Confocal Microscope
A1 Simple Instruction Manual (Ver.4.10)

Nikon Corporation

This document is subject to change for improvement without prior notice.

CONTENTS

1 Introduction

Thank you for purchasing a Confocal Microscope A1.

We are sure that the A1 will greatly contribute to your research with its many excellent functions. Read this manual carefully to maximize the effectiveness of these excellent functions.

This manual also describes optional components. Since these are selective based on system configuration, some of them may not be provided for the system you purchased. Furthermore, the software is sequentially upgraded and therefore descriptions in this manual may not match the actual equipment in some cases. If you have any questions, contact us or the dealer from whom you purchased the product.

This system is highly advanced and there are procedures and conditions specified for its operation. Be sure to follow them.

From the perspective of customer satisfaction, we are striving to improve product quality while constantly listening to customers' opinions. If you have any opinions and requirements, please let us know.

This system is designed for use as a confocal microscope or a fluorescence microscope. Do not use this system for other purposes.

Basic Operation

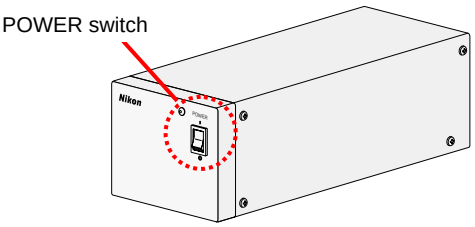
A1 / Ni-E /
Motorized Stage / Piezo Z Stage / Intensilight

This edition may have unavailable functions depending on model in use and option settings.

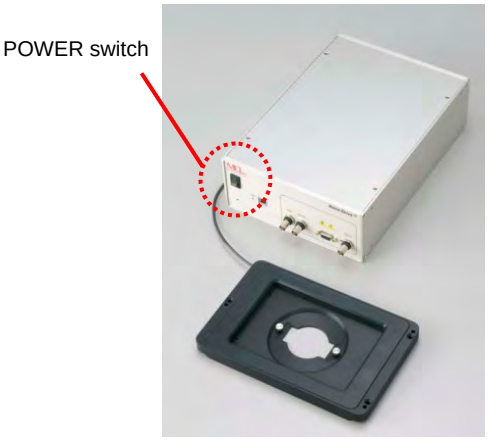
2 Startup

2.1 Turn on the power to the microscope.

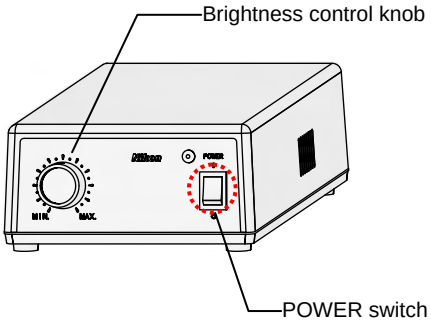
(1) Turn on the power to the motorized stage.



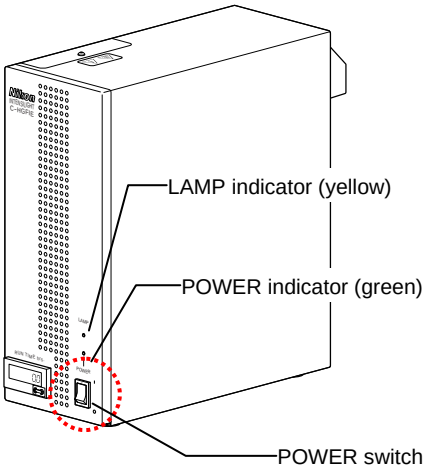
(2) Turn on the power to the piezo Z stage.



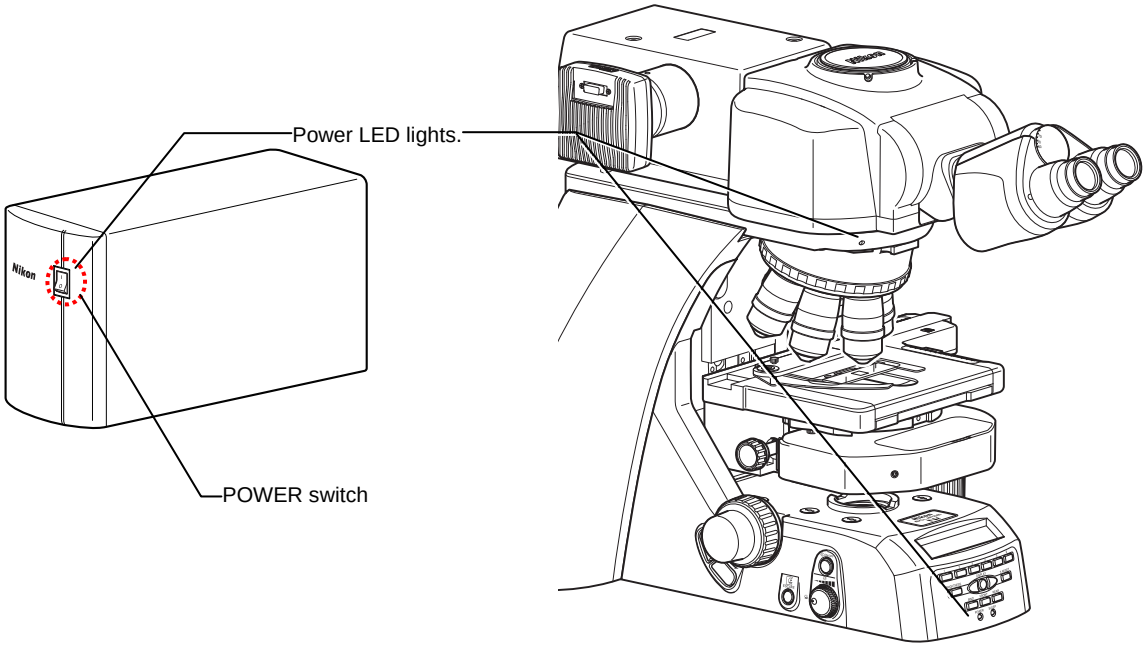
(3) Turn on the power to the halogen lamp (for visual diascopic microscopy).



(4) Turn on the power to the mercury lamp (for visual fluorescence microscopy).

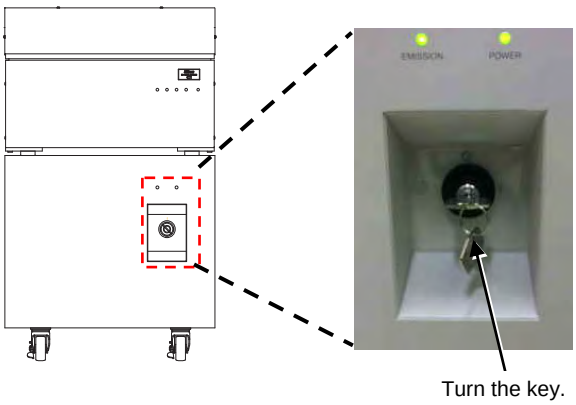


(5) Turn on the power to control box A.
(Pressing the power switch to the [|] side turns on the power.)



2.2 Turn on the power to the laser.

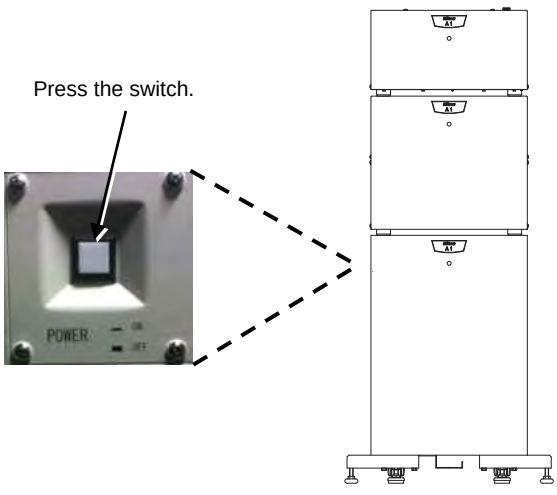
Turn the key 90 degrees clockwise from the vertical position (off).



2.3 Turn on the power to the controller.

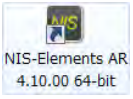
Press the switch on the side of the controller.

Note: The depressed state of the switch is power-on state.



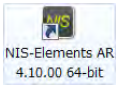
2.4 Start the PC.

2.5 Run the NIS-Elements software.

(1) Click the 

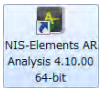
icon to run the NIS-Elements software.

[Icons for acquisition and analysis]



"For acquisition"

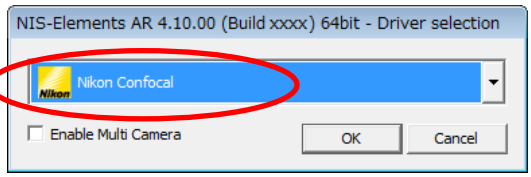
Use this icon for image acquisition.
This icon consists of the acquisition function and analysis function.



"For analysis"

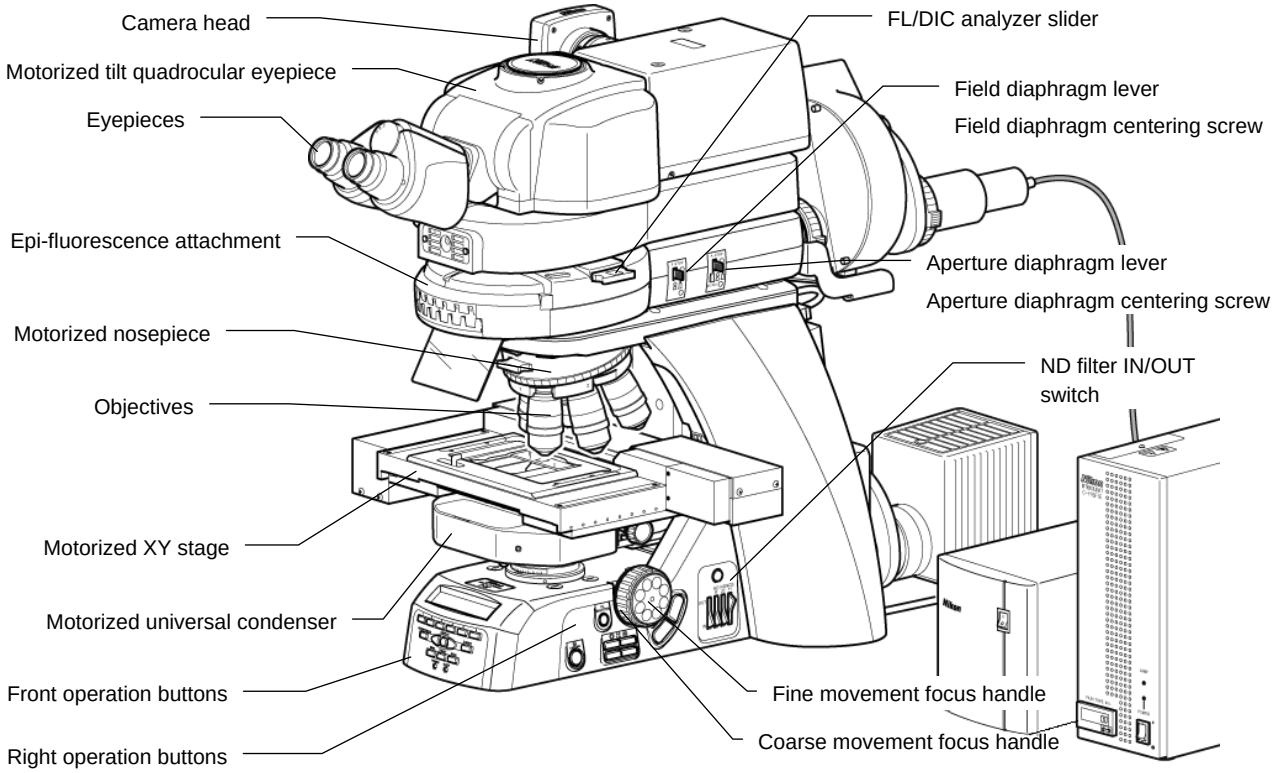
Use this icon for brightness analysis or others.
This icon consists of only the analysis function.

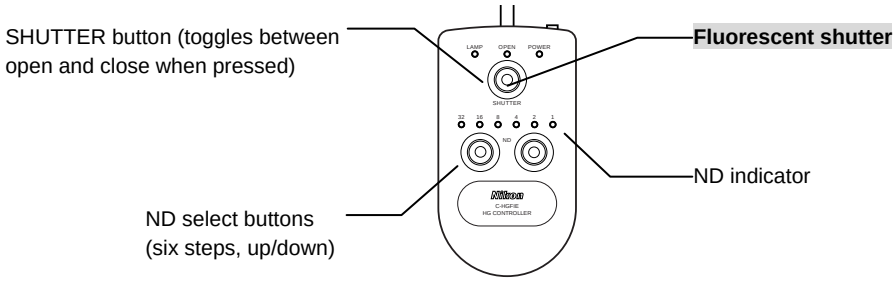
Note: When not only a confocal microscope is connected but also a camera, the Driver selection dialog box opens to select a driver.
Select “Nikon Confocal” in the Driver selection dialog box and click the [OK] button.



3 Operation of the Microscope

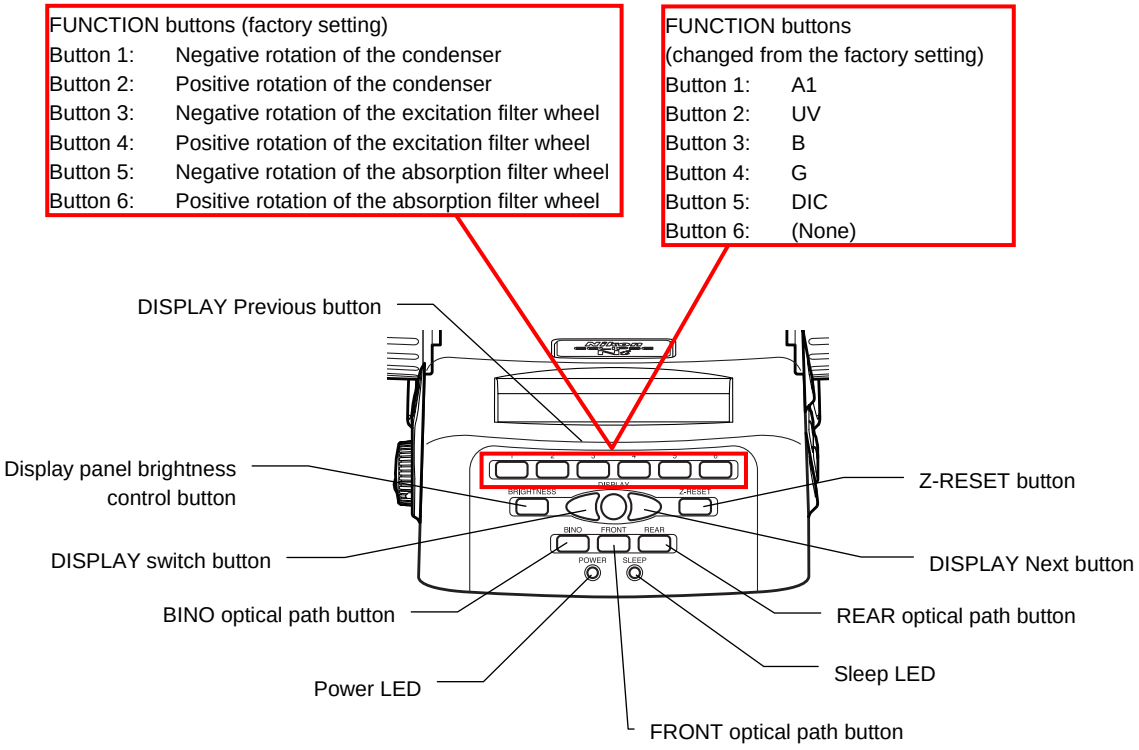
3.1 Microscopy Setting



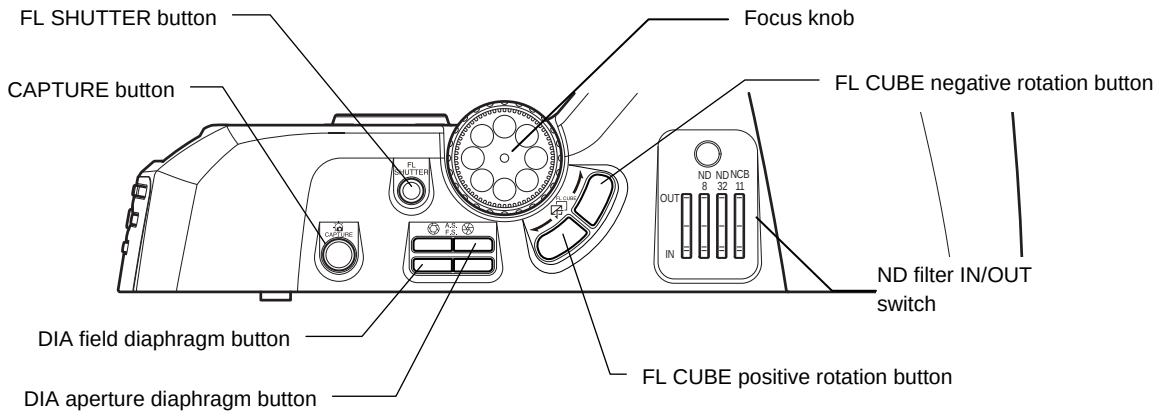


		Diascopic microscopy (DIC)	Fluorescence microscopy	Confocal microscopy
Microscope	Optical path	BINO	BINO	FRONT
	Condenser	N1, N2, NR	Unnecessary (No problem if provided)	N1, N2, NR
	Fluorescent filter	No filter	DAPI, etc.	No filter
	Fluorescent shutter	Close	Open	Close
	Polarizer	Necessary	Necessary	Unnecessary (No problem if provided)
	Analyzer	IN	IN	OUT
	ND filter	Necessary	Necessary	Unnecessary - Be sure to remove it when acquiring a DIC image using a laser
	D filter NCB filter	Necessary	Unnecessary (No problem if provided)	Unnecessary - Be sure to remove it when acquiring a DIC image using a laser
HG controller	Fluorescent shutter	Close	Open	Close
Diascopic illumination		On	Off	Off

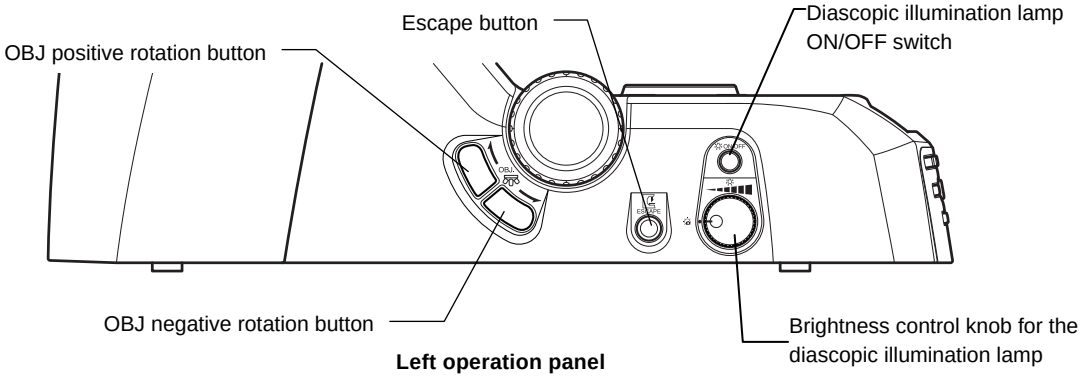
3.2 Operation Panel on the Microscope Ni Main Body



Front operation panel



Right operation panel



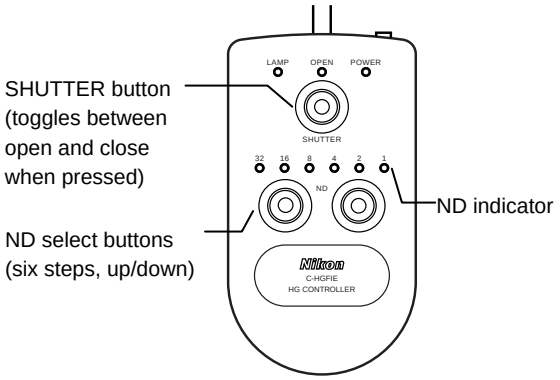
Left operation panel

3.3 HG Controller Operation (Shutter Controller for Visual Fluorescence Microscopy)

(1) Press the SHUTTER button to open the shutter and start fluorescence microscopy.
(Open: LED lights, Close: LED lights out)

(2) If the sample is severely faded, press the ND select up button to reduce the excitation light and start microscopy.
ND values are in the range from 1 to 32. The larger the value, the darker the excitation light becomes.

