Acquiring image

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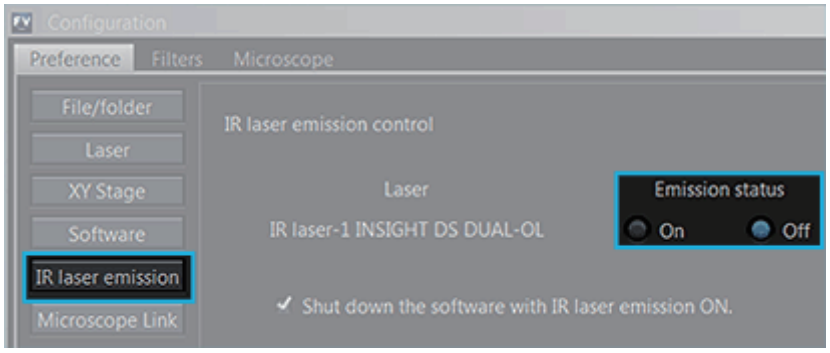
Acquiring image

Setting IR Laser

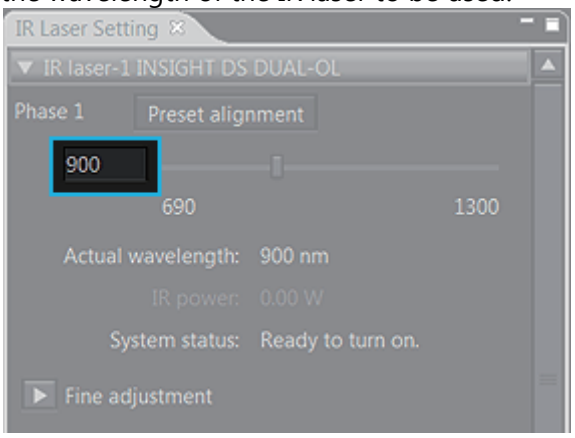
1. Select [Configuration] in the [Tools] menu on the software screen. The [Configuration] dialog box appears.

2. On the [Configuration] dialog box, press the IR laser emission button in the [Preference] tab to display the [IR laser emission control] screen. Select "On" in [Emission status] of the laser to be used.

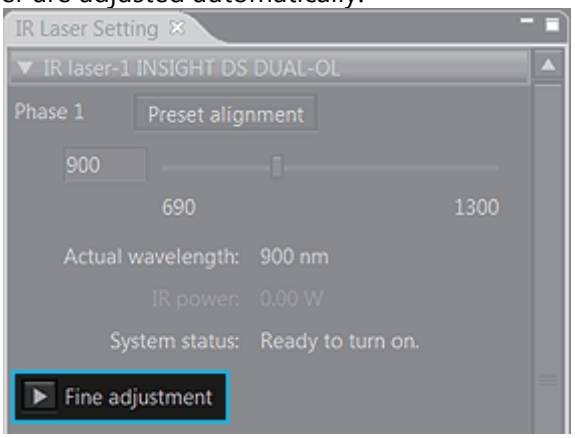


3. Press the OK button to close the [Configuration] dialog box.

4. On [IR Laser Setting] Tool Window, set the wavelength of the IR laser to be used.

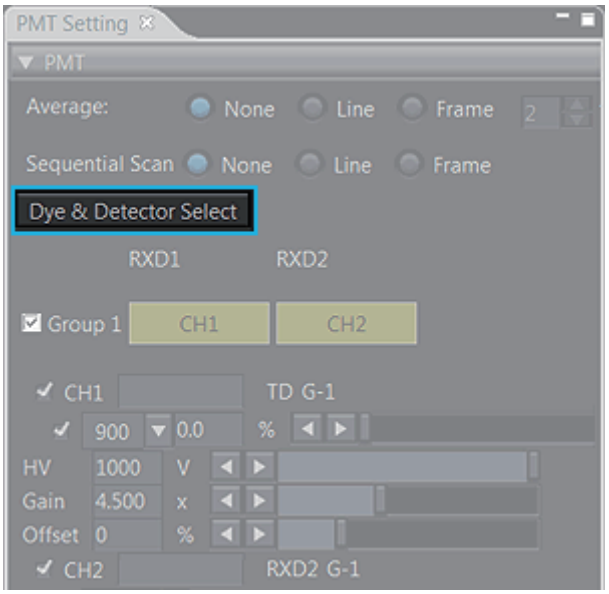


5 Press the ▶ button of [Fine adjustment] to display the screen, and press the Start active alignment button.
The optical guide systems for the IR laser are adjusted automatically.

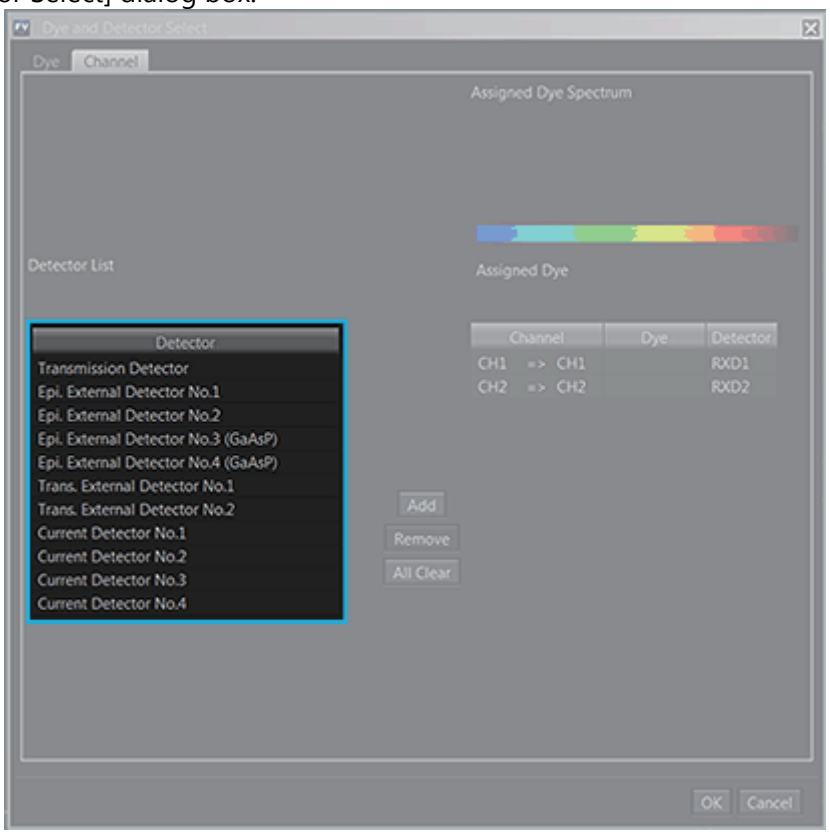


Assigning the detector to channel

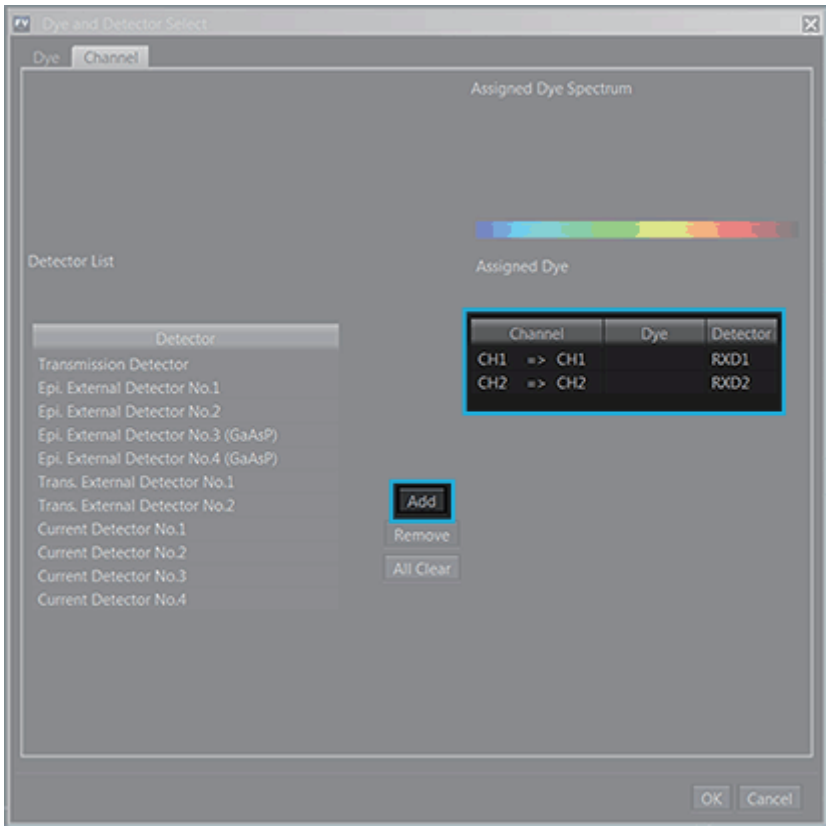
1 Press the Dye & Detector Select button on [PMT Setting] Tool window. The [Dye & Detector Select] dialog box appears.



