choose “Add Channel” or “New Image” in [Output Type]. 。

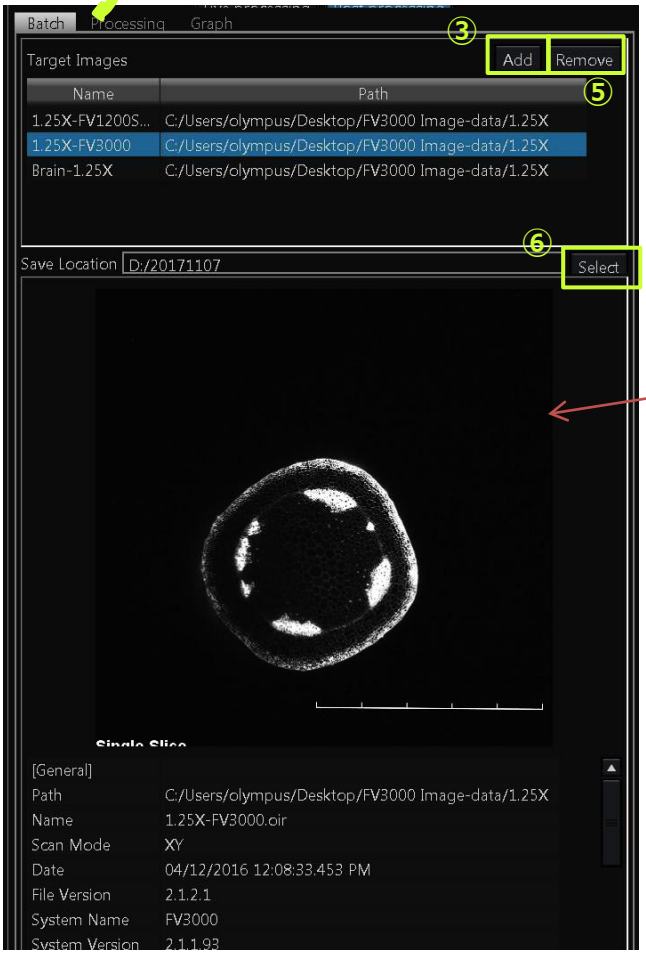


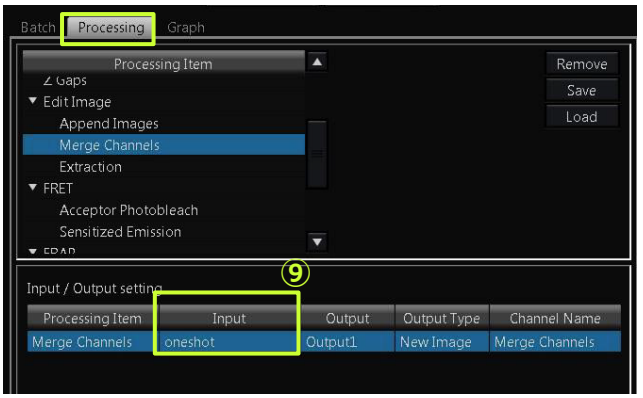
⑤ Start by [Process].



BATCH :
Batch processing for multiple images







■Available condition
→Select the [Single process] and [Post Processing] in the [Analysis] tool window.

① Select the [Single process] and [Post processing].

② Select the [Batch] tab.

③ Press the [Add] to display the dialog box.

④ Specify the folder or file to be processed.

⑤ If there is an unnecessary image, delete it with [Remove].
※when the date is selected , image and acquisition condition are displayed.

⑥ Press the [Select] to specify the location folder.

⑦ Select the [Processing] tab and return to the processing menu selection.

⑧ Select the process to be performed, a list is added to “Input/Output setting”.

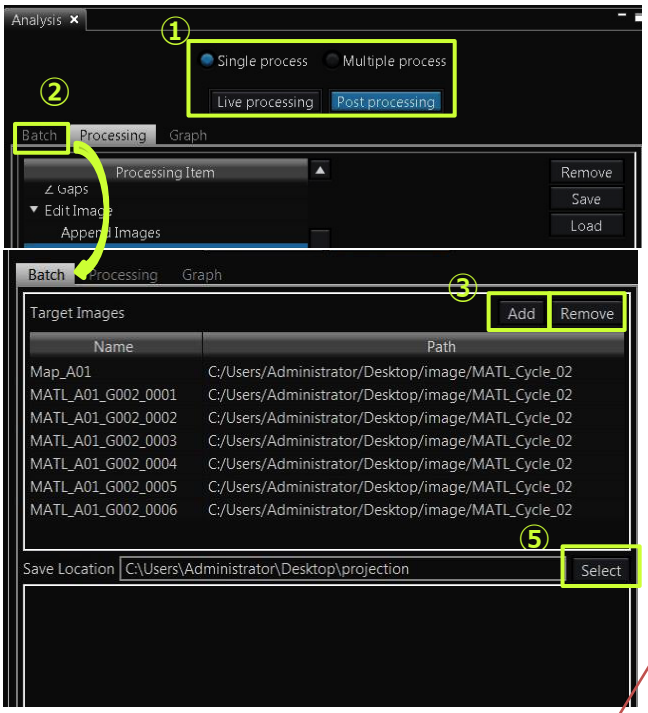
⑨ Click the [Input], and select the [BATCH].

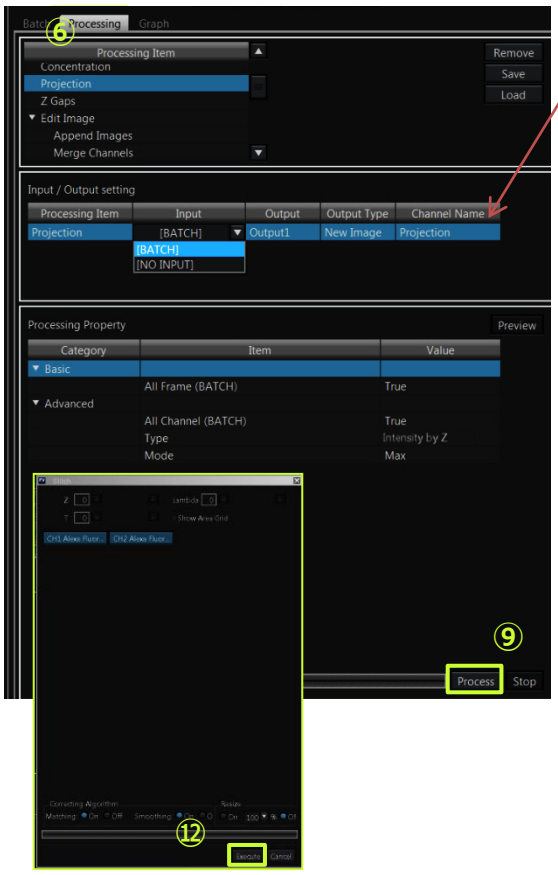
⑩ Press the Process button.

