FV4000-BX63L Operation Manual





Ver.4.1 2025/2

(cellSens-FV ver3.2.1)

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Image Acquisition



<u>Starting the system</u>



Controller

See the next page for observation via eyepieces





Observation via eyepieces



Change light path to binocular 100%

★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".

🛛 Ocular 🗙 🖻 Obs	ervation Method 💈	LSM Imaging 🖬 Acquire		
Cular CLSM				
EPI Light Path				
Cube turret #1:	8:IX3-FDICT			
EPI Shutter:	🔵 Open 💿 Clo	se		
EPI ND Filter:	1:0.0			
▼ DIA				
TD Shutter:				
Trans Lamp:	💿 On 🔵 Off			
9	240 🖣 🕨 —	•		
Polarizer:	💿 In 🔵 Out			



*Display may differ depending on the configuration.

★After observation via eyes, click "LSM"

이 Ocular 🗙 🛃	SM Imaging	Setector S
Ocular O	LSM	
▼ EPI Light Path		
Cube turret #1:	1:MIRROR	
EPI Shutter:		
▼ DIA		
TD Shutter:		

XY Image Acquisition(1)

S Detector Setting × Super-resolution □ TruSight imaging
▼ Detector
Mode : 💿 Standard 💿 Lambda
Average: None Line Frame 3 Times
Accumulate: None Line Frame 8 Times
Sequential Scan: None Line Frame Dye & Detector Select Phase1 Confocal Aperture Auto 219 um
Airy Disk x 1.00
BSD1 RSD3 TD Variable confocal aperture
Group 1 CH1
Group 2 CH2 CH3
Laser ND Filter: 🔵 None 💿 10% Variable detection wavelength
One-time laser power calibration: Execute 8
P1 CH1 Acin ▼ BSD1 500 - 540 nm G-1 ✓ 488 ▼ 0.00 % ► Laser Intensity P1 CH2 Alexa Fluor 568 ▼ RSD3 570 - 620 nm G-2 ✓ 561 ▼ 0.00 % ► P1 CH3 ▼ TD G-2 ✓ 561 ▼ 0.00 % ► HV 150 V ► Sensitivity of TD Offset 0 % ► ● ▼ Spectral Setting

 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.
- Adjust LUT via Range: _ = = or vertical bar on the right.

XY Image Acquisition (2)

Ocular Observation	n Method 록 LSM Ir	naging 🗙 🖻 Acquire
▼ Scan Settings		
$\Diamond \circ \Box \circ \land$	<u>, </u> 2 + ⊞	4
Scanner: 10		
Туре:	💿 Galvano 🔵	Resonant
Mode:	💿 OneWay 🔵	Roundtrip
Interlace:	💿 OFF 🛛 🔵	2x 🔵 4x
Speed: 1.0 us/pixel	🕄 🛡 <u></u>	
Image Size:	inter and	13
Aspect Ratio:	1:1 4:3	
Scan Size: 10)24x1024 🔻	
Pixel: 1.0 usec Lin	e 2.113 msec - Fi	- ramo: 1220 coc
		ame. 4.550 sec
▼ Area Settings		ame. 4.550 Sec
▼ Area Settings	2	
▼ Area Settings	2 Rotation :	0.0 ‡ deg Reset
▼ Area Settings	2) Rotation :	0.0 ‡ deg Reset
▼ Area Settings	2 Rotation : Pan X :	0.0 ‡ deg Reset
▼ Area Settings	Rotation : Pan X :	0.0 deg Reset
▼ Area Settings	2 Rotation : Pan X : Pan Y :	0.0 ‡ deg Reset 0.00 ‡ um Reset 0.00 ‡ um Reset
▼ Area Settings	Rotation : Pan X : Pan Y : x0.9 x1 @	0.0 ‡ deg Reset 0.00 ‡ um Reset 0.00 ‡ um Reset Optimize
▼ Area Settings	2 Rotation : Pan X : Pan Y : x0.9 x1 @	0.0 ‡ deg Reset 0.00 ‡ um Reset 0.00 ‡ um Reset Optimize
✓ Area Settings ✓ Area Settings ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓	Rotation : Pan X : Pan Y : x0.9 x1 @	0.0 + deg Reset 0.00 + um Reset 0.00 + um Reset Optimize

Ocular	r 🖻 Observation Method 🖻 LSM Imaging	Acquire ×
Norma	al Sync Sequence MATL	
_ Imag	ging15	Bleach
r.	LSM Start	
SEF		
APF	END NEXT Append 1	
LSM	D:\Data	
	1K-XY	
🕨 Exp	eriment Info.	
Current	scan condition	
Total o	canning time : 0:00:04.22	
Remain	ning time : -	
Next so	can start at : -	

Setting the scanner in [LSM Imaging]

 ${\scriptstyle \textcircled{10}}$ 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 D 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.