(3) Press the SHUTTER button to close the shutter and finish the fluorescence microscopy.



3.4 Joystick Controller Operation (Controller for Driving the Motorized Stage)

[1] Joystick

Use the joystick to move the motorized stage in the X and Y directions.
The direction of movement of the stage varies according to the direction at which you tilt the joystick. The speed of movement of the stage varies according to the angle at which you tilt the joystick.

[2] XY Stage Operation Mode Switch

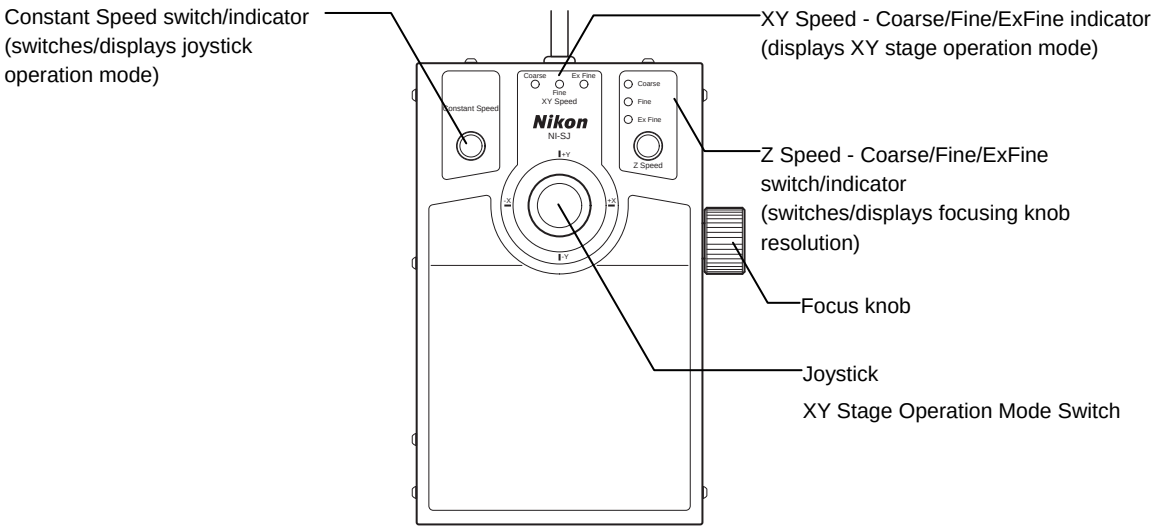
This rotary switch is on the tip of the joystick.
Use it to change the operation mode (Coarse/Fine/ExFine) of the XY stage when controlled via the joystick.

[3] Constant Speed Switch

Use this switch to store the XY stage movement speed and switch to constant speed mode.
Press this switch while moving the XY stage via the joystick to store the current movement speed as the constant speed.
To cancel constant speed mode, press this switch again.

[4] Focus knob

This knob has the same function as the focus knob supplied with the microscope.
Turning this knob varies the focus of the microscope.
Pressing the Z speed switch changes the mode among Coarse, Fine and ExFine.

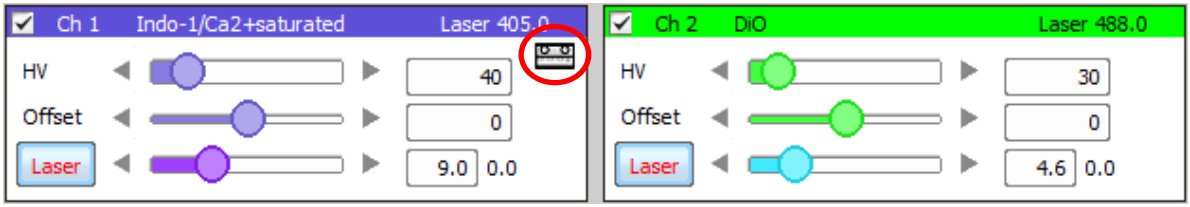


Joystick Controller

3.5 Remote Controller for A1

This remote controller enables you to control laser power and detector sensitivity adjustments required for confocal image adjustment.

(1) Select the channel to be controlled with the **Channel Select buttons**.
The currently selected channel can be checked with the  icon in the [Ch] of the A1plus setting window of the NIS-Elements.



(2) Press the **Start/Stop button** to start scanning.

(3) Adjust the live image while checking it.

Laser Power dial:
Use this dial to adjust the laser power. Turning it clockwise increases the power and turning it counterclockwise decreases the power.
Pressing the dial selects coarse motion or fine motion alternately.

PMT gain dial:
Use this dial to adjust the detector sensitivity (HV). Turning it clockwise increases HV and turning it counterclockwise decreases HV.
Pressing the dial selects coarse motion or fine motion alternately.

(4) Adjust the scan speed as needed.

Scan Speed buttons: Use these buttons to adjust the scan speed. Pressing the [+] button increases the speed and pressing the [−] button decreases it.

(5) Zoom the image as needed.

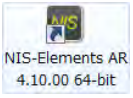
Zoom buttons: Use these buttons to change the zoom magnification. Pressing the [+] button increases the zoom magnification and pressing the [−] button decreases it.

(6) Press the **Start/Stop button** to stop scanning.

Remote Controller for A1

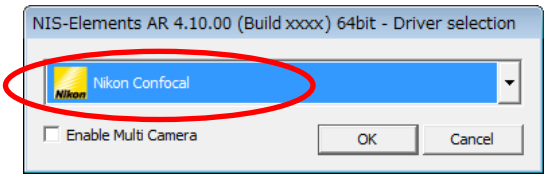
4 Capturing Color Images (Standard Detector)

4.1 Run the NIS-Elements software.

(1) Click the 

icon to run the NIS-Elements software.

Note: When not only a confocal microscope is connected but also a camera, the Driver selection dialog box opens to select a driver.
Select "Nikon Confocal" in the Driver selection dialog box and click the [OK] button.

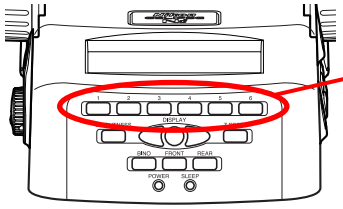


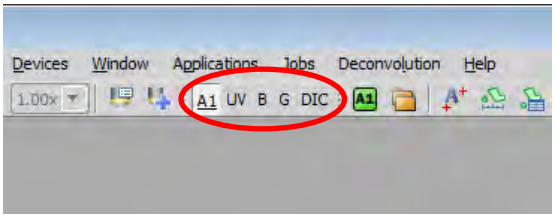
4.2 Observe the sample through the microscope.

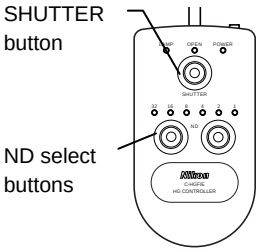
(1) Select microscopy.
When the assignment of the function buttons of the microscope main body is changed from the factory setting, select microscopy and the [A1] button.
If the desired microscopy and the [A1] button have been registered for the Optical Configuration button (hereafter called O.C button) on the NIS-Elements software beforehand, click the O.C button.

- **Optical Configuration button**
  Buttons for which the optical path has been recorded in advance
  The buttons can be customized so the number of buttons and their names vary depending on the customer's preference.

Note: To prevent fading, close the fluorescent shutter frequently. Use the ND filter to look for the sample.



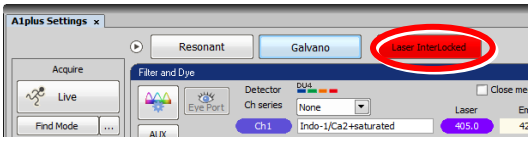




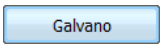
4.3 Switch the optical path to A1.

4.4 Click the [Laser InterLocked] button to reset blinking and to enable laser oscillation with the software.

Note: If the optical path is not switched to A1, blinking cannot be reset even though the button is clicked.



4.5 Select a scan mode.

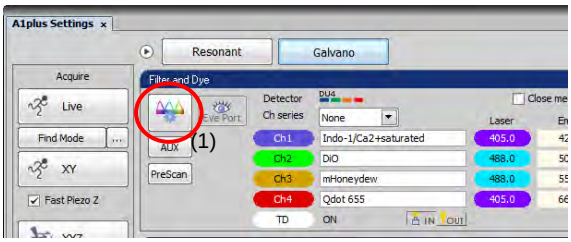
Select 

[Galvano].

4.6 Set the optical path. (Optical path setting for the confocal system required for acquiring images)

Check the settings.

(1) Click  to open the Optical path window.

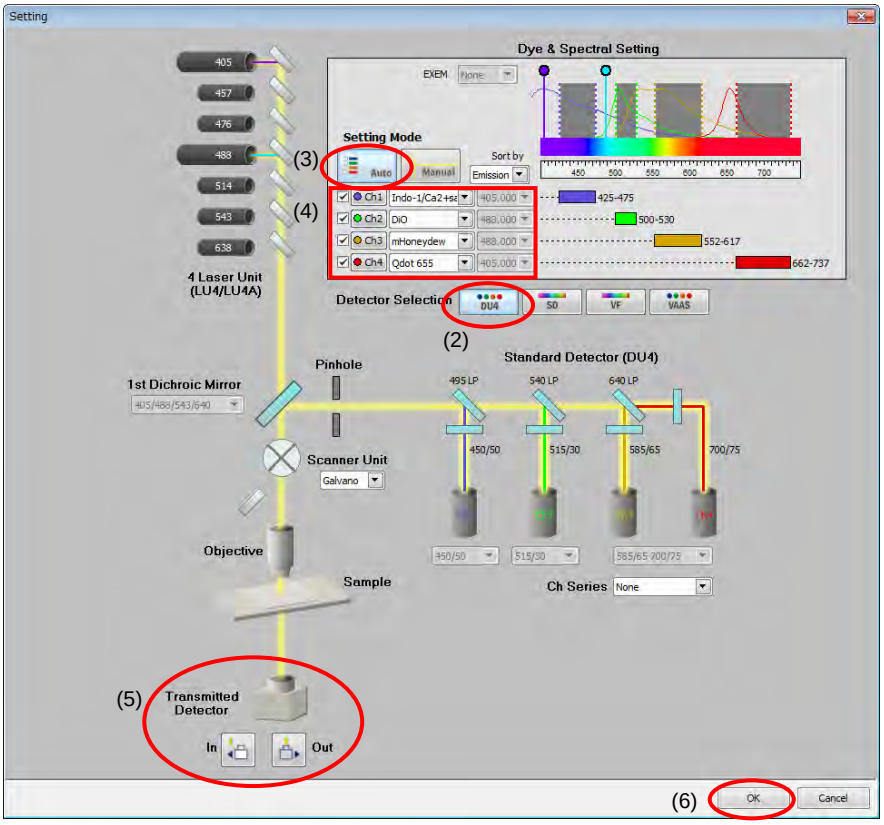


(2) Click the  [DU4] button to select the standard detector.

(3) Click the  [Auto] button to set the optical path in the Auto mode.

(4) Check the checkboxes of channels to be used.
Select a reagent name.
Click  of each channel and select a pseudo-color.

(5) If acquiring a transmitted image together with a confocal image, click  to bring  into the optical path.

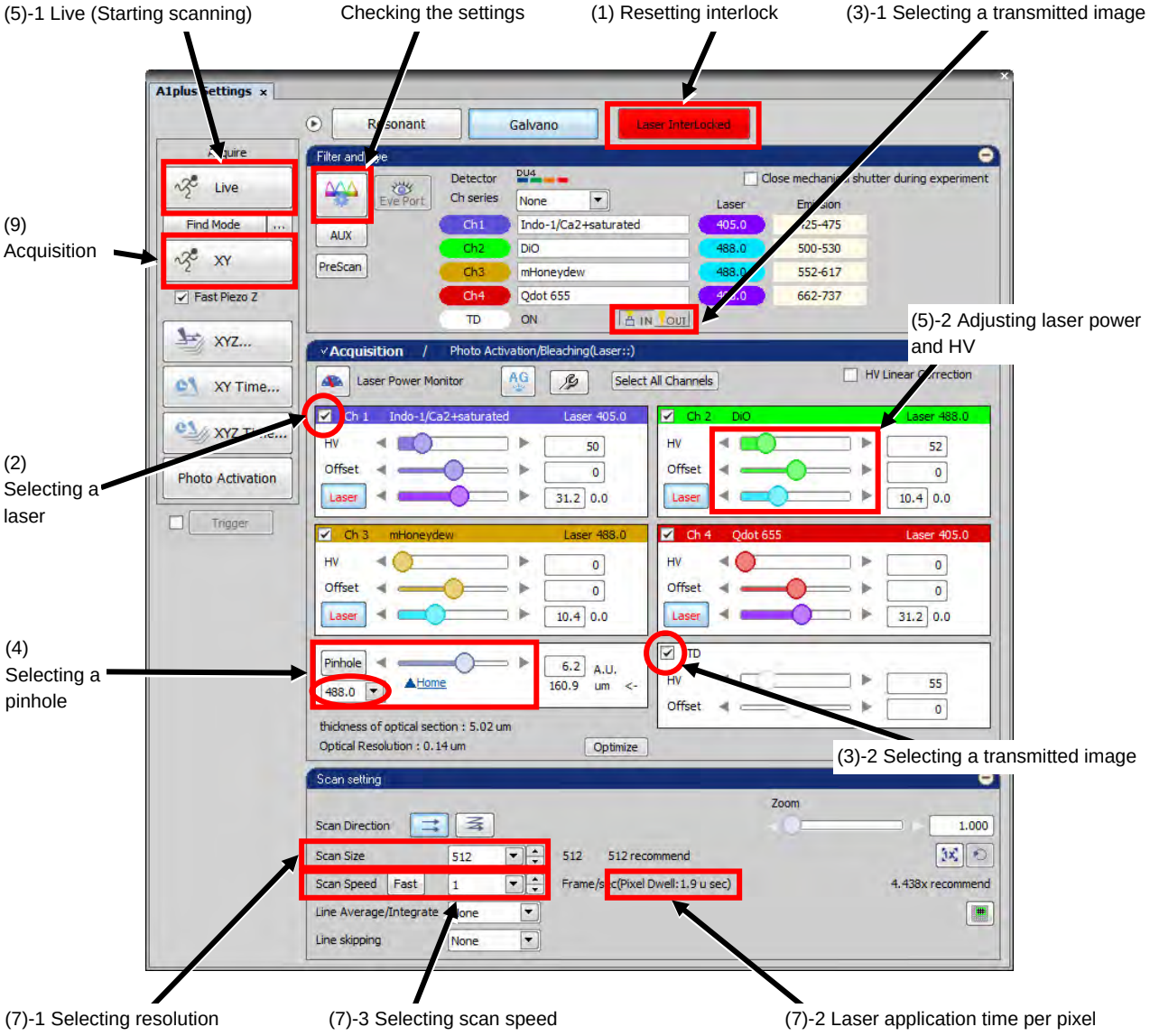


Note: Before acquiring a transmitted image, turn off the light above the microscope.

Note: Because the transmitted light detector is placed in front of transmitted light, transmitted images (differential interferences (DIC)) cannot be observed visually while putting the transmitted light detector into the optical path.
To observe transmitted images visually, remove the transmitted light detector from the optical path.

(6) Click the [OK] button to set the optical path automatically.

4.7 Determine image acquisition conditions and acquire images.



(1) Click the Laser InterLocked [Laser InterLocked] button to reset blinking and to enable laser oscillation with the software.

Note: If the optical path is not switched to A1, blinking cannot be reset even though the [Laser InterLocked] button is clicked.

(2) Select the laser and channel to be used.

(3) If you want to acquire a transmitted image together with a confocal image, click the TD [IN] button and check the TD checkbox.

Note: Before acquiring a transmitted image, turn off the light above the microscope.

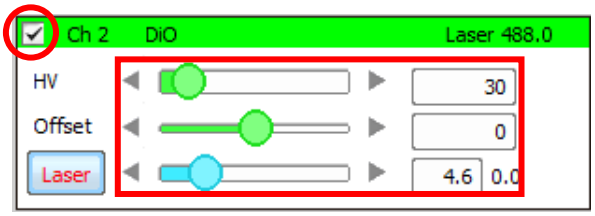
(4) Select the laser wavelength to be used from [Pinhole].
Select a pinhole size best suited for the objective with [▲Home].

(5) Click the [Live] button and adjust [Laser] (laser power) and [HV] (detector sensitivity) while checking the image.

HV: 4ch detector sensitivity

Offset: Signal cutoff (standard: 0)

Laser: Laser power



Note: Use Offset "0" as the standard setting.

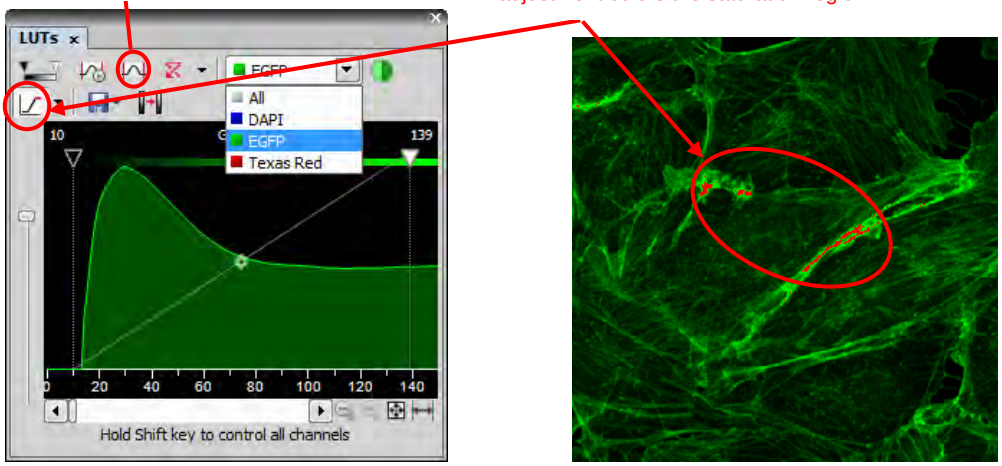
Note: Using the [Pixel Saturation Indication] button in the LUTs tab during adjustment makes it easy to adjust the sensitivity.

