FV4000-IX83 Operation Manual





Ver.2

(cellSens-FV ver3.1.1)

Table of contents

Image Acquisition	
Starting the system	6
Controller / Objective lens	7
Observation via eyepieces	8
XY Image Acquisition	9
Virtual channel scan	11
XYZ Image Acquisition	12
Bright Z	14
XYT Image Acquisition	15
Exiting the system	16
2D view and operation	17
3D view and operation	18
Creating the movie	20
Processing: Projection	21
Series Analysis	23
1D profile	24
Exporting the image	25
Reloading and saving Observation Methods	26
Configuration	27

ZDC-----

ZDC system	29
Configuration :ZDC	30
XYT Image Acquisition	31

Table of contents

-Motorized Stage*-

Option : Motorized Stage	33
Multi Area Time Lapse Imaging Acquisition	34
Multi Area Time Lapse using Map Image(1)	35
Multi Area Time Lapse using Map Image (2)	36
Multi Area Time Lapse (with the Z drift compensation)	37
Overlap section at tiling imaging	38
Acquiring the stitched image using Map Image	39
Stitching	41
Adjusting that tilt in Z direction ~Focus Map~	42
Setting of the well plate	43
Calibration of the well plate	44
Map Image display area for well plate	45
Registration of Image Acquisition area	47

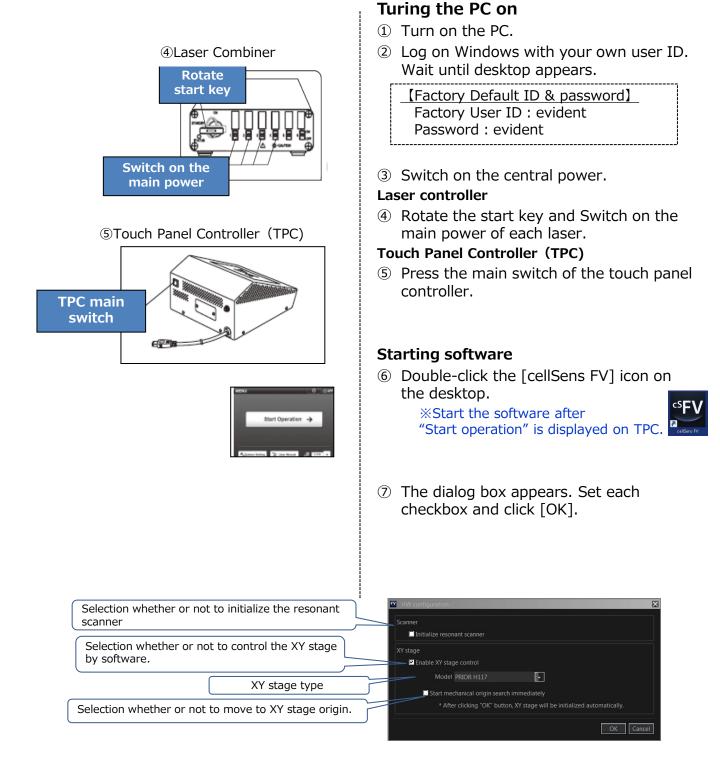
-Advanced-

Stimulation	49
Super resolution : FV-OSR	51
Append Image	52
Extraction	53
Merge Channel	54
Colocalization	55
3D Deconvolution	56
TruAI Noise Reduction	57
BATCH \sim batch processing for multiple images \sim	58
[for large volume of data] Stitching after projection	59
Sequence Manager	60
New registration of Dye	61
—Spectral Imaging—	
Lambda scan	64
Unmixing (1) Blind Unmixing	67
Ummixing (2) Normal Unmixing	68
Unmixing (3) Spectral Image Unmixing	69
—Appendix—	
Laser power correction (LPM)	71
Font size / Reset the layout / Trouble shooting	72
How to create Log file	73
Maintenance IX3-SSU	74

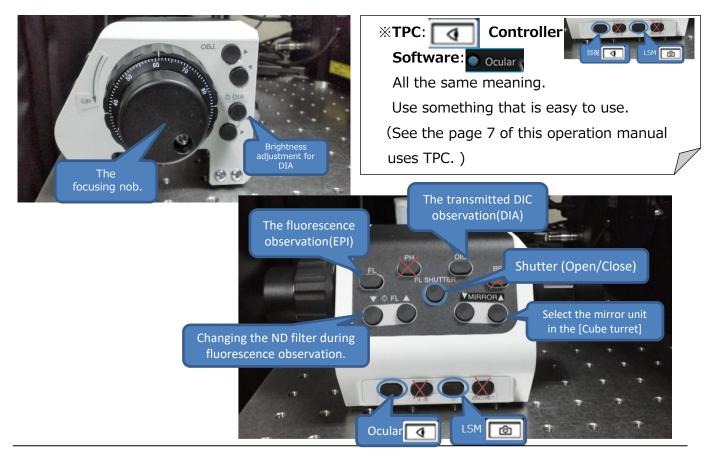
Image Acquisition

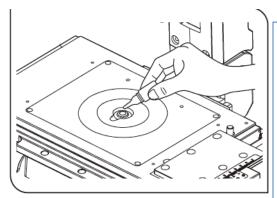


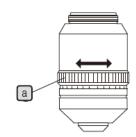
<u>Starting the system</u>



<u>Controller/Objective lens</u>







How to use the immersion objective lens

- ① Set the immersion objective lens in the optical path.
- 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.

XClean the objective lens and specimen after use.

[a]Adjustment of Objective Correction collar

- 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.
- Click "DIA".
- ③ Adjust the brightness via slider in Trans Lamp.

Ocular × ⊠ Obs Ocular	ervation Method 록	LSM Imaging 🖻 Acquire 👘 💻
EPI DIA 2		
🔻 EPI Light Path	ı	
Cube turret #1:	8:IX3-FDICT	
EPI Shutter:	🔵 Open 💿 Clo	se
EPI ND Filter:	1:0.0	
▼ DIA		
TD Shutter:		
Trans Lamp: 3	• On • Off	
Polarizer:	240 • • -	

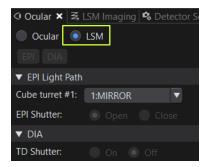
★ Fluorescence

- * 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".

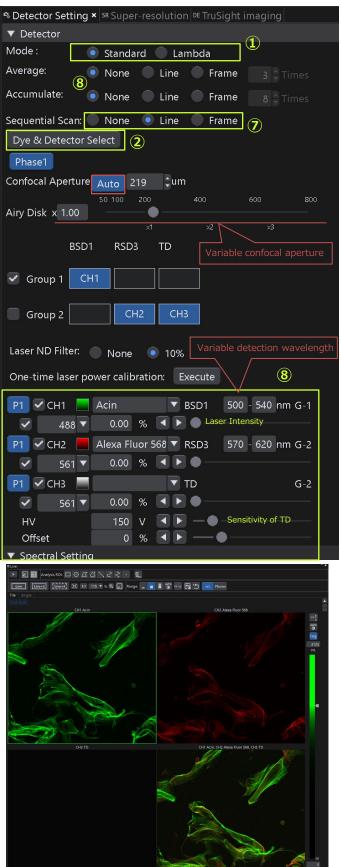
📼 Imagant 🔍 O	Cular 🗙 🖾 LSM Imaging	
💿 Ocular 🔵 L	SM	
EPI 😥		
▼ EPI Light Path		
Cube turret #1:	3:U-FBNA 3	
EPI Shutter:	🖲 Open 🔵 Close 👍	
EPI Lamp:	● On ● Off	
5	30 🖌 🕨 🔴 🔤	
▼ DIA		
TD Shutter:		
Trans Lamp: (On 💿 Off	
Polarizer:	🔵 In 💿 Out	

*Display may differ depending on the configuration.

★After observation via eyes, click "LSM"



XY Image Acquisition(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 Adjust focus and Laser Intensity. (HV and Offset on TD.)
- 9 Use "Average" or "Accumulate" to get better S/N.
- ① Adjust LUT via Range: _ = = or vertical bar on the right.

XY Image Acquisition (2)

Ocular Obse Obse	ervation Method 🖻 LSM Imaging × 🖻 Acquire
🔻 Scan Setting:	
ØOZ	0 N 2 + 🕮 🎝 🗖
Scanner:	
Туре:	💿 Galvano 🔵 Resonant
Mode:	💿 OneWay 🔵 Roundtrip
Interlace:	• OFF • 2x • 4x
Speed: 1.0 us/p	ixel 🔳 🛡 🕨
Image Size:	(3)
Aspect Ratio:	• 1:1 • 4:3
Scan Size:	1024x1024 🔻
Pixel: 1.0 usec	Line: 2.113 msec Frame: 4.330 sec
▼ Area Settings	s
6	·↓ ①
	Rotation : 0.0 🖨 deg Reset
	Pan X: 0.00 🗘 um Reset
	Pan Y: 0.00 🕻 um Reset
• _	x0.9 x1 🔍 Optimize
Zoom : 1.00	

Ocular	r ◙ Observation Method 록 LSM Imaging	🖬 Acquire ×
Norma		
_ Imag	ging15	Bleach
	LSM Start T Z C L	
	NES DONE Append 1	
LSM	D:\Data	
	1K-XY	
🕨 Exp	eriment Info.	—
Current	scan condition	
	canning time : 0:00:04.33	
	ning time : -	
Next so	can start at 🛛 : -	

Setting the scanner in [LSM Imaging]

③ Select the type of scanner and mode.

- 1 Set "Scan Size" and "Aspect Ratio".
- ② Set "Zoom" and "Rotation".

Clicking "Optimize", Zoom changes to make pixel size to $\frac{1}{2}$ of optical resolution.

 $\ensuremath{\textcircled{B}}$ 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

④Select [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 " $\times \times \times _0001$ " and " $\times \times \times _0002$ ".

Final check

- $\checkmark\,$ DO NOT use "%" to the file name.
- ✓ Setting such as Timelapse, Z stack, etc. are recognized via icons.



(15) Press the USM Start button to start acquiring the image.