Is Press the 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				
	50 100 200	400	600	
Airy Disk x 1.00	_●			
	x1 x2	x3 x4	x5 x6	
BSE	01 BSD2			
Group 1	H1			
Group 2	CH2			
Laser ND Filter:	None	70%		
One-time laser p	ower calibration:	Execute		
P1 ✓ CH1		SD1	430 - 470 nm	
			500 540 pm	
PT CH2			500 - 540 nm	
	ECED		460 - 500 nm	
			1400 [000] IIIII	
P2 ✓ CH4	EVEP	BSD2	530 - 580 nm	
			550 100 mm	

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.
- 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.
- ID 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	Ì	
Series: 1 min 10 sec R	eset: Reset to initia	l values.
► Time Lapse		
▼ Z Section	2	1
Motor: 💿 Start/End 🔵 Rai	nge	Reset
Near limit:1950.00	Origin: 0.00	4050.00
Current:9.80	Register	Move
±		
	Start: 8.85	4058.85
Start	Register	Move
End	End: 0.00	4050.00
• ~	Register	Move
	START <=> E	ND
	Slices: 16	5
	💼 Step size: 0.59	Optimize



* 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	Res	et: Res	set to initia	al values.
Time Lapse		_		1
▼ Z Section		2		
Motor: Start/End	💿 Range			Reset
Near limit:1946.40		Origin:	0.00	4053.60
Current:0.00		<u></u>	Register	Move
★		9		
		Start:		4046.10
	End	\diamond	4	Move
💾 1	Chard	Range:	15.00 ‡ .	m
¥	Start	End:	7.50 ≑	4061.10
				Move
			START <=> EN	D
	s	lices:	31 🗘	(5)
	à s	tep size:	0.50 🗘	Optimize

🚡 Acquire 🗙	
Normal Sync Sequence MATL	
	Bleach
😱 LSM Start	✓ Stimulation Start
SERIES DONE 8 SR 👻 🖳	
APPEND NEXT Append	
	6
LSM C:\Users\10015580\Desktop\fv	
image	
Experiment Info.	
Current scan condition	

 \ast 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 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

<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

	ir 🖻 Observation Method ጃ LSM Imaging	🖻 Acquire 🗙	
Norm	ial Sync Sequence MATL		
- Ima	ging (4) LSM Start T Z 52 L RIES DONE DE SR 🔅 🖶	Bleach	
	PEND NEXT Append 1	3	
LSM	D:\Data		
	<u>1К-ХҮТ</u>		
🕨 Exp	beriment Info.		
Curren	t scan condition		
Total s	canning time : 0:19:34.33		
Remai	ning time :-		
Next s	can start at : -		
© Series	×		
LSM - Time : Z : Series: ∠	ON OFF ON OFF		
▼ Time	lanse		
LSM Tot	tal: 0:19:34.33		
	Interva <mark>i: 0:00:30.0</mark>		
🖵 Sca	n: 0:00:04.33 2 Rest: 0:00:25.67	<u> </u>	
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

*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



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 . \times 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

<u>Mouse wheel</u>:Zooms toward the center of the 3D image displayed. <u>Shift key+mouse wheel</u>: 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			
		Acquisition	Viewer cellSens
'Live Fucci3day-small 🗙	Analysis × Graph Ta	ble	
	3	Single process	C Multiple process
● T 1 🛊 /88 🗰 🔍 🕨 🗰 🛋 🕨 MAX 👻 Loop 👻 🌇 Start: 1 - End: 88		Live processing	Post processing
JI 11 149 ▼ × Q HHu P	Batch Processing	Graph	
Tile Single ThreeSides Volume	Series Analysis		
CH1_Extraction CH2_Extraction PhaseContrast_E_ CH1_Extraction , CH2_Extraction	1D Profile Multi 1D Profile		
	Graph List		
	No. N	lame Inc	5
	1 Series Analy	Fucciaday	
	Property		Preview
	Category	Item	Value
		x	TIMELAPSE
14		Y Apply All Frame Range	Average Ver
		Apply All Channel	Yes
	▼ Advanced	Apply All ROI	Yes 🔻
	▼ X Axis Range		
	V Avie Studie	Apply Auto	Yes 🔻
	in the style		Yes 🔻
		Label	TIMELAPSE
		Line Style	SOLID V
	V Avic Scalo		RGB {255, 255, 255}
			7 Apply Stop
Size:512x512 0.415[um/pixel] xy=(58,257) Int:132, 178	L		

- 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 save 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

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



Saving images with the photon numbers

cellSens FV		
File Tool Window Tools	W	indow Help
Open	×	
Save	•	- 🗆 🗣 De
Save As	Þ	Image
Export Ctrl+	·Е	Photon Image
Export multiple files		Projection Image
Exit		Sequence Manager Protocol
Type: Oalvand	5	

Send To Clipboard		
Save		
Save As	•	Image
Export		Photon Image
Save Display		Projection Image
Save Animation	1	
Run TruSight		
Send to cellSens		
Send to cellSens macro	×	
Select All ROIs		
Delete All ROIs		
Paste ROI		
 Show Overlay 		
 Show Overlay ID 		
add canvas		
remove selected canvas		

- ① From the File menu, select SaveAs/PhotonImage, or right-click on the image and select SaveAs/Photon.
- ② New OIR file that reflects the photon numbers will be created.