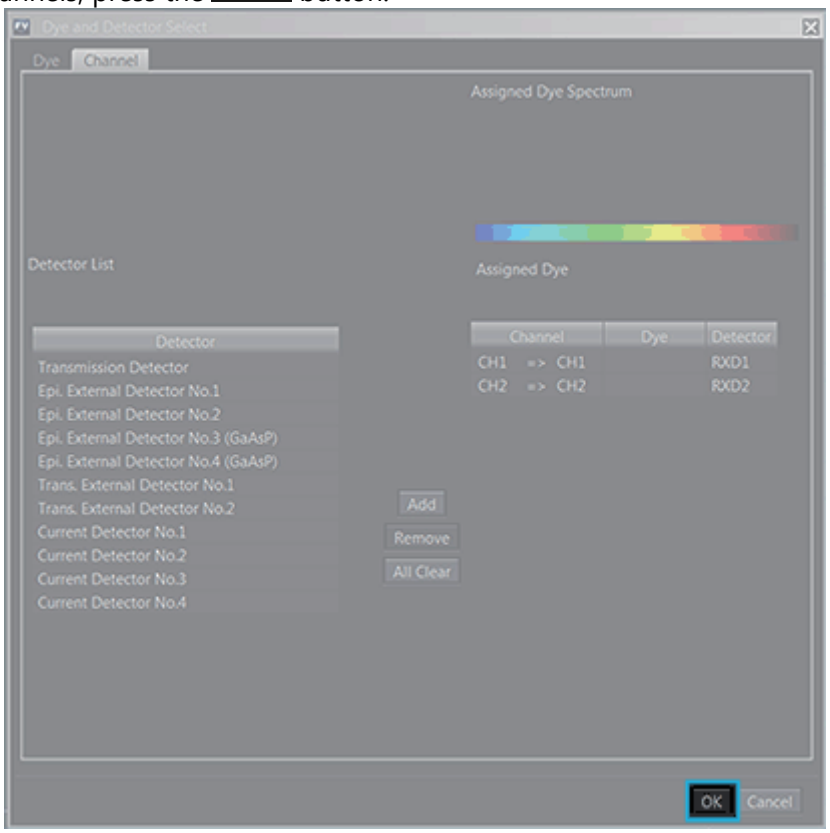
2 Select the detector to be assigned to the observation channel in [Detector List] on the [Dye & Detector Select] dialog box.



3 Press the Add button. The observation channel number and the abbreviation (physical channel name) of the assigned detector are displayed in the observation channel list.

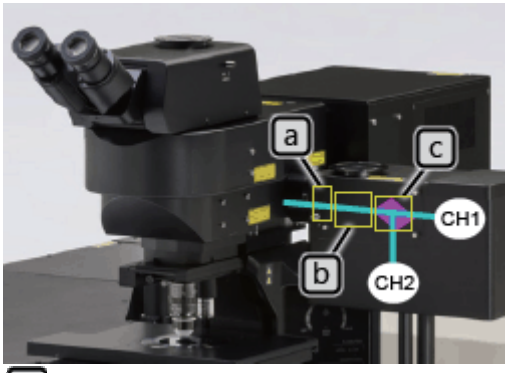


4 After setting all channels, press the OK button.

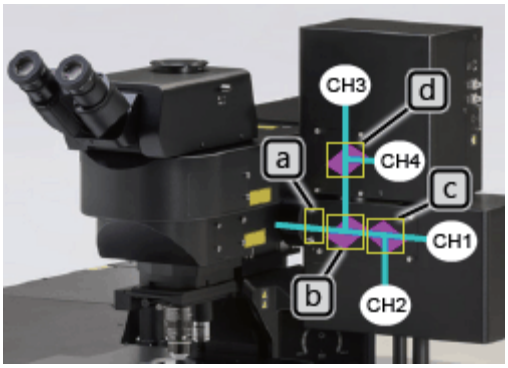


The following table shows the examples of the configuration of detectors connected and the assignments to observation channels.

Configuration examples of detectors and optical parts			CH	Detector	Abbr.
Reflected: 2CH			CH1	Epi. External Detector No.1	RXD1
a Filter	BA685	BA750			
b Cube	Out of light path				
c Cube	FVG FCY FGR	FRCY5	CH2	Epi. External Detector No.2	RXD2



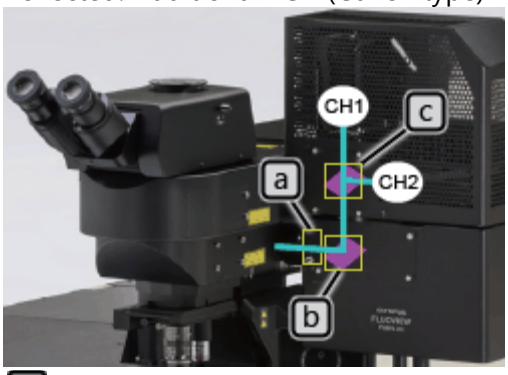
Reflected: 2CH + additional 2CH



a Filter	BA750 (BA685)	
b Cube	SDM570	
c Cube	FRCY5	
d Cube	FVG	

CH1	Epi. External Detector No.1	RXD1
CH2	Epi. External Detector No.2	RXD2
CH3	Epi. External Detector No.3	RXD3
CH4	Epi. External Detector No.4	RXD4

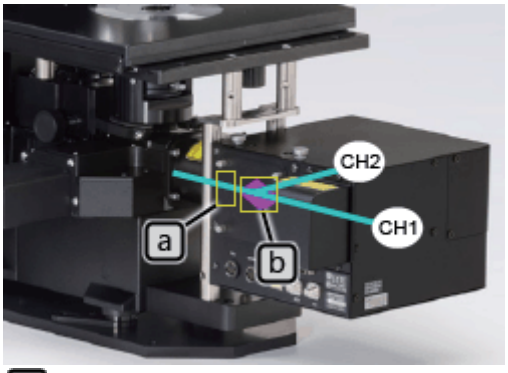
Reflected: Additional 2CH (GaAsP type)



a Filter	BA685	BA750
b Cube	SDM-M	
c Cube	FVG FCY FGR	FRCY5

CH1	Epi. External Detector No.3 (GaAsP)	RXD3G
CH2	Epi. External Detector No.4 (GaAsP)	RXD4G

