

Outline of acquisition procedures

! GaAsP-PMT is required to use this function. FV30-AGAPD or FV31-HSD must be installed on the system.

- 1 Attach the objective lens applicable for super-resolution to the microscope and register this objective lens in the [\[Microscope\] tab in the \[Configuration\] dialog box](#).

Objective lens applicable for super-resolution	
FVMPE-RS	FV3000
XLPLN25XWMP	UPLSAPO60XW
XLPLN25XWMP2	UPLSAPO60XO
XLPLN25XSVMP	UPLSAPO60XS
XLPLN25XSVMP2	UPLSAPO60XS2
XLPLN25XSVMP	UPLSAPO100XO
XLPLN25XSVMP2	UPLSAPO100XS
XLPLN25XGMP	PLAPON60XO
UPLSAPO30XS	PLAPON60XOSC
UPLSAPO30XSIR	PLAPON60XOSC2
UPLSAPO40XS	APON60XOTIRF
UPLSAPO60XW	APON100XHOTIRF
UPLSAPO60XS	UAPON100XOTIRF
UPLSAPO60XS2	UPLSAPO30XS
LUMPLFLN60XW	UPLSAPO40XS
LUMFLN60XW	UPLSAPO40X2


- 2 Set the [super-resolution mode to OFF](#), and adjust the live image and set the basic observation conditions.

- [Setting IR Laser](#) **FVMPE-RS**
- [Assigning the detector to channel](#)
- [Adjusting the live image](#)

! Adjust the position so that the observation target comes to the center of the live image.
When the super-resolution mode is set to ON according to the following procedures, the live image is zoomed-in (enlarged). Therefore, if the observation target deviates from the center of the live image significantly, the observation target is not visible when the live image is zoomed-in.

- 3 Set the [super-resolution mode to ON](#).
- 4 [Adjust the scan conditions](#) while performing the repeat scan ^{*1}.
- 5 Set for acquiring the image in the [super-resolution mode](#).
- 6 [Acquire the super-resolution image](#).

^{*1} Repeat Scan means to acquire the live image continuously by pressing the  ,  or

 button in the [\[Live\] window](#).

Setting the super-resolution mode to ON

- 1 Select "ON" in [Super-resolution imaging] in the [\[Super-resolution\] tool window](#) to set the super-resolution mode to ON.

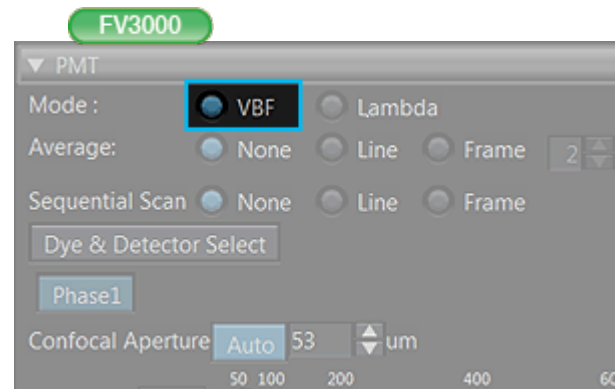
When the super-resolution mode is set to ON, following functions are set automatically.

Tool window name	Function	Setting contents
[LSM Imaging] [LSM Stimulation]	ROI	All ROIs are deleted. While the super-resolution mode is ON, only one ROI for imaging the rectangle can be specified.
[LSM Imaging]	[Type:]/ [Mode:] in [Scanner:]	"Galvano"/"OneWay" is set.
[LSM Imaging]	[Scan Size:] in [Image Size:]	The maximum image size acquirable by the super-resolution is 4096*4096. The size close to the image size which is set before setting the super-resolution mode to "ON" is set.
[LSM Imaging]	[Zoom:]	Based on the magnification of the current objective lens and the image size, the zoom magnification is calculated so that the pixel pitch (pixel resolution) becomes the specified pixel pitch, and this zoom magnification is set.
[PMT Setting]	[Average:]	The accumulation (Averaging process when acquiring the image) is set to "None".
[LSM Imaging]	[Speed:]	"8.0 us/Pixel" is set.
[PMT Setting]	[HV] of GaAsP channel	The value close to the HV value which is set before setting the super-resolution image to "ON" is set in the range between 350 and 500 V.
[PMT Setting]	[Confocal Aperture]	The value optimal for acquiring the image in the super-resolution mode is set. FV3000

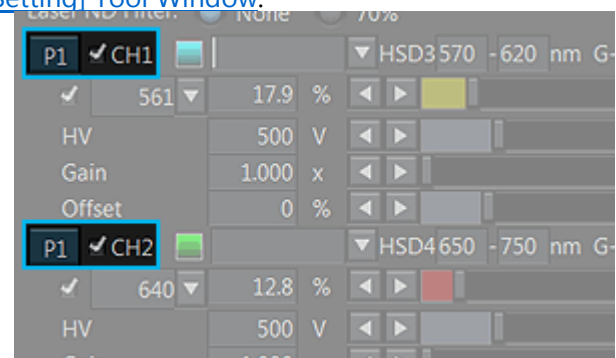
-  When acquiring the image in the super-resolution mode, it is recommended to set [Rotation] in [\[Area Settings\]](#) in the [\[LSM Imaging\] tool window](#) to 0°.