*"_photon" will be displayed at the end of the file name and will be saved.

* This image can be opened with cellSens, cellSensFV, ImageJ or Fiji.



Opened with cellSens FV

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.
- 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



			_		_	_	_	_	X
Preference									
ObjectiveLens	Nosepiece:IX3-D6REA								
MirrorTurret1	Objective lens								
Condenser	PLAPON1.25X	•		DRY(1.000)					•
ZDC				DRY(1.000)	0.40			IX2-DIC10	•
									•
									•
								IX2-D1C60	•
	UPLSAPO 30XS	Y						IX2-DIC100	۳
	UPLSAPO 30XSIR	-					J		
		а.							
	UPLSAPO 40XS			E: Glass and plastic are					
	UPLSAPO 60XS								
	UPLSAPO 100XO								
	UPLSAPO 100XS								
	PLAPON 60XOSC								
	PLAPON 60XOSC2							6	
	UPLFLN 10X2							OK	
	UDEFEN 2007								
Configuration	042 052 ····								X
Preference Micro									
EPittelder									
(2)									
2	Micro plate settings Select micro plate					_			
2 XY Stage	Micro plate settings Select micro plate) Comi	1g-6	Corning-12 Corn	ning-24	٦			
XY Stage Plate	Micro plate settings Select micro plate None Custom plate	Comii Comii	1g-6 1g-48	Corning-12 Corn Corning-96 Corn	ning-24 ning-384]	4		
XY Stage Plate Software Microscope Life	Micro plate settings Select micro plate O None Custom plate	Comii Comii	1g-6 1g-48	Corning-12 Corr Corning-96 Corr	ning-24 ning-384]	4		
XY Stage Plate Software Microscope Life Keyboard	Micro plate settings Select micro plate None Custom plate Scan order ● A1→A2→A3 81-) Comii) Comii +82→83	ng-6 ng-48 ⊙ /	Corning-12 Corr Corning-96 Corr A1→81→C1 A2→82→C2	ning-24 ning-384]	4		
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XY Stage Plate Software Microscope Link Keyboard	Micro plate settings Select micro plate ↓ Kone ↓ Custom plate ↓ Scan order ↓ A1→A2→A3 B1→ Custom plate setting None) Comii) Comii •B2→B3	ng-6 ng-48	© Corning-12 © Corr © Corning-96 © Corr A1.−B1.−C1 A2.−B2.−C2 Edit Create	ning-24 ning-384 Dete	te	4		
XY Stage Pate Software Microscope Ling Keyboard	Micro plate settings Select micro plate None Custom plate Scan order A1-A2-A3B1- Custom plate setting None) Comii) Comii •B2-+B3	ng-6 ng-48 • J	Corning-12 Corn Corning-96 Corn A1B1C1 A2B2C2 Edit Create	ning-24 ning-384 Dete	rte	4		
XY Stage Plate Software Microscope L	Micro plate settings Select micro plate None Scan order A1-A2-A3. B1- Custom plate setting None	Comii Comii +82-+83	ng-6 ng-48	Corning-12 Corring-96 Corring-96 Corr A1-B1C1 A2-B2C2 Edit Create	ning-24 ning-384 Dete	te	4		
XY Stage Pate Software Microscope Ly Keyboard	Micro plate settings Select micro plate ● None ● Cutom plate ● A1+A2-A3 81- Cutom plate setting None	Comii Comii •B2-+B3	ng-6 ng-48	Coming-12 Coming-12 Coming-6 Coming-6 Coming-6 Coming-6 Coming-6 Coming-6 Coming-6	ning-24 ning-384 - Dete	te	4		
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XY Slage Pate Software Microscope Lu Keyboard	Micro plate settings Select micro plate None Curstom plate Scan order A1→A2→A3 81- Custom plate setting None) Comii) Comii B2→B3	ng-6 ng-48	Coming-12 Con Coming-96 Con A1-B1-CL A3-B2-C2 Eait Create	ning-24 ning-384 - Dele	te	4		
XY Slage Pate Software Microscope Luce Keyboard	Micro plate settings Select micro plate None Custom plate A1A2A3 B1 Custom plate setting None) Comii) Comii +B2-+B3	ng-6 ng-48	Coming-12 Corr Coming-96 Corr A1-B1-C1 A7-B2-C2. Exit Create	ning-24 Dele	te	4		
XY Stage Piar Software Microscope U Keyboard	Micro plate settings Select micro plate None Custom plate A1-A2-A3. B1- Cutom plate setting None	Comii Comii +B2-+B3	ng-6 	Coming-12 Coming-12 Coming-56 Coming-56 Coming-56 Coming-56 Coming-56 Coming-56 Create	ling-24 ling-384	te	4		
XY Slage Point Software Microscope L Keybeard	Micro plate settings Select micro plate Noce Scan order A1-A2-A3 81- Custom plate setting Noce) Cornin	ng-6 ng-48	Coming-32 Com Coming-36 Com Coming-36 Com A1-B1-CL A2-B2-C2. Eat Create	ling-24 - Dele	te	4		5
XY Slage Pote Microscope List Keyboard	Micro plate settings Select micro plate None Scan order Custom plate setting None	Cornii Cornii +B2→B3	ng-6 ng-48	Coming-32 Com Coming-96 Com A1-81-CL A3-82CL Eat Create	bele	te	4		S