Note: If the displayed image is dark, click the [Auto Scale All] button to adjust the contrast of the channel automatically to make the image clear.

Note: Turning on and off the [Live] button (scan ON/OFF) during adjustment minimizes fading.

Auto Scale All

Using the [Pixel Saturation Indication] button during adjustment colors the saturation region.



Note: If the LUTs tab is not displayed, right-click the gray area of the software and select [Visualization Control] - [LUTs] from the displayed menu to call it.

(6) To use Auto Gain, a function that automatically adjusts the detector sensitivity (HV) based on the set rate of saturated pixels, click the [AG] button.

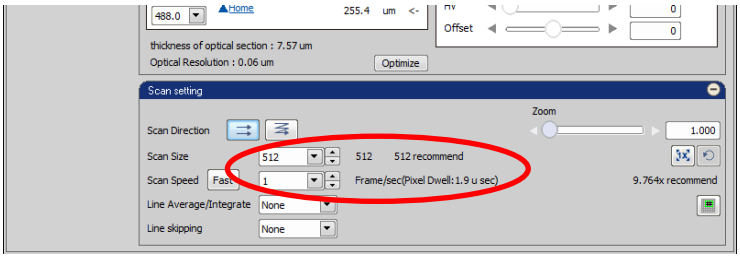
"NG" is displayed for channels that failed in Auto Gain and the HV values are returned to the previous values. Use the [Auto Gain setting] button to change the rate of saturated pixels. Set the maximum value and minimum value of the rate.

Notes:

- **Auto Gain is disabled during scanning.**
- **Auto Gain is disabled during 2Ex1Em or 1Ex2Emx2 line sequence.**
- **Auto Gain is disabled when line scan is set.**
- **Do not make a manual adjustment in the Acquisition window and do not make an adjustment using the remote controller during Auto Gain.**

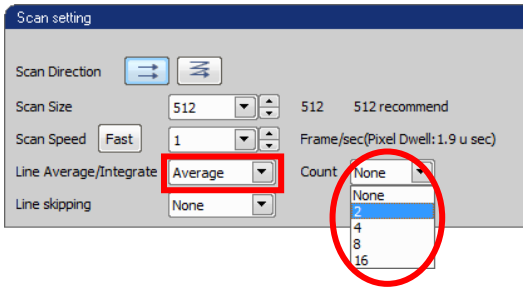
(7) Set the number of pixels to the necessary resolution. (e.g. 512 x 512)
If the image is dark, reduce the scan speed.

Note: Check the pixel dwell for when the resolution is changed.
Pixel dwell indicates laser application time per pixel.
The larger the value, the brighter the image that can be acquired.

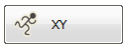


Note: When selecting the Band scan area in Galvano scan mode, the scan speed that is displayed in the Scan Speed pull-down menu is displayed with a decimal point. In such a case, the scan speed is just an approximation, and may differ from the actual scan speed.

(8) Apply Line Average as needed.
Average is a function to scan the same image multiple times and average it to remove noises.
Select [Line Average/Integrate] - [Average] in the Scan setting window and select an average scan frequency in [Count].



Note: Averaging reduces noise, but decreases the frame rate (number of images acquirable per second).

(9) Click the 

[XY] button to acquire an image.

4.8 Save an image.

Make the image you want to save active and select [File] - [Save As] to save it.

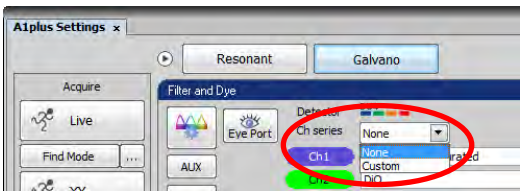
Note: We recommend that the image be saved in nd2 file format. Conditions such as parameters are also saved.

5 Capturing Multistained Images: Cross Talk Reduction (Standard Detector)

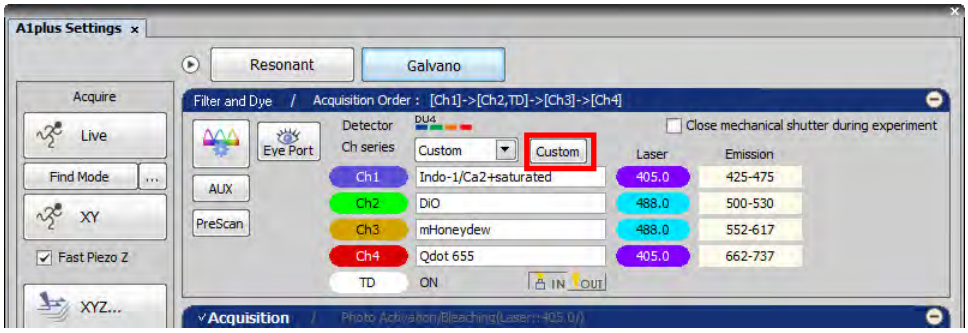
5.1 Perform Steps 4.1 to 4.6 in Chapter 4, “Capturing Color Images”.

5.2 Set a channel series.

(1) Select [Custom] from the [Ch series].



(2) Click the [Custom] button to open the Line Channel Series Setup dialog box.



(3) In the Line Channel Series Setup dialog, set the order of scanning for each channel.

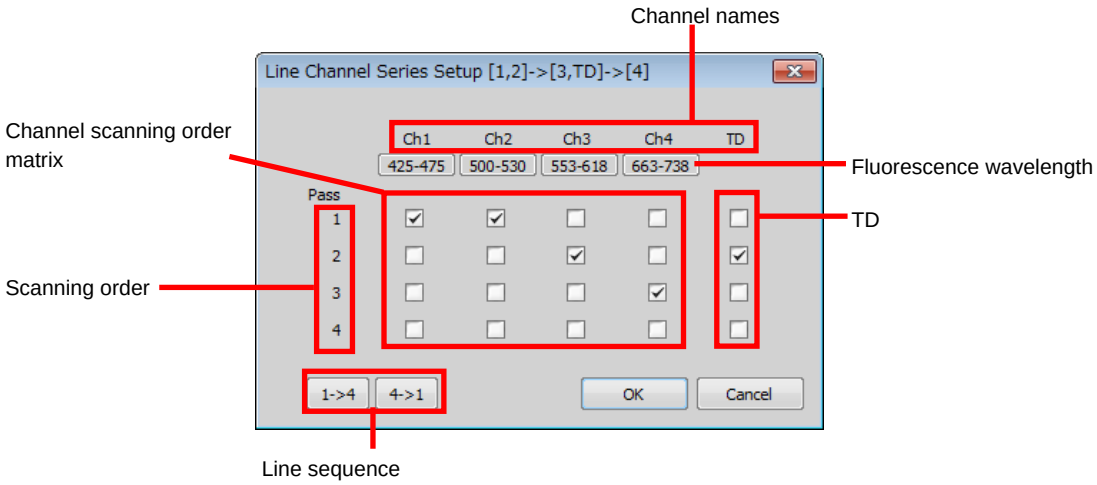
Setting the order of scanning arbitrarily:

Set the order of scanning channels using the channel scanning order matrix.

Using line sequence:

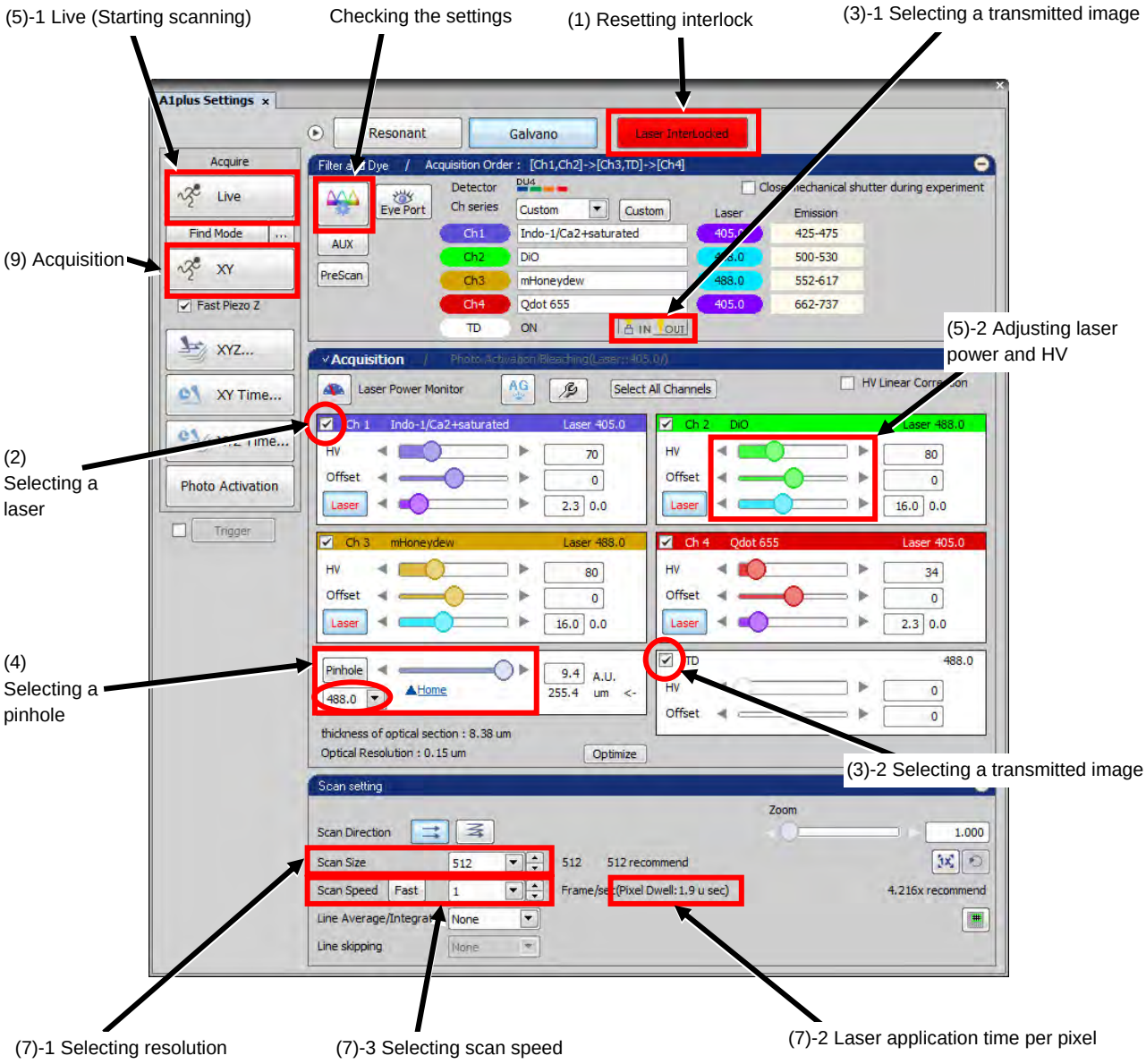
Select the [1->4] or [4->1] button.

Apply lasers to each scan line and start scanning.



Note: Be sure to set the TD (transmitted image) scanning order so that the scanning order comes together with other channels because single TD scan is disabled. (Example: Laser is applied to Ch3 and TD by second scanning.)

5.3 Determine image acquisition conditions and acquire images.



(1) Click the Laser InterLocked [Laser InterLocked] button to reset blinking and to enable laser oscillation with the software.

Note: If the optical path is not switched to A1, blinking cannot be reset even though the [Laser InterLocked] button is clicked.

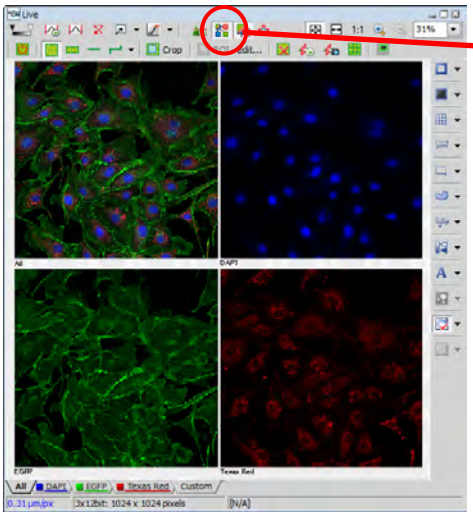
(2) Select the laser and channel to be used.

(3) If you want to acquire a transmitted image together with a confocal image, click the TD [IN] button and check the TD checkbox.

Note: Before acquiring a transmitted image, turn off the light above the microscope.

(4) Select the laser wavelength to be used from [Pinhole].
Select a pinhole size best suited for the objective with [▲Home].

(5) Click the Live [Live] button and adjust [Laser] (laser power) and [HV] (detector sensitivity) while checking the image.



Click the [Split Channels] button to display channels in division which facilitates adjustment of each channel. Reclicking the button returns to the previous display.

Note: Use Offset "0" as the standard setting.

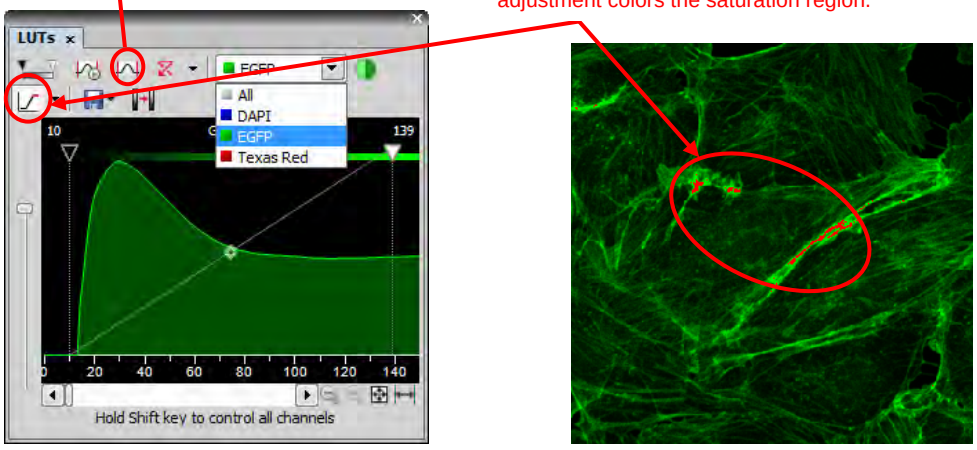
Note: Using the [Pixel Saturation Indication] button in the LUTs tab during adjustment makes it easy to adjust the sensitivity.

Note: If the displayed image is dark, click the [Auto Scale All] button to adjust the contrast of the channel automatically to make the image clear.

Note: Turning on and off the [Live] button (scan ON/OFF) during adjustment minimizes fading.

Auto Scale All

Using the [Pixel Saturation Indication] button during adjustment colors the saturation region.



Note: If the LUTs tab is not displayed, right-click the gray area of the software and select [Visualization Control] - [LUTs] from the displayed menu to call it.

(6) To use Auto Gain, a function that automatically adjusts the detector sensitivity (HV) based on the set rate of saturated pixels, click the [AG] button.

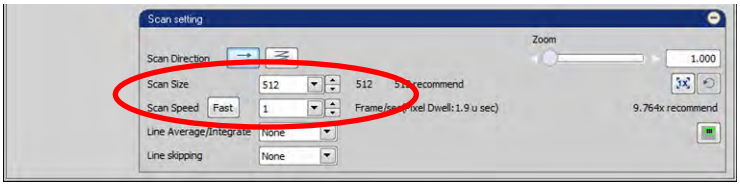
"NG" is displayed for channels that failed in Auto Gain and the HV values are returned to the previous values. Use the [Auto Gain setting] button to change the rate of saturated pixels. Set the maximum value and minimum value of the rate.

Notes:

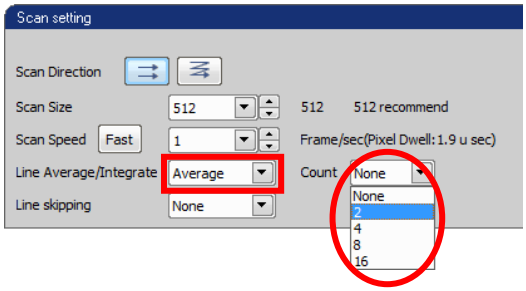
- **Auto Gain is disabled during scanning.**
- **Auto Gain is disabled during 2Ex1Em or 1Ex2Emx2 line sequence.**
- **Auto Gain is disabled when line scan is set.**
- **Do not make a manual adjustment in the Acquisition window and do not make an adjustment using the remote controller during Auto Gain.**

(7) Set the number of pixels to the necessary resolution. (e.g. 512 x 512)
If the image is dark, reduce the scan speed.

Note: Check the pixel dwell for when the resolution is changed.
Pixel dwell indicates laser application time per pixel.
The larger the value, the brighter the image that can be acquired.



(8) Apply Line Average as needed.
Average is a function to scan the same image multiple times and average it to remove noises.
Select [Line Average/Integrate] - [Average] in the Scan setting window and select an average scan frequency in [Count].



Note: Averaging reduces noise, but decreases the frame rate (number of images acquirable per second).

(9) Click the [XY] button to acquire an image.

5.4 Save an image.

Make the image you want to save active and select [File] - [Save As] to save it.

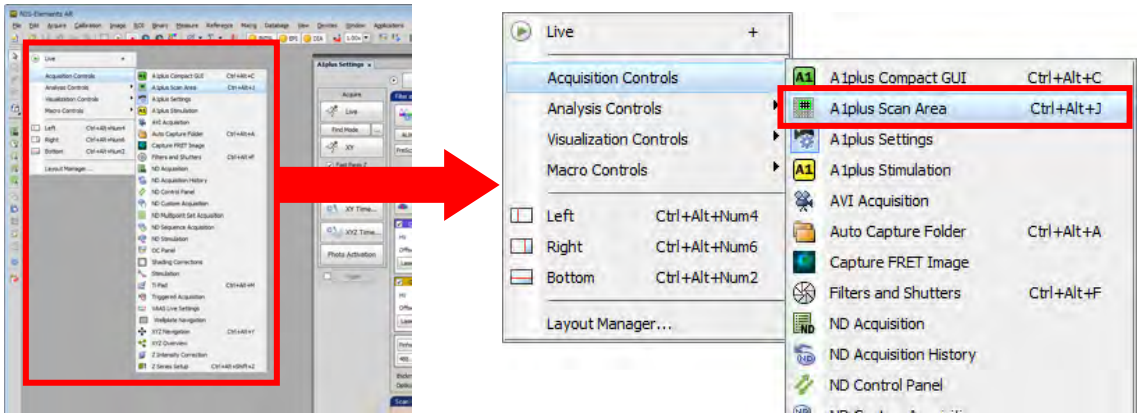
Note: We recommend that the image be saved in nd2 file format. Conditions such as parameters are also saved.