※Only the file processed last remains.
If you check the image after processing , please reopen the file.

Stitching after projection

: For large volumes of date, time can be reduced.





① Select the [Analysis] in the [Tool window] menu. And select the [Single process] and [Post processing].

② Select the [BATCH] tab.

③ Press the [Add] to display the dialog box.

④ Select the folder that tiling was acquired.

⑤ Press the [Select] to open the dialog box. And specify the folder to save/

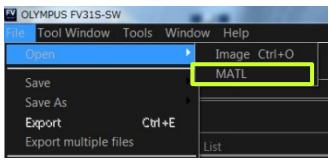
⑥ Open the [Processing] tab.

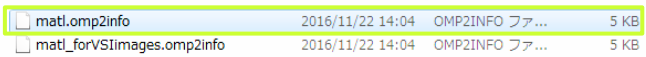
⑦ Select “projection” in the [▼Edit Images].

⑧ Click the [Input], and select the [BATCH].

⑨ Press the Process button.

⑩ Select File>Open>[MATL] to open the folder, and select ”***.omp2info”.

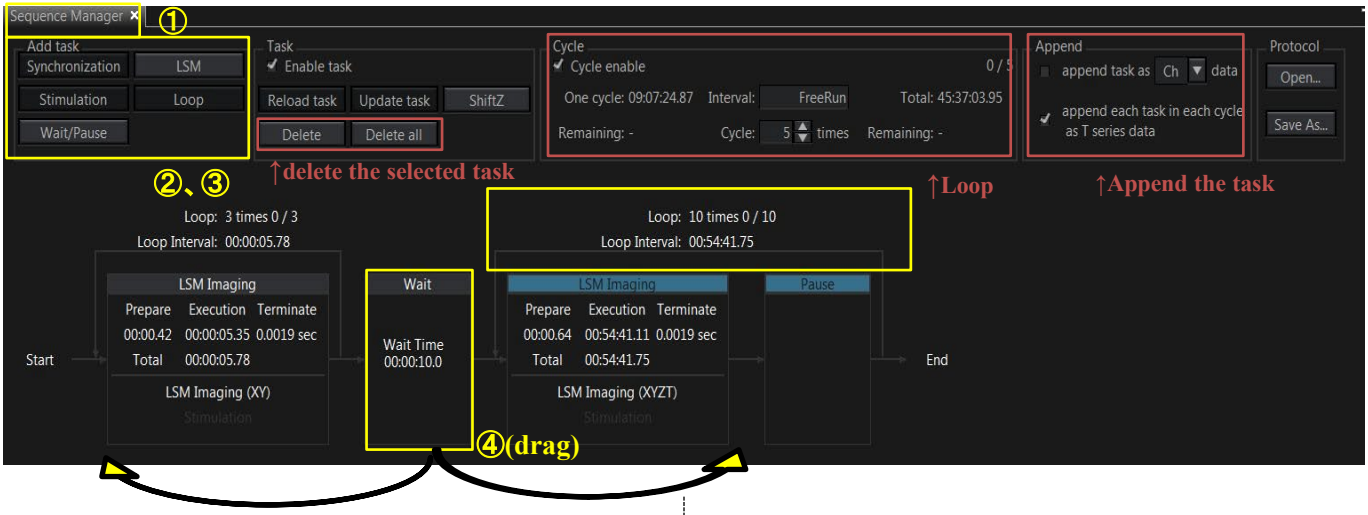




⑪ Press the > and select [Map] tab.

⑫ Press the to display the image processed.
(Not displayed when the number of tiling is large)
Confirm that the image is displayed, then press Execute to execute the process.

Sequence Manager
(making protocol of acquiring)



Making the protcol

① Select the [Sequence Manager] in [Tool Window] menu.

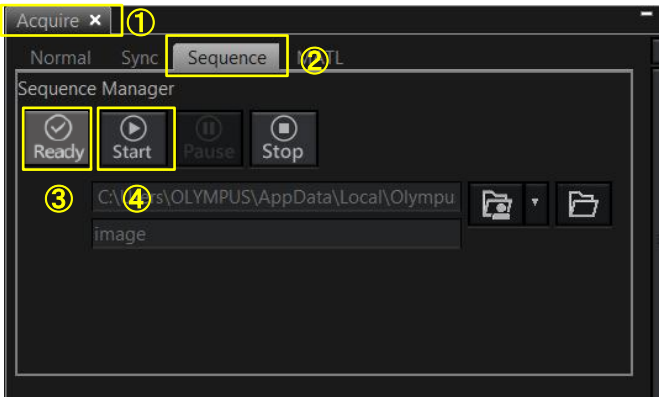
② Press the button in [Add task] to add the task.
※before adding the task, make adjustments for setting.

LSM	Acquiring the XYZT
Synchronization	Imaging and the light stimulation
Stimulation	A light stimulation
Wait/Pause	Wait or Pause

→Pause : stops the protocol that is running until you press the Resume button
→Wait: stop the protocol that is running until the time specified in [wait time].

③ Resister the Loop task
1. Selecting a task registered in the protocol to repeat the selected task.
2. Press the Loop button.
3. Sspecify the repeat count in [Loop setting] dialog box.

④ Move the task
select the task and drag .



Execute the protocol

① Select the [Sequence] in the [Acquire] window.

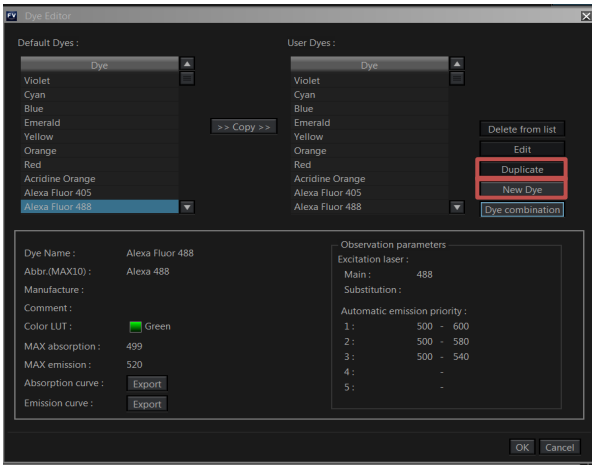
② Select the folder to save image and file name.