■ 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.

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

example) $30X \rightarrow IX2$ -DIC30 $100X \rightarrow IX2$ -DIC100

6 Press the K 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 <u>mic</u>ro plate to be used.
- 5 Press the OK button.

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 data 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 **b**utton 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)

Live 0617xyz 20Xxyz	
Analysis ROI:	\backslash $<$ $<$
Live [Livex2] [Live×4]	[其 1:1 2
Reference LUT Grid/Profile Property Map	🖌 < 🛛 Analysis ROI: [
Stop stage 🖆 🕲 Calibration	
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Method LSM Imaging Stimulation	Sec. 18
Synchrotration	
▼ Registered Area List	
✓ Display order on map Protocol Map	
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Display order on map Protocol Map	
Open Clear	
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Enable X pos Y pos Area Method Time Image: Comparison of the state of	Stitching CrerlayMap
32 \	

Scroll to the right

* 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

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

Only for

motorized stage

- 2 Press the **I** 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.

		-
quire	×	
lorma	Sync Sequence MATL	
ilti are	a time lapse	
€ Start	(II) Pause Stop	
	C:\Users\olympus\Desktop	
	matl_moto	

s ÷times

5 Cycle interval: FreeRun

Remaining time: -

Cycle:

Total time: 00:04:15.47

Overlap section at tiling imaging



- 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	
Preference Filters	
File/folder	Speed of XY stage
Laser	High Medium Low
IR laser emission	Enable drag and drag XV stage move on live window
Detector	
XY Stage	Escape objective lens when the stage position changes in MATL.
Plate	Overlap section at tiling imaging
Software	10 %
LUT Microscopo Link	
Keyboard	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
	Intelligent shading correction: On Off
L L	

Matching/Smoothing : On \rightarrow Enables matching and smoothing processing even during automatic stitching.

Intelligent shading correction \rightarrow Stitched image quality will be better.

Acquiring the stitched image using Map Image(1)

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- 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.

<u>B. Drawing a rectangular ROI in the</u> <u>Map</u>

- 1. Refer to page 30, create a map image.
- 2. Press the subutton.
- 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 30, 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 set button to start acquiring the image. Finishing the acquisition, map image is displayed in [Map] tab.

Acquire >	
Normal	Sync Sequence MATL
Multi area	time lapse
€ Start	Ause Stop
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[matl_moto

<u>Stitching</u>



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

🗷 Live	Pri	path	20)X 🖬	20X	_Сус	le			-					_
Refer		LUT	Gr	id/P	rofile	e F	rope	rty	Ma	ip					
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- Before starting the following procedure, make adjustment for XY imaging.
- 2 Setting the acquiring area (more than 2X2). (p.39)
- ③ Click "FocusMap" button . The 3 red focus points will be displayed in MATL ROI.
- ④ Click "Three point" to change the density of focus points.
- ⑤ Click "Edit" and click on the MATL ROI to add/delete focus points. Finally, click "Edit" again.
- 6 Click ► to move to the 1st reference position.
- $\ensuremath{\textcircled{}}$ Click "Live" and adjust focus.
- 8 Repeat 6 and 7 to all points. Once focus is adjusted, F mark is added on the point.
- 9 Start MATL.

Setting of the Well Plate

Preference Micros	cope	
2)r XY Stage	Micro plate settings Select micro plate	comina-12 Comina-24
Plate Software	Custom plate Corning-48 Co	Corning-96 Corning-384
Microscope Link Keyboard	Scan order ● A1→A2→A3 B1→B2→B3 ○ A1→B	31CL A2B2C2
		Create Delete
	/	
	/	(4)
		OK Cancel
💟 Custom p	ate settings	×
Plate nam		
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Number o	f column 2 🔷	
Well diam	eter 9.00 mm	
Well spac	ng 19.00 mm	
		Plate length
		OK Cancel

ojectiveLens	Noraniace/V2-D6REA				

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 (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

- 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
 - 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





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 Sutton.
- 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 $\textcircled{\mbox{ }}$ button.