Virtual Channel Scan

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



Sequential Scan: None Line Frame Dye & Detector Select 2 Phase1 Phase2 8 Confocal Aperture Auto 112 um				
50 100 200 400 600				
Airy Disk x 1.00				
BSD1 BSD2				
Group 1 CH1				
Group 2 CH2				
Laser ND Filter: 💿 None 💿 70%				
One-time laser power calibration: Execute				
P1 ✓ CH1 DAPI ▼ BSD1 430 - 470 nm				
✓ 405 ▼ 0.0 % < ► ●				
P1				
P2 CH3 ECFP BSD1 460 - 500 nm				
P2 CH4 EYFP BSD2 530 - 580 nm				
✓ 514 ▼ 0.0 % ◀ ▶				

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

Adjusting the live image

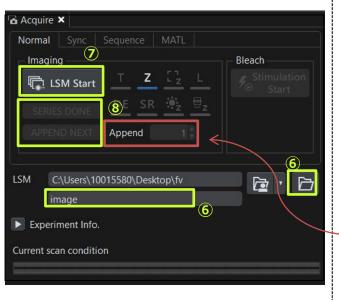
- 8 Press Phase1 Phase2 Or P1 P2 to switch phase.
- Adjust the acquisition setting. Refer the previous pages.
- ③ Set "XYZ" and "XYT" setting. Refer the following pages.

Setting Acquisition

- 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 " $\times \times \times _0001$ " and " $\times \times \times _0002$ ".
- IS Press the LSM Start button to start acquiring the image

XYZ Image Acquisition(Start/End)

Series × LSM Time : ON OFF Z : ON OFF)	- •
Series: 1 min 10 sec Res	set: Reset to initi	ial values.
► Time Lapse		
▼ Z Section	_ 2	\mathbf{V}
Motor: 💿 Start/End 🔵 Range	e	Reset
Near limit:1950.00	Origin: 0.0	4050.00
Current:9.80	Register	Move
★ ▼ ↓ Start End	Start: 8.85 Register End: 0.00 Register START <=>	Move 4050.00 Move



* 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 P to display the dialog box, then select the folder to save the image.
- ⑦ Press the R LSM Start button to start acquiring the image .

Finishing Acquisition

⑧ Finishing acquisition,



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)

© Series × LSM Time : ON OFF Z : ON OFF	1			
Series: 2 min 16 sec	Rese	et: Res	set to initia	al values.
► Time Lapse				
▼ Z Section		2		1
Motor: 🔵 Start/End 💿	Range			Reset
Near limit:1946.40		Origin:	0.00	4053.60
Current:0.00		3	Register	Move
	ind	Start:	-7.50	4046.10 Move
	tart >	Range:	<mark>15.00</mark> 🗘 I	m
		End:		4061.10
				Move
			START <=> ENI	D
	s	lices:	31 🛱	5
	💼 S	tep size:	0.50 🖨	Optimize

Acquire ×		
Normal Sync	Sequence MATL	
Imaging		Bleach
🕞 LSM Start	T Z Z L	F _☉ Stimulation Start
SERIES DONE	<u>8</u> E <u>SR</u> <u>₩</u> z <u></u>	
APPEND NEXT	Append 1	
		<u> </u>
LSM C:\Users\10	015580\Desktop\fv	
image	6	
Experiment Info.		
Current scan conditio	on	

* 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 D 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 buttons blink .

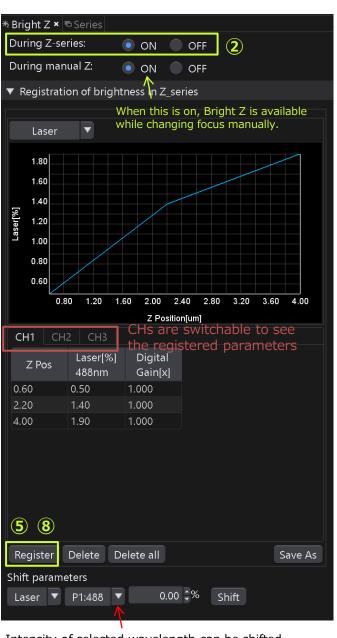
* Press the SERIES DONE to finalize the image acquisition. If you want additional images from -end position, enter the number of additional acquisition and press the APPEND NEXT

After acquiring, press the series done

<u>Bright Z</u>

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] .
- 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.
- $\ensuremath{\textcircled{}}$ Acquire as same way of XYZ.

XYT Image Acquisition

	ar 🖻 Observation Method 🌫 LSM Imaging 🖬 Acquire 🗙	
Norm		
_ Ima	agingBleach	
F	LSM Start \underline{T} \underline{Z} $\underline{C}_{\underline{Z}}$ \underline{L} $\mathcal{T}_{\underline{S}}$ Stimulation DE SR $\underline{\oplus}_{\underline{S}}$ $\underline{\oplus}_{\underline{S}}$	
SE		
API	PEND NEXT Append 1	
LSM	D:\Data 🔂 ' 🔂	
	1K-XYT	
🕨 Exp	periment Info.	
Curren	nt scan condition	
Total s	scanning time : 0:19:34.33	
	ining time : -	
Next s	scan start at 🛛 : -	T
Series	; x	
LSM - Time : Z :	ON OFF	
Series: 4	4.330 sec	
▼ Time	e Lapse	
LSM Tot	tal: 0:19:34.33	
	Interva <mark>:</mark> 0:00:30.0	
ے۔ Sca	an: 0:00:04.33 2 Rest: 0:00:25.67	
Cycle:	40 🗘	

* 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

 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, 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) **3Touch** the "OFF" **④TPC main** switch C B - an 7)Central power **5**Laser unit **Rotate the Key** Turn to OFF

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

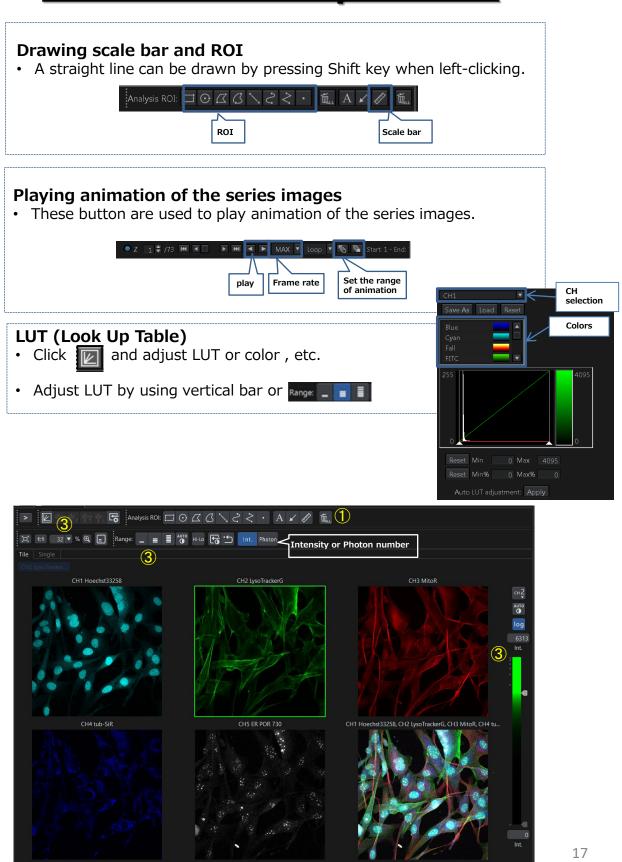
5 Turn the laser combiner to OFF.
 ※Rotate start key of the power.
 Supply and set the switch to OFF.

Central power

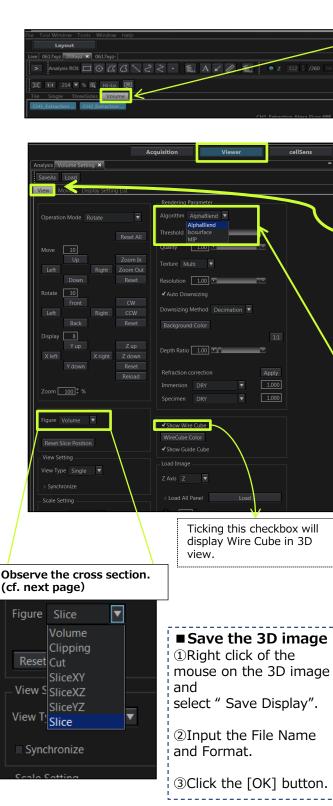
6 Turn off the central power.

<u>* When using immersion oil , clean the objective lens.</u>

2 D view and operation



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 <u>Viewer</u> 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.

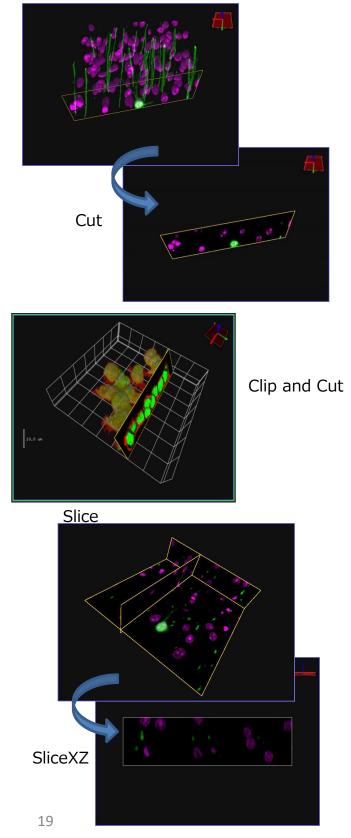
3) AlphaBlend

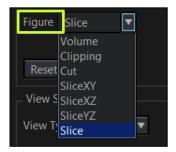
The a blend method reflects the intensity on the top surface of the object preferentially to the image.

Therefore , the context of the object is displayed properly.

3D view and operation (2)

Clipping





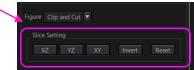
■ Clipping/Cut

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

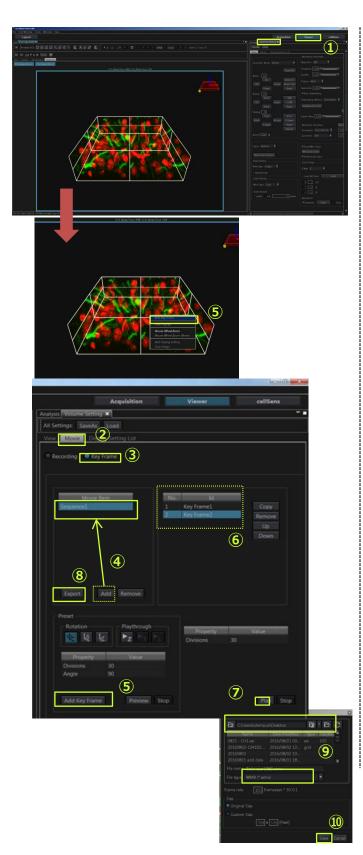
Create the cross-sectional view sliced in XY/XZ/YZ directions in the image constructed in 3D image, and displays the image sliced in each direction.