Transmitted: 2CH



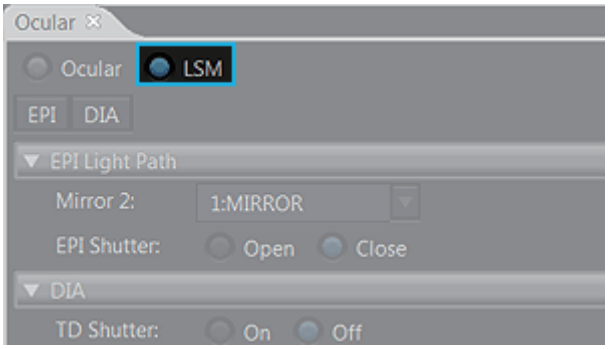
a Filter	BA685	BA750

CH1	Trans. External Detector No.1	TXD1
CH2	Trans. External Detector No.2	TXD2

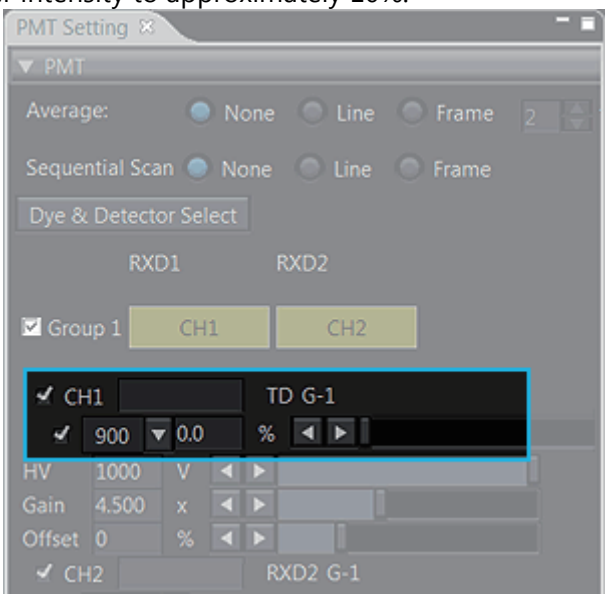
bCube	FVG FCY FGR	FRCY5

Adjusting the live image

1. Select "LSM" in [Ocular] Tool Window.

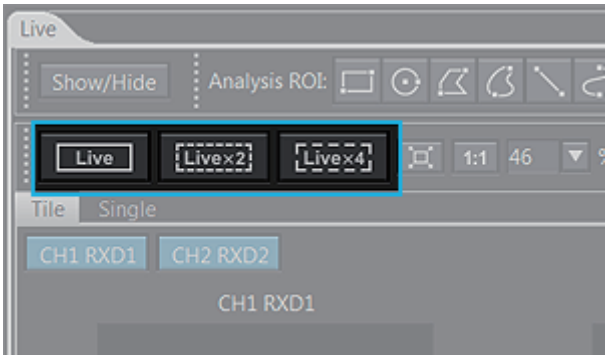


2. On [PMT Setting] Tool Window, tick the checkboxes of the channel to acquire the image and the laser, and set the laser intensity to approximately 10%.

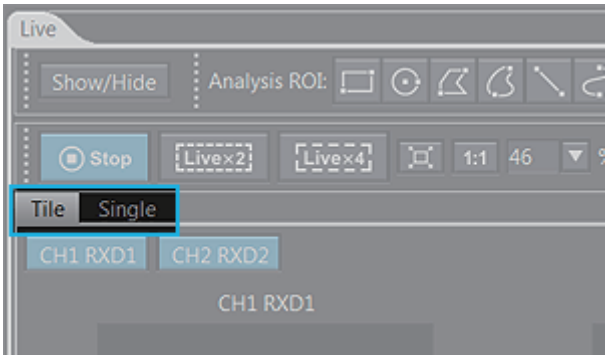


3 Press the Live button on the [Live] window. (The button view is changed to Stop .)
The live image (repeat scan image) is displayed on the LSM live area.

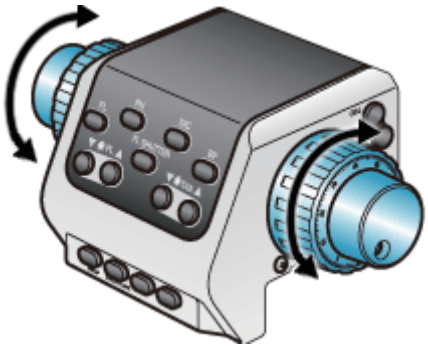
Pressing the Live×2 button or the Live×4 button will display the live image whose repeat interval is reduced by subtractive scan. This function is useful when searching the cells or observing with moving the focus.



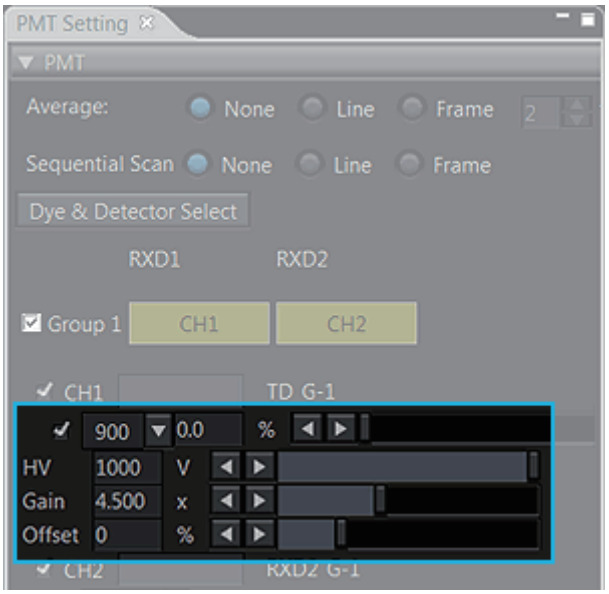
4 Select the display method of the live image.
[Tile] : The LSM live area is divided, and the image of each channel and the image composing all channels are displayed side by side.
[Single] : The image composing all channels is displayed over the entire LSM live area.



5 With viewing the live image, rotate the focusing knob of U-MCZ to bring the specimen into focus.

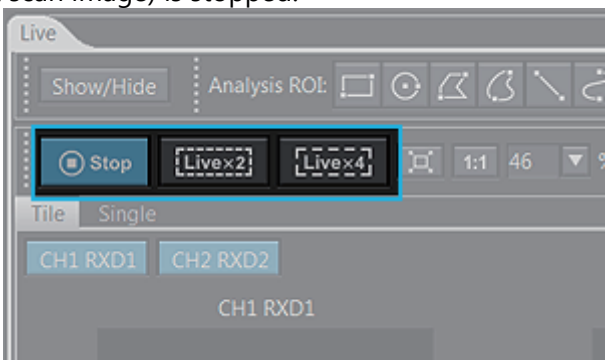


6 On [PMT Setting] Tool Window, set Laser Intensity (%), Sensitivity (HV), Gain and Offset to adjust the live image.

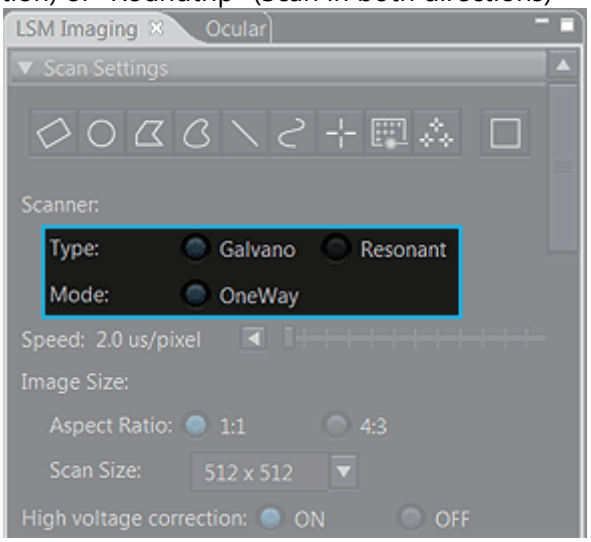


7 Press the 

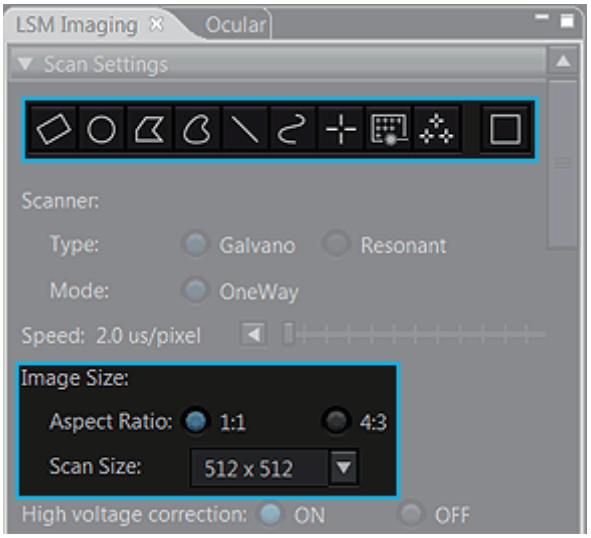
button on the [Live] window.
The display of the live image (repeat scan image) is stopped.



8 On [LSM Imaging] Tool Window, select the scan method in [Type] and [Mode].
[Type] : "Galvano" (High definition scan) or "Resonant" (High speed scan)
[Mode] : "OneWay" (Scan in one direction) or "Roundtrip" (Scan in both directions)



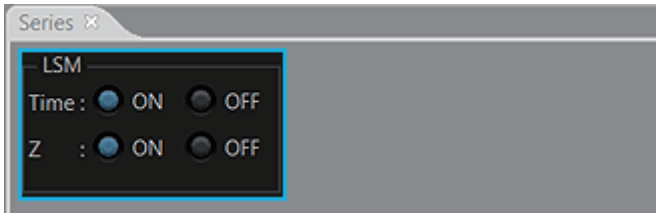
9 Specify ROI or [Image Size] to set the scan area.



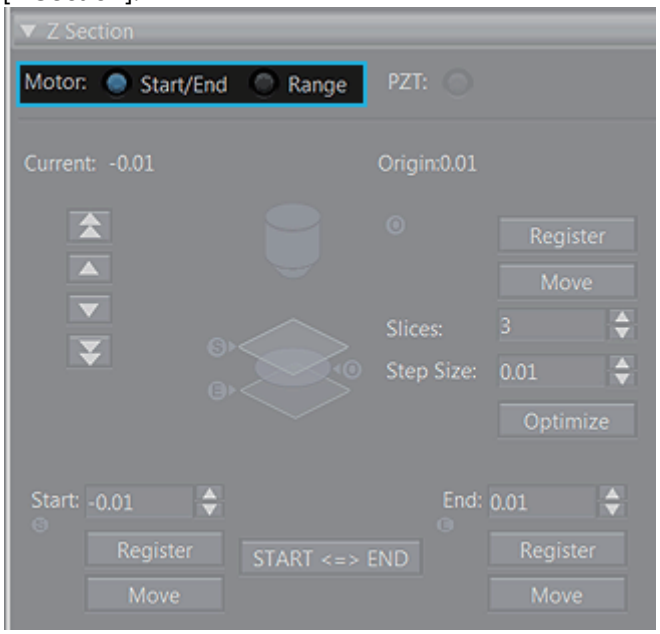
Acquiring the series image

Setting Z series (Specifying start/end positions)

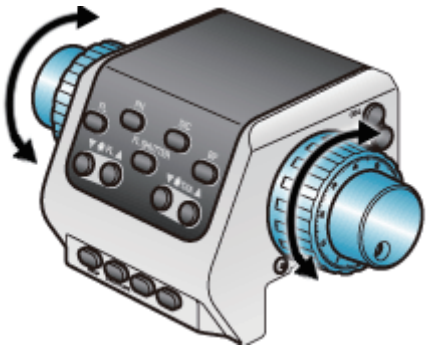
1 Select "ON" in [Z] on [Series] Tool Window.



2 Select "Start/End" in [Motor] on [Z Section].

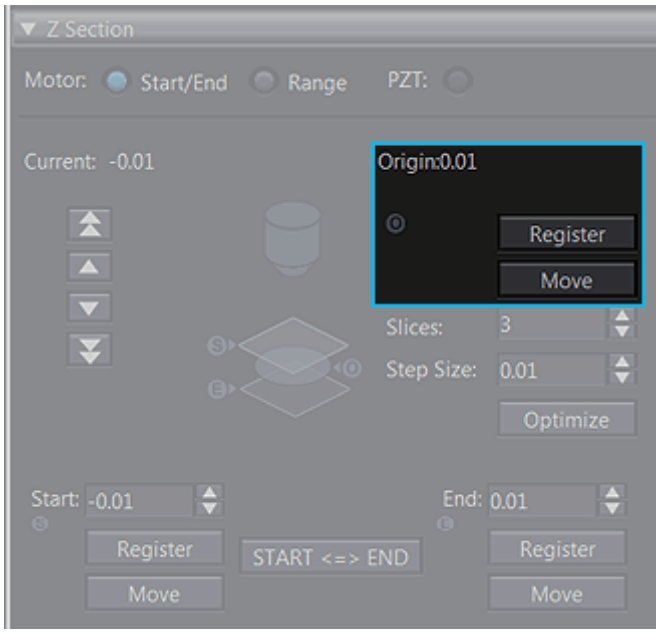


As described below, set Z series with changing the Z position by rotating the focusing knob of U-MCZ.



3 Press the Register button in [Origin] at the Z position which is the reference position to acquire the images.

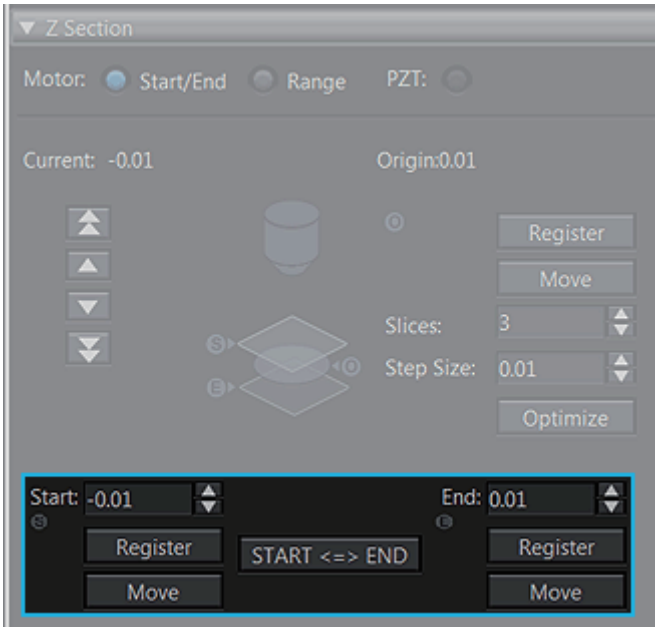
The distance from the Z position registered in [Origin] to the current Z position is displayed in [Current].
If you register the top surface of the specimen in [Origin], the depth from the top surface to the observation position is always displayed in [Current], which is very useful.



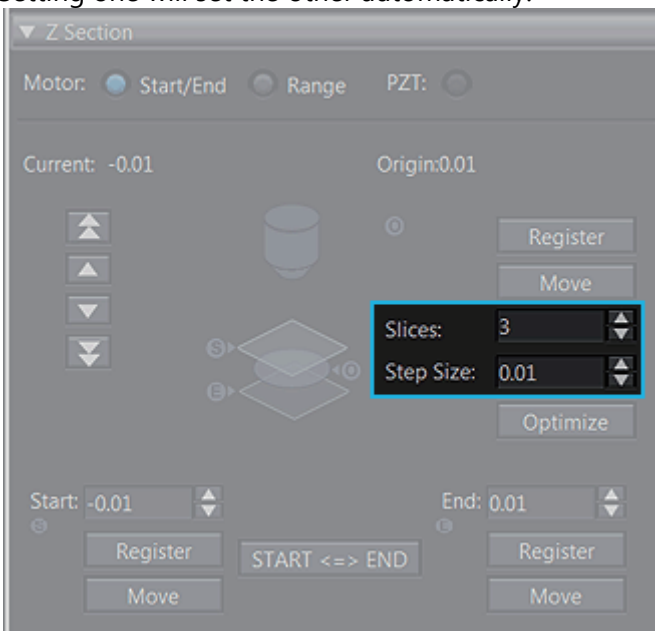
4 Press the Register button in [Start] at the Z position to start acquiring the image.

Then, go on to press the Register button in [End] at the Z position to end acquiring the image.

Press the START <=> END button to reverse the start position and the end position.



5 Set either [Slices] or [Step Size]. Setting one will set the other automatically.

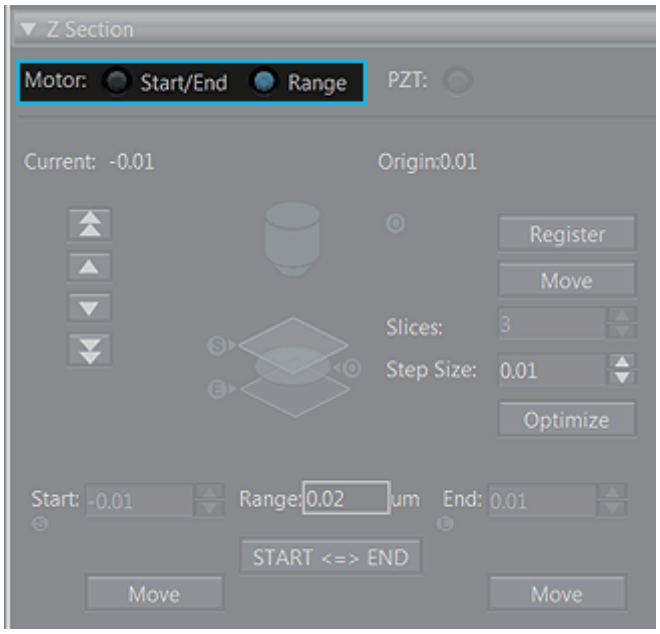


Setting Z series (Specifying the center position and the range)

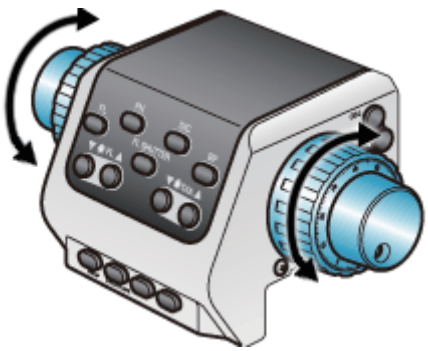
1. Select "ON" in [Z] on [Series] Tool Window.