Stimulation

LSM Stimulation ×
Speed: 10.0 us/pixel
Pixel: Line: Frame:
Duration : 0.000 msec 🗸 Continuous
Main
Laser ND Filter: 10%
■ 405 10.00 % ▲ ▶ 8 405 (3)
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MAP TEST
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 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: 🗖 🖸 🖾 🔇 🔪 🕹 🗧 🖬 🖬 🗛 🖉 🛍 🖽

- 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.
- 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

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

•Zoom

 \rightarrow 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 guality.)
- 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 .
- 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 start button.

Append Images

Analysis X Single process Live processing Processing Processing Item Z Gaps Vedit Image Append Images Material 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
Merge Channels Extraction FRET Acceptor Photobleach Sensitized Emission EDAN Input / Output setting Processing Item Input Output Output Type Channel Name Append Images timelapse 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.
S Processing Property Preview Category Item Value ▼ Advanced Dimension T Interval Type Auto	Input / Output setting Processing Item Input Output Output Type Channel Name Append Images I timelapse Cutput1 New Image Append Images timelapse oneshot2 timelapse2 Zstack Zstack2 [NO INPUT]
Appended Series Uniterapsez	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".
	Processing Property Category ▼ Advanced Dimension Interval Type Auto Appended Series Appended Series None Open File timelapse2 oneshot oneshot2 timelapse2 timelapse2
Progress Process Stop	 Press the Process button.

Extraction

Analysis ×	 Select the [Analysis] in the [Tool window] menu. Select the [Single process] and [Post processing] in the [Analysis] tool window.
Processing Item Remove 2 Gaps Save Edit Image Append Images Merce Channels Extraction FRE1	Single process Multiple process
Acceptor Photobleach Sensitized Emission CDAD Input / Output setting Processing Item Input Output Output Type Channel Name Extraction Itimelapse Output New Image Extraction	 ③ Open the [Processing] tab. ④ Select "Extraction" in the [▼Edit Images]. ⑤ Click the [Input], and select the image for image processing.
Processing Property Preview Category Item Value ▼ Advanced TIMELAPSE Step Size[15] TIMELAPSE Rance 2.4	Input / Output setting Processing Item Input Output Output Type Channel Name Extraction I timelapse V Output New Image Extraction timelapse2 Zstack Zstack Zstack2 oneshot2 (NO INPUT)
▼ Selected Channels CH1, CH2 ✓ CH1 ✓ CH2 □ CH3 □ All	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→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.
Progress Process Stop	⑦ Press the Process button.

<u>Merge Channels</u>

Analysis X Single process Live processing Post.processing B(G) Processing Graph Processing Item Z Gaps Edit Image Coard Load	 Select the [Analysis] in the [Tool window] menu. Select the [Single process] and [Post processing] in the [Analysis] tool window. Single process Multiple process
Append Indues Merge Channels Extraction FRET Acceptor Photobleach Sensitized Emission coAn Input / Output setting Processing Item Input Output Output Output Type Channel Name Merge Channels Merge Channels	 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. Select the different image and channel to be compared in the [Discossing Durport 1]
Processing Property Preview Category Item Value Advanced Selected Channels (oneshot) CH1, CH2 CH3 All CH3 Merged Series oneshot2 CH1, CH2 CH3 All oneshot2 CH4, CH2 CH3 All Merged Series Oneshot2 CH4, CH2 CH3 All CH3 All	Processing Property Preview Category Advenced Selected Channels (oneshot) CH1, CH2 CH2 CH3 All Merged Series None Open File oneshot2 timelapse2 Zstack2
Progress Process Stop	⑦ Press the Process button.

Colocalization



- 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.
- \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.
- Numerical data 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



- 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…)
- (5) Press the \underbrace{Next} button.
- 6 Choose the "Laser Scanning Confocal" or "FLUOVIEW FV3000" in Modality.
- ② Set the Algorithm and parameters.

8 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.

R Analysis ×	
Single process	Multiple process
Live processing	Post processing
Batch Processing Graph	
Processing Item	Remove
Spectral Deconvolution	Save
Rolling Average/Accumulate	load
Ratin	Load
Concentration	
Projection	
Z Gaps	
▼ Edit Image	
Append Images	
Merge Channels	
Extraction	
▼ Noise	
Noise Reduction	
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