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
- Press the <u>Viewer</u> 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.

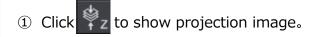
- 6 Repeat 5 and register the statuses you want to display as Key Frames.
- ⑦ When you press the Play button, the image between Key Frame is interpolated automatically to play back the movie.

Exporting movie

- 8 Select "Sequence xx" in [Movie Item] and select the movie you want to export. Press the Export button.
- Press the button to select the folder of the save destination.
 Set the [File name] and [Frame rate] by entering them directly.
- IPress the same button.

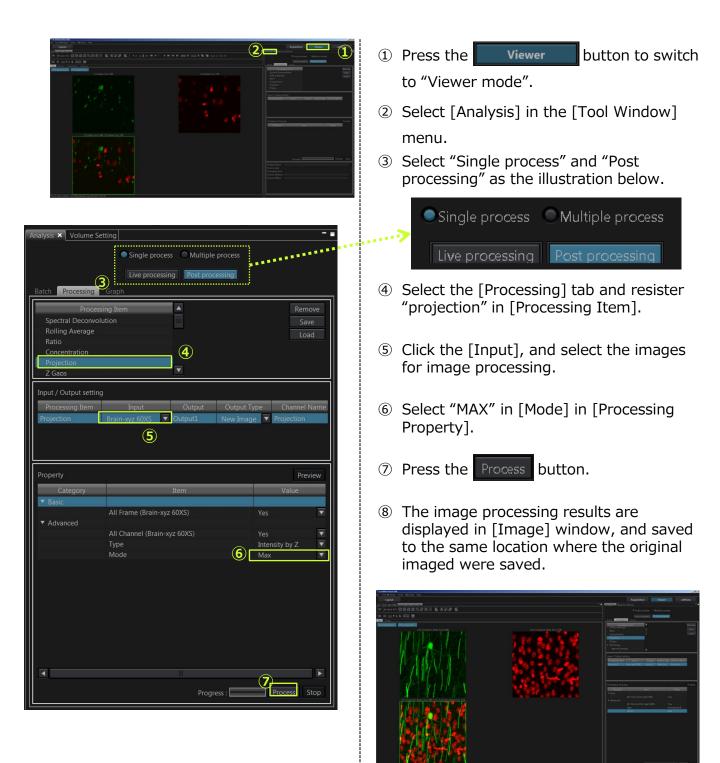
Projection(1)





- Projection Image OR
 right-click on the image > Save As >
 Projection Image.
- ③ Save the image (.OIR is the extension only available)

Projection(2)



Drawing a Intensity Graph : Series Analysis (Specifying ROI on the series image)

File Tool Window Tools Window Help		
Layout 2	Acquisition	Viewer cellSens
*Live Fucci3day-small ×	Analysis × Graph Table	
	3 Single process	Multiple process
● T 1 🕏 /88 🗰 🔍 🕨 💓 🛋 🕨 MAX 💌 Loop 💌 🎭 🖄 Start: 1 - End: 88		Post processing.
II HI 149 ▼ % @ HELO P	Batch Processing Graph	Add
Tile Single ThreeSides Volume	Series Analysis	Remove
CHI_Extraction CH2_Extraction PhaseContrast_E_ CHI_Extraction_CH2_Extraction	1D Profile Multi 1D Profile	
	Graph List	
	No. Name In	
	Series Analysis1 Fucci3day	esmall
	Property	Preview
	Category Item	Value
	x	TIMELAPSE
JA	Y Apply All Frame Range	Average 💌 Yes 💌
	Apply All Channel Apply All ROI	Yes 👻
	Advanced	Yes
	 X Axis Range Apply Auto 	Yes
이 이 이 것 같은 것 같은 것 같은 것 같은 것 같은 것 같이 있는 것 같이 있는 것 같이 없는 것 같이 않는 것 않는 것 같이 않는 것 않는 것 같이 않는 것 같이 않는 것 같이 않는 것 않는 것 같이 않는 것 않는 않는 것 않는 않는 것 않는	▼ X Axis Style	and the second
	Label Is Visible	Yes 🔽 TIMELAPSE
	Line Is Visible	Yes
	Line Style Line Color	SOLID RGB (255, 255, 255)
	▼ X Axis Scale	
		Apply Stop
Size512x512 0.415(um/pixel) xy=(58,257) Int:132,178		

- 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)

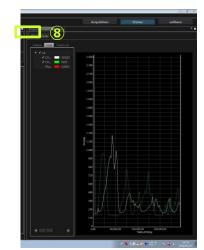
⑦ Press the Apply button allows you to draw all graphs registered in [Graph List].

▼[Graph] tab

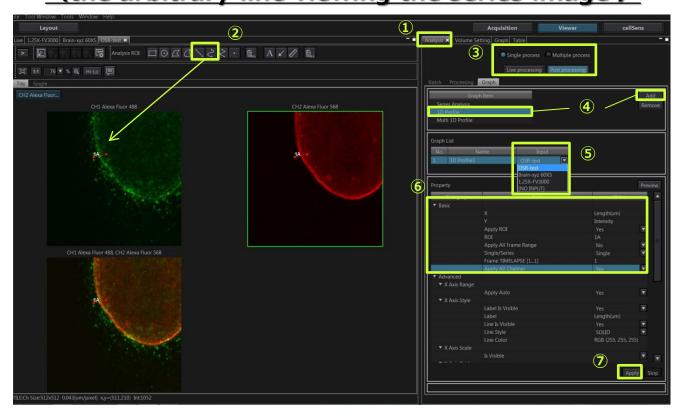
- You can change the color in [Line] tab.
- Press the save Display button to save the graph.

▼[Table] tab

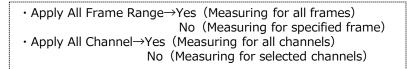
• Press the SaveAs button to save the results as CSV file.



Drawing average intensity profile : <u>1D profile</u> (the arbitrary line viewing the series image)



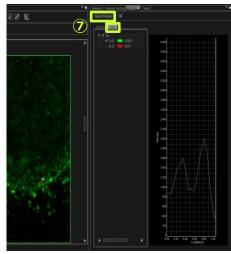
- ① Select [Analysis] in the Tool window menu.
- ② Select the ROI tool in [Analysis ROI], and specify the area you want to measure on the item.
- ③ Select "Single process" and "1D profile".
- ④ Select the [Graph] tab, and Select [Series Analysis], and press the button.
- ⑤ Click the [Input], and select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



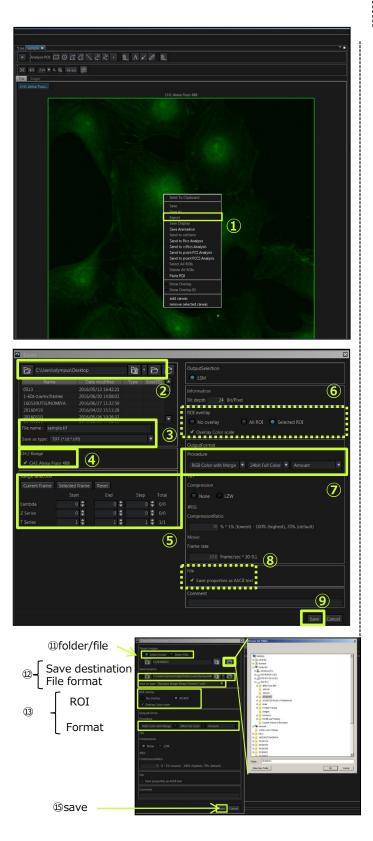
⑦ Press the Apply button, [Graph] tab and [Table] tab appears.

▼[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

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

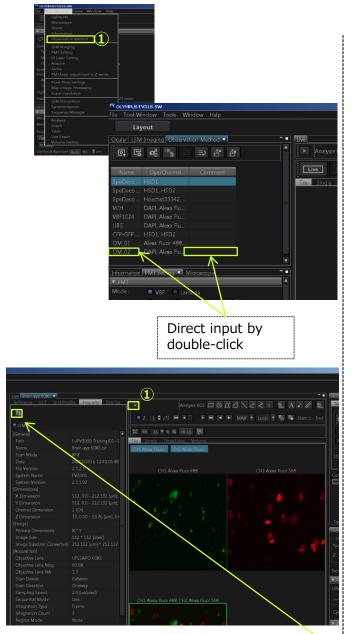


- ⑧ Ticking this checkbox will output the properties in the text.
- 9 Press the Save button. The image will be exported.

B. Exporting Multiple images

- In the [File] menu on the software screen, select [export multiple files].
 The [Export multiple files] dialog box appears.
- ① Select the method to export images.
- Press the button to select the folder or the file to be exported.
- ③ Select the save destination and the file format to be exported in [Save as Type].
- (4) Same as 4~8 of A.
- Is Press the Save button. The image will be exported.

Reloading and saving Observation Method



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.
- 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.

 \ast Select the DIC which is same number as the objective lens.

example) 30X→IX2-DIC30 100X→IX2-DIC100

6 Press the or button.

■ Specifying the micro plate

- Select [Configuration] in [Tools] menu. The [Configuration] dialog box appears.
- ② Select [Preference] tab.
- ③ Select [Plate].
- ④ Select the <u>mic</u>ro 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.



EVIDENT

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

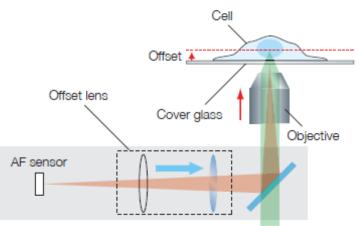
ZDC (Z Drift Compensator)

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

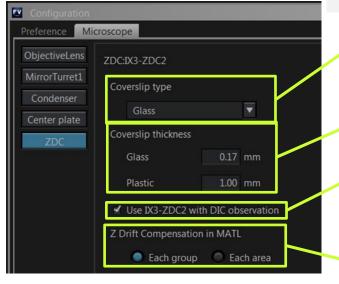


■ Objective lens for ZDC2

Objective lens		Glass bottom dis	h(t=0.17mm)	Plastic dish	(t=1.0mm)
		Continuous	One shot	Continuous	One shot
Dry	UPLXAPO4X	×	×	-	-
	UPLXAPO10X	0	0	-	-
	UPLXAPO20X	0	0	-	-
	UPLXAPO40X	0	0	-	-
	UCPLFLN20X	0	0	0	0
Long working distance	LUCPLFLN20X	0	0	0	0
	LUCPLFLN40X	0	0	×	0
	LUCPLFLN60X	0	0	×	0
silicone	UPSAPO30XS	0	0	-	-
	UPLSAPO40XS	0	0	-	-
	UPLSAPO60XS	0	0	-	-
	UPLSAPO100XS	0	0	-	-
oil	UPLXAPO60XO	0	0	-	-
	UPLXAPO100XO	0	0	-	-
	PLAPON60XOSC	0	0	-	-
water	UPLSAPO60XW	0	0	-	-

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 \rightarrow The DIC prism is placed in the light path Uncheck \rightarrow 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.