6 Capturing Confocal Zoom

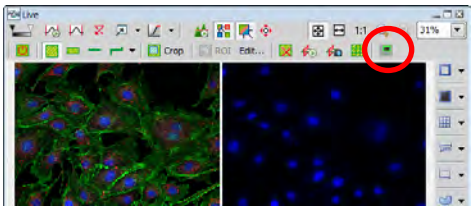
6.1 Perform Steps 4.1 to 4.7 in Chapter 4, “Capturing Color Images” to determine image acquisition conditions.

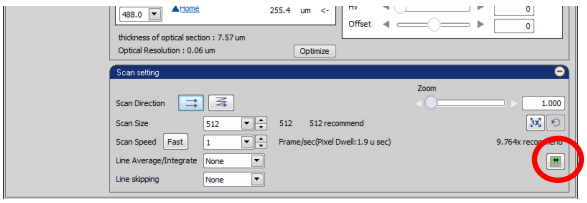
6.2 Call the Scan Area window.

Right-click the gray area of the software and select [Acquisition Controls] - [A1plus Scan Area] from the displayed menu to call it.



* This window also opens by clicking the button shown below that is displayed in the Live window or A1plus Settings window.



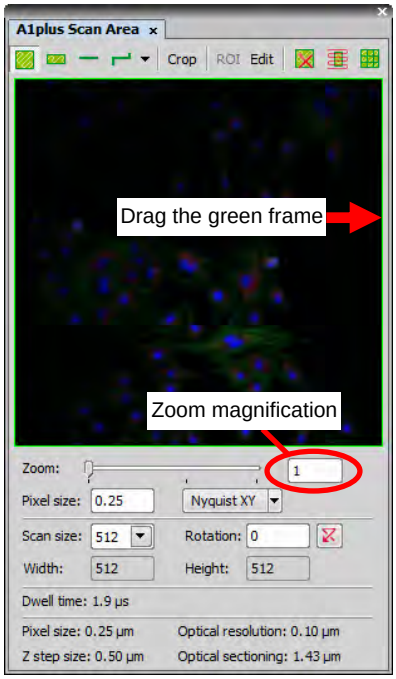


6.3 Select the zoom area.

Click  in the Scan Area window and drag the green frame to reduce the scan area.

The frame can be moved with the mouse cursor set in the cross arrow state. The scan position can be determined by right-clicking.

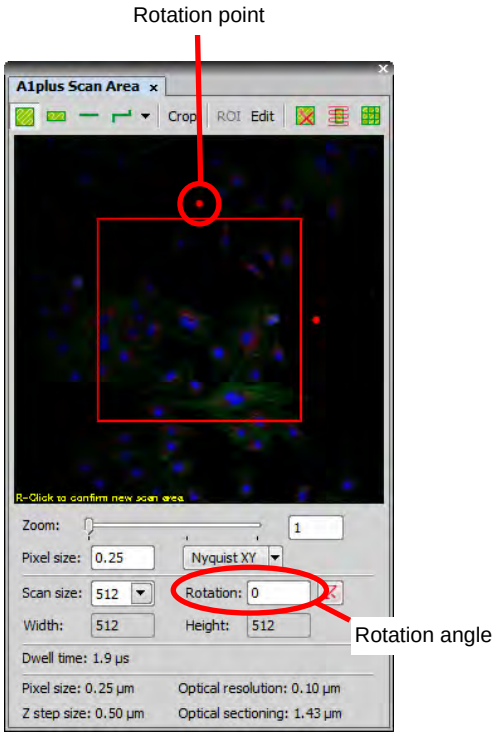
Zoom magnification is shown in [Zoom].



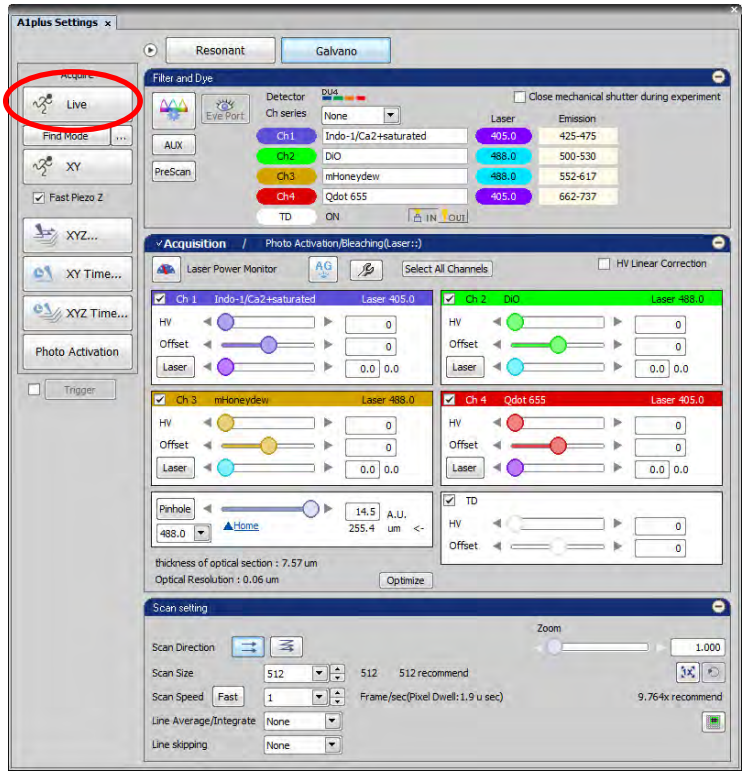
6.4 Rotate the scan area as needed.

The scan area can be rotated by dragging the mouse at the rotation point above the green frame.

The set rotation angle is shown in [Rotation]. The rotation range is -90 to +90 degrees.



6.5 Click the [Live] button to readjust image acquisition conditions.

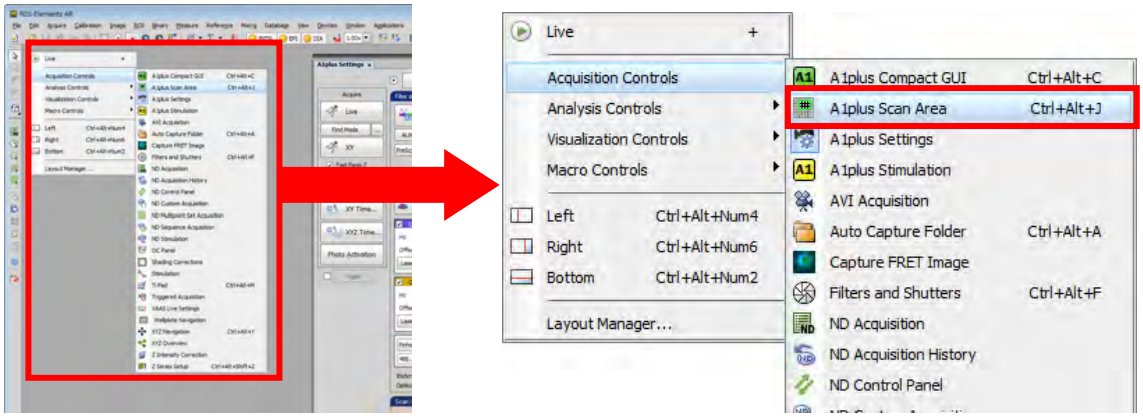


7 Capturing ROI Scan and CROP Scan

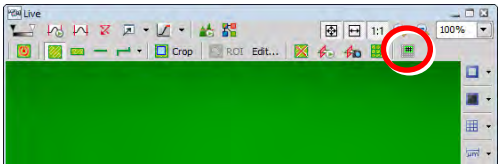
7.1 Perform Steps 4.1 to 4.6 in Chapter 4, “Capturing Color Images” to determine image acquisition conditions.

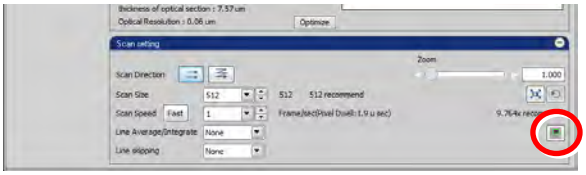
7.2 Call the Scan Area window.

Right-click the gray area of the software and select [Acquisition Controls] - [A1plus Scan Area] from the displayed menu to call it.



* This window also opens by clicking the button shown below that is displayed in the Live window or A1plus Settings window.



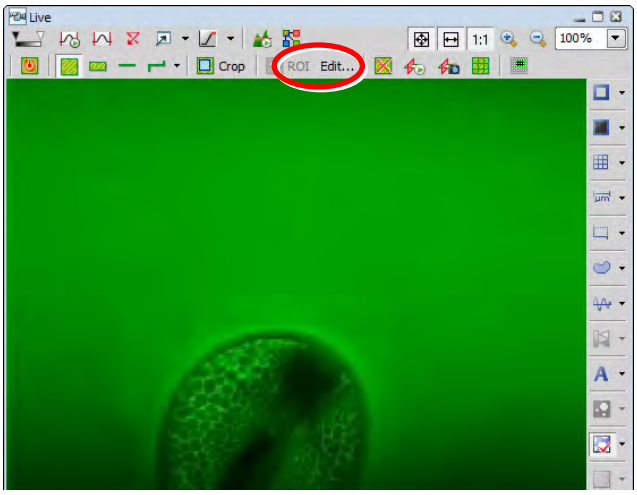


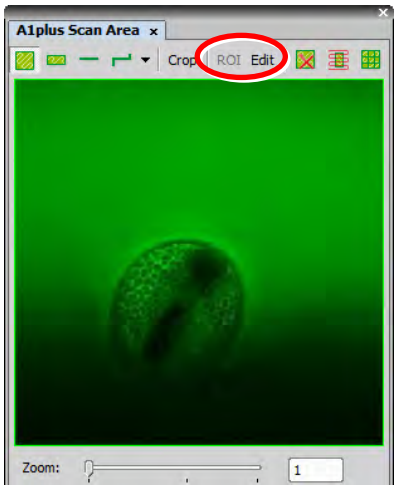
7.3 Select the area to be scanned.

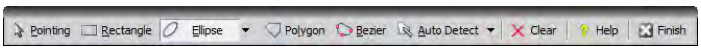
1) ROI scan images the entire window but does not cause photo damage to outside the ROI range.

Note: The Ch series is disabled during ROI scan.

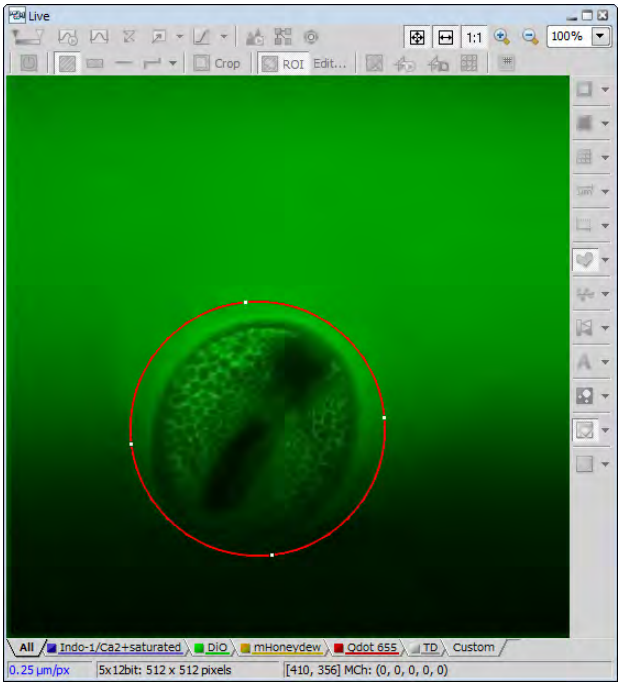
(1) Click the ROI Edit [Edit] button in the Live window or Scan Area window to open [ROI Editor].

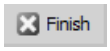




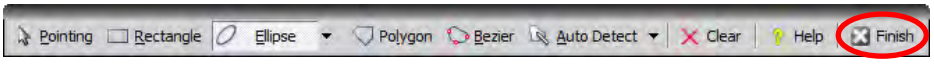
(2) Draw a ROI in the Live window using the 

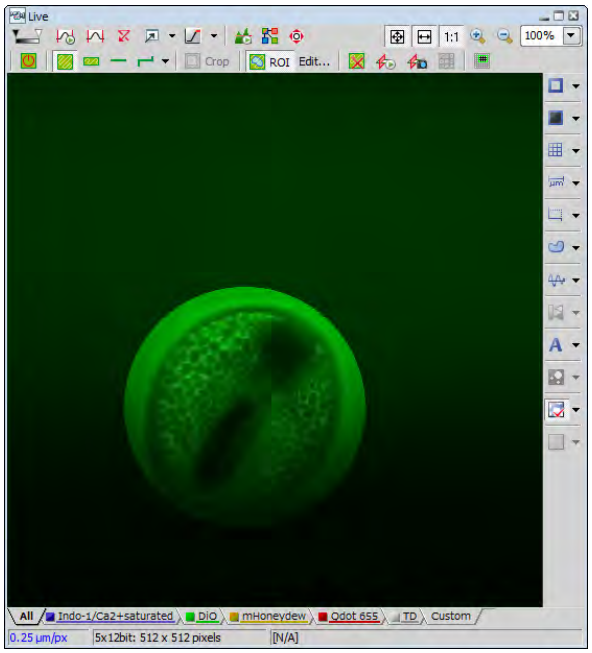
[ROI Editor].
Right-clicking commits the ROI being edited.

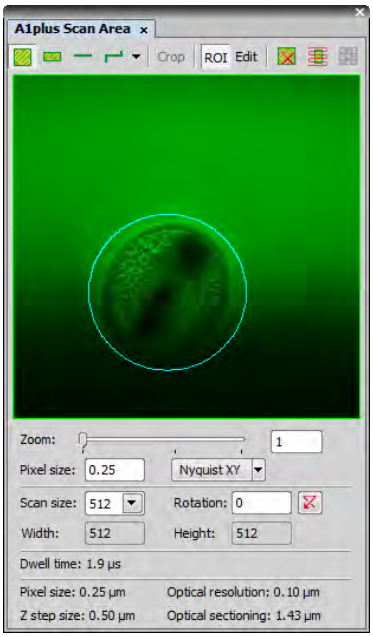


(3) Click the 

[Finish] button to close the [ROI Editor]. The ROI drawn in the Live window is hidden and the scan position is determined.
The drawn ROI is displayed in the Scan Area window with a light blue frame.



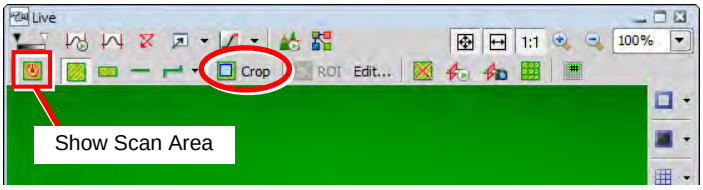


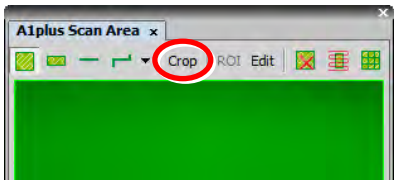


Note: If a ROI is drawn on Frozen images, click the Live button to start ROI scanning.

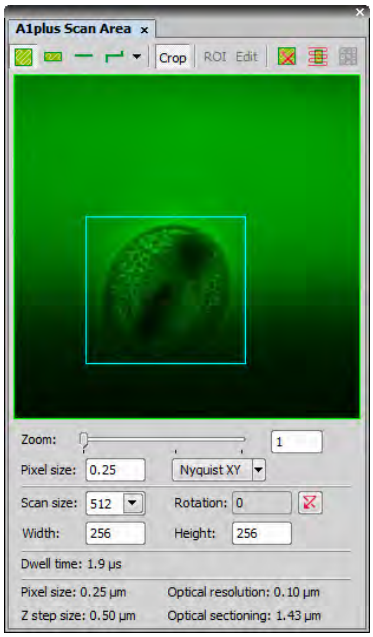
2) CROP scan extracts the selected area (pixel) and images it.

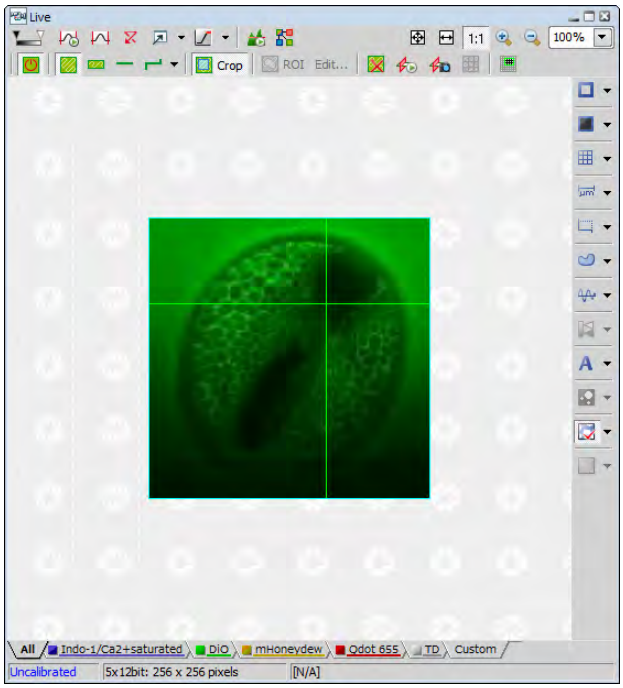
(1) Click the Crop [Crop] button in the Live window or Scan Area window.
To draw a crop in the Live window, turn on the [Show Scan Area] button beforehand.





(2) In the Scan Area window, drag the light blue frame to reduce its size.
The frame can be moved with the mouse cursor set in the cross arrow state. (When the size or position of the frame is changed, the light blue frame turns to red.)
The scan position can be determined by right-clicking.





Note: If a crop is drawn on Frozen images, click the Live button to start crop scanning.

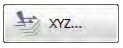
7.4 Readjust image acquisition conditions while checking the live image.

8 Capturing Z Series Images

8.1 Perform Steps 4.1 to 4.7 in Chapter 4, “Capturing Color Images” to determine image acquisition conditions.

Note: We recommend that image acquiring conditions (for laser power and detector sensitivity) be adjusted on the brightest focus plane among sample thicknesses to be acquired to prevent images at each focus from saturating.

8.2 Determine the range for capturing Z series images.

(1) Click the 

[XYZ...] button to open the Capture Z-Series dialog box.

Note: The Z image acquisition mode is switched according to whether or not [Fast Piezo Z] is checked.

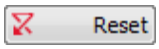
Checked: Speed priority mode
Images are acquired giving a higher priority to speed regardless of the specified Z stroke. (This setting is enabled only when "Nikon A1 Piezo Z Drive" is selected as Z drive.)

Not checked: Stroke priority mode
Images are acquired in accordance with the specified Z stroke. (Stroke priority mode needs a BNC cable.)