Analysis × 1 Single process Multiple process Live processing Post processing Batch Processing Graph	■Availa →Selec Process
C Gaps ▼ Edit Image Append Inages Batch Brocessing Granb	① Sele proc
Target Images Add Remove Name Path 5	② Sele
1.25X-FV12005 C:/Users/olympus/Desktop/FV3000 Image-data/1.25X 1.25X-FV3000 C:/Users/olympus/Desktop/FV3000 Image-data/1.25X Brain-1.25X C:/Users/olympus/Desktop/FV3000 Image-data/1.25X	③ Pres
Save Location D:/20171107 Select	④ Spe
	⑤ If th with ※w
	acqu
	6 Pres folde
	⑦ Sele proc
Einala Eliaa [General] Path C:/Users/olympus/Deskton/EV3000 Imane-data/1.25X	8 Sele adde
Name 1.25X-FV3000.oir Scan Mode XY Date 04/12/2016 12:08:33.453 PM	9 Click
File Version 2.1.2.1 System Name FV3000 System Version 2.1.1.93	10 Pres
Batch Processing Graph	
∠ Gaps ✓ Edit Image Append Images Extraction ✓ FRET Acceptor Photobleach Sensitized Emission ✓ EDAn	※Only If you please
Input / Output setting Processing Item Input Output Output Type Channel Name	
Merge Channels oneshot Output1 New Image Merge Channels	

Available condition

 \rightarrow 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.
- If there is an unnecessary image, delete it with [Remove].
 *when the data is selected , image and acquisition condition are displayed.
- 6 Press the [Select] to specify the location folder.
- $\ensuremath{\textcircled{}}$ 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".
- 1 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 data, time can be reduced.

Analysis × 1 Single process Multiple process Live processing Post processing Batch Processing Graph	 Select the [Analysis] in the [Tool window] menu. And select the [Single process] and [Post processing].
∠ Gaps ▼ Edit Image	② Select the [BATCH] tab.
Apper I 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	 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/
MATL_A01_G002_0002 C:/Users/Administrator/Desktop/image/MATL_Cycle_02 MATL_A01_G002_0003 C:/Users/Administrator/Desktop/image/MATL_Cycle_02 MATL_A01_G002_0004 C:/Users/Administrator/Desktop/image/MATL_Cycle_02 MATL_A01_G002_0005 C:/Users/Administrator/Desktop/image/MATL_Cycle_02 MATL_A01_G002_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.
Batcy Processing Graph	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] TOUTput New Image Projection [BATCH] [NO INPUT]	Molecular Projects Tool Window Tools Window Help Open Image Ctrl+O Save MATL Save As List
Processing Property Preview Category Item Value Basic All Frame (BATCH) True Vadvanced	Imatl.omp2info 2016/11/22 14:04 OMP2INFO ファ 5 KB Imatl_forVSIimages.omp2info 2016/11/22 14:04 OMP2INFO ファ 5 KB
All Channel (BATCH) True Type Intensity by Z Mode Max Z C I I Intensity I Max	IPress the and select [Map] tab.
SoldAmentatics (Shi American)	 Press the to display the image processed. (Not displayed when the number of tiling is large) Confirm that the image is displayed, then press to execute the process.
Matter grape of Mattering ● Or = OF = Smoothing ● Or = O = To = 100 ● % ● O(~~

Execute Cance

Sequence Manager (making protocol of acquiring)



New registration of Dye



New registration of Dye

	_		_			
Default Dyes :			User Dyes			
Dye	4			Dye	A	
Violet			Violet		=	
Cyan			Cyan			
Blue			Blue			
Yellow		>> Copy >>	Yellow			Delete from list
Orange			Orange			Edit
Red			Red			Duplicate
Acridine Orange			Acridine	Orange		New Dve
Alexa Fluor 405	•	1	Alexa Flu	or 488	•	Dye combination
Dye Name :	Alexa Fluor 48	8		Observation pa	rameters ——	
Abbr.(MAX10) :	Alexa 488			Excitation laser :	100	
Manufacture				Substitution :	488	
				Substitution.		_
College LUT				Automatic emis	sion priority :	
Color LUI :	Green			1:	500 - 600	
MAX absorption :	499			2:	500 - 580	
MAX emission :	520			4:	-	J
Absorption curve :	Export					
Emission curve :	Export					
V (405) : 43 C (440) : 46 B (488) : 56 5	30-470 50-500 00-600 00-580 00-540					
E (514) : 53	30-630					
:5	30-580					
Y (561) : 57	/0-6/0					
: 5	/0-620					
0 (594) : 6	10-710					
R (640) : 6	50-750					
■ selecting Ex + the main laser	- automa waveleng	atically select oth.	S			
	tl	nina other				

Spectral Imaging



Lambda series(1) (Ch setting)