▼ Z Limit Setting Current Z position: 3112.55 Register current position as near limit Near limit: 3200.12 ♣ Refresh		
Apply Entered parameter to near limit		
Apply to an Real matching 1:PLAPON1.25X 3200.12		
2:UPLSAPO 10X2 3200.12 3:UPLSAPO 20X 3200.12		
4:UPLSAPO 40X2 3200.12		
 5:UPLSAPO 60XO 6:UPLSAPO 100XO 3200.12 		



XYT Image Acquisition *2 (with the Z drift compensation)

Z Limit Setting Current Z position: 0.00 Register current position as near limit Near limit: 6500.01 Refresh Apply Entered parameter to near limit
▼ Z Drift Compensator Control
ZDC DM: 💽 In 💿 Out 💈
Search Zone Upper Limit 100.00 — um Lower Limit -1000.00 — um
Continuous ZDC
Start continuous ZDC 6
Single shot ZDC ZDC position 3196.93 um Register ZDC position 5
Register ZDC position ZDC during series scan
ON OFF
Focus check
Drift Value 0.76 um Reset
AF Status Off
▼ Time Lapse LSM Total: 0:00:37.17 Interval: 0:00:35.0 Scan: 0:00:02.17 Rest: 0:00:32.83 Cycle: 2 LPM:Enabled
Acquire Normal Sync Sequence MATL Imaging ELSM Start LSM DA\Images\Demo data at TOBIC\2014

[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

 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 $[\lor Z \text{ limit Setting}].$

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".
- 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 <u>Start continuous ZDC</u> 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
- $\rightarrow \! \mathsf{You}$ had better not use Continuous AF
- Press LSM Start in [Normal] tab in [Acquire] Tool Window to start acquisition.

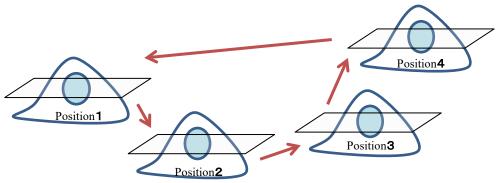
Mortorized Stage

*Option



Option : Motorized stage

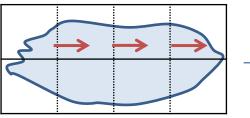
Multi Area Time Lapse

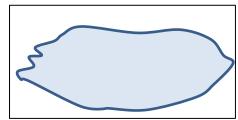


- Register multiple areas and repeatedly acquire images .
- It is also possible to set the interval.
- Time lapse date of multiple area can be acquired at once.

Acquiring stitched image

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





only for motorized

Map image

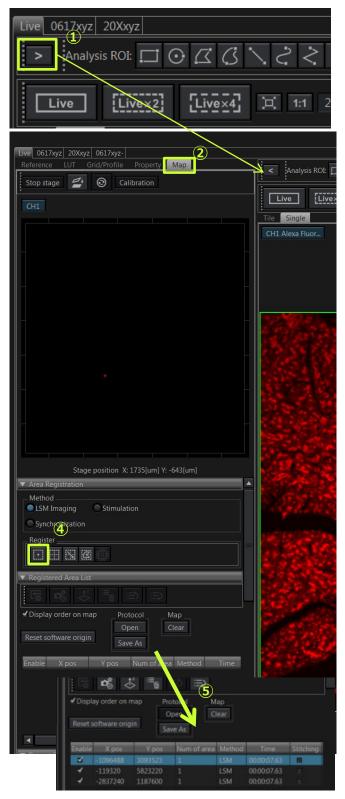
- Display wide field of view image acquire with low magnification objective lens.
- And it can be used as a guide when looking for the field of view.

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





Multi Area Time Lapse Motorized stage **Imaging Acquisition**



* 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.

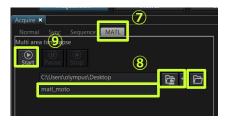
[Attention]

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].

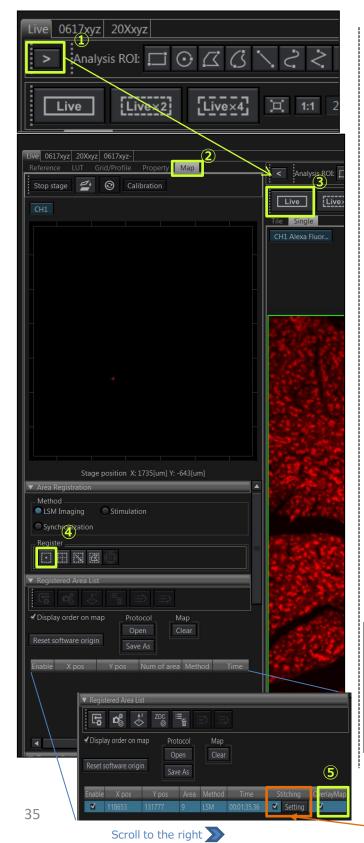


- ⑦ Select [MATL] tab in [Acquire] Tool Window.
- 8 Press the button to display the dialog box, and select the folder to save the images.
- 9 Press Start button to start acquiring the image.



♦Only for motorized stage ◆

<u>Multi Area Time lapse</u> using Map Image (1)



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

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

Create the map

- ③ Bring the image into focus and adjust acquiring parameters using the low magnification objective lens.
- 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

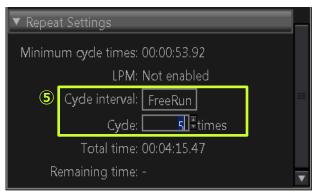
- 6 Select [Acquire] Tool Window in [Tool Window] menu and select [MATL] tab.
- ⑦ Press the button to display the dialog box, and select the folder to save the images.
- 8 Press button to start acquiring the image. Finishing the acquisition, map image is displayed in [Map] tab.





<u>Multi Area Time lapse</u> using Map Image(2)







Registering the multiple area

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

Only for

motorized stage

- 2 Press the **s** 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. ____
- 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.

<u>Multi Area Time Lapse</u> (with the Z drift compensation) *****only for mechanical stage *****

▼ Z Drift Compensator Control	* Before starting the following procedure, make adjustments for XY imaging.
ZDC DM: Out 2	ZDC setting (See page 24 for details.)
Search Zone Upper Limit 100.00 🔷 um Lower Limit -1000.00 🖨 um	 In [▼Z Limit Setting], press the <u>Register current position as near limit</u> button to set the Near limit to all objective lenses.
Continuous ZDC Start continuous ZDC Single shot ZDC	 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]
ZDC position 3196.93 um Register ZDC position 5	in [Search Zone] using the Origin coordinate of Z as a base point.
ZDC during series scan	④ Set [ZDC during series scan] to "ON".
ON OFF	S Adjust focus and Press the Register ZDC position button of [Coverslip position]. The coverslip top surface position is acquired.
Drift Value 0.76 um Reset AF Status Off	*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.
Stage position X: 1735[um] Y: -643[um]	 6 Press the button to register areas. ⑦ Set [Cycle interval] and [Cycle].
Area Registration Method LSM Imaging Stimulation	⑧ Select [MATL] tab in [Acquire] Tool Window.
Synchronization	Press the solution to display the dialog box, and select the folder to save the images.
Repeat Settings Minimum cyde times: 00:00:53.92	IPress button to start acquiring the image.
LPM: Not enabled Cyde interval: FreeRun	*Update the setting of ZDC position Registered Area List
Cyde: Stimes Total time: 00:04:15.47 Remaining time: - Acquire Sequence MATL Multi area timplege Start Long Cylicestolympus/Desktop matt. noto	✓ Display order on map Protocol Map ✓ Display order on map Protocol Clear Reset software origin Save As Clear Enable X pos Y pos Num of area Method Time Stitching OverlayMap ✓ 483594 302443 16 LSM 00:01:53:78 ✓
	Apply to : Selected group All group

Overlap section at tiling imaging

Configuration	
Preference Micro	scope
File/folder XY Stage Plate	Speed of XY stage • High • Medium • Low
Software	
Microscope Link Keyboard	
	Overlap section at tiling imaging.
	Initialize XY stage (search mechanical oligin). Start initialization
	Resolution setting for map Low ::Recommend for 4- or lower may objective lens. (Physical memory consumption will be small.)
	Moddle : Recommend for 10× objective lent High : Recommend for 30× or higher many objective lens. (Physical memory consumption will be large.)
	OK Cancel
7	
0	verlap section at tiling imaging.
	10 %

- 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.

<u>~Setting of automatic</u> <u>stitching process</u>~

Configuration	ascope
File/folder XY Stage	Speed of XY stage High Medium Low
Software	Enable drag and drop XY stage move on live window.
Microscope Link Keyboard	Escape objective lens when the stage position changes in MATL
	Overlap section at tiling imaging.
	Initialize XY stage (search mechanical origin). Start initialization
	Resolution setting for map Low : Recommend for 4× or lower mag. objective lens. (Physical memory consumption will be small.) Middle : Recommend for 10× objective lens. High : Recommend for 30× or higher mag. objective lens. (Physical memory consumption will be large.)
	Correcting Algorithm of Auto Stitch Matching: On Off Smoothing: On Off

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

Acquiring the stitched image using Map Image(1)

Tive XrT Cyde XrT Cyde 01 XrT Cyde 02 XrT Cyde 03 XrT Cyde UT Grid/Profile Property Map T T T	4e.04 XYT_Cycle.05 XYT_Cycle.06 XYT_Cycle.07 XYT_Cycle.08
Stop stage 🖆 🞯 Calibration	ः □ ○ द ८ \ २ २ · ६
CH1 Alexa Ruor CH2 Alexa Fluor	
CH1 Alexa Fluor.	
₹ 	CH1 Alexa Fluor 568 CH2 Alexa Fluor 633
	ALL
· · · · · · · · · · · · · · · · · · ·	🗧 🔤 Defini Matria 🖾 🎽 🖉 🖉 🖉
Stage position X: -11011[um] Y: 126[um]	Column 2: Row 2:
Method LSM Imaging Stimulation	Rew 31: Advanced settings Skip Column 11:
Synchroniz	Row 1
	OK Carcel
Registered Area List G CH1 Alexa F CH1 Alexa F	
Display order on map Display order on map	
Save As	
Time StitchingOverlayMac Comment	🔼 Define Metric 🛛 🔀
	10
	Column 3:
Minimum cycle times: 00:00:05:24 LPM: Not enabled	Row 2
Cycle interval: FreeRun Cycle: 1: times	
Size:640x640 0.99	Advanced settings
	Skip
	Column0‡
	Row a
T	OK Cancel
B. [52]	
<u> </u>	8
	24
	Rs
Carrier S	
1 12 WW	
4°	
13 - 7 - 8 4	

- 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.
- 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)

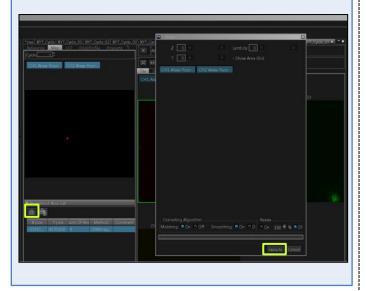
Processing to stitch after acquiring

the image.