③ Press the Ready button.
→the protocol are resistered to the system

④ Press the Start button.

Pause	The running protocols are paused.
Stop	The running protocols are stopped.

New registration of Dye



① Select the [Dye Editor] in Tools.

② Press the New Dye to display the dialog box.

Duplicate :edit based on the data of the "User Dye" selected in [User Dyes]

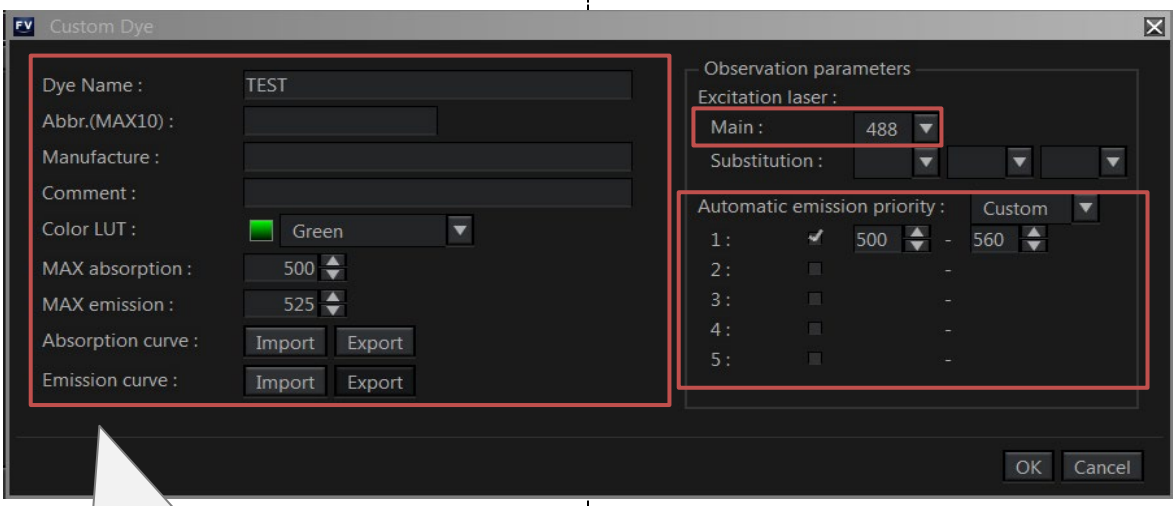
③ Enter each items.

○Excitation peak/fFuorescence peak

: Airy Disk

○Automatic emission priority

: refer to next page

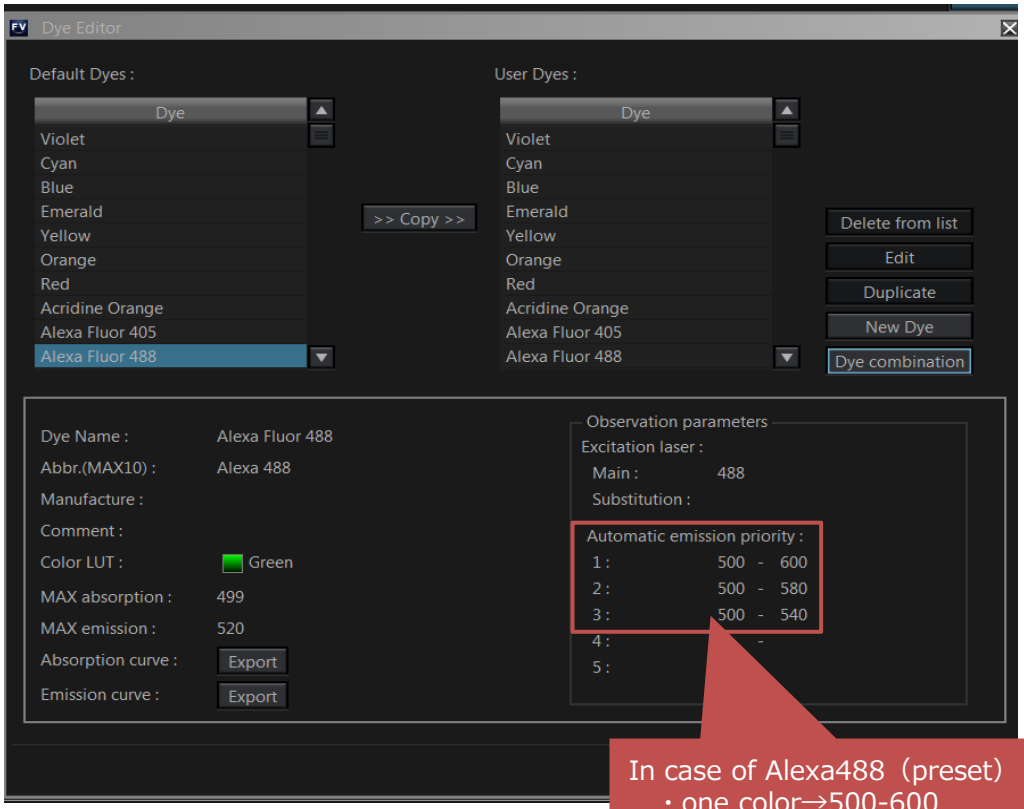


Absorption curve / Emission curve is Imported.
It can be read from a CSV file.
※Curve Import is not required.

④ Press the OK button to add “User Dye”

⑤ Press the OK button to close the dialog box.

New registration of Dye



In case of Alexa488 (preset)
- one color→500-600
- 488/640→500-580
- 488/568→500-540

Emission Priority

■set automatically

V (405) : 430-470
C (440) : 460-500
B (488) : 500-600
500-580
500-540
E (514) : 530-630
: 530-580
Y (561) : 570-670
: 570-620
O (594) : 610-710
R (640) : 650-750