OLYMPUS FV318-SW	
File Tool Window Tools Window Help	
Layout	
Ocular LSM Imaging × Observation Method -	Acquire ×
▼ Scan Settings	Normal Sync Sequence MATL
Scanner:	LSM Start
Type: 🔍 Galvano 🔍 Resonant	SERIES DONE Annual
Mode: 🥥 OneWay 🔿 Roundtrip	
Interlace: OFF O 2x O 4x	APPENDINEAL
Speed: 2.0 us/pixel 🗐 🕛 🕂	ISM Cilling data and Darking Data
Image Size:	L3M C. LUSers Lolvin Dus LDesktop
Aspect Ratio: 11 43	
Scan Size 512x512	Current scan condition
High voltage correction: VON VOIF	
Pixel: 2.0 usec Line: 2.118 msec Frame: 1.087 sec	
✓ Area Settings	
e a	
Rotation 0.0 7 deg Reset	
Pan X: 0.00 🕏 um Reset	Remaining time : -
Page Victoria Page Page	Next scan start at
Pan T. 0.00 Willin Reset	PMT Setting *
1	
200m 1.00	Mode : 🔍 VBF 🔍 Lambda
x1 Optimize @	Average: O None O Line O Frame 3 🕆 Times
Dound tele constition	Dye & Detector Select
 Round trip correction 	Confocal Aperture Autor 205 \$um
Series × Microscope Information -	50 100 200 400 800 800
LSM ON OFF	Ary Disk x 100 x1 x2 x3 x4
	Laser ND Filte 🔿 None 🔎 10%
Z : ON OFF	= 405 0.00 % 🕷 💌 🖿
	= 445 0.00 % 🗷 💌
Series: 17.822 sec	₹ 488 1.00 % ₹ ₹
▼ Time Lapse	= 514 0.00 % ★ ►
LSM Total: 0.00.17.82	504 0.00 % M M
Interval: FreeRon	640 0.00 % *
- Scan: 0:00:17.82 Rest: 0:00:00.00	
Cycle. 2 V LEWINGLENabled	
▼ Z Section	Gain 1.000 × 🔍 🕨
Motor: Start/Eni Range	Offset 0 %
Curren4026.59 Origin: 200.00	Spectral Setting
Register	
Move	
Slices: 13	
Step Size: 0.56 🗣	ato son eon rott son
Optimize	Pand Midth 100 🛎 nm
Start: 0.00 🗘 End: 6.72 🕏	Step Size 5 \$ mm
Register Register	
Move START <=> END Move	CHI 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
 →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	Lambda
Average: 💿 None	🔘 Line 🔘 Frame 📑 🔚 Times
Dye & Detector Select	
Confocal Aperture Aut	o 219 ♦um
Airy Disk x 1.00	
Laser ND Filte 🔵 None	x1 x2 x3 • 10%
405 0.00	% • •
445 0.00	%
⊻ 488 <u>1.00</u>	%
□ 514 <u>0.00</u>	% Change laser, band width or step size
≤ 561 0.00	%
594 0.00	% Change laser, band width or step size
640 0.00	
⊻CH1	HSD1 35 🔷 - 545nm
HV 390	
Offset 0	%
⊻ СН2 📃	▼ HSD2 85 🗣- 595nm 🕕
HV 470	$\forall \bullet \bullet$ (12)
Gain <u>1.000</u>	
	%
 Spectral Setting 	
	R
400 500	0.05 0.05 0.03
Band Width 10 ♣ nm	Linked
CH1: 00 ₹ - 5 CH2: 57(₹ - 3	5(〒nm 9 〒 steps 10 ♦ nm 11 ♦ steps
Total:	20 steps
Acquire ×	
Normal Sync	Sequence MATL
Imaging	Bleach
류 LSM Start	Stimulation Start
SERIES DONE	Append
APPEND NEXT	1 -
LSM D:\Images Image001	a\Demo data at TOBIC\2014 📴 🕐 🗗

Setting the wavelength

 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

- ⁽¹⁾ 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 Volume Setting Graph Table	1 Press the Viewer putton to switch
Single process Multiple process	to "Viewer mode".
Live processing Post processing	② Select [Analysis] in [Tool Window]
Processing Item	menu. [Analysis] Tool Window appears.
Rolling Average Ratio	③ Select [Single process] mode, press the
Concentration Projection Z Gans	[Post Processing] button.
Edit Image Append Images	Single process
Input / Output setting Processing Item Input Output Output Type Channel Name Spectral Deconvo Tambda Output1 New Image Spectral Deconvo	Live processing Post processing
5	④ Press the Remove button to reset the assigned item and select [Spectral
Processing Property Preview	Deconvolution] in [Processing Item].
Category Item Value 🔺	⑤ In [Input] in [Input / Output setting],
Au Frame (Jamoda) True Advanced Target Channel CH1	select the image for image processing.
Mode Blind Unmixing 6	6 In [Mode] in [processing Property], select
DYEO Dye Profile Save Folder DYEL Dye Profile Save Folder	"Blind Unmixing.
DYE2 Dye Profile Save Folder DYE3 Dye Profile Save Folder Background Correction False	Category Item Value
Progress : 8 Process Stop	All Frame (lambda) True Advanced Double click
Preview Object: lambda	Target Channel CH1 CH1 CH1 CH1
Frame Index: L 1 - /11 Processing Item:	Mode Blind Unmixing Number of SpectralData[1_11]
Channel (Before):	DYED Dye Profile Save Folder OVE1. Dye Profile Save Folder
	DYE2 Dye Profile Save Folder DYE3 Dye Profile Save Folder
	Background Correction False

- Set the number of dyes in [Number of Spectral data].
- (8) Press the **Process** button to start the fluorescent separation process.