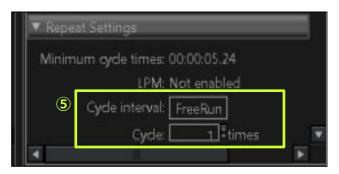
- ① Open the image.
- Press the button.

The [Stitch Dialog box] appears.

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



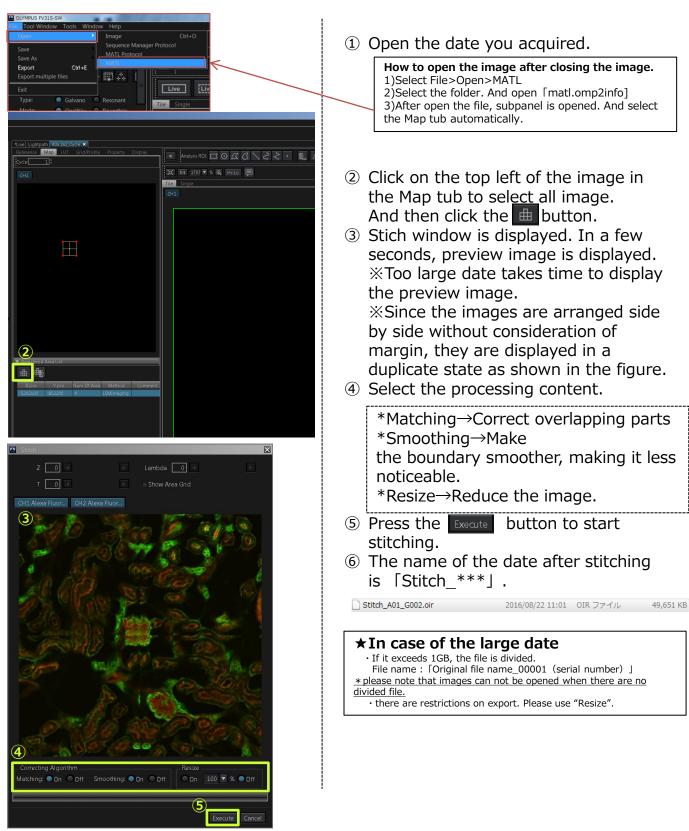
- 3 Check the registered area List whether it is ticked to "Enable" at the area which you want acquire images.
- If you want to see the image on map in real-time, tick the "OverlayMap".
- ⑤ If necessary, set [Cycle interval] and [Cycle].



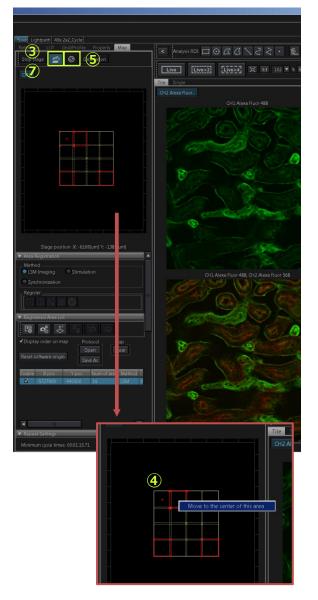
- 6 Select [MATL] tab in [Acquire] Tool Window.
- Press the button to display the dialog box, and select the folder to save the images.
- 8 Press the Register button to start acquiring the image. Finishing the acquisition, map image is displayed in [Map] tab.

Acquire ×				
Normal	Sync Sequence	MATL		
Multi area t	ime lapse			
) Start				
			6	h • 🕞
	iatl_moto			

<u>Stitching</u>



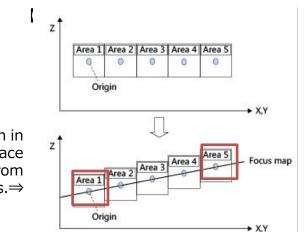
Adjusting the tilt in Z direction <u>~Focus Map~</u>



- Before starting the following procedure, make adjustment for XY imaging.
- 2 Setting the acquiring area (more than 2X2). (p.39)
- ③ Click the solution . 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].
- 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.
- Click the button again.
 Then the focus point is disappeared.
- [®] Start acquiring MATL.



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

Setting of the Well Plate

Configuration Preference Micros	spe	X
XY Stage	Aicro plate settings Select micro plate	
Plate Software	None Corning-6 Corning-12 Corning-24 Custom plate Corning-48 Corning-96 Corning-384	
Microscope Link Keyboard	Scan order ● A1→A2→A3 B1→B2→B3 ● A1→B1→C1 A2→B2→C2	
Reyboard	Custom plate setting	
	None Edit Create Delete	
	/	
	/	
	/	
	/	
	/	4
		OK Cancel
EV Custom p	ate settings	
Plate nam		
Well shap		4
Number o		
Number o Well diam	row 3 € ³ BOOC eter 9.00 mm COOC	
Well spac	$\Gamma = \Gamma =$	' ¥ 'ell spacing
	Pla	te length
		OK Cancel

bjectiveLens					
ienter 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.

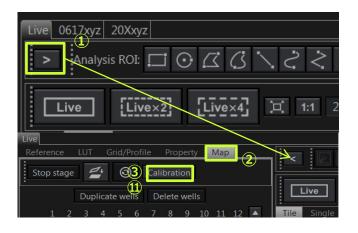
Register plate in Custom plate setting

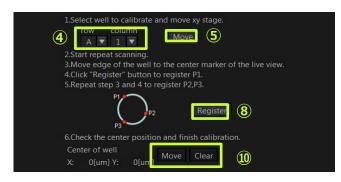
(ex : circle well)
Plate name
Well shape : circle or square
Number of column : Lateral well(line)
Number of row : Vertical well(column)
Well diameter
Well spacing : distance between the center of the well

■ Stage : IX3-SSU

- \rightarrow 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 S matches the well specified with A (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

- Select [Live] window. And select the
 button to display the tool on the left side of the LIVE image.
- 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)
- S Press the [Move] button to move the stage for the center of the well to be adjusted.
- 6 Press the **Image** 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.

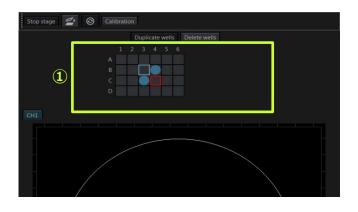
Attention !

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

 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.

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

<u>Map Image display area</u> for well plate(1)

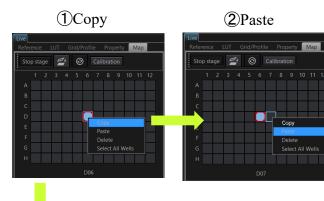


When selecting plate type in [Plate], it is displayed for microplate.

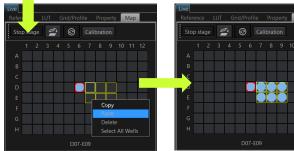
Pattern diagram for well plate

- ① Line is alphabet , column is number.
 - : well in the stage operation range click \rightarrow selected double click \rightarrow 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".



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
 - \cdot Delete $\,$ acquisition settings of the well $\,$
 - Select All Wells in the plate
- ② Select "Paste" on the target well

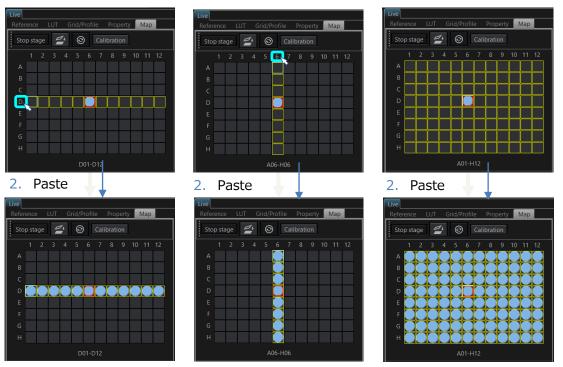
*Dragging on the wells to select the Multiple wells.

Yellow frame means selected wells. It is possible to paste it there.

<u>Map Image display area</u> 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
- 1. Select one column
- 1. Select all wells



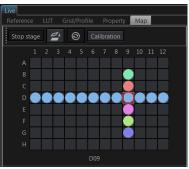
Color labeling of well

- 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.

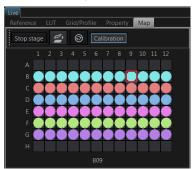
Different setting in eack well



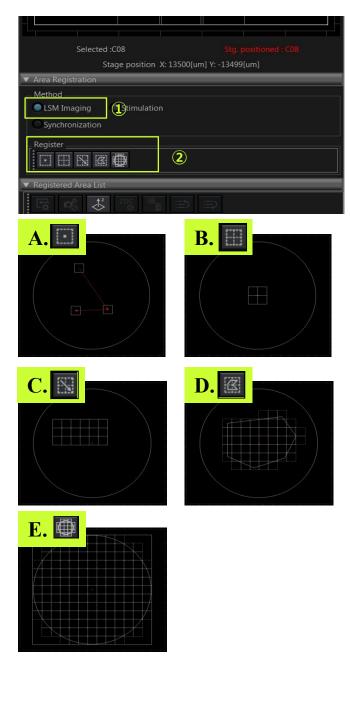
Setting of D9 was paste to all wells in rowD



Same setting in each row



Registration of Image Acquisition area



- 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.
- 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 \square 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.