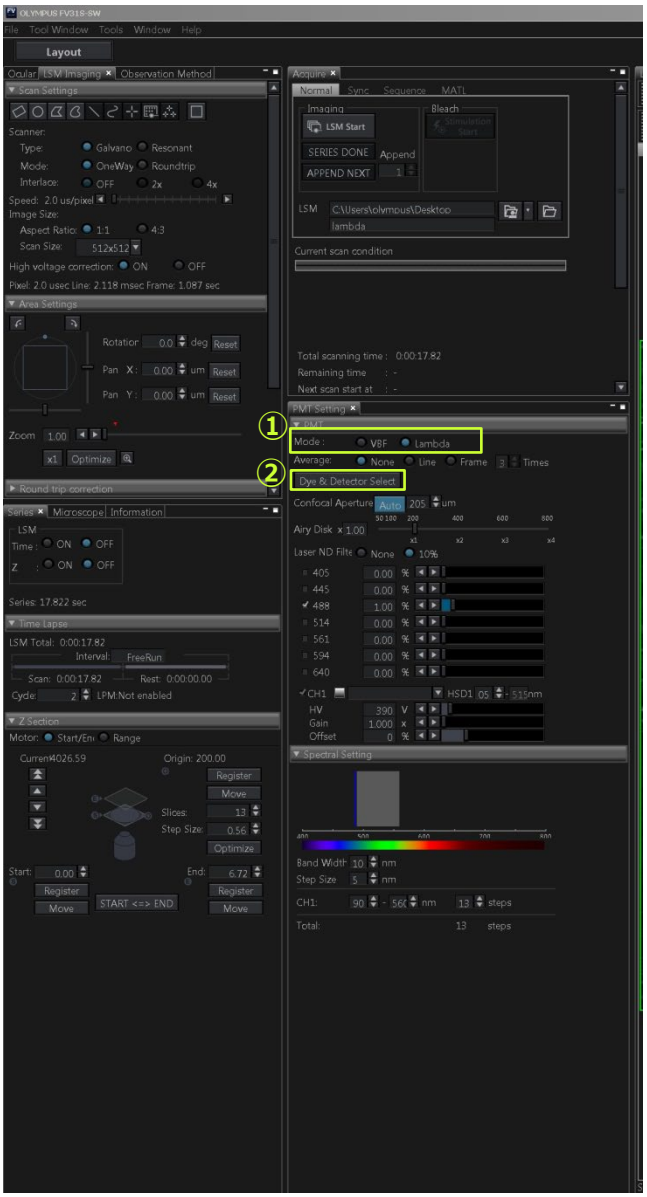
■selecting Ex+ automatically selects the main laser wavelength.

■If you enter something other than the above,
Select "Custome" and enter the key.
→ Please set Wide Band is upper, Narrow Band is lower.
(One kind is also acceptable)

Spectral Imaging

EVIDENT

Lambda series(1)
(Ch setting)



＊Before starting the following procedure, make adjustments for imaging.

Changing to Lambda mode

① Select “Lambda” in [Mode] in [Detector setting] Tool Window

Select Channel

② Press the Dye & Detector Select button. The [Dye & Detector Select] dialog box appears.

③ Press the All Clear button to remove the previous setting.

④ Double click and apply the detectors. Then, click [OK].

▼Lambda scan with single channel

: Acquire lambda series with single laser.

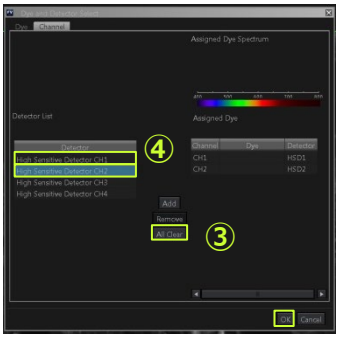
Select BSD1

e.g.) Try to separate GFP and Auto FL→ excited by LD 488

▼Lambda scan with multi channels

: Acquire lambda series with multiple lasers.

e.g.) Try to separate GFP, RFP and Auto FL→ excited by LD 488 and 561



Lambda series(2) (LightPath setting)

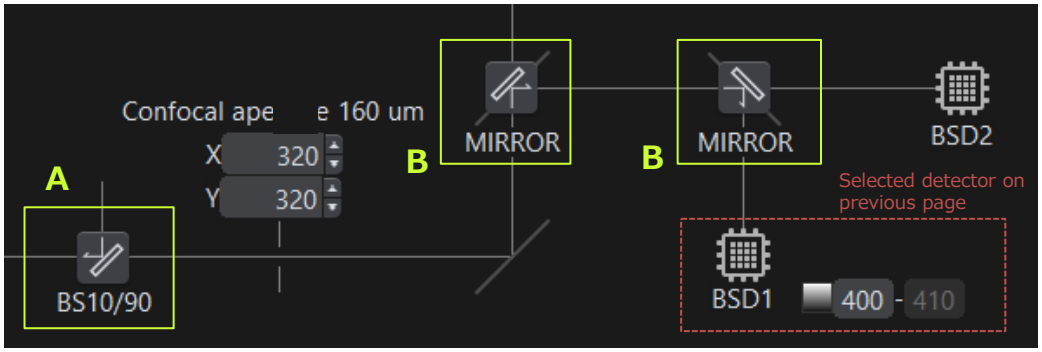
⑤ Select [LightPath] in Tool Window.
⑥ Select “LSMScanner” tab at bottom of LightPath tool window.
⑦ Select DMs to guide fluorescent light to detectors.
DMs are selectable by clicking mirror icon.

A. Selecting Excitation DM
・To acquire accurate spectrum
→Select BS10/90

・To acquire bright spectrum
→Select DM with same wavelength of excited lasers.

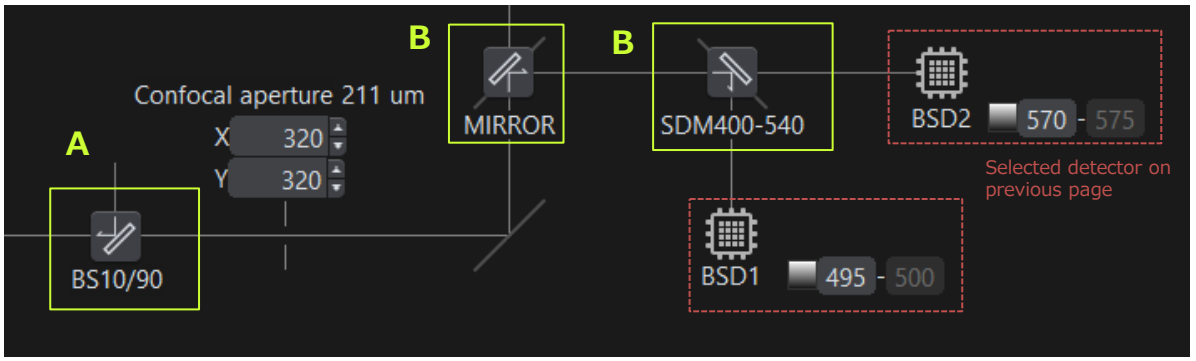
B. Selecting SDMs
MIRROR→Reflected totally.
GLASS →Transmit
SDM →Reflected particular wavelength.
例）SDM400-540 :
Reflected :400nm-540nm
Transmitted :540nm-

■Example of single channel.