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

Analysis X Volume Setting Graph Table	 Press the viewer button to switch to "Viewer mode".
Live processing Fost processing Batch Processing Graph Processing Item Spectral Deconvolution Rolling Average Ratio Concentration Projection	 Select [Analysis] in [Tool Window] menu. [Analysis] Tool Window appears. Select [Single process] mode, press the [Post Processing] button.
Z Gaps • Edit Image Append Images Input / Output setting Processing Item Input Output Output Type Channel Name Spectral Deconvo Iambda Output New Image Spectral Deconvo	Single process Multiple process Live processing Post processing
5	④ Press the Remove button to reset the assigned item and select [Spectral Deconvolution] in [Processing Item].
Category Item Value Category Item Value All Frame (lambda) True Advanced Tamet (based Ch1	 In [Input] in [Input / Output setting], select the image for image processing.
Mode IDYED Dye Profile Load file Background Correction False	In [Mode] in [processing Property], select"Nomal Unmixing"
Progress : Stop	Processing Property Category Item Value * Basic All Frame (lambda) True * Advanced * Tarmet Channel CH1
Frame Index: L 11 /11 Processing Item: Channel (Before): Channel (After):	Mode Normal Unmixing ▼ IDVIG Dye Nvotife Isad File Background Correction 6 Normal Unmixing Normal Unmixing
	⑦ In "!DYE0 Dye Profile Load File", select the first dye profile among from dye data.

- Select the all data.
- 9 Press the Process button to start the fluorescent separation process.

<u>Processing : Unmixing(3)</u> <u>Spectral Image Unmixing</u> <u>~by specifying dyes~</u>

Analysis × Volume Setting Graph Table -	 Press the <u>Viewer</u> button to switch to "Viewer mode".
Live processing Batch Processing Graph Rehove Spectral Deconvolution Save Load Ratio Concentration	 Select [Analysis] in [Tool Window] menu. [Analysis] Tool Window appears. Select [Single process] mode, press the [Post Processing] button
Projection Z Gaps * Edit Image Append Images Input / Output setting Processing Item Input Output Output Type Channel Name Spectral Deconvol. Iambda Output New Image Spectral Deconvol.	Single process Multiple process
Processing Property	④ Press the Remove button to reset the assigned item and select [Spectral Deconvolution] in [Processing Item].
Category Item Value Category Item Value Category Item Value Category Item Value Category Fasc Catego	⑤ In [Input] in [Input / Output setting], select the image for image processing.
Target Channel CH1 ✓ CH1 Mode Spectral Image Unmi_ DYE0 ROI File Name Open File. Remove DVF	 In [Mode] in [processing Property], select "Spectral Image Unmixing.
DYE0 ROI Index DYE0 Dye Profile Save Folder Iambda.0002 Progress: Progress: Proview Object: Iambda.0001 Proview Object: Iambda	Company Imm Verain Company Imm Verain Imm Imm Imm Imm Imm Imm Imm Imm Imm
Frame Index 1 1 (6)Select the mode Processing Item: (8)select the file Channel (After): (1)	⑦ Specify multiple ROIs on the regions where only the target fluorescence dye locates to acquire the spectral data for image processing.
CHI (197) Jones Theor 568	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. Image: specified in ⑦. Image: speci
	 10 Defendes See Todar 10 Repeat (8)(9) to register all ROIs. Press 10 Repeat (b)(9) 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 Laser	Software settings
XY Stage	Font size Default Large
Plate Software	Sample
IR laser emission	

Reset the layout

Window Help Full Screen Mode Alt+Shift+Enter Close all images Elive 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 Purpo	ose.				
This	wizard need	is to know	which pur	pose to exp	ort.	
	Select th	e purpore t	then click N	evt		
	Jaectan	e parpose, t	anen cilot n		7	
	For an	alysis of pro	oblems	2	1	
			Back	Next	Ca	ncel

FV Log Exporter - Export Options Export Options. This wizard needs to know which type of informations to expo	× ort.	FV Log Exporter - Custom Selection X Custom Selection. Select options to export informations.
Select the option, then click Next. Standard Export all system informations. Custom You may choose individual options to be exported.		Choose an option and then click Next. IF OS setting and error event files IF PV hardware data and ROM data files IF FV hardware data and ROM data files IF FV software configuration and log files
O S setting and error event files O S setting and arror event files O Hardware data and ROM data files O Hardware configuration and log files Back Next Cance	el	Back Next Cancel

Export for La	st DDD HH:MM
	7
C. Event in ran	ae of Date
Start	End
MM/DD/W	HH:MM MM/DD/YY HH:MM
07/21/10 -	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.



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