Adjusting scan conditions

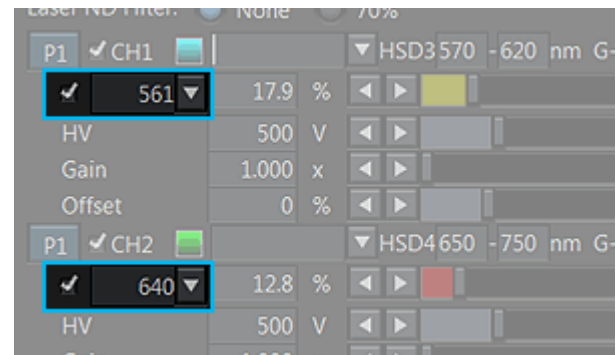
- 1 Select "VBF" in the [\[PMT Setting\] Tool Window](#).



- 2 Select the GaAsP channel you will use in the [\[PMT Setting\] Tool Window](#).



- 3 Assign the laser to all GaAsP channels you will use.

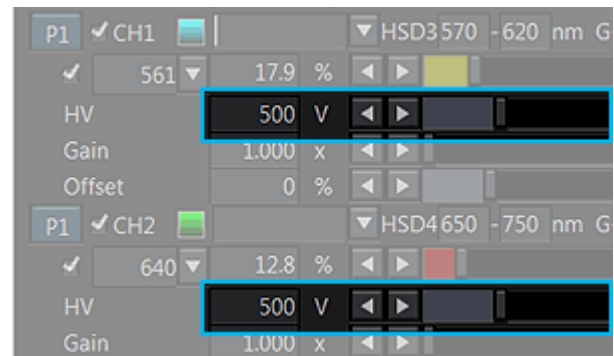


! Even though "ON" is selected in [Super-resolution imaging] in [\[Super-resolution\] Tool Window](#), if the laser is not assigned to the GaAsP channel, the super-resolution image cannot be acquired.

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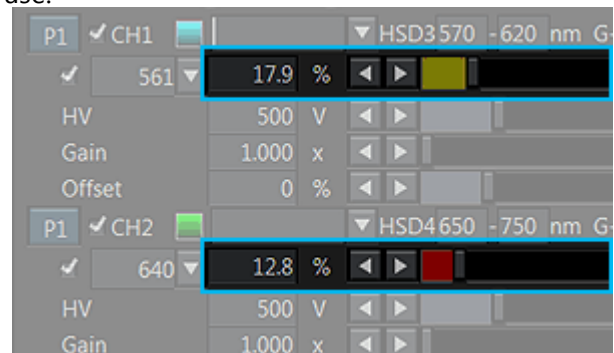
Adjust [HV] of the GaAsP channel you will use according to the purpose.

- 500 V: The discoloring is prevented. (The laser power is reduced, but the noise elements may be increased.)
- 350 V: The super-resolution is prioritized. (The noise elements are decreased. Increase the laser power to adjust the brightness.)



! For acquisition of the super-resolution image, HV can be set in the range between 350 and 500 V.

- 5** Adjust the laser level of the GaAsP channel you will use.

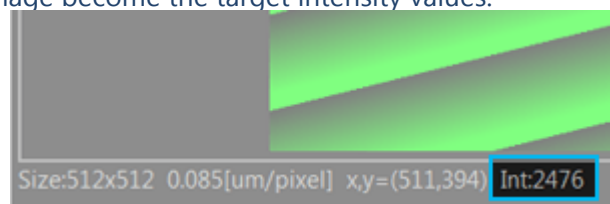


📖 It is recommended to increase the intensity value of the image to the following target intensity values.

Standard mode: Approx. 2400

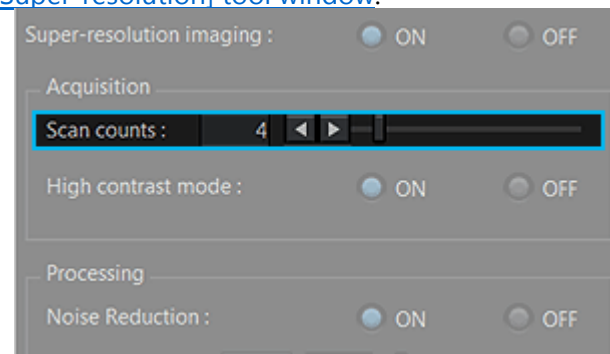
High Contrast mode: Approx. 1800

The intensity value can be confirmed by moving the mouse pointer on the image in the [Live window](#). The intensity value indicated by the mouse pointer is displayed in "Int:" in the lower area of the window. Use this method to confirm the intensity value, and adjust the laser level so that the most of intensity values of the image become the target intensity values.