Set mirrors to all fluorescence are guided to HSD1.

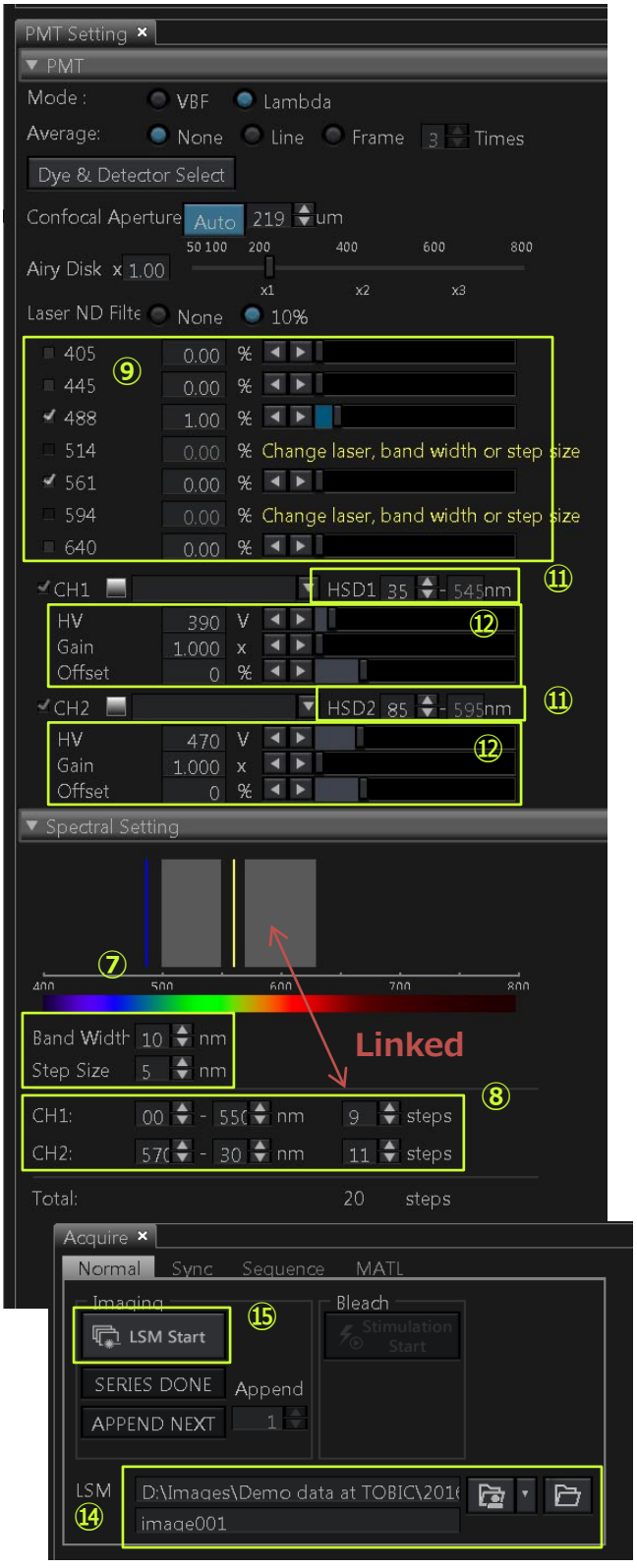
■Example of 2 channels.



FL are separated by SDM400-540

Shorter than 540nm to BSD1
Longer than 540nm to BSD2

Lambda series(3)
(Scan setting)



Setting the wavelength

⑦ Set “Band Width” and “Step size” in [Spectral setting].

Bandwidth
Recommend : 15nm or so.
When the image is dark, enlarge bandwidth.

Step Size
Recommend :5nm or so.
Small step size leads to accurate spectrum.

⑧ Set range of lambda series.

＊You cannot select the laser which includes ±5nm of the excitation wavelength in the range of each channel.

Adjusting the live image

⑨ Check the laser to use. Adjust the laser power not to 0%.

⑩ Click “Live” to show live image.

⑪ Detection wavelength can be changed for each channels.

⑫ Adjust the laser power.

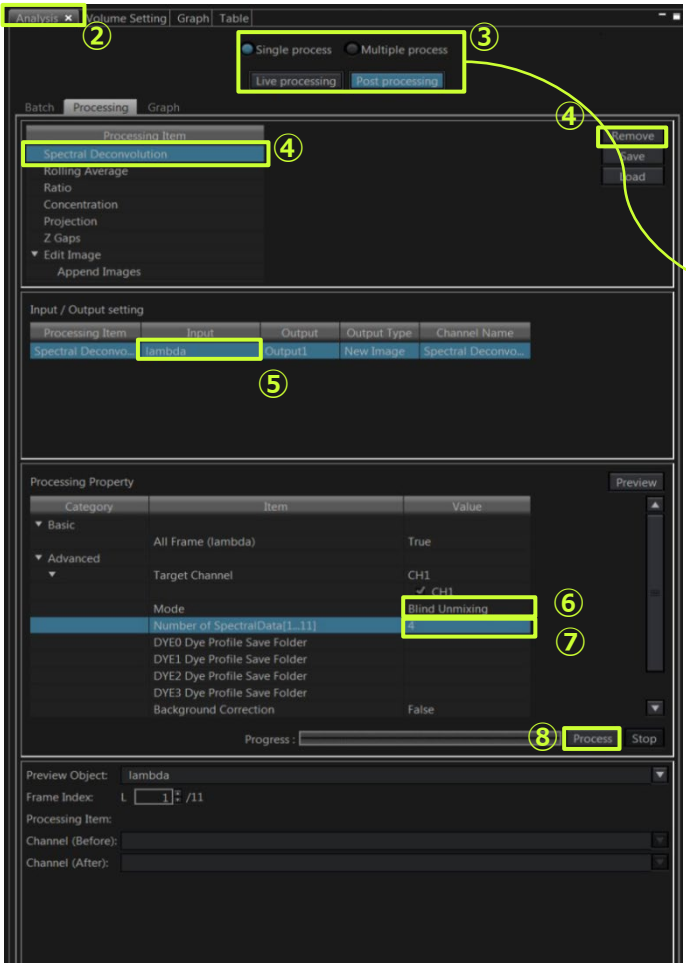
Start acquisition

⑬ Set Z and/or T series when needed.