Stimulation

LSM Stimulation ×
Speed: 10.0 us/pixel
Pixel: Line: Frame:
Duration : 0.000 msec 🖌 🖌 Continuous (5)
Main Recommendation
Laser ND Filter=None
405 10.00 %
■ 445 0.00 % ► ✓ 488 10.00 % ►
561 0.00 %
■ 594 0.00 % ▲ ▶
640 0.00 %
Synchronization ×
Base method: LSM Imaging 🔻 2
Imaging Rest Imaging
LSM Imaging:
Vait Stimulation
Time
Rest in stimulation: ON OFF 3
Parameters
Stimulation
Wait: 1 Frames 4
Serie X Microscope -
Time: ON OFF Z : ON OFF
Series 65.779 msec
Y Time Lapse LSM Total: 0:00:00.66
2 Interval: FreeRun
Sran 0:10:10:00.06 → Rest: 0:100:10.00 → Cycle: 10 \$
Acquire ×
Normal Sync Sequen(a) MATL Synchronization
Start 3
D:\mukai\20170807
MAP TEST
(2)
Current scan condition

Condition setting

- 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).
- S 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.)
- 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.

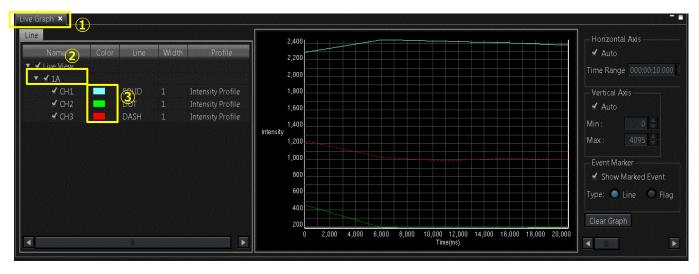
Set the T series(Total number of the frame)

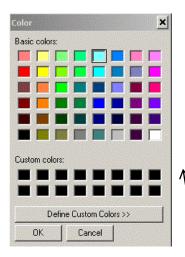
- 1 Select "ON" in [Time] on [Series] tool window.
- ② Set the interval to acquire the image in [Interval] and [Cycle] on [▼Time Lapse].

Execution of TimeLine

- Select [Sync] tab in [Acquire] window.
 Press the b button to display the dialog box, and select the folder to save the images.
- Press the Start button to start acquiring the image.

<u>Stimulation</u>





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].

Analysis ROI: 🗔 🖸 🖾 🖒 之 之 · 🛱 🚛 A 🖌 🖉 🛍 📖

- 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.

SOLID

(5) change the color in the box.

🗹 CH1

- 6 Press the Start button to start imaging and drawing the intensity graph.
- 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** \rightarrow refer to p.22 "Creating a luminance graph: Series Analysis"

Super Resolution:FV-OSR

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

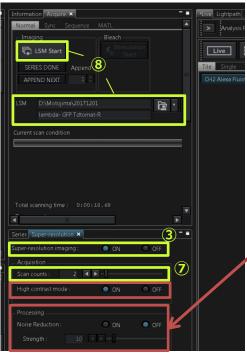
- Adjusting focus and set Laser Intensity(%), Sensitivity(HV), Gain and Offset on [PMT setting] Tool Window.
- O Select the [Super-resolution] in the [Tool Window].
- ③ When "Super-resolution imaging" is turned on, the following items are set automatically.
 - Following parameters are set automatically
 - ·Scanner : Galvano / Oneway
 - Pinhole size

 \rightarrow The image will be darker because it is squeezed smaller.

•Zoom

 \rightarrow Lager scan size leads lager field of view.

- Remove the DIC slider from the light path and set ZDC DM to "Out" in the "Microscope" window.
 (It affect the image quality.)
 - (It affect the image quality.)
- S 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 .
- "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 Start button.

Append Images

Analysis X Single process Live processing Batch Processing Processing Processing Processing Append Images Merge Channels Merge Channels	 Select the [Analysis] in the [Tool window] menu. Select the [Single process] and [Post processing] in the [Analysis] tool window. Single process Multiple process Live processing Post processing
Extraction FRET Acceptor Photobleach Sensitized Emission Coversing Input / Output setting Processing Item Input Output Output Type Channel Name Append Images Itimelapse Dutput1 New Image Append Images	 ③ Open the [Processing] tab. ④ Select "Append Images" in the [▼Edit Images]. ⑤ Click the [Input], and select the image for image processing.
Image: Solution of the system Preview Processing Property Preview Category Item Value Value ✓ Advanced Dimension Interval Type Auto Appended Series timelapse2	Input / Output setting Processing Item Input Output Output Type Channel Name Append Images timelapse Itimelapse Oneshot Oneshot Utimelapse2 Zstack2 Zstack2 Zstack2 [NO INPUT]
Appended Series None	 Select the axis for series to connect images when adding the image in "Dimension" and select the image to be added in "Append Series".
Progress Process Stop	Dimension T Interval Type Auto Appended Series timelapse2 ▼ Appended Series Open File timelapse oneshot oneshot2 timelapse2 Zstack Zstack2
	⑦ Press the Process button.

Extraction

Analysis X Single process Live processing Batch Processing Processing Item Z Gaps Edit Image Append Images Merca Channels Extraction FREI Acceptor Photobleach Sensitized Emission Could be an	 Select the [Analysis] in the [Tool window] menu. Select the [Single process] and [Post processing] in the [Analysis] tool window. Single process ● Multiple process Live processing Post processing Open the [Processing] tab. Select "Extraction" in the [▼Edit Images]. Click the [Input], and select the image for image processing.
Processing Property Preview Category Item Value Advanced 6 1 TIMELAPSE Step Size[15] 1 TIMELAPSE Range 2.4 Selected Channels CH1, CH2 CH2 CH3 All	 Input / Output setting Processing Item Input Output Output Type Channel Name Extraction Itimelapse Output New Image Extraction Itimelapse Zzstack Zstack Zoneshot Oneshot Zoneshot Onesho
■ Progress Process Stop	 i. Setting the interval among frames (ex: 1) ii. Setting the section to extract the image from the series image. (ex:2~4→24) ※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.

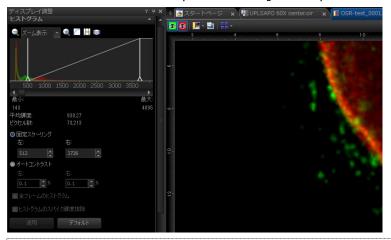
<u>Merge Channels</u>

Analysis X Single process Live processing Processing Graph Processing Item Processing Item Append Images Append Images Load Merge Channels Extraction FRET Acceptor Photobleach Sensitized Emission Coan Input / Output Setting Processing Item Input Output Output Type Channel Name Merge Channels Output New Image Merge Channels	 Select the [Analysis] in the [Tool window] menu. Select the [Single process] and [Post processing] in the [Analysis] tool window. Single process ● Multiple process Live processing Post processing Open the [Processing] tab. Select "Merge Channels" in the [▼Edit Images]. Click the [Input], and select the image for image processing.
Processing Property Preview Category Item Advanced CH1, CH2 CH1 CH1 CH2 CH1 CH3 All oneshot2 CH1, CH2 CH3 All Oreshot2 CH1, CH2 CH3 All Oreshot2 CH1, CH2 CH3 All Oreshot2 CH1, CH2 CH1 CH2 CH3 All	6 Select the different image and channel to be composed in the [Processing Property]. Processing Property Preview Advanced Selected Channels (oneshot) CH1, CH2 Classing Property CH1 CH2 Advanced Selected Channels (oneshot) CH1 Merged Series None Open File Oneshot Unrelapse2 Zstack Zstack Zstack Zstack
Progress Process Stop	⑦ Press the Process button.

Colocalization



- ① Open the Image on FV software and Right-click to select [Send to cellSens].
- 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.
- \bigcirc In the scatterplot, define the intensity range.
- 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.
- ① 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

Select the [View]>[ToolWindow]>[Colocalization]
 Adjust the brightness and Backgroung with [Adjust Display] to see the signal.
 the 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].
- 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...)
- 6 Choose the "Laser Scanning Confocal" or "FLUOVIEW FV3000" in Modality.
- ② Set the Algorithm and parameters.

Press the <u>Finish</u> button to start the process.
 (press the <u>Verify</u> 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.

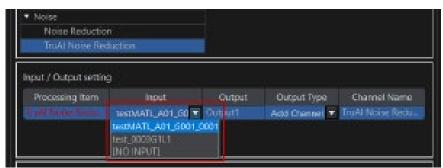
TruAI Noise Reduction ** FV40S-AINR option

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

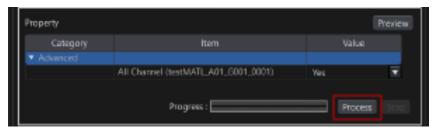
- 1 Open the images.
- ② Show Analysis tool window by Tool Window > Analysis.
- ③ Select [Single process] and [Post processing] then select [TruAI Noise Reduction] in Processing Item.

L Analysis X			-
	Single process	Multiple process	
	Live processing	Post processing	
Batch Processing Graph			
Processing Item			Remove
Spectral Deconvolution			Save
Rolling Average/Accumulat	e		Load
Ratin			
Concentration			
Projection			
Z Gaps			
▼ Edit Image			
Append Images			
Merge Channels			
Ediaction			
▼ Noise			
Noise Resurtion			
TruAl Noise Reduction			

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

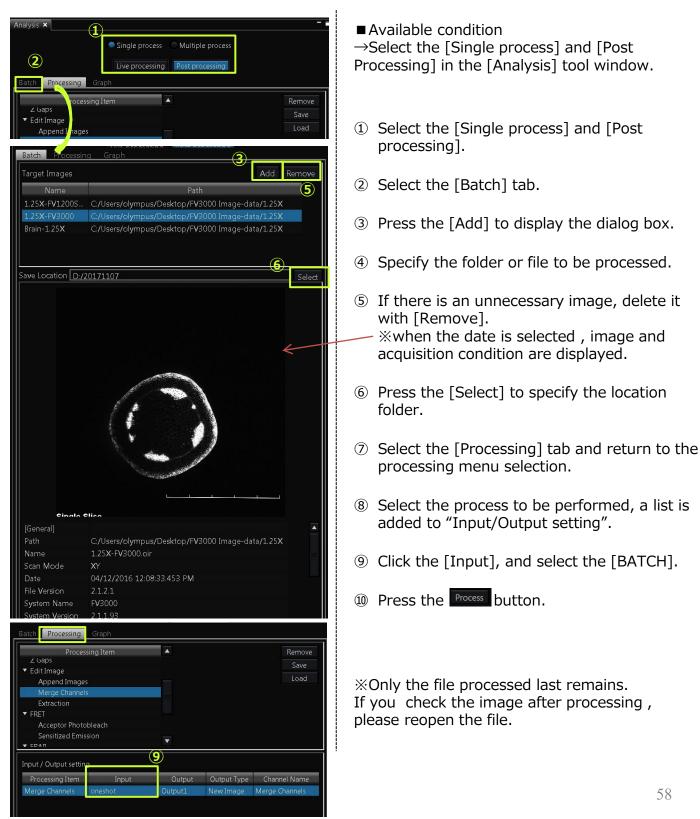


5 Start by [Process].



BATCH :