Note:

(1)

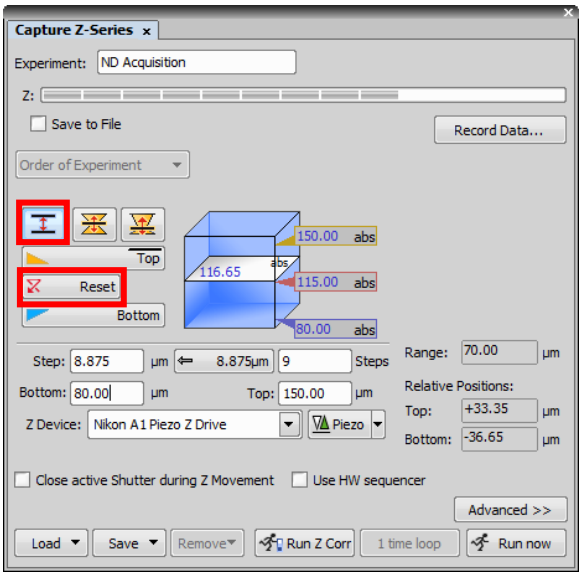
(2) Click the  [Defined top & bottom] button.

(3) Click the 

[Reset] button.

(2)

(3)

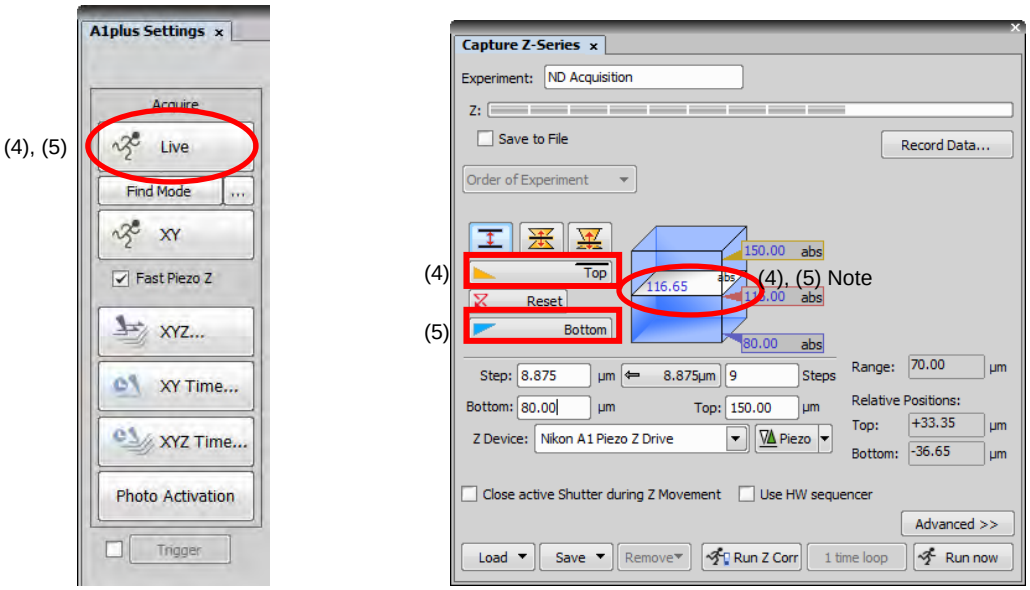


(4) Click the [Live] button and move the focus knob (fine motion mode) of the microscope while checking the image, and then click the [Top] button to determine the top position.

Note: Move the focus knob in the direction where the value of the plane in the cube increases.

(5) Click the [Live] button and move the focus knob (fine motion mode) of the microscope while checking the image. Click the [Bottom] button to determine the bottom position.

Note: Move the focus knob in the direction where the value of the plane in the cube decreases.

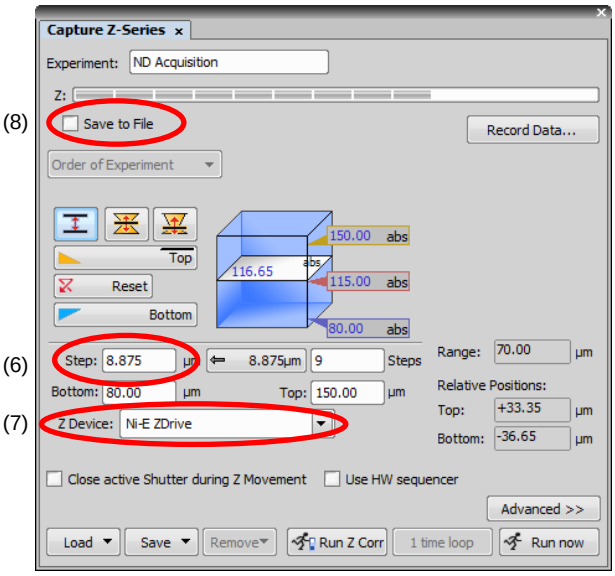


(6) Determine [Step].

(7) Select “Ni-E ZDrive” for [Z Device].

(8) Check the Save to File checkbox as needed, and acquire images while saving them.

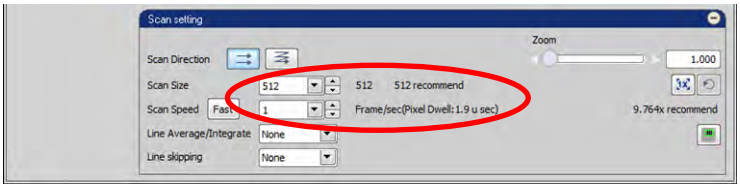
Note: Images are saved in nd2 file format.



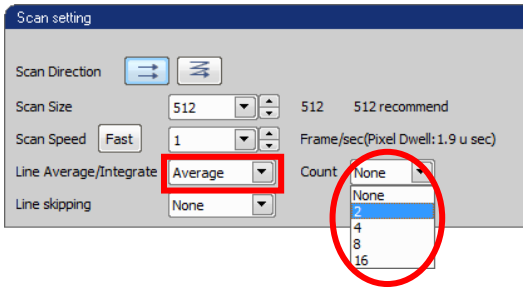
8.3 Acquire Z series images.

(1) Set the number of pixels to the necessary resolution. (e.g. 512 x 512)
If the image is dark, reduce the scan speed.

Note: Check the pixel dwell for when the resolution is changed.
Pixel dwell indicates laser application time per pixel.
The larger the value, the brighter the image that can be acquired.

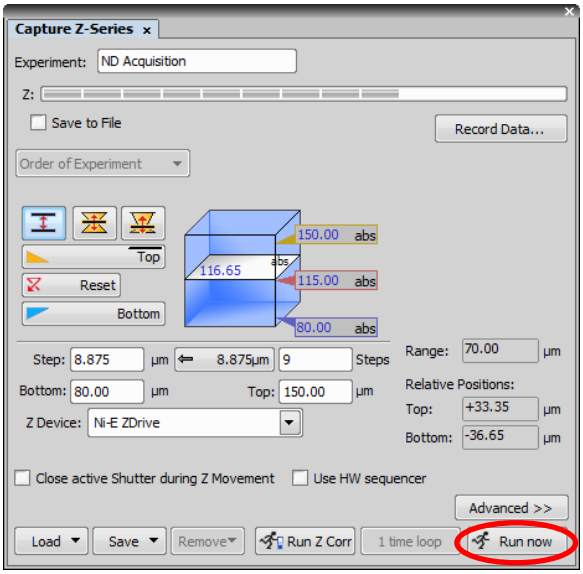


(2) Apply Line Average as needed.
Average is a function to scan the same image multiple times and average it to remove noises.
Select [Line Average/Integrate] - [Average] in the Scan setting window and select an average scan frequency in [Count].



Note: Averaging reduces noise, but decreases the frame rate (number of images acquirable per second).

(3) Click Run now [Run now] button to acquire Z series images.

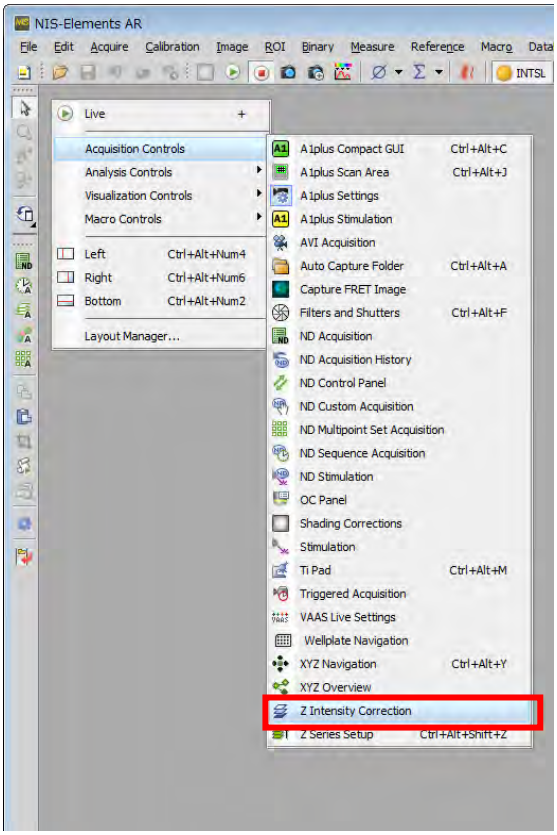


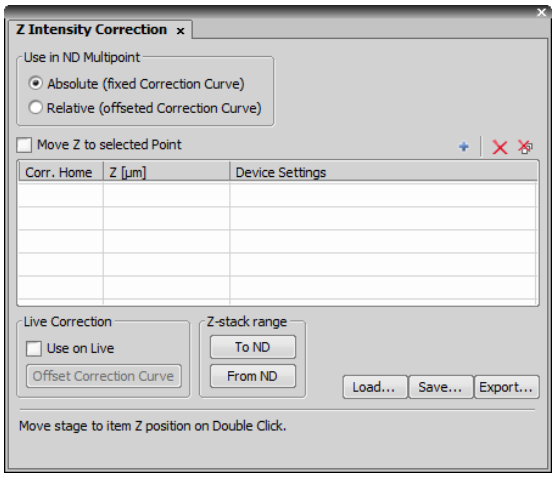
9 Capturing Z Series Images (While Changing Brightness)

9.1 Perform Steps (1) to (5) in Section 8.2, in Chapter 8, “Capturing Z Series Images”.

9.2 Call the Z Intensity Correction dialog box.

Right-click the gray area of the software and select [Acquisition Controls] - [Z Intensity Correction] from the displayed menu to call it.

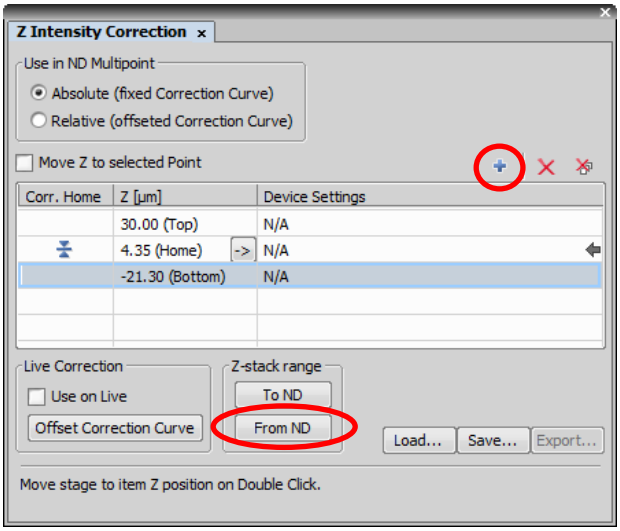




9.3 Specify the Z position and set brightness at that position.

(1) Click From ND to register the Z points as Z items.

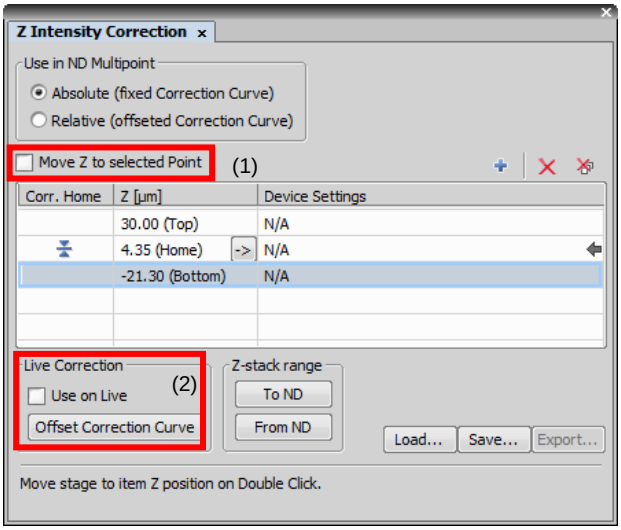
(Clicking this button also registers three points, top, home, and bottom, automatically. To register additional points, click + while checking the image in the Live window.)



9.4 Register laser power, HV, or others at each Z position.

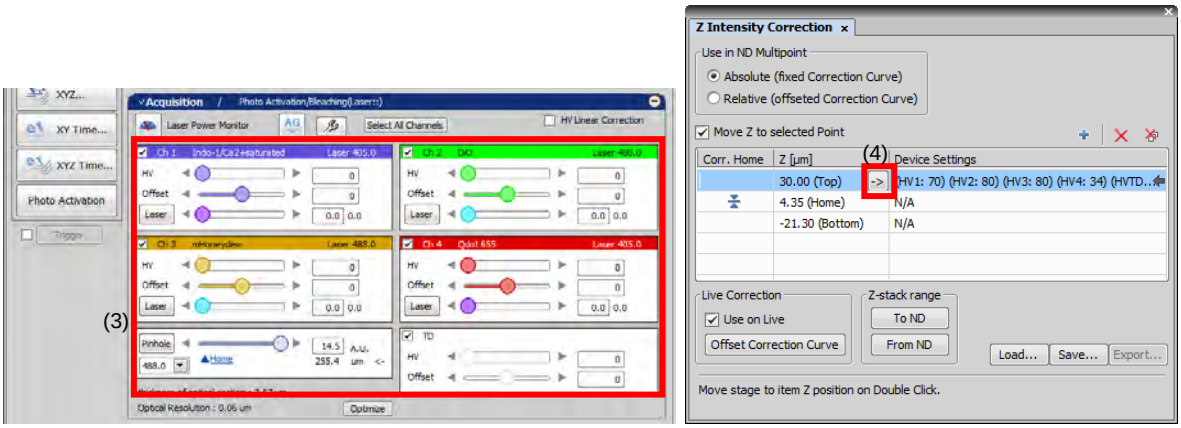
(1) Check the [Move Z to selected Point] checkbox and double-click the Z position in the Z [µm] column to move the focus to the displayed Z position.

(2) Check the [Use on Live] checkbox under [Live Correction].
When this checkbox is checked, changing the Z position displays the Live image with the set HV and laser power.
After you finish checking, uncheck the checkbox.

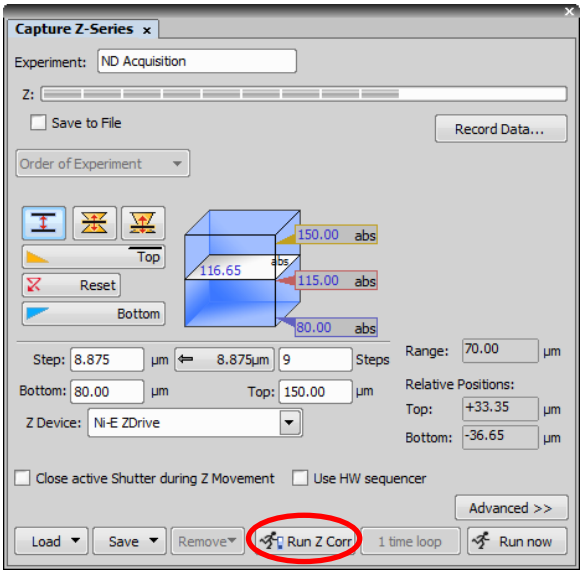


(3) After the Z position is moved to where you want to adjust brightness, adjust HV and laser power.

(4) To register the adjusted HV and laser power, click [->].
After you finish adjusting brightness at each Z position, you can close [Z Intensity Correction].



(5) After you finish all registrations, click the [Run Z Corr] button in the Capture Z-Series dialog box to start image acquisition.



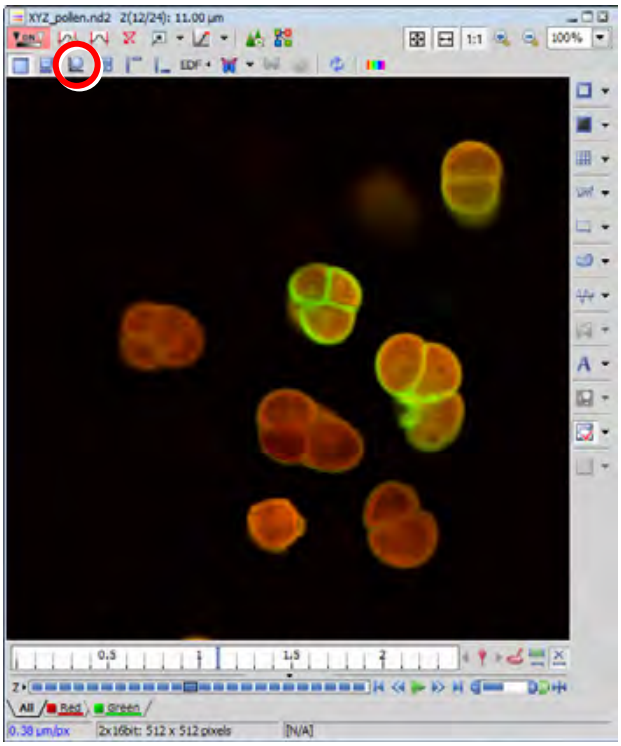
10 Creating Three-Dimensional Image

10.1 Create a three-dimensional image.

(1) Click  to open the Z series image.

(2) Click the  [Show Volume View] button on the image frame to create a three-dimensional image.

Note: It takes several to 30 minutes for creation depending on the image data size.



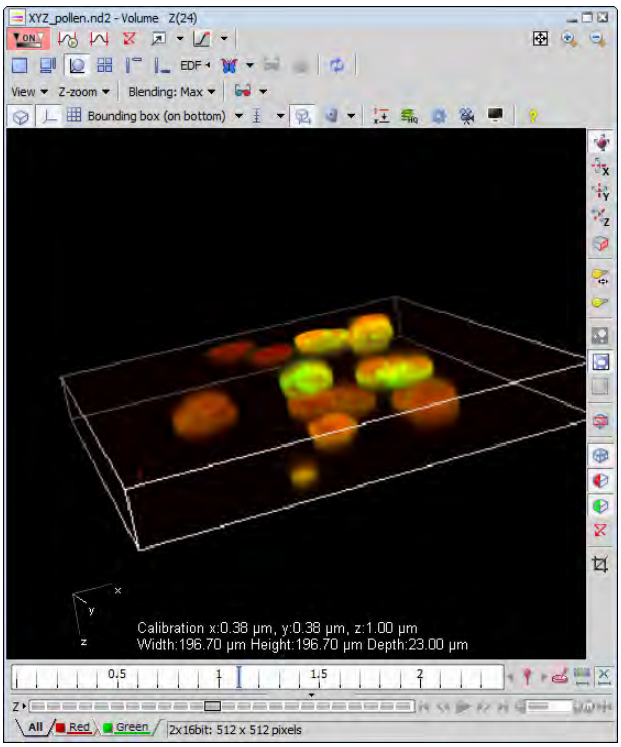
10.2 Save the three-dimensional image of a desired angle as an image.