⑭ Determine the file location and file name.

⑮ Click “LSM Start” .

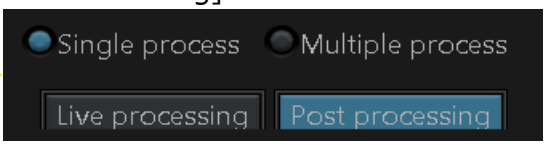
Processing: Unmixing(1)
Blind Unmixing
~by setting the number of dyes~



① Press the Viewer button to switch to “Viewer mode”.

② Select [Analysis] in [Tool Window] menu. [Analysis] Tool Window appears.

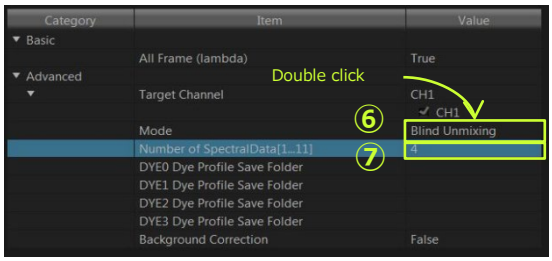
③ Select [Single process] mode, press the [Post Processing] button.



④ Press the Remove button to reset the assigned item and select [Spectral Deconvolution] in [Processing Item].

⑤ In [Input] in [Input / Output setting], select the image for image processing.

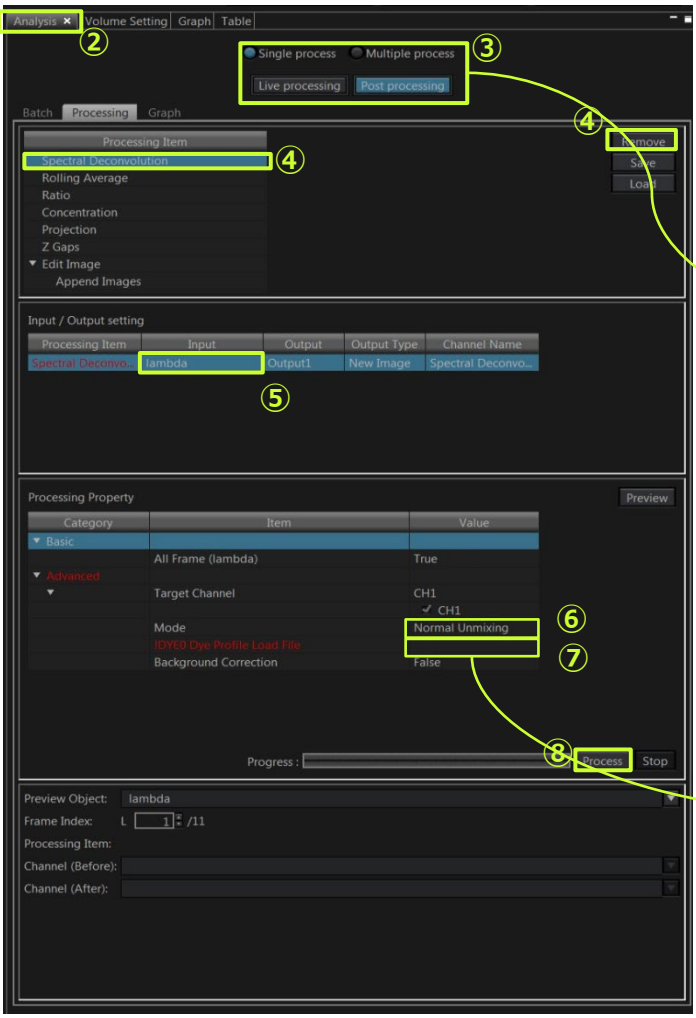
⑥ In [Mode] in [processing Property], select “Blind Unmixing.



⑦ Set the number of dyes in [Number of Spectral Date].

⑧ Press the Process button to start the fluorescent separation process.

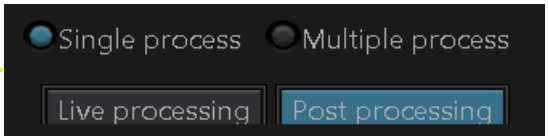
Processing :Unmixing(2)
Normal Unmixing
~by specifying dye dates~



① Press the Viewer button to switch to “Viewer mode”.

② Select [Analysis] in [Tool Window] menu. [Analysis] Tool Window appears.

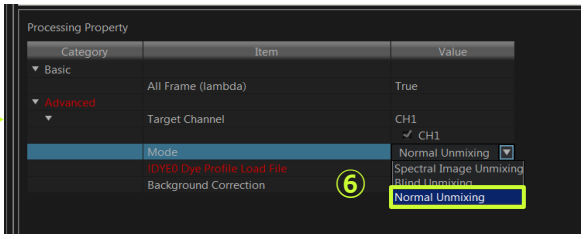
③ Select [Single process] mode, press the [Post Processing] button.



④ Press the Remove button to reset the assigned item and select [Spectral Deconvolution] in [Processing Item].

⑤ In [Input] in [Input / Output setting], select the image for image processing.

⑥ In [Mode] in [processing Property], select “Nomal Unmixing”