Batch processing for multiple images



Stitching after projection

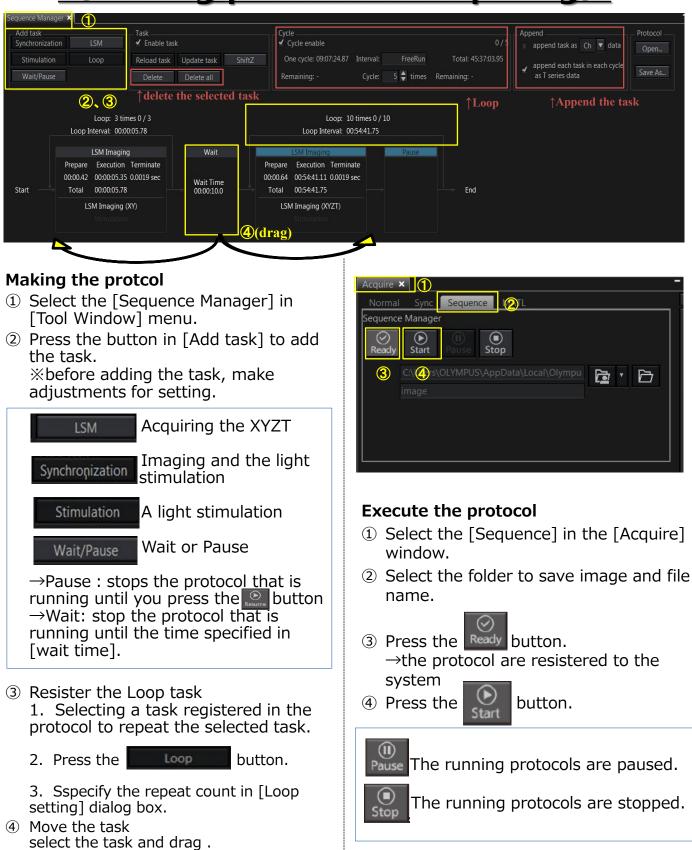
: For large volumes of date, time can be reduced.

Analysis × 1 Single process Multiple process Live processing Post processing	 Select the [Analysis] in the [Tool window] menu. And select the [Single process] and [Post processing].
Batch Processing Graph Processing Item ∠ Gaps ✓ Edit Image	② Select the [BATCH] tab.
Apper Images Load Batch Processing Graph Target Images Add Remove Name Path Map_A01 C:/Users/Administrator/Desktop/image/MATL_Cycle_02 MATL_A01_G002_0001 C:/Users/Administrator/Desktop/image/MATL_Cycle_02 MATL_A01_G002_0002 C:/Users/Administrator/Desktop/image/MATL_Cycle_02	 Press the [Add] to display the dialog box. Select the folder that tiling was acquired. Press the [Select] to open the dialog box. And specify the folder to save/
MATL_A01_0002_0002 C:/Users/Administrator/Desktop/image/MATL_Cycle_02 MATL_A01_6002_0003 C:/Users/Administrator/Desktop/image/MATL_Cycle_02 MATL_A01_6002_0004 C:/Users/Administrator/Desktop/image/MATL_Cycle_02 MATL_A01_6002_0005 C:/Users/Administrator/Desktop/image/MATL_Cycle_02 MATL_A01_6002_0006 C:/Users/Administrator/Desktop/image/MATL_Cycle_02	 ⑥ Open the [Processing] tab. ⑦ Select "projection" in the [▼Edit Images].
Save Location C:\Users\Administrator\Desktop\projection Select	[®] Click the [Input], and select the [BATCH].
	9 Press the Process button.
Bate Processing Graph Projection Save Z Gaps Load ✓ Edit Image Append Images Merge Channels ▼	③ Select File>Open>[MATL] to open the folder, and select "***.omp2info".
Input / Output setting Processing Item Input Output Output Type Channel Name Projection [BATCH] Output1 New Image Projection [BATCH] [NO INPUT] Image Projection	Correct Correc
Category Item Value Basic All Frame (BATCH) All Frame (BATCH) True	matl.omp2info 2016/11/22 14:04 OMP2INFO 77 5 KB matl_forVSIimages.omp2info 2016/11/22 14:04 OMP2INFO 77 5 KB
All Channel (BATCH) True Type Intensity by Z Mode Max 2 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	① Press the and select [Map] tab.
Proces Stop	 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.
Considering Approxim Metademain Scher Coll Strongthrop (\$22, 10) Con 120 * \$ \$ Coll (12)	50

Execute Cancel

is

Sequence Manager (making protocol of acquiring)



New registration of Dye

	 Select the [Dye Editor] in Tools. Press the <u>New Dye</u> to display the dialog box. <u>Dusinate</u> : edit based on the data of the "User Dye" selected in [User Dyes] Enter each items. OExcitation peak/fFuorescence peak
Color LUT: Green 1: Store MAX absorption: 499 2: 500 580 MAX emission: 520 3: 500 540 Absorption: 420 4: - 5: - Emission curve: Export 5: - - - Concell OK Cancell - - -	: Airy Disk OAutomatic emission priority : refer to next page
✓ Custom Dye Dye Name : TEST Abbr.(MAX10) : Manufacture : Comment : Color LUT : MAX absorption : 500 ◆ MAX emission : 525 ◆ Absorption curve : Import Export	Cobservation parameters Excitation laser : Main : 488 ▼ Substitution : ▼ ▼ ▼ ▼ Automatic emission priority : Custom ▼ 1: ▼ 500 ♥ - 560 ♥ 2: - 3: - 4: - 5: -
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

Dye Editor						
Default Dyes :			User Dyes			
Dye Violet			Violet	Dye	A	
Cyan Blue			Cyan Blue			
Emerald Yellow		>> Copy >>	Emerald Yellow			Delete from list
Orange			Orange			Edit
Red			Red			Duplicate
Acridine Orange Alexa Fluor 405			Acridine Alexa Flu			New Dye
Alexa Fluor 488	▼		Alexa Flu	ior 488	▼	Dye combination
Dye Name :	Alexa Fluor 488			Observation pa		
Abbr.(MAX10) :	Alexa 488			Excitation laser Main :	: 488	
Manufacture :				Substitution :	400	
Comment :				Automatic emi	ssion priority .	- 1
Color LUT :	Green			1:	500 - 600	
MAX absorption :	499			2:	500 - 580	
MAX emission :	520			3:	500 - 540	
Absorption curve :	Export			4: 5:		
Emission curve :	Export					
Emission Pri set automati V (405) : 4	cally			• 4	00/300-	÷500-540
C (405) : 4 C (440) : 4						
B (488) : 5	60-500 600-600 500-580					
5	500-540					
	30-630					
	530-580					
· · · · · ·						
: : 0 (594) : 6	570-620					
R (640) : 6						
K (0+0) . 0	50 7 50					
■ selecting Ex- the main laser		,	s			
■ If you enter than the abov Select "Custo → Please set Narrow Band	<u>/e,</u> me" and e Wide Band	nter the k				
(One kind is a		<u>able)</u>				

Spectral Imaging



Lambda series(1) (Ch setting)

OLYMPUS FV318-SW	
File Tool Window Tools Window Help	
Layout	
	Acquire *
💌 Scan Settings 🔷	ASSUMENT SYNC. Sequence work t
	LSM Start
Mode: 💿 OneWay 🔍 Roundtrip	APPEND NEXT 1
Speed: 2.0 us/pixel 🖲 🗋	15M Cillinged along und Dadatas
	LSM C:\Users\olympus\Desktop 📴 * 🖻
Scan Size: 512x512	Current scan condition
High voltage correction: ON OFF	
Pixel: 2.0 usec Line: 2.118 msec Frame: 1.087 sec	
▼ Area Settings	
Rotation 0.0 🖨 deg Reset	
Pan_X:0.00 ♀ umReset	
	Remaining time : - Next scan start at : -
Pan Y: 0.00 🕏 um Reset	PMTSetting ×
	PMT Setting ★
Zoom 1.00	Mode: VBF Lambda
x1 Optimize @	Average: None Line Frame 3 Times
(2	Dye & Detector Select
▶ Round trip correction	
Series × Microscope Information -	Confocal Aperture Auto 205 \$ um
	Airy Disk x 1.00
Time : ON OFF	x1 x2 x3 x4 Laser ND Filte O None O 10%
Z : ON OFF	= 405 0.00 % ◀ ►
	445 0.00 %
	✓ 488 1.00 % ▲ ►
▼ Time Lapse	■ 514 0.00 % ▼
LSM Total: 0:00:17.82	561 0.00 %
	= 594 0.00 % ◄ ►
Scan: 0.00:17.82 Rest: 0.00:00.00	= 640 0.00 % ◀ ▶
Cyde: 2 2 LPM:Not enabled	✓CH1 🔤 🔍 HSD1 05 🗣-515nm
▼ Z Section	HV 390 V S S S S S S S S S S S S S S S S S S
▼ Z Section Motor: ● Start/En: ◎ Range	Gain 1.000 x ◀ ▶ Offset 0 % ◀ ▶
	▼ Spectral Setting
Curren4026.59 Origin: 200.00	a position of a straining
Kegister Move	
Slices: 13 \$ Step Size: 0.56 \$	
Optimize	400 S00 600 700 800
	Band Width 10 🗘 nm
Start: 0.00 \$ End: 6.72 \$	Step Size 5 🖨 nm
Register Register	CH1: 90 🗣 - 56(🏶 nm 13 🗣 steps

* 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

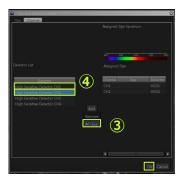
: Acquire lambda series with single laser. Select BSD1

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



▼Lambda scan with multi channels

: Acquire lambda series with multiple lasers. e.g.) Try to separate GFP, RFP and Auto $FL \rightarrow$ excited by LD 488 and 561



Lambda series(2) (LightPath setting)

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

A. Selecting Excitation DM

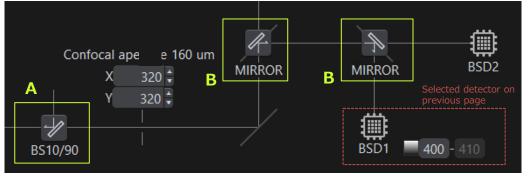
- To acquire accurate spectrum \rightarrow Select BS10/90
- To acquire bright spectrum \rightarrow Select DM with same wavelength of excited lasers.