(1) Drag the white frame of the three-dimensional image with the mouse and adjust it to a desired angle.

Note: Ctrl + mouse left-click + drag: Cut cross section
Mouse right-click+ drag: Move three-dimensional image display position
Turning mouse scroll wheel: Zoom

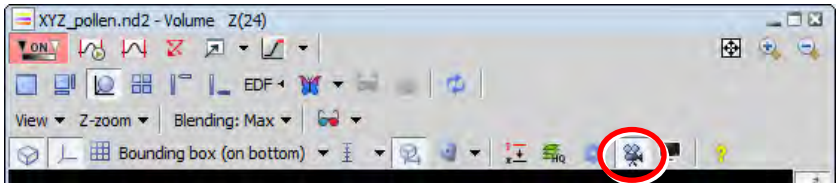
(2) Select [Edit] - [Create View Snapshot (8bit RGB)] from the menu bar to capture a screen.

(3) Select [File] - [Save As] from the menu bar to save the image in a desired file format (jpg, tiff, bmp, etc.)

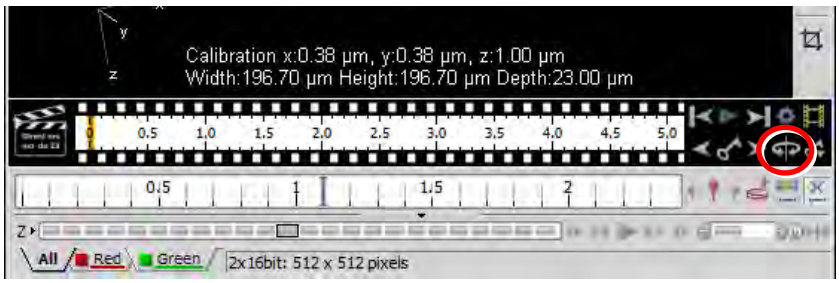


10.3 Create a rotation image of the three-dimensional image.

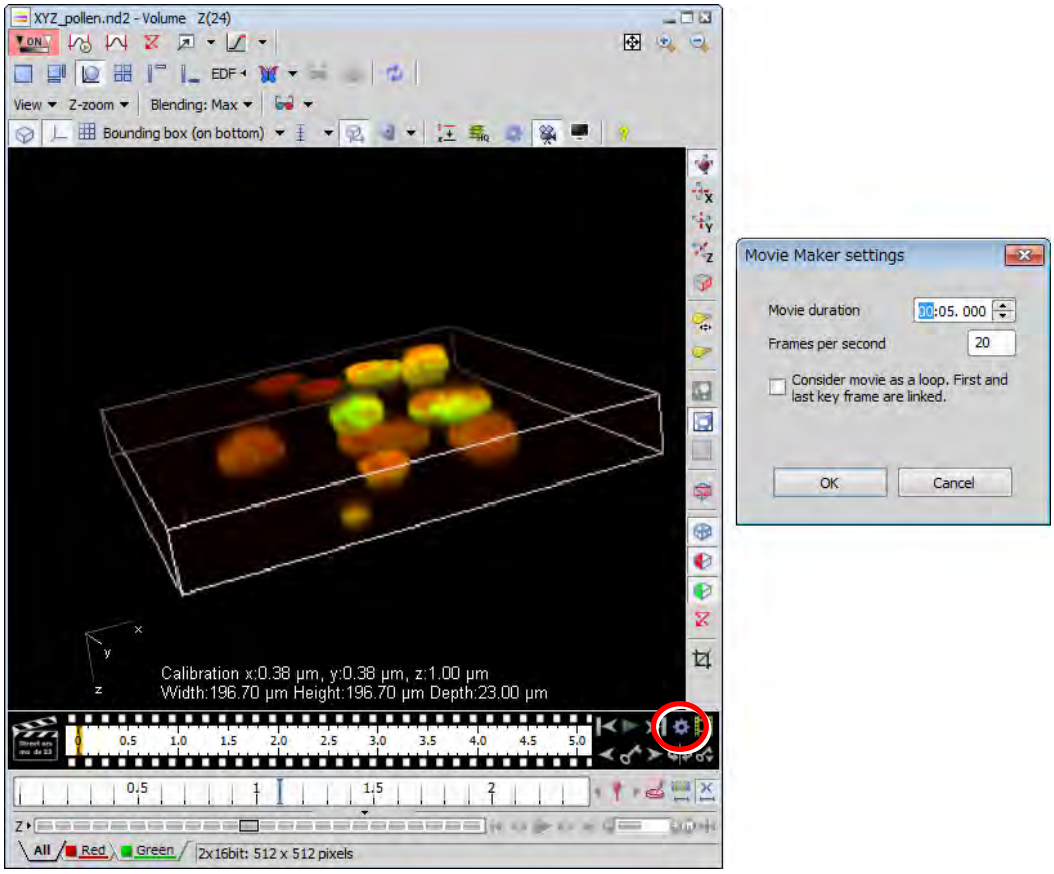
(1) Turn on the [Show Movie Maker] button.



(2) Select the rotating direction with the [Presets] button.

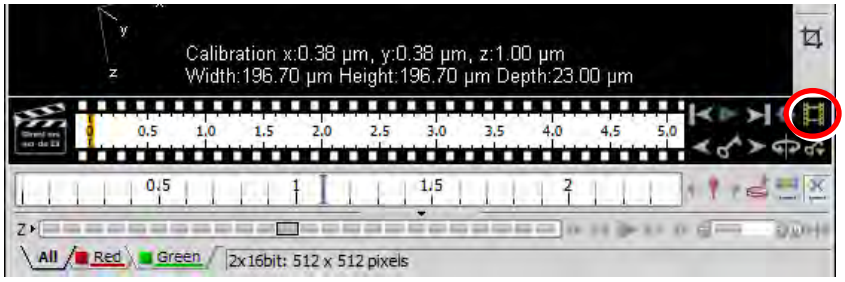


(3) Click the [Settings] button to open the Movie Maker settings dialog box.
Set [Movie duration] (playback time) and [Frames per second] (playback speed) and click the [OK] button.

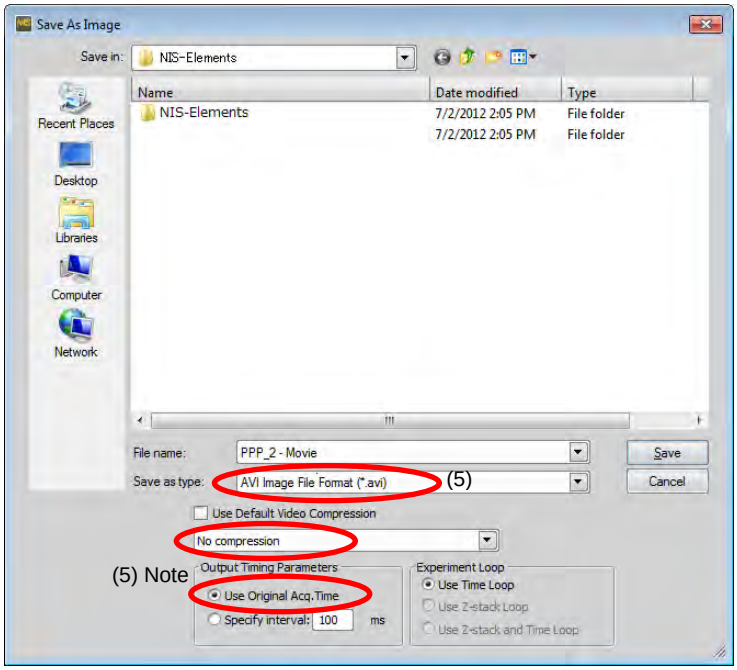


Note: Frames per Second (fps) is the number of images displayed per second. Moving picture becomes smoother as the number of images increases. Use 3 to 10 fps as a guideline.

(4) Click the [Create Movie] button to create a movie.



(5) Select the acquired rotation image and select [File] - [Save As] from the menu bar to save the image in the AVI file format.



Note: We recommend that the avi image file be saved with “No Compression”.

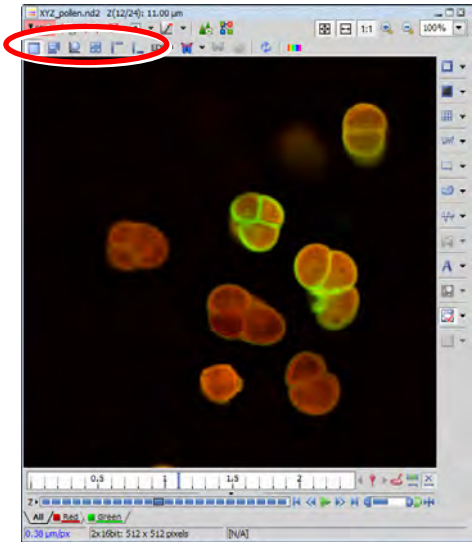
Note: Select "Use Original Acq. Time" when saving the file.

11 Creating a Slice View Image and a Projection Image

11.1 Create a slice view image or a projection image.

(1) Click  to open the Z series image.

(2) Click the  or  button at the side of the image frame to create an image.

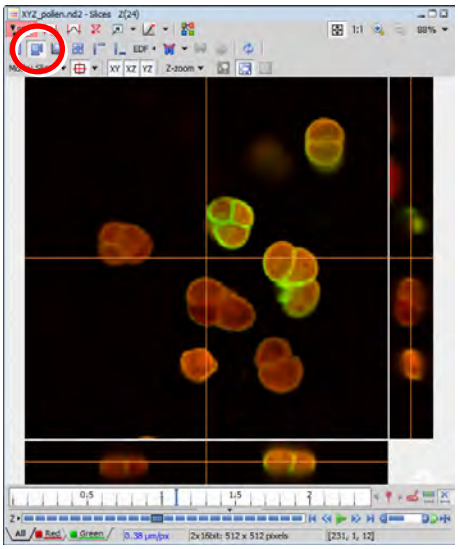


 [Show Slices View] button:

Click this button to display XZ and YZ cross sections of any position.

Clicking  displays the selected position.

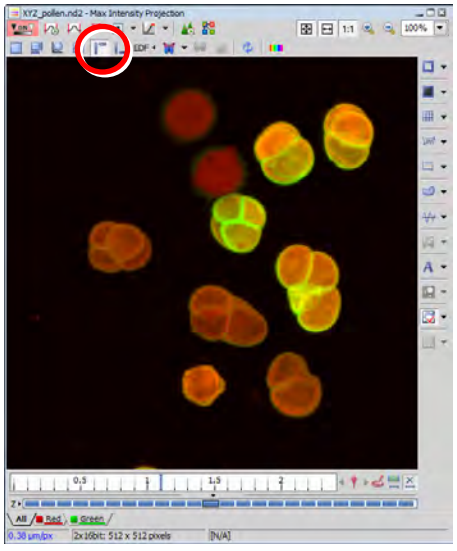
Drag the cross with the mouse.



 [Show Maximum Intensity Projection] button:

Clicking this button detects the maximum-brightness pixel from all frames to build an image.

This button is useful when capturing a thick sample as a plane.

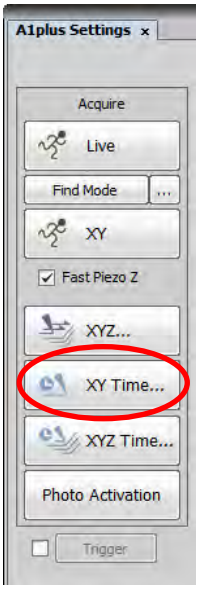


12 Capturing Time Series Images

12.1 Perform Steps 4.1 to 4.6 in Chapter 4, “Capturing Color Images” to determine image acquisition conditions.

12.2 Set the time series time settings.

(1) Click the XY Time... [XY Time] button to open the Capture Timelapse dialog box.



(2) Determine [Interval] (time interval) and [Duration] (duration time).

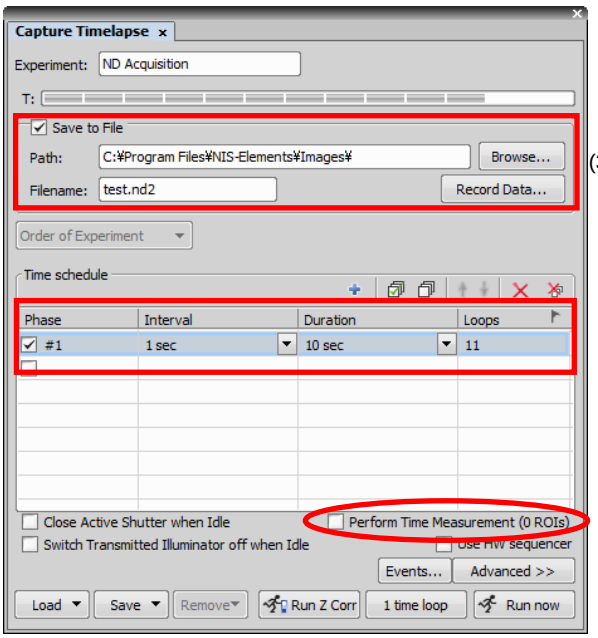
Note: Select “No Delay” for the [Interval] to acquire images at the highest speed.

Note: Two or more phases can be created. Selecting two or more phases allows variable time lapse (time lapse that changes the interval during the process).

(3) Check the [Save to File] checkbox to acquire images while saving them.

Note: We recommend acquiring time series images while saving them.

Note: Images are saved in nd2 file format.



(2)

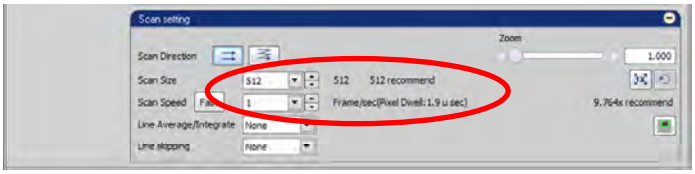
(3)

Time measurement can be performed while acquiring time series images.

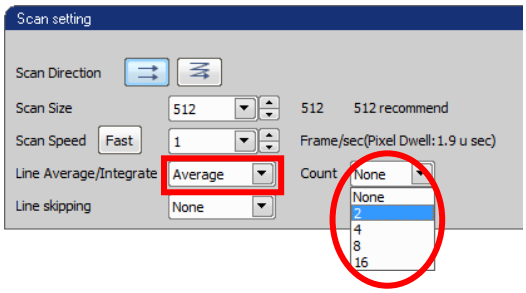
12.3 Acquire time series images.

(1) Set the number of pixels to the necessary resolution. (e.g. 512 x 512)
If the image is dark, reduce the scan speed.

Note: Check the pixel dwell for when the resolution is changed.
Pixel dwell indicates laser application time per pixel.
The larger the value, the brighter the image that can be acquired.

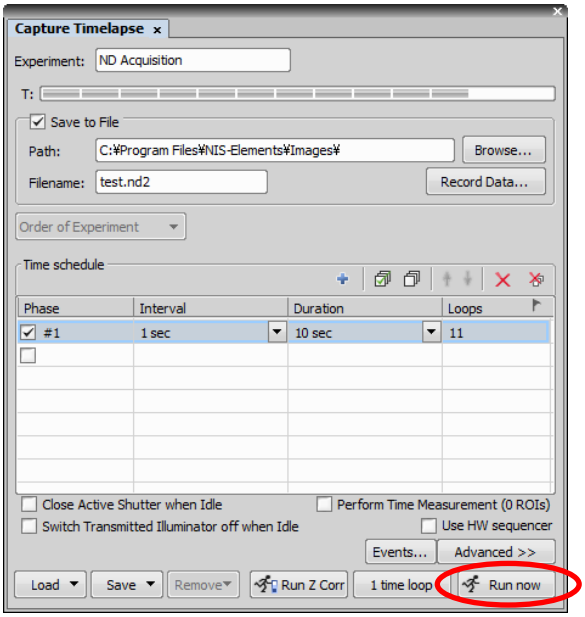


(2) Apply Line Average as needed.
Average is a function to scan the same image multiple times and average it to remove noises.
Select [Line Average/Integrate] - [Average] in the Scan setting window and select an average scan frequency in [Count].



Note: Averaging reduces noise, but decreases the frame rate (number of images acquirable per second).

(3) Click the Run now [Run now] button to acquire a time series image.

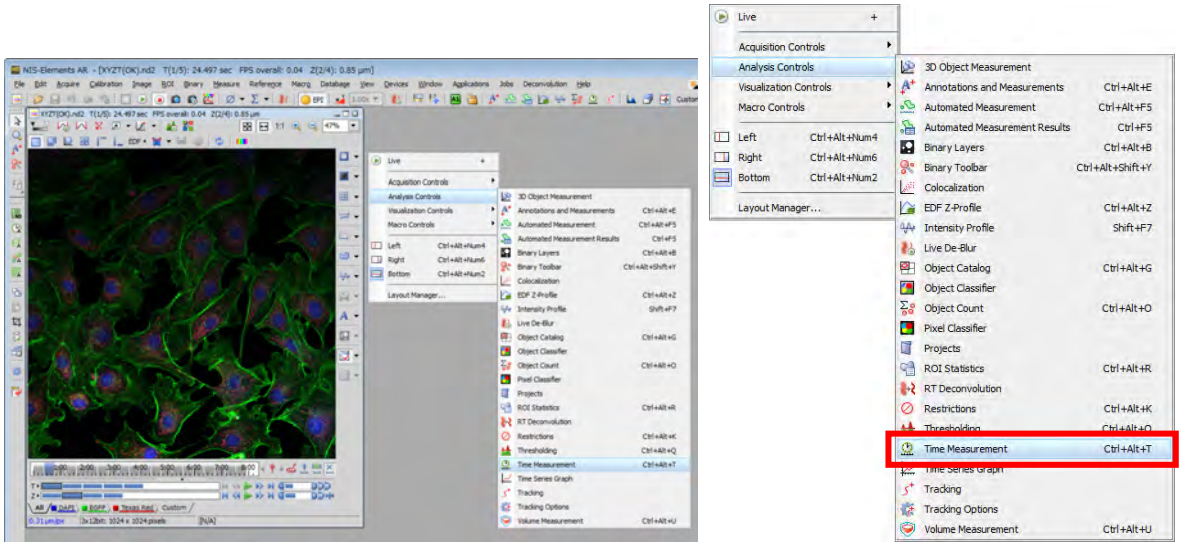


13 Time Measurement

13.1 Click to open the time series image.

13.2 Display [Time Measurement].

If [Time Measurement] is not displayed on the software, right-click the gray area of the software and select [Analysis Controls] - [Time Measurement] from the displayed menu to call Time Measurement.

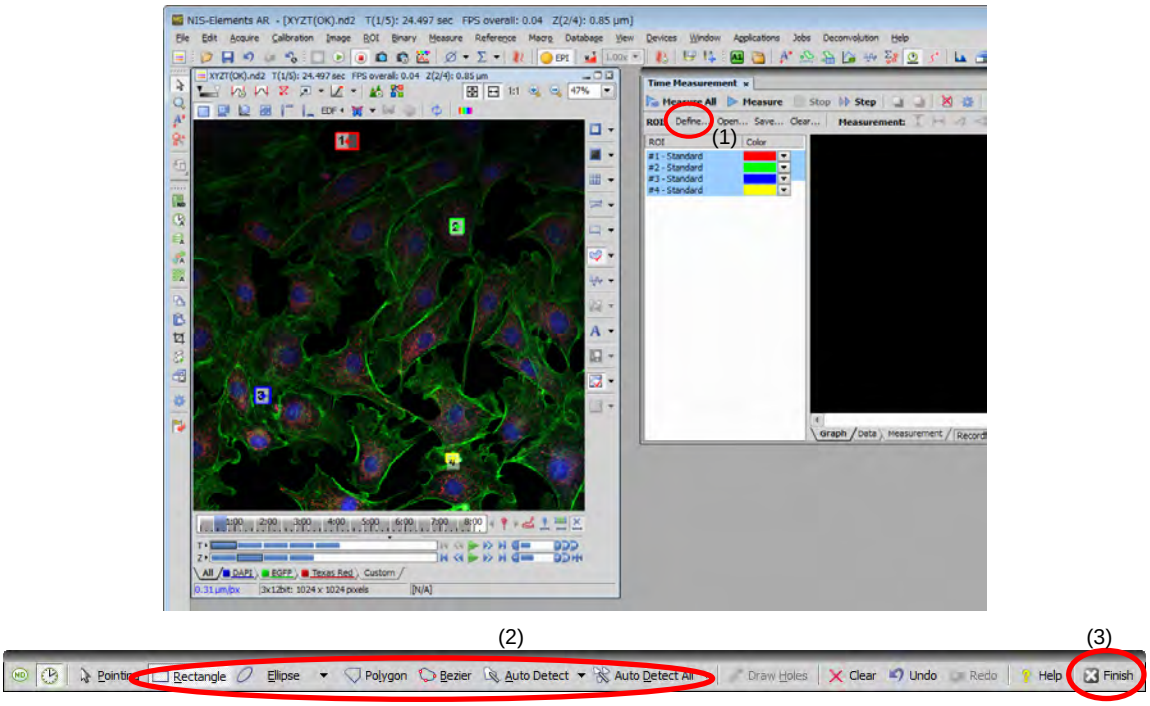


13.3 Set a ROI (Region of Interest) on the image.

(1) Click the Define... [Define] button to open the Define ROI window.

(2) Set a ROI on the image.

(3) Click the Finish [Finish] button to finish the setting.



13.4 Perform time measurement.

(1) Click the Measure [Measure] button to draw a graph.

(2) Use either of ROIs Channels measurement modes.

Multi ROIs: Displays change with time of multiple ROIs.
(Only a single channel can be selected.)

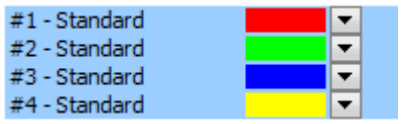
Multi Dyes: Displays change with time of multiple channels ("ALL" or "Custom").
(Only a single ROI can be selected.)

(3) Click Export and select “Export Data”, and then click the Export [Export] button to save the export data as text data.

Note: If the data cannot be saved as text data, select [Edit] - [Options] - [Data export] - [Global Settings] from the menu bar, and check [Export text files into folder], and then specify the save destination folder.

(1) (3) (2)

13.5 How to Use Time Measurement



Select the ROI to be displayed on the graph.

Click the ROI to be displayed.
Two or more ROIs can be selected by clicking the mouse while pressing SHIFT.

Measurement:

Time interval between two lines can be measured.

Brightness difference between two lines can be measured.

Left: Texas Red

Select the channel to be displayed on the graph.

14 Capturing Photo Activation Imaging (Galvano Scanner / Time-Series Activation)

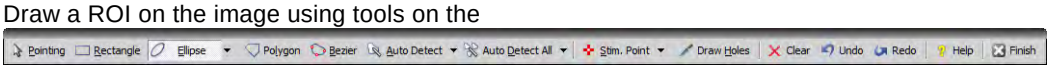
14.1 Perform Steps 4.1 to 4.7 in Chapter 4, “Capturing Color Images” to determine image acquisition conditions.

Note: When using Ch series, photo activation cannot be set. Select [None] in Ch series.

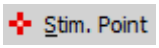
14.2 Set the area where photo activation is to be performed.

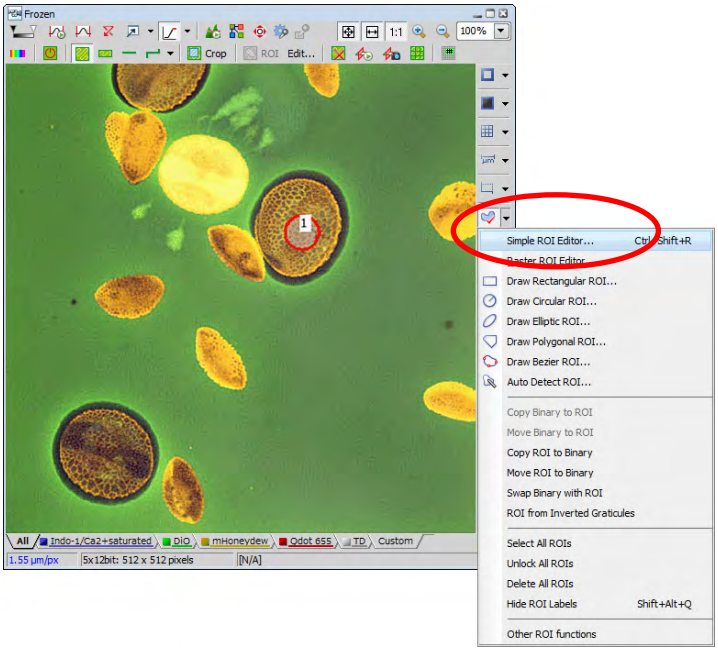
(1) Click  at the side of the image frame and select “Simple ROI Editor”.

Draw a ROI on the image using tools on the



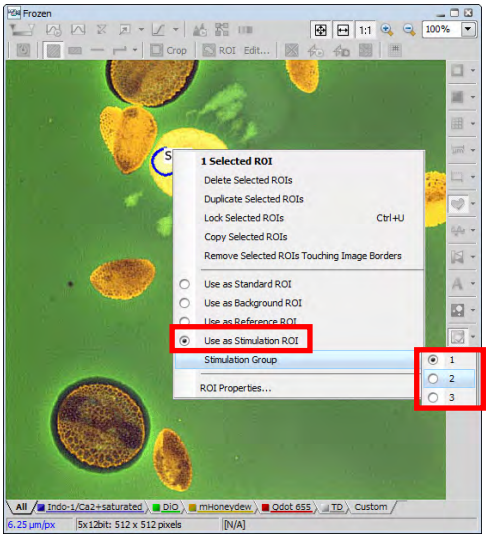
tool bar.

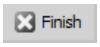
Note: Using  **allows point activation.**



(2) Right-click on the ROI and select [Use as Stimulation ROI] from the displayed menu, and then select [Stimulation Group].

Note: ROIs can be divided into up to three groups. A group can contain two or more ROIs.
Different activation conditions can be set by grouping ROIs.



(3) Click the  [Finish] button of “Simple ROI Editor” to finish the setting.

14.3 Set the laser light for activation.

(1) Click [Photo Activation] to switch the setting window.

(2) Click Tab 1 (Stimulation Group 1 setting).

(3) Select lasers used for activation.

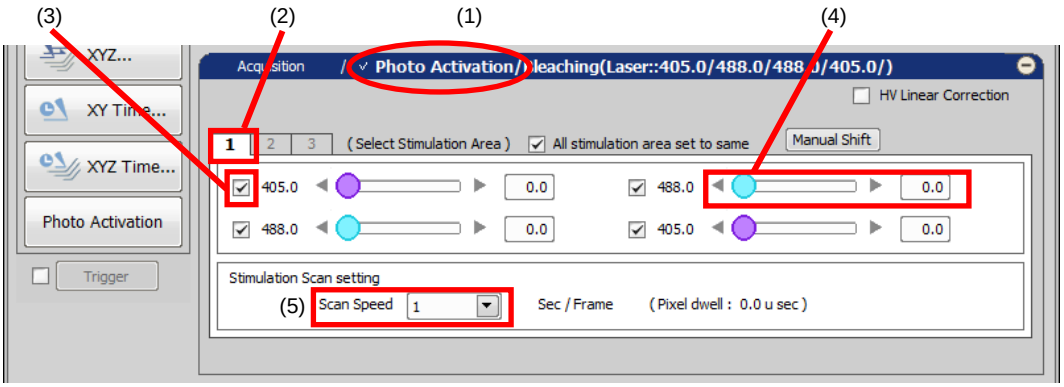
Note: All lasers can be used for activation.

(4) Move the laser bar to select the laser power for activation.

(5) Select [Scan Speed] for activation.

Note: Consider that Scan Speed is the time required for a single activation.
When "1 Sec/Frame" is selected, the time for a single activation is one second.

(6) When there are Stimulation Groups 2 and 3, repeat Steps (2) to (5).



14.4 Set time series for photo activation imaging.

(1) Click the [Photo Activation] button to open the ND Stimulation window.

(2) Set the photo activation imaging time settings.

Acq/Stim: Set whether to perform image acquisition or photo activation.

ROIs: Set stimulation groups used for activation.

Interval: Set the time interval of image acquisition or photo activation.

Duration: Set the duration time of image acquisition or photo activation.
When [Loops] is set, duration is automatically determined.

Loops: Set the number of image acquisition or photo activation execution times.

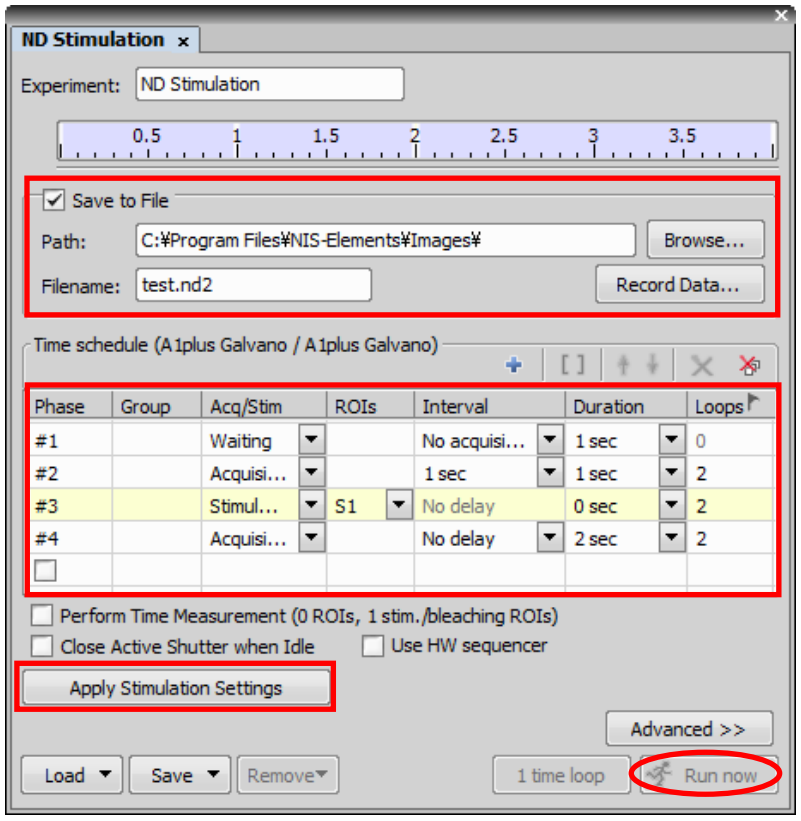
(3) Check the [Save to File] checkbox to acquire images while saving them.

Note: Images are saved in nd2 file format.

Note: We recommend acquiring time series images while saving them.

(4) Click Apply Stimulation Settings to read the settings for photo activation simultaneous imaging.

(5) Click the Run now [Run now] button to start photo activation imaging.



15 Image Display Function

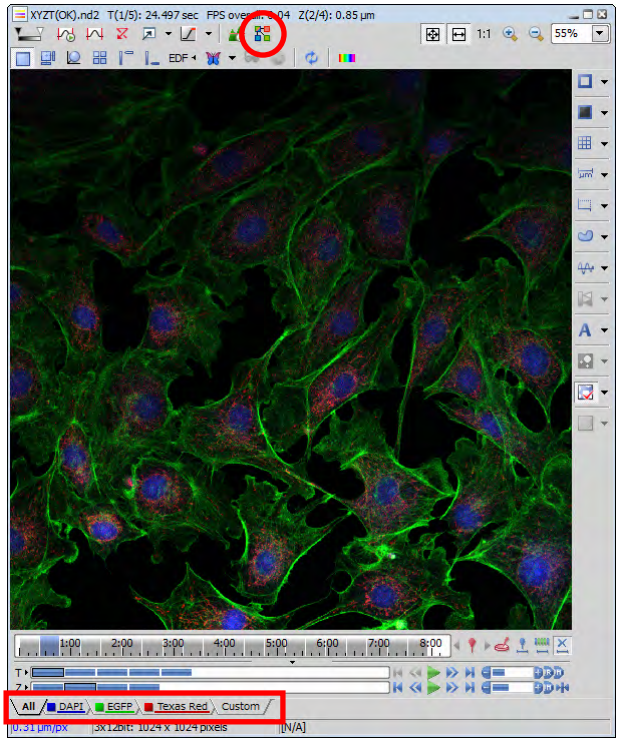
15.1 Channel display switch

(1) Click a tab on the 

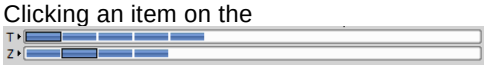
task bar to switch channels to be displayed.

The [Custom] tab allows you to freely select images for overlapping.
Right-click on the [Custom] tab and select images from the list.

(2) Clicking the [Split Components] button displays all channels at the same time. Reclicking this button restores the previous display.

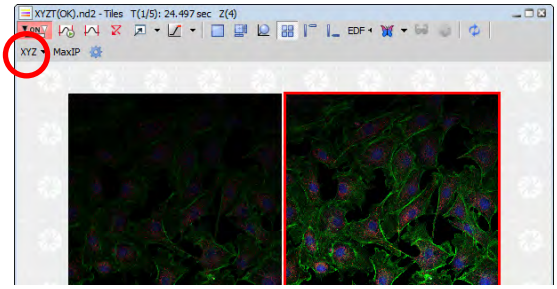


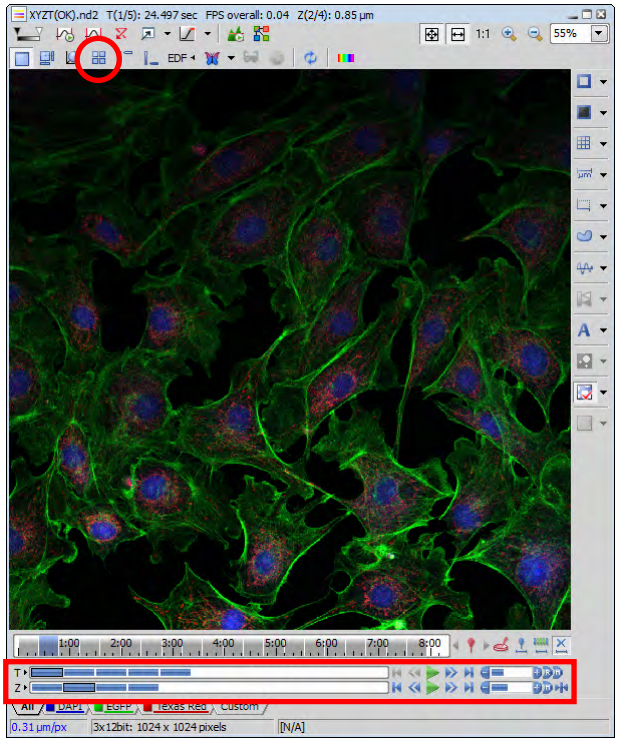
15.2 Multidimensional image display switch

(1) Clicking an item on the 
scale bar allows you to view each series image. Clicking or shifts the image to the next one.

(2) Use to play back the image. Reclicking this button stops playback. Use to adjust the playback speed.
Use for real-time playback (at the speed of actual image acquisition) and use for fastest playback.

(3) Clicking the [Show Tiled View] button displays series images in tiling mode.
Use XYZ to select series images for tiling.



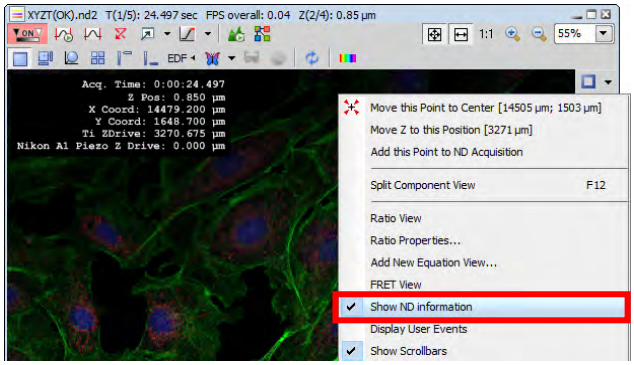


15.3 Inserting a scale

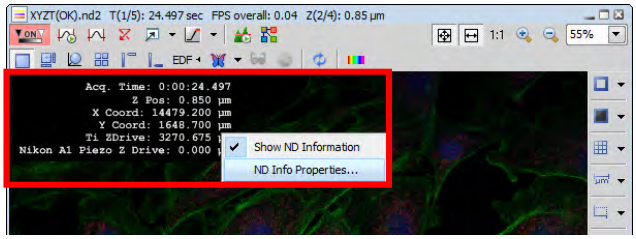
(1) Clicking the [Show Scale] button displays a scale on the image. The scale is hidden by reclicking this button.
Right-clicking on the scale allows you to edit the scale.
Unchecking the [Automatically adjust size] checkbox on the Scale tab from [Scale Properties] allows you to change the scale size to be displayed in [Size].
In addition, the scale bar and text color and size can be changed.

15.4 Inserting acquisition information into time-lapse image

(1) Right-click on a time-lapse image and select [Show ND Information] from the displayed menu to display the acquisition information on the image.



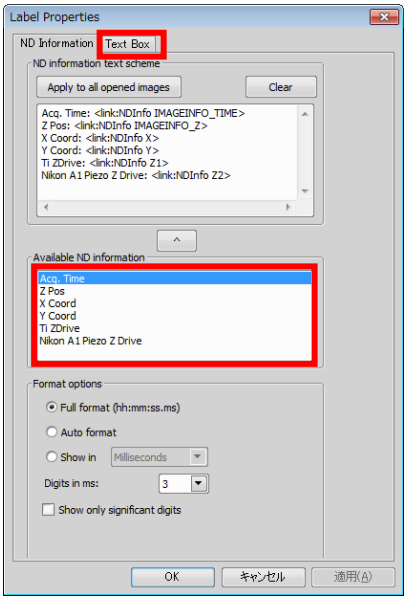
(2) Right-click on the displayed acquisition information and select [ND Info Properties] from the displayed menu to open the Label Properties dialog box.



(3) In the [Text Box] tab, you can add or delete information to be displayed, and change the display font, size, and color.

* When an image is saved as a moving picture file (by selecting [File] - [Save As] from the menu bar, and then selecting the AVI file) while the acquisition information is displayed, a moving picture file that contains acquisition time is created.

You can select information to be inserted.



15.5 Saving images

In the following cases, images cannot be saved in the normal manner (selecting [File] - [Save As] from the menu bar).

(a) When you save images with a scale or measurement result
(b) When you save images in a display method using Z stack images, such as Slice View or Projection

Save these images as follows.

(1) Select [Edit] - [Create View Snapshot (8bit RGB)] from the menu bar.

(2) An image is converted into an 8-bit file, and then saved temporarily.

(3) Select [File] - [Save As] from the menu bar to save the image in a desired file format.

15.6 Reading image acquisition conditions

Capturing conditions (such as information of the Optical path window and the frequency of averaging) at the time of acquisition can be read from the acquired image.

(1) Click  to open an image (nd2) whose acquisition conditions are to be read.

(2) Right-click on the image and select [Reuse Camera Setting] from the displayed menu.

(3) The setting conditions are read.

15.7 Reading the control state of the microscope

The control state of the microscope at the time of acquisition can be read from the acquired image.

(1) Click  to open an image (nd2) whose acquisition conditions are to be read.

(2) Right-click on the image and select [Reuse Device Setting] from the displayed menu.

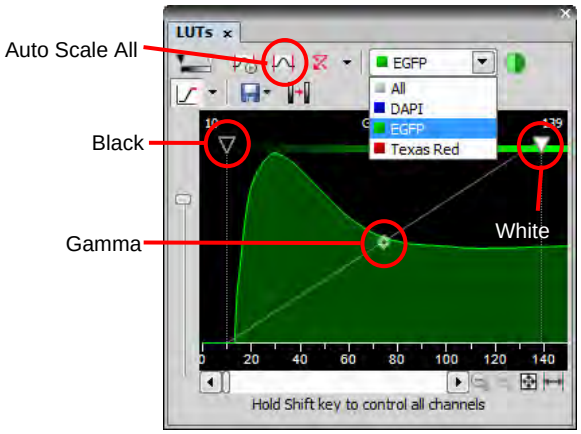
(3) The control state is read.

15.8 Adjusting Contrast

Right-click the gray area of the software and select [Visualization Control] - [LUTs] from the displayed menu to call the LUTs tab.

(1) Clicking the [Auto Scale All] button adjusts the contrast of all channels automatically.

(2) To adjust the contrast for each channel, select a channel from the list and drag the [Black], [White], and [Gamma] buttons to adjust the contrast.

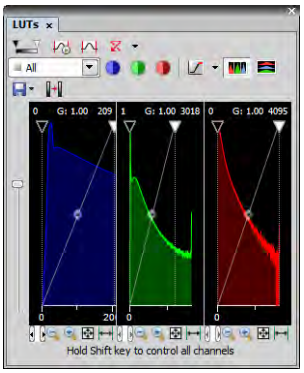


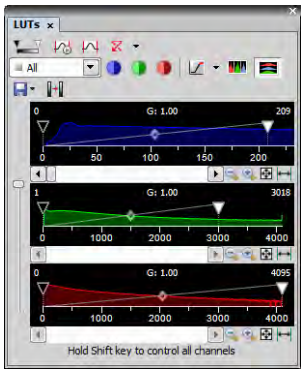
Lookup Table (LUT)

Display of the LUTs varies with the number of channels.

Up to three channels: All channels are displayed simultaneously.

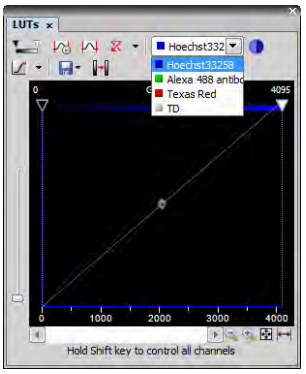
Vertical display or horizontal display can be selected with .





Four channels or more: A single channel is displayed.

Display channel can be selected from the pull-down menu.



(3) Clicking the [Reset LUTs] button returns the display without contrast adjustment.

(4) Clicking the [Pixel Saturation indication] button displays the saturated image area in red.

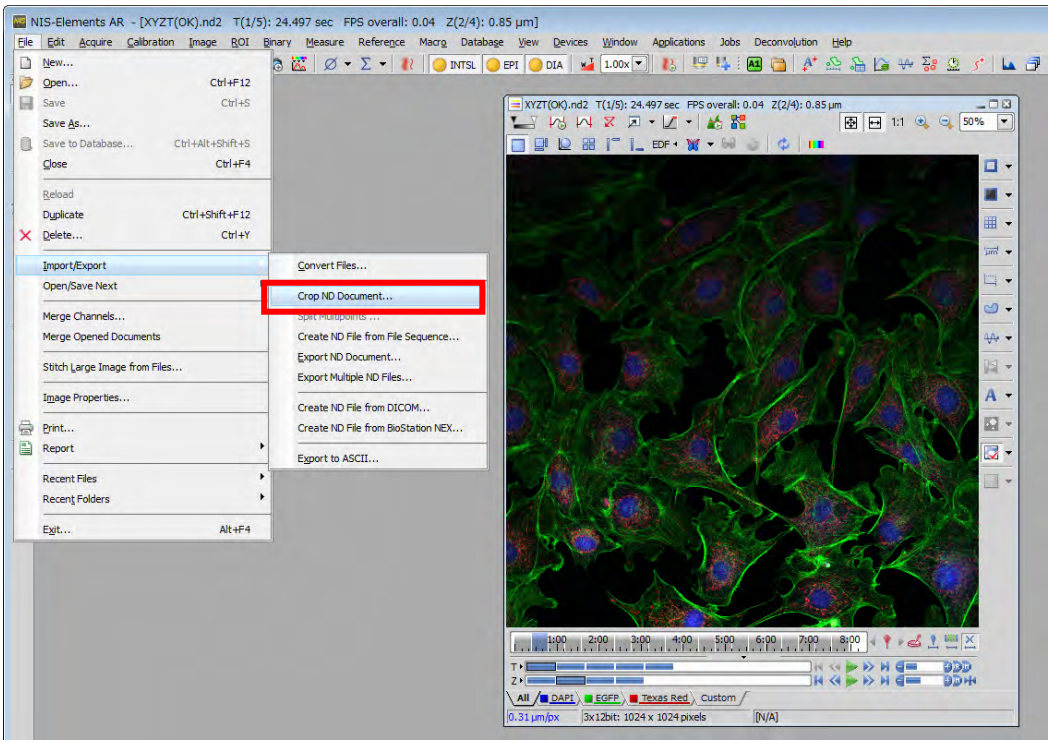
Note: Turning on and off the [Live] button (scan ON/OFF) during adjustment minimizes fading.

16 Extracting ND2 Files

Multidimensional images are managed with the nd2 file format. Files of only arbitrary dimension and range can be cropped.

(1) Click  to open the nd2 file.

(2) Select [File] - [Import/Export] - [Crop ND Document] from the menu bar.



(3) Specify the dimension and range of files you want to crop.

(4) Click the [Crop] button to create a new nd2 file.

Time axis

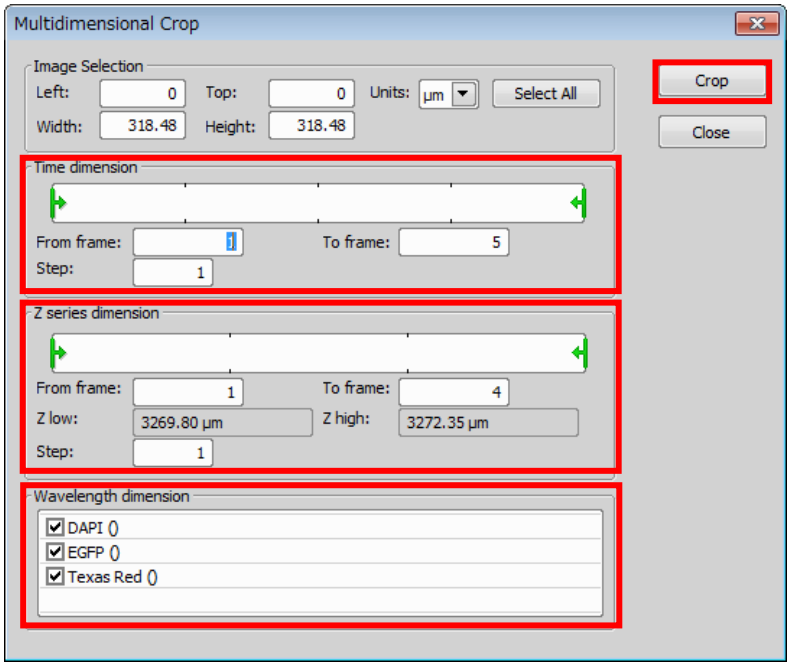
Only arbitrary time axis range is selectable.

Z axis

Only arbitrary Z axis range is selectable.

Wavelength

Only arbitrary channels are selectable.

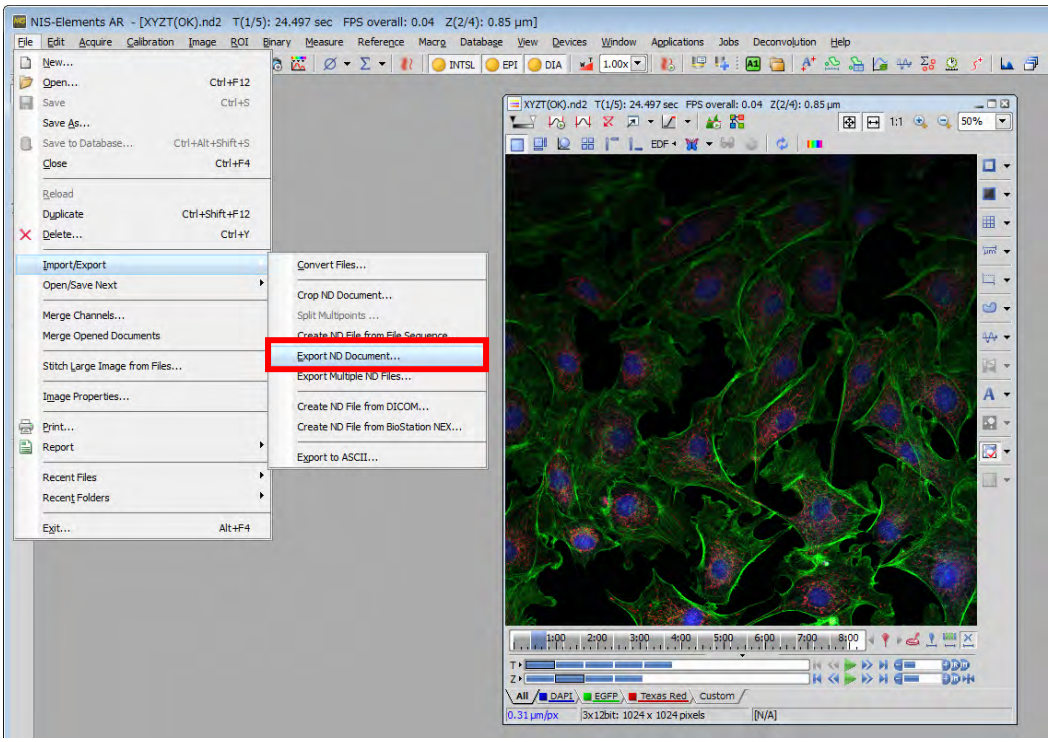


17 Exporting ND2 Files

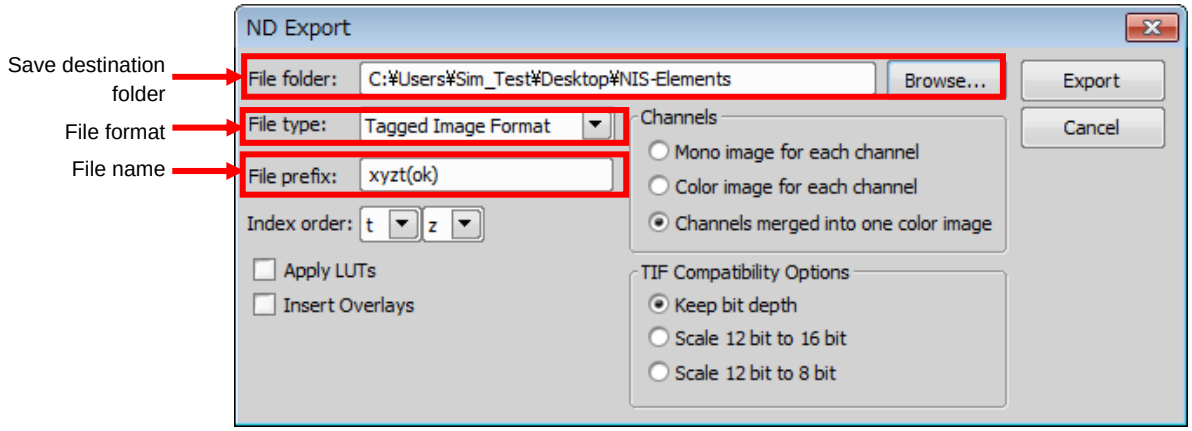
Multidimensional images are saved in nd2 file format specific to NIS-Elements. To view such images by other software, they must be exported in TIF file format.

(1) Click  to open the nd2 file.

(2) Select [File] - [Import/Export] - [Export ND Document] from the menu bar to open the ND Export window.



(3) Specify the save destination folder, file name, and file format.



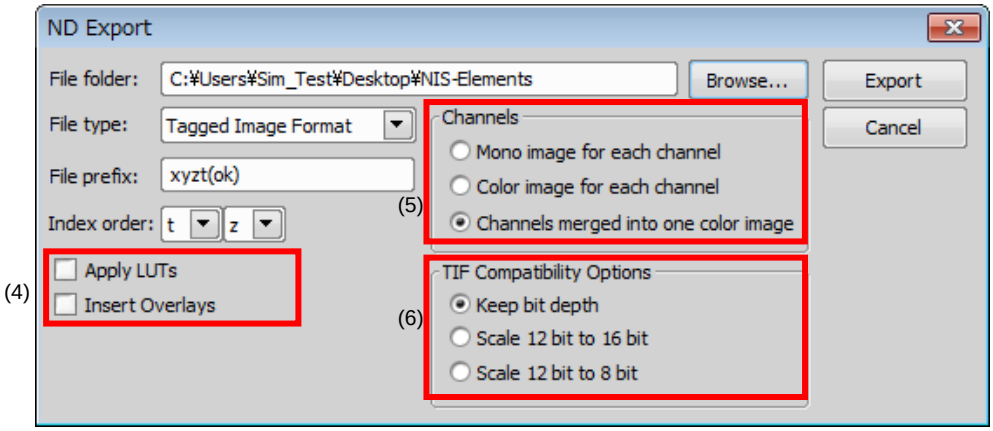
(4) Select details about conversion.

[Apply LUTs]:
Select this when you want to convert an image whose contrast is edited by [LUTs].

[Insert Overlays]:
Select this when you want to convert an image that contains a scale bar or measurement result.
Only overlapping images can be saved.

(5) Select a channel to be saved.
[Mono image]: A single channel is saved in monochrome.
[Color image]: A single channel is saved in color.
[Channels merged into color image]: Overlapping images are saved.

(6) To save an image as a TIFF file, specify TIF Compatibility (bit conversion).
[Keep bit depth]: Saved as a 12-bit file.
[Scale 12bit to 16bit]: Saved as a 16-bit file.
[Scale 12bit to 8bit]: Saved as an 8-bit file.



<Combination of Channel, TIF Compatibility Options, and Insert Overlay>

	Depth	12 bit to 16 bit	12 bit to 8 bit
Mono image	12 bit	16 bit	8 bit
Color image	12 bit RGB	16 bit RGB	8 bit RGB
Channel merged	Multi tif 12 bit	Multi tif 16 bit	Multi tif 8 bit
Channel merged + Insert overlay	12 bit RGB	16 bit RGB	8 bit RGB

Image: Image on a single channel Merged: Overlapping images
Light yellow: Output in color

Exporting a tif file that contains time lapse images with acquisition time

(1) Right-click on a time lapse image and select [Show ND Information] - [Acq.Time] from the displayed menu to display acquisition time on the image.

(2) Click 1:1 at the side of the image frame to set the image display size to 100%.

(3) Select [Edit] - [Create Full View Snapshot] from the menu bar to capture the screen.

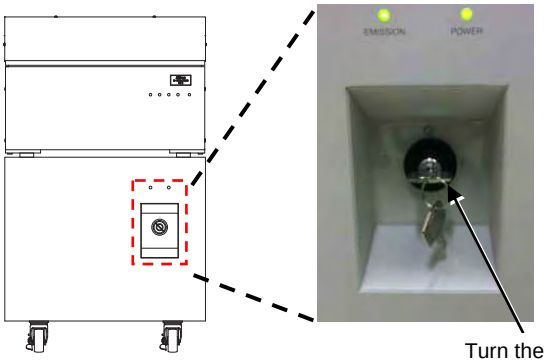
(4) Perform Steps (1) to (3) in Chapter 17, “Exporting ND2 Files” to export images as a TIF file.

18 Shutdown

18.1 Exit the NIS-Elements software.

18.2 Turn off the power to the laser.

Turn the key 90 degrees counterclockwise from the horizontal position (on).



Turn the key.

18.3 Turn off the power to the controller.

18.4 Turn off the power to the microscope.

(1) Turn off the power to control box A.

(2) Turn off the power to the microscope main body.

(3) Turn off the power to the mercury lamp (for visual fluorescence microscopy).

(4) Turn off the power to the halogen lamp (for visual diascopic microscopy).

(5) Turn off the power to the piezo Z stage.

(6) Turn off the power to the motorized stage.

18.5 Shut down the PC.

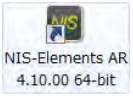
Spectrum Imaging

A1 / Ni-E /
Motorized Stage / Piezo Z Stage / Intensilight

This edition may have unavailable functions depending on model in use and option settings.

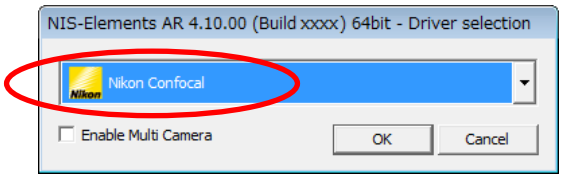
19 Capturing Spectral Images (Spectral Detector)

19.1 Run the NIS-Elements software.

(1) Click the 

icon to run the NIS-Elements software.

Note: When not only a confocal microscope is connected but also a camera, the Driver selection dialog box opens to select a driver.
Select "Nikon Confocal" in the Driver selection dialog box and click the [OK] button.

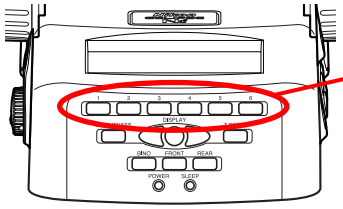


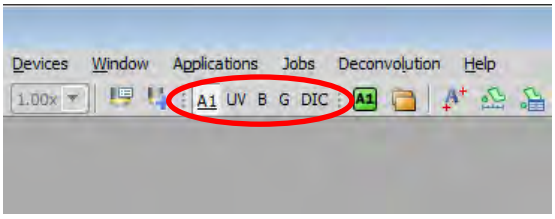
19.2 Observe the sample through the microscope.