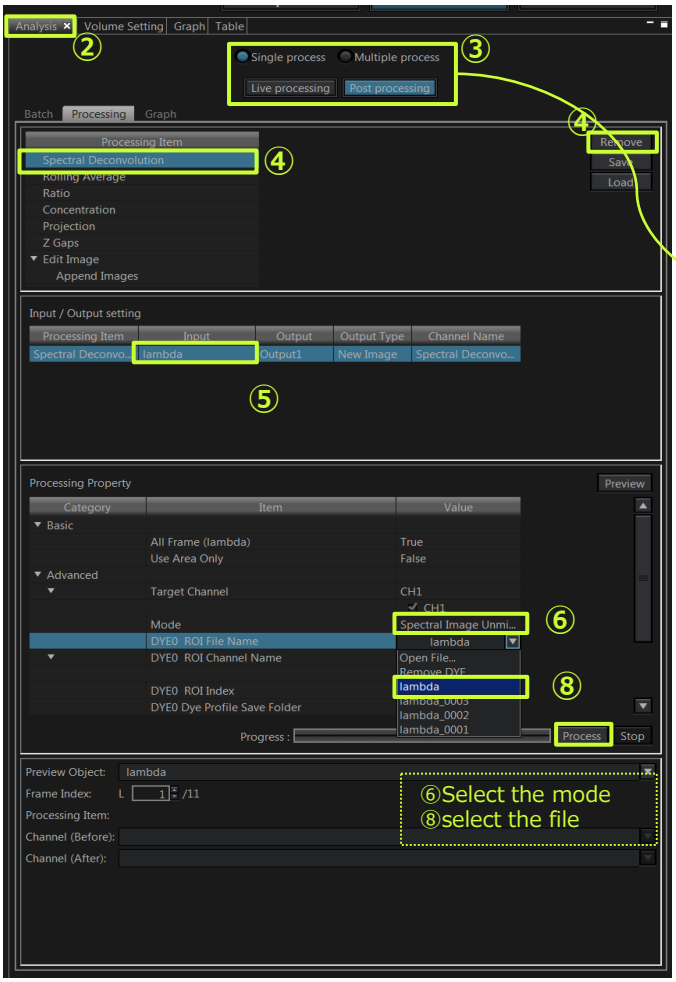


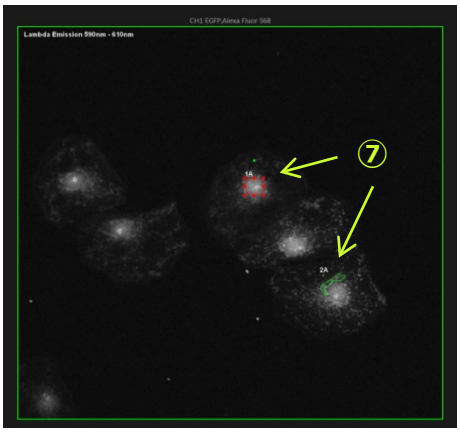
⑦ In “!DYE0 Dye Profile Load File”, select the first dye profile among from dye data.

⑧ Select the all date.

⑨ Press the Process button to start the fluorescent separation process.

Processing : Unmixing③
Spectral Image Unmixing
~by specifying dyes~

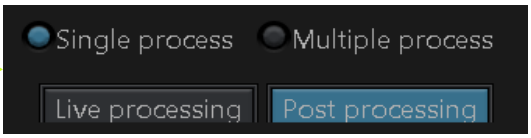




① Press the **Viewer** button to switch to “Viewer mode”.

② Select [Analysis] in [Tool Window] menu. [Analysis] Tool Window appears.

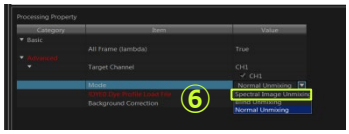
③ Select [Single process] mode, press the [Post Processing] button.



④ Press the **Remove** button to reset the assigned item and select [Spectral Deconvolution] in [Processing Item].

⑤ In [Input] in [Input / Output setting], select the image for image processing.

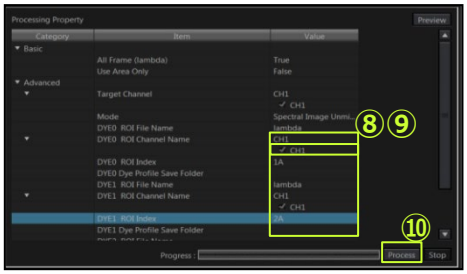
⑥ In [Mode] in [processing Property], select “Spectral Image Unmixing.



⑦ Specify multiple ROIs on the regions where only the target fluorescence dye locates to acquire the spectral data for image processing.

⑧ In [DYE0 ROI File Name], select the file name of the image on which the ROI was specified in ⑦.

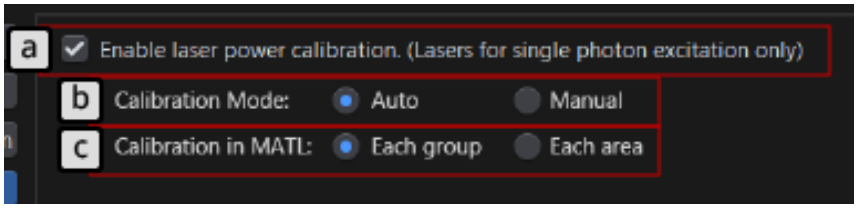
⑨ In [DYE0 ROI Index], select the first ROI specified in ⑦.



⑩ Repeat ⑧⑨ to register all ROIs. Press the **Process** button to start the fluorescent separation process.

Appendix

Laser power correction（LPM）



Setting of Laser power correction

① Select [Tools] > [Configuration] > [Preference] tab >[LPM] .

② a. Laser power correction can be used when checked.
b.
-Auto: Laser power correction works during scanning. There will be delay before scan starts.
-Manual : Laser power correction will not work automatically. Delay doesn't exist before scan. Manual correction available by [One-time laser power calibration] in [Detector Setting].
c. Set the correction timing on MATL.
-Each group: Correct before each groups in MATL.
-Each area: Correct before each area in MATL.

Laser Power Monitor window

① Open by [Tool Window] > [Laser Power Monitor]

※Available only administrator.

②

a. Check the all lasers available in LPM.

b. Power check: Log is shown by clicking a.
Power correction: Log is shown when correction worked.

c. Ratio or Absolute value

d. Select the laser wavelength.

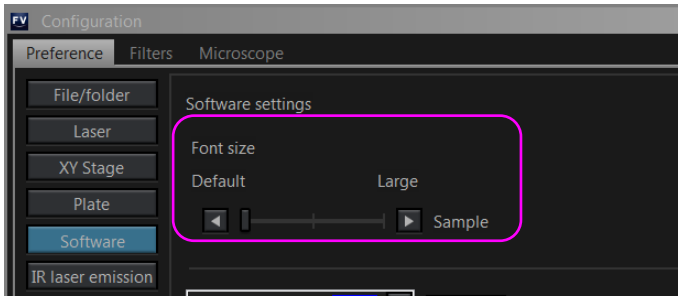
e. Graph

f. Table of laser power values.

g, Delete value of h and f.

i. CSV output based on selection of b and c.

Font size setting

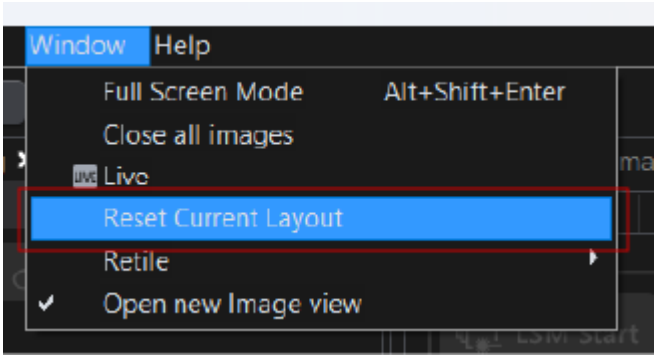


1. Open the [Configuration] > [Preference]
2. Select the ”Font size” in [Software] .
3. Font size can be adjusted in3 types.
4. Restarting the software.