■ Example of single channel.

B. Selecting SDMs

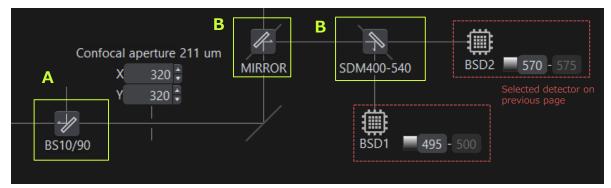
 $\begin{array}{l} \text{MIRROR} \rightarrow \text{Reflected totally.} \\ \text{GLASS} \rightarrow \text{Transmit} \\ \end{array}$

- SDM \rightarrow Reflected particular wavelength.
 - 例)SDM400-540:
 - Reflected :400nm-540nm Transmitted :540nm-



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)

PMT Setting	×				
▼ PMT					
Mode :	🔘 VBF	🔍 Lambd			
Average:	None	🔘 Line	🔘 Frame	3 🗧 Times	
Dye & Det	ector Select				
Confocal Ap	perture Auto		um 400	600 80	וח
Airy Disk x		I			
Laser ND Fil	te 🔘 None	×1 10%			
405	0.00	% ◀ ▶			
· 445 🤇	0.00	% ◀ ▶			
ᢞ 488	1.00	% ◀ ▶			
= 514		% Change	e laser, band	d width or st	ep <mark>s</mark> ize
🗹 561	0.00	% ◀ ▶			
□ 594			e laser, band	d width or st	ep <mark>s</mark> ize
· □ 640	0.00	% 🔹 🕨			
🗹 CH1 📃			HSD1 35	🗣 - 545nm	1
HV	390	V		12	
Gain Offset	1.000	x ◀ ▶ % ◀ ▶			
≤CH2		7			11
HV	470	VIL	H3D2 85		
Gain	1.000	x I		(12)	
Offset	0	% ◀ ▶			
▼ Spectral S	letting				
(7)		N			
400					
	500	600	700		n
					n
	500 10		⁷⁰⁰	ked	ņ
Band Width	10 🔷 nm		Link		n
Band Width Step Size	- 10 🕈 nm 5 🗘 nm	5(♦ nm	Link	teps	n
Band Width Step Size CH1:	10 🕈 nm 5 🗘 nm 00 🖨 - 5	5(♦ nm	2 Link 9 ♦ s ¹	teps	n
Band Width Step Size CH1: CH2:	10	5(♦ nm	2 Link 9 ♦ s ¹	teps teps	n
Band Width Step Size CH1: CH2: Total: Acquire Norm	10	5(♦ nm	9 ♦ s ⁻ 11 ♦ s ⁻ 20 st	teps teps	n
Band Width Step Size CH1: CH2: Total: Acquire Norm	10	5(♦ nm 10 ♦ nm Sequence	9 ♦ st 11 ♦ st 20 st • MATL Bleach	teps teps teps	n
Band Width Step Size CH1: CH2: Total: Acquire Norm	10	5(♦ nm 10 ♦ nm	9 ♦ st 11 ♦ st 20 st Bleach	teps teps teps	n
Band Width Step Size CH1: CH2: Total: Acquire Norm	10	5(🕈 nm 10 🔹 nm Sequence	9 ♦ st 11 ♦ st 20 st • MATL Bleach	teps teps teps	n
Band Width Step Size CH1: CH2: Total: Acquire Norm Ima. SER	10	5(♦ nm 10 ♦ nm Sequence	9 ♦ st 11 ♦ st 20 st Bleach	teps teps teps	n
Band Width Step Size CH1: CH2: Total: Acquire Norm SER	10	5(♦ nm 0 ♦ nm Sequence 15 Append 1 ♦	9 ♦ st 11 ♦ st 20 st Bleach	teps teps teps	

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

- O Check the laser to use. Adjust the laser power not to 0%.
 O
- ① Click "Live" to show live image.
- Detection wavelength can be changed for each channels.
- ② Adjust the laser power.

Start acquisition

- ③ Set Z and/or T series when needed.
- () Determine the file location and file name.
- IS Click "LSM Start" .

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

Analysis X Jolume Setting Graph Table	① Press the Viewer putton to switch
2 Single process Multiple process Batch Processing Post processing Processing Item 4 Spectral Deconvolution 4 Ratio Concentration Concentration Projection Z Gaps 4	 to "Viewer mode". 2 Select [Analysis] in [Tool Window] menu. [Analysis] Tool Window appears. 3 Select [Single process] mode, press the [Post Processing] button.
Append Images Input / Output setting Processing Item Input Output Output Type Channel Name Spectral Deconvo Iambda Oxutput New Image Spectral Deconvo 5	 Single process Multiple process Live processing Post processing Press the Remove button to reset the
Processing Property Preview Category Item Value * Basic All Frame (lamibda) True * Advanced CH1 Image: CH1 * Target Channel CH1 Image: CH1 Mode Blind Unmixing Image: CH1 DVED Dye Profile Save Folder Image: CH1 Image: CH1	 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.
DYE3 Dye Profile Save Folder DYE3 Dye Profile Save Folder DYE3 Dye Profile Save Folder Background Correction False Progress : Progress : Stop Preview Object: lambda Frame Index: L 1 Processing Item: Channel (Before): Channel (After):	Category Item Value * Basic All Frame (lambda) True * Advanced Double click True Mode 6 Blind Unmixing Number of SpectralData[1_11] 0YED Dye Profile Save Folder Blind Unmixing DYED Dye Profile Save Folder DYE2 Dye Profile Save Folder False

- 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~

Analysis X Volume Setting Graph Table -	 Press the <u>viewer</u> button to switch to "Viewer mode". Select [Analysis] in [Tool Window] menu. [Analysis] Tool Window appears. Select [Single process] mode, press the [Post Processing] button.
Append Images Input / Output Setting Processing Item Input Output Output Type Channel Name Spectral Deconvo Ismbda Output1 New Image Spectral Deconvo Processing Property Processing Property Preview Category Item Value Basic All Frame (lambda) True All Frame (lambda) CH1	 Single process Multiple process Live processing Post processing Press the Remove button to reset the assigned item and select [Spectral Deconvolution] in [Processing Item]. In [Input] in [Input / Output setting], select the image for image processing.
Preview Object: Iambda Preview Object: Iambda Frame Index: 1 Channel (Before): Channel (After):	 In [Mode] in [processing Property], select "Nomal Unmixing" In [Cocessing Property Them (ambda) In Category Target Channel (ambda) Target Channel (CH1) (CH1)

- (8) Select the all date.
- 9 Press the Process button to start the fluorescent separation process.

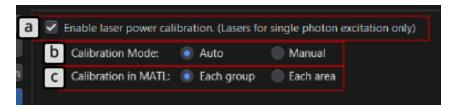
Processing : Unmixing(3) Spectral Image Unmixing ~by specifying dyes~

Volume Setting Graph Table	① Press the <u>Viewer</u> button to switch to "Viewer mode".
Batch Processing Graph	 ② Select [Analysis] in [Tool Window] menu. [Analysis] Tool Window appears.
Processing Item Rebove Spectral Deconvolution Sav Rolling Average Load Concentration Projection Z Gaps	 ③ Select [Single process] mode, press the [Post Processing] button.
Edit Image Append Images Input / Output setting Processing Item Input Output Output Type Channel Name Spectral Decomo_ Iambda Output New Image Spectral Decomo_	 Single process Multiple process Live processing Post processing
5	④ Press the Remove button to reset the assigned item and select [Spectral
Processing Property Preview Category Item Value	Deconvolution] in [Processing Item].
▼ Basic All Frame (lambda) True Use Area Only False	⑤ In [Input] in [Input / Output setting],
Advanced Target Channel CH1	select the image for image processing. 6 In [Mode] in [processing Property], select
Mode Spectral Image Unmi_	"Spectral Image Unmixing.
▼ DYE0 ROI Channel Name Open File Remove DYF DYE0 ROI Index Iambda 0003 DYE0 Dye Profile Save Folder Iambda_0002 Progress : Iambda_0001 Process Stop	Protecting Respective Company Incom Variat Respective Respective Company Incom Variat Respective Com Variat Co
Preview Object: lambda	Encount Connection (6) Linear Instance Instance
Processing Item: Channel (Before): Channel (After):	⑦ Specify multiple ROIs on the regions where only the target fluorescence dye locates to acquire the spectral data for image processing.
Crit (CPA Jona Tuer 508	⑧ In [DYE0 ROI File Name], select the file name of the image on which the ROI was specified in ⑦.
	 In [DYE0 ROI Index], select the first ROI specified in ⑦.
	Processing Property: Contempory Contemporation Con
	① Repeat ⑧ 0 to register all ROIs. Press the provide button to start the fluorescent
	the Process button to start the fluorescent

separation process.



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

Configuration	
Preference Filter	s Microscope
File/folder	Software settings
Laser	Font size
XY Stage	Default Large
Plate	
Software	Sample
IR laser emission	

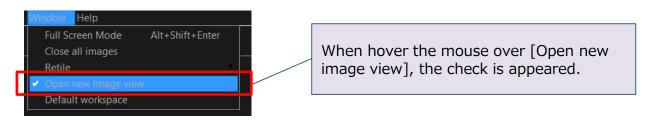
Reset the layout

Window Help Full Screen Mode Alt+Shift+Enter Close all images Live Reset Current Layout Retile V Open new Image view

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].



【2】

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

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

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"
- 4. Select [Custom] , click "Next" Check all then click "Next"

Exp	ort Purpose.
	rizard needs to know which purpose to export.
	Select the purpose, then click Next.
	For analysis of problems

Select the option, then click Next. C Standard Export all system informations. C Custom	event files
Export Options. This wizard needs to know which type of informations to export. Select options to export options to export options to export options to export options and the option op	

Export for La	st DDD		нн:мм	
	7	÷.	00:00	•
Export in ran Start	ge of Date		End	
MM/DD/W	HH:MM	мм	/DD/W	HH:MM
07/31/19 -			1/19 -	11:30

- 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

\Rightarrow 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.