2. Select "Range" in [Motor] on [Z Section].

"Range" is suitable when you set the acquisition area in the Z direction assuming the displacement of the focus position during acquiring T series images.

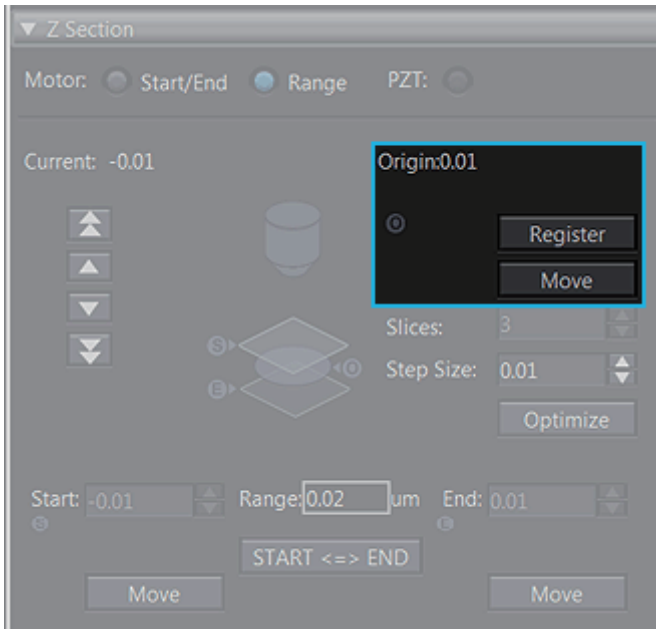


As described below, set Z series with changing the Z position by rotating the focusing knob of U-MCZ.



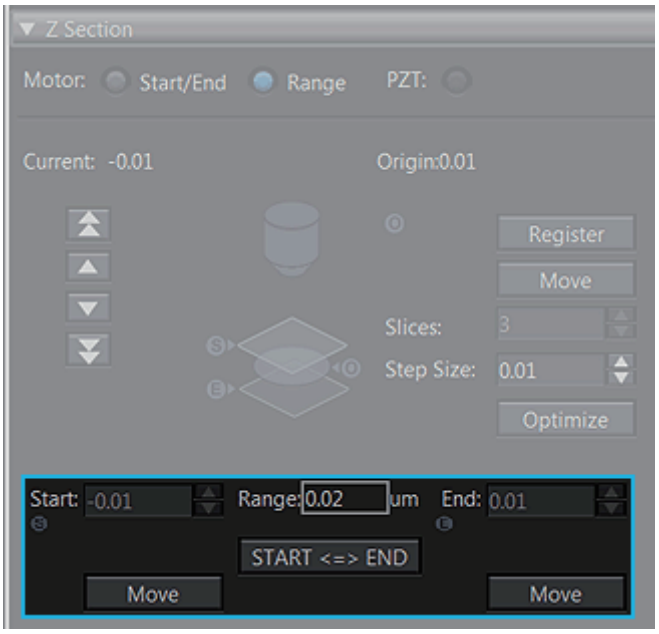
3 Press the Register button in [Origin] at the Z position which is the reference position to acquire the images.

The distance from the Z position registered in [Origin] to the current Z position is displayed in [Current].
If you register the top surface of the specimen in [Origin], the depth from the top surface to the observation position is always displayed in [Current], which is very useful.

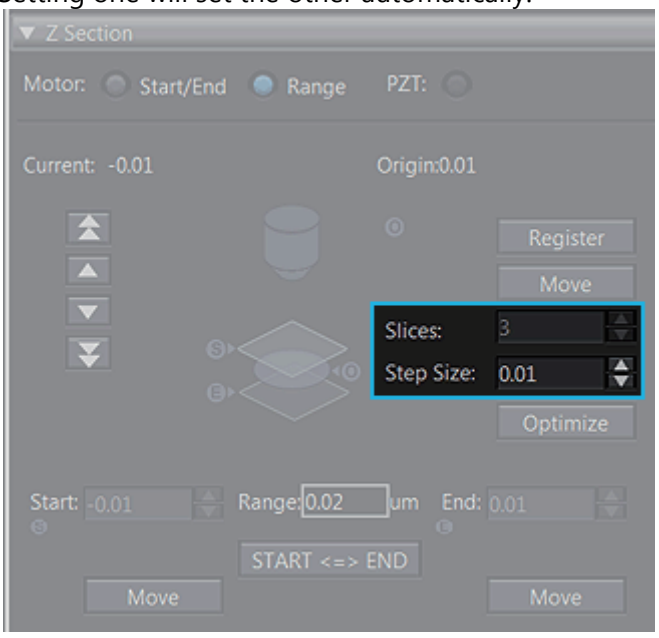


4 Set the range to acquire the image in [Range]. The start position and the end position to acquire the image are set automatically.

Press the START <=> END button to reverse the start position and the end position.

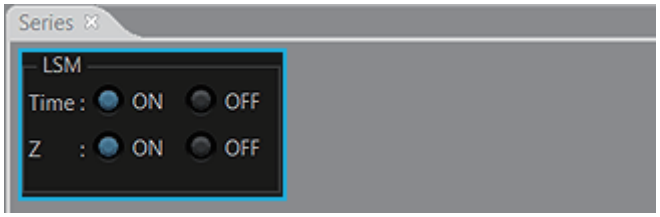


5 Set either [Slices] or [Step Size]. Setting one will set the other automatically.



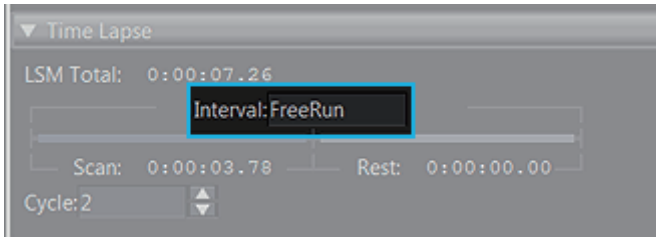
Setting T series

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



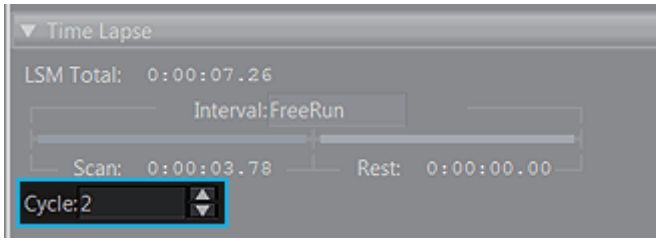
2. Set the interval to acquire the image in [Interval] on [Time Lapse].

   If you attempt to set the time shorter than the time displayed in [Scan] (time taken for acquiring 1 cycle image) in [Interval], "FreeRun" appears. In this case, the interval to acquire the image is the time displayed in [Scan].



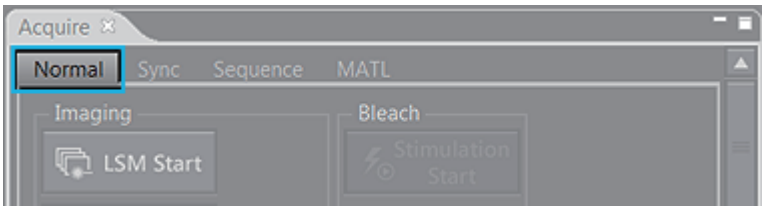
3. Set the number of image acquisitions in [Cycle].

   The time from the start of the T series image acquisition to the end of the T series image acquisition is calculated and displayed in [LSM Total]. Set [Cycle] so that [LSM Total] becomes an appropriate time.



Starting acquisition

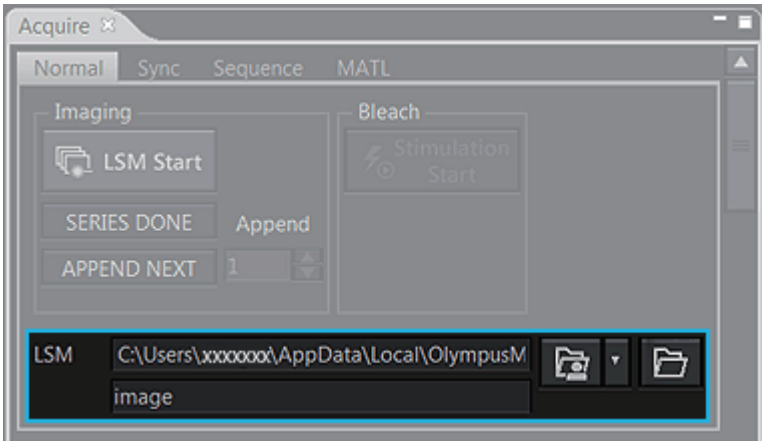
1 Select [Normal] tab in [Acquire] Tool Window.



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

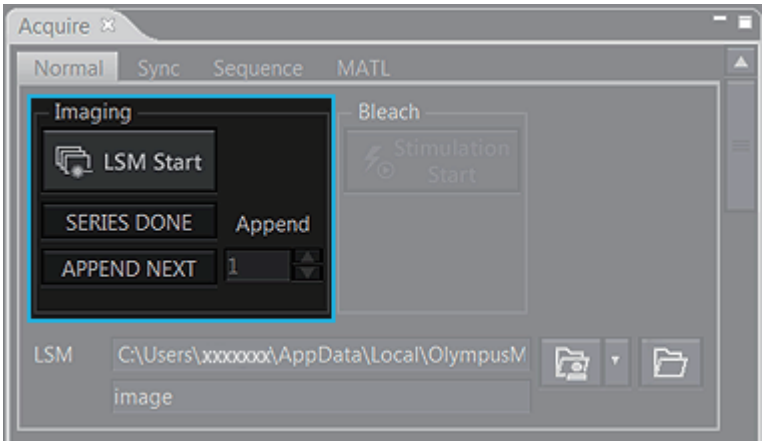
In order to organize files easily after acquiring the images, it is recommended to create a new folder before acquiring the images and specify that folder as the save destination of the images.

The acquired images are saved automatically.



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

When the image acquisition starts, the [Image] window opens and the image in process of acquisition is displayed.



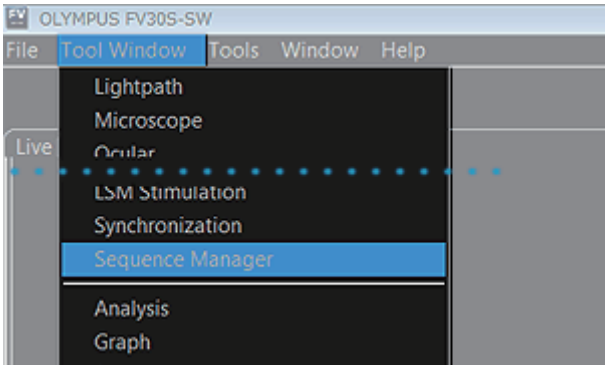
4 After the image is acquired, pressing the APPEND NEXT button allows you to perform the image acquisition repetitively under the same condition. Press the SERIES DONE button to compete the image acquisition.

Automation of acquisition

Planning acquisition (Creating protocols)

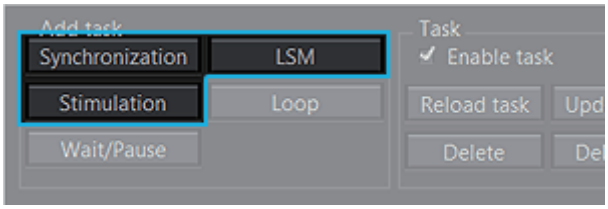
The tasks, such as to acquire, stop, loop images or etc. are registered as a series of flow. This series of flow is called Protocols. The protocols are created in [Sequence Manager] Tool Window .

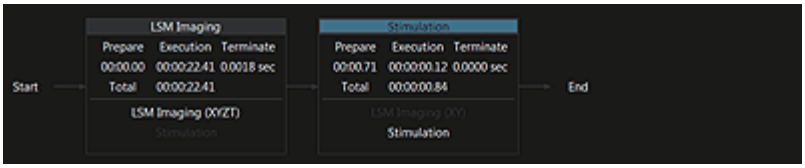
1. Select [Sequence Manager] from the [Tool Window] menu. [Sequence Manager]M Tool Window appears.



Registering Acquisition task

1. Press the [Add task] button to register tasks.
   If there are tasks which were registered, the new task is added to the last task.





Registering Wait task or Pause task

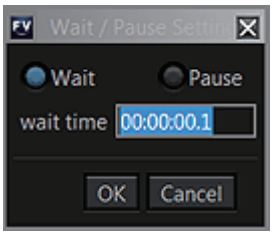
1 Press the Wait/Pause button in [Add task] to display the [Wait/Pause Setting] dialog box.

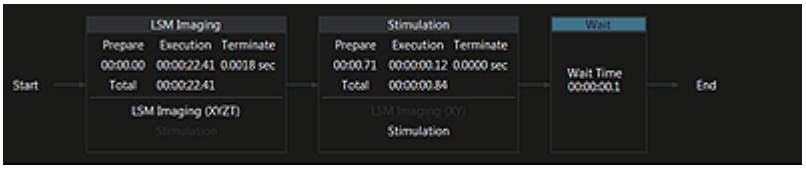
2 Select "Wait" or "Pause".

"Wait" : The execution of protocols is paused until the time specified in [wait time] elapses.

"Pause" : The execution of protocols is paused until the Resume button in [Acquire] Tool Window is pressed.

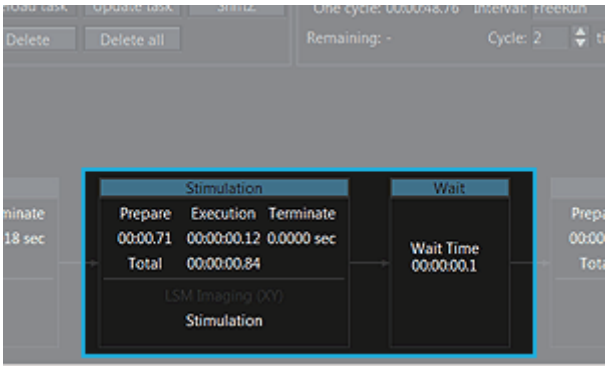
Press the OK button to register the Wait task or the Pause task.





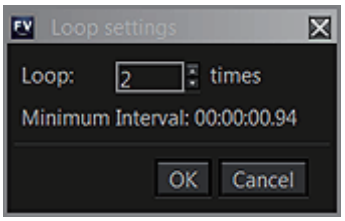
Registering Loop task

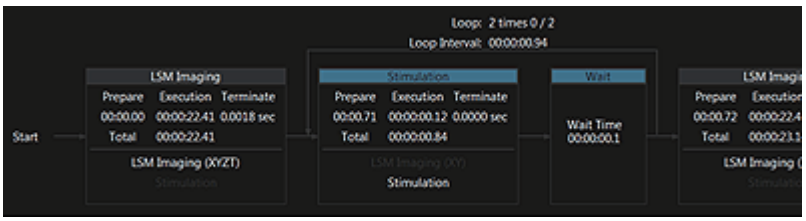
1. Click the task to be repeated. If you want to select multiple tasks, click them with pressing Ctrl key or Shift key.



2. Press the Loop button in [Add task] to display the [Loop Setting] dialog box.

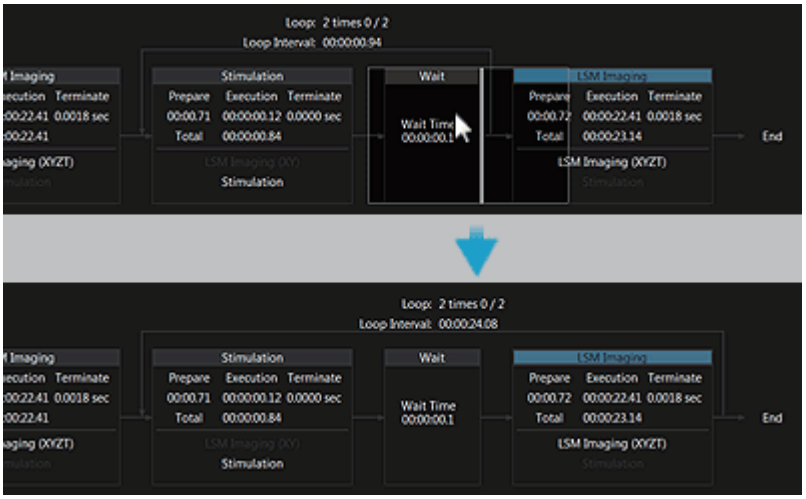
3. Set the number of repetitions in [Loop], and press the OK button. The Loop task is registered.





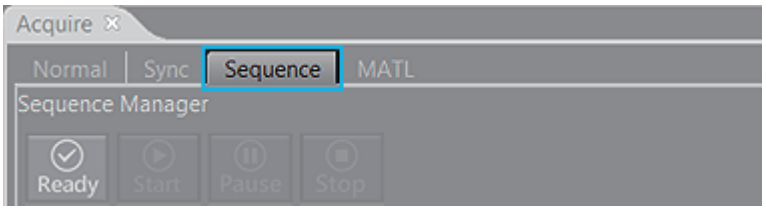
Moving the task position

1. When you drag the task you want to move, the vertical line appears to show the position to insert the task. Drag the task so that this line comes to the destination.



Executing and registering protocol

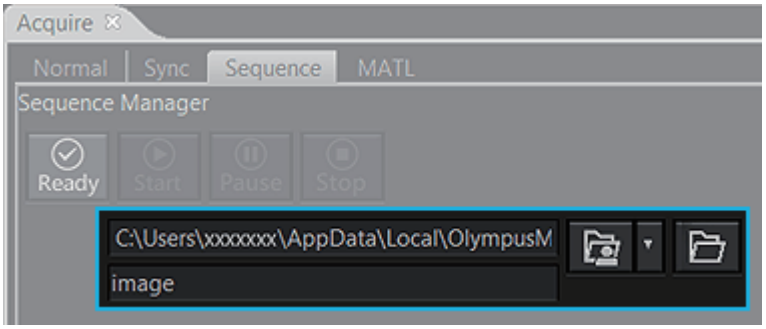
1. Select [Sequence] tab in [Acquire] Tool Window.



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

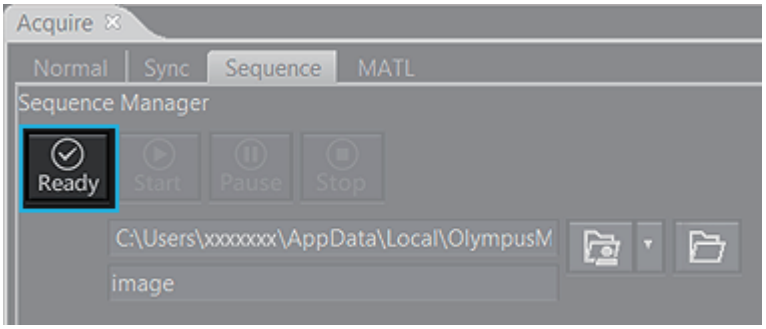
In order to organize files easily after acquiring the images, it is recommended to create a new folder before acquiring the images and specify that folder as the save destination of the images.

The acquired images are saved automatically.



3 Press the 

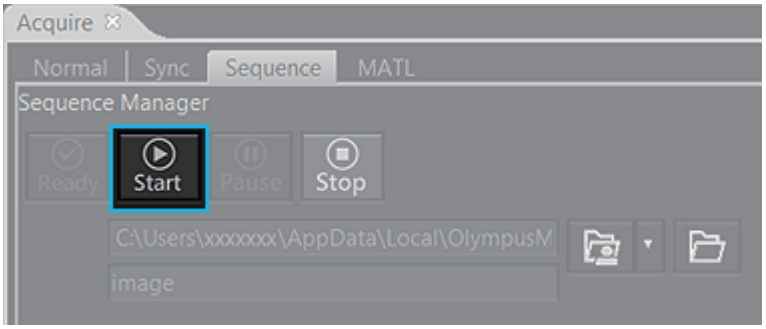
button. The protocols set in [Sequence Manager] Tool Window are registered to the system of the laser microscope.

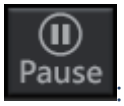


4

Press the 

button. The protocols start (are executed).



: The running protocols are paused.

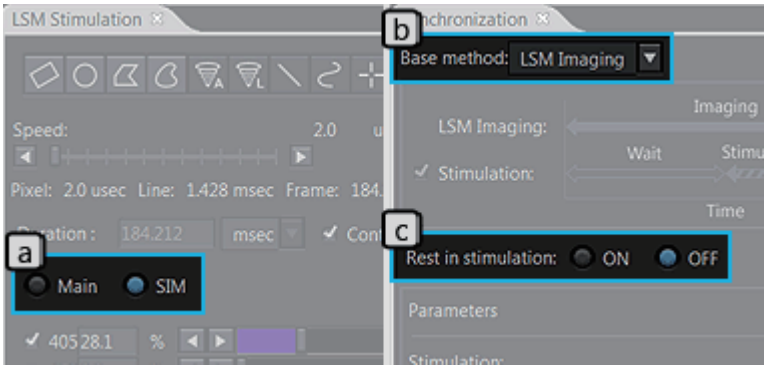
: The running protocols are stopped.

If you started the protocol by setting ON [append task as Ch/λ data] or [append each task in each cycle as T series data] in [Sequence Manager] Tool Window, do not close the window of the acquired image until the protocol finishes.
If the window of the acquired image is closed during executing the protocol, the append process is not executed.

Timing of image acquisition and light stimulation

Creating timeline

This function allows you to create the timeline of the image acquisition and the light stimulation.
The image acquisition is set in [Series] Tool Window and the light stimulation in [LSM Stimulation] Tool Window respectively in advance.
The timeline is created in [Synchronization] Tool Window, but the setting items differ depending on the combination of a, b or c to be selected.

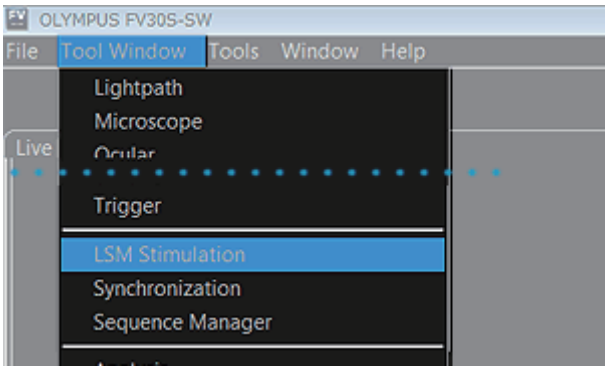


This section describes the operating procedures by using the following selection as an example. For other combinations, refer to Synchronization Tool Window in the Functional explanation section.

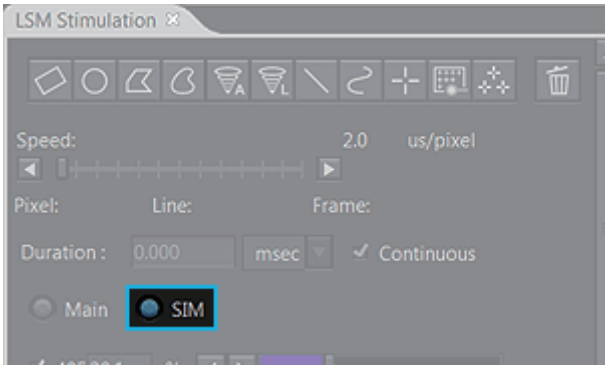
a: "SIM" (The SIM scanner is used for the light stimulation.)
b: "LSM Imaging" (The image acquisition becomes the reference of the start time.)
c: "OFF" (The image acquisition is not stopped during the light stimulation.)

Setting light stimulation

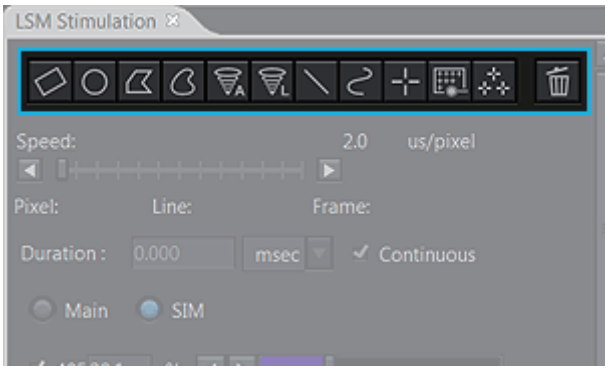
1. Select [LSM Stimulation] in [Tool Window] menu. [LSM Stimulation] Tool Window appears.



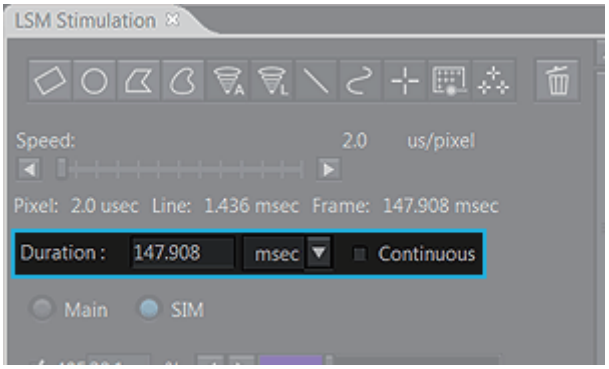
2. Set the scanner to be used for the light stimulation to "SIM".



3. Select ROI, and specify the light stimulation range in the LSM live range on [Live] Window.

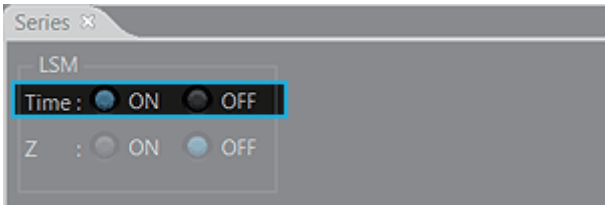


4 Set [Continuous] in [Duration] to "OFF", and enter the duration of the light stimulation in the box.



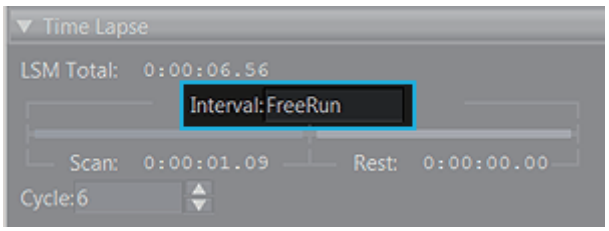
Setting T series image acquisition

1 Set [Time] to "ON" on [Series] Tool Window.



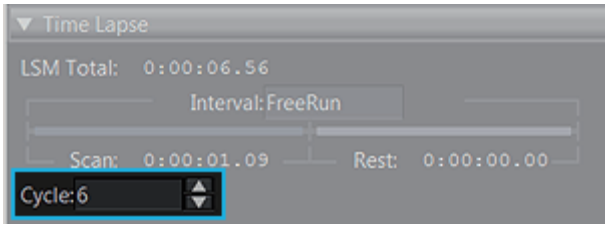
2 Set the interval to acquire the image in [Interval] on [Time Lapse].

If you attempt to set the time shorter than the time displayed in [Scan] (time taken for acquiring 1 cycle image) in [Interval], "FreeRun" appears. In this case, the interval to acquire the image is the time displayed in [Scan].



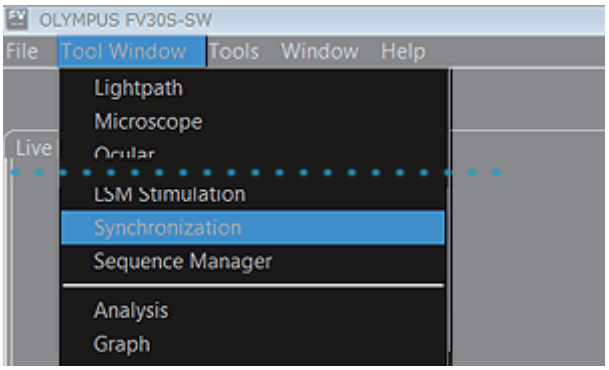
3 Set the number of image acquisitions in [Cycle].

The time from the start of the T series image acquisition to the end of the T series image acquisition is calculated and displayed in [LSM Total]. Set [Cycle] so that [LSM Total] becomes an appropriate time.

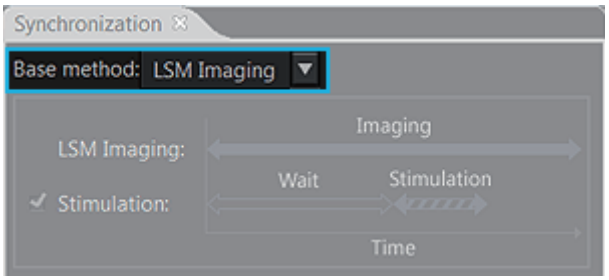


Creating timeline

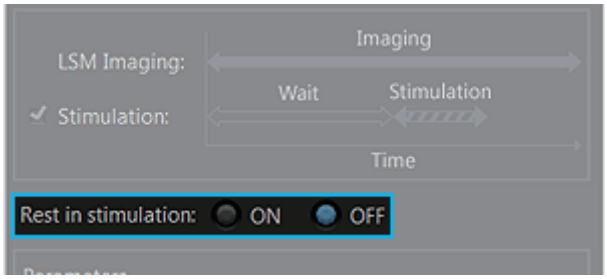
1 Select [Synchronization] in the [Tool Window] menu. [Synchronization] Tool Window appears.



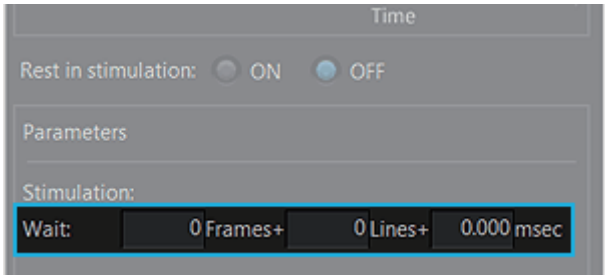
2 Select "LSM Imaging" in [Base method]. The image acquisition becomes the reference of the start time.



3 Select "Off" in [Rest in stimulation]. The image acquisition continues during the light stimulation.



4 Double-click the [Wait] box, and enter the time from starting the image acquisition until starting the light stimulation.



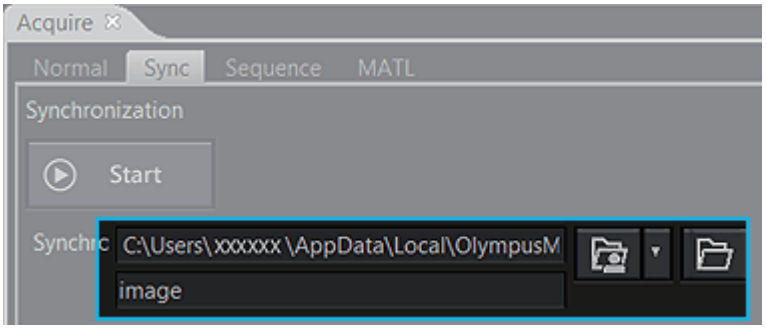
Executing timeline

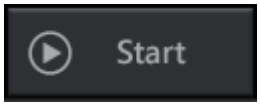
1 Select [Sync] tab in [Acquire] Tool Window.

2 Press the  button to display the dialog box, and select the folder for saving the image.

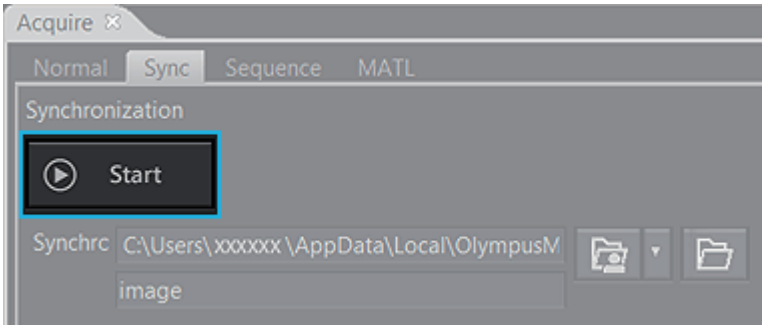
In order to organize files easily after acquiring the images, it is recommended to create a new folder before acquiring the images and specify that folder as the save destination of the images.

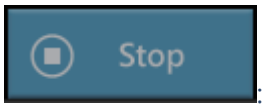
The acquired images are saved automatically.



3 Press the 

button. The image acquisition or the light stimulation starts according to the timeline set in [Synchronization] Tool Window.





: Stops the image acquisition or the light stimulation.