Font size below cannot be changed

- Launch display
- Title of the software/tool window
- Dialog of Windows
- About FV31S-SW dialog
- cellSens
- On line help

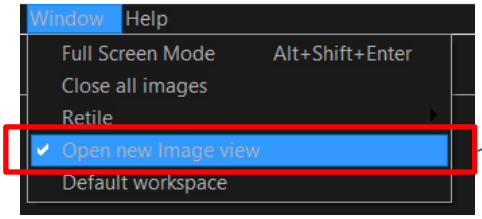
Reset the layout



1. Click [Window] > [Reset Current Layout]

Trouble shooting

【1】
Q : the acquired image doesn’t appeared in the [Live] tab.
A : confirm if it is checked in [Window]>[Open new Image view].



When hover the mouse over [Open new image view], the check is appeared.

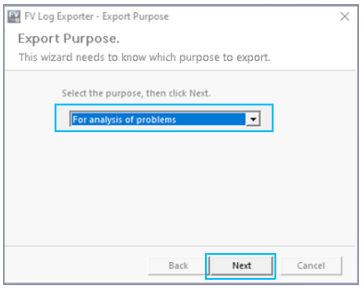
【2】
Q : the tab has been minimized or maximized.
A : Double-click the item part of the tab to return to the original.

How to create Log file

※When trouble occurred, create log file and make a note when it happened.
※When software is frozen, create log file with remaining software.
※Log file can be created only when logging in with Administrator.

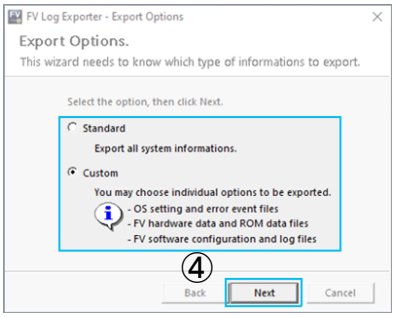
1. Click Start on windows and launch
   OLYMPUS cellSens FV > FVLogExporter

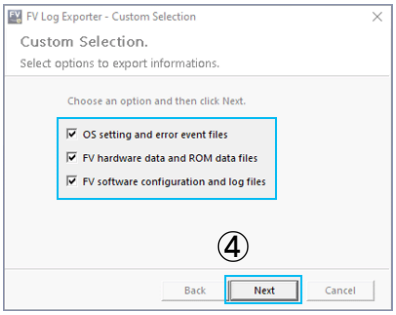
2. Click “Next”.
3. Select [For Analysis of problems] , click “Next”



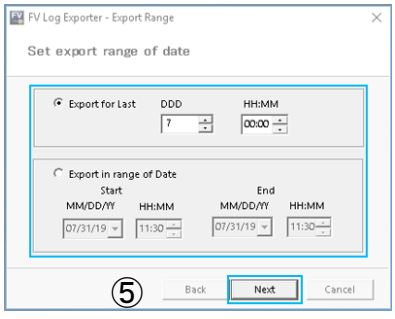
③

4. Select [Custom] , click “Next”
   Check all then click “Next”





5. Set date and time to include when the trouble occurred then click “Next”
   ※[Export for last] Collection period by days from the present.
   ※[Export in range of Date] Collection period by the start and end date.

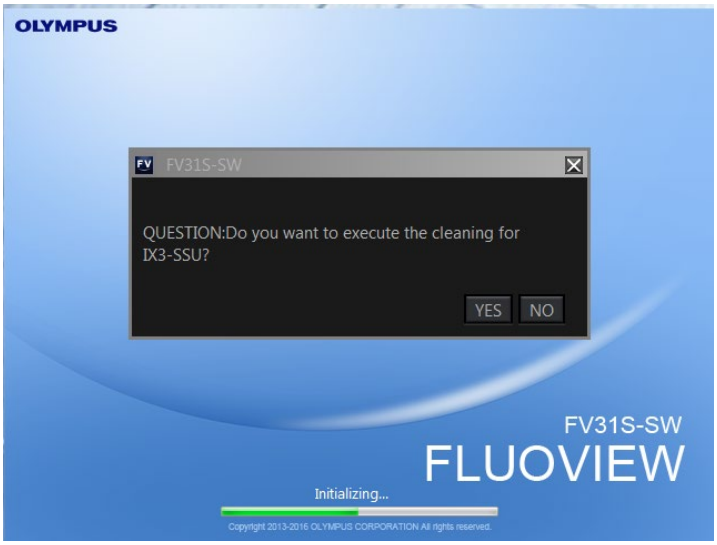


6. Make sure the file output is Desktop, then click “Next” .
7. Follow the dialogue when it appears, then click “Next”.
8. Wait until “Finish” appears, then click “Finish”.
   ZIP file will be created on desktop.

■Maintenance IX3-SSU

When using this stage for a long time period, the stage movement may be deteriorated due to the wear of the ultrasonic drive unit.

Clean the units as follows on regular basis.



① The auto cleaning

⇒Approx, once a week

If the IX3-SSU is selected as a XY stage, the message to select whether or not to perform the auto-cleaning appears.
Click the [OK] button.

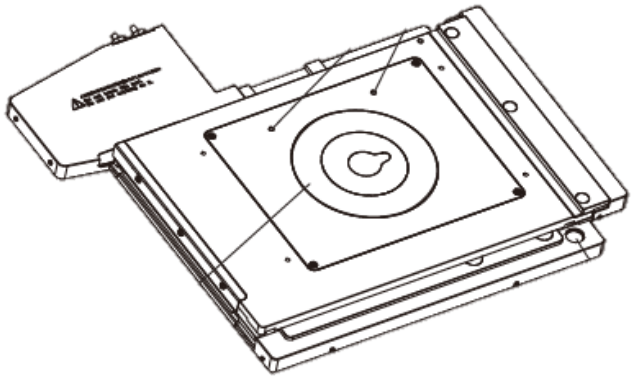
② The manual cleaning

⇒Approx, once a year(depending on frequency of use)

《Cleaning Method》

1) Set the main switch of the controller for ultrasonic stage to OFF.
2) Then, move the stage manually in front/back/right/left directions for 10 reciprocations each within the full stage movable range one second for one reciprocation.

※Pleas don't move the stage manually except for cleaning.

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