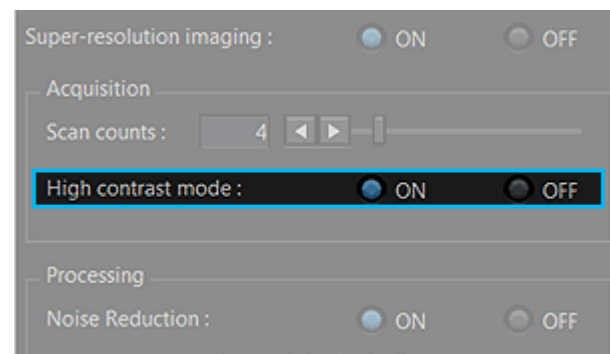
Setting the super-resolution

- 1 Set the cumulative number in [Scan counts] in the [\[Super-resolution\] tool window](#).



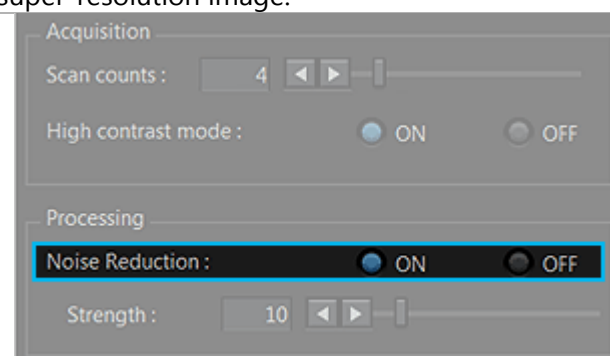
- 2 Select the super-resolution processing mode in [High contrast mode].

ON	High Contrast mode
OFF	Standard mode




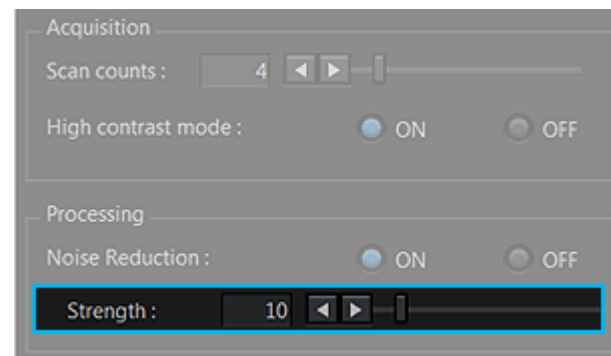
- 3 In [Noise Reduction], set whether or not to reduce the noise concurrently with acquiring the image.

ON	The noise is reduced while acquiring the super-resolution image and it is saved in an image different from the super-resolution image. This is used when the noise is noticeable on the super-resolution image.
OFF	The noise is not reduced while acquiring the super-resolution image.



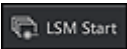

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If "ON" is selected in , set the strength of the noise reduction in [Strength].





Even though you acquire the super-resolution image after "OFF" is selected in **3** to set not to reduce the noise concurrently with acquiring the image, you can reduce the noise from the super-resolution image afterwards. In this case, set each parameter in the [\[Processing\] tab in the \[Analysis\] tool window](#) after the super-resolution image is acquired and click the [Process] button. The noise is reduced.

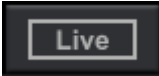
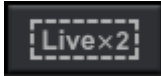
Acquiring the super-resolution image


- 1 Click  or  in the [\[Acquire\] tool window](#) to acquire the super-resolution image. When the image is acquired, the super-resolution image is displayed in the [\[Image\] window](#) and the live image in the [\[Live\] window](#) is cleared. At the same time, the super-resolution image is saved to the hard disk. (Images before processing the super-resolution are not saved.)

 **The acquisition of the super-resolution image may discolor the specimens quickly because the time to irradiate the laser to the specimens is longer than that of the normal acquisition.**

-  During the acquisition in the super-resolution mode, the accumulation is performed regardless of the settings in [Average] in the [\[PMT Setting\] tool window](#). However, during the repeat scan^{*1}, the integration is not performed.

 **While acquiring the super-resolution image, the light stimulation is not available. During the Rest time (waiting time) while acquiring the T series image, the repeat scan^{*1} is not available.**

^{*1} Repeat Scan means to acquire the live image continuously by pressing the  ,  or

 button in the [\[Live\] window](#).

^{*2} MALT is an abbreviation of Multi Area Time Lapse and it means to observe the chronological changes in the multiple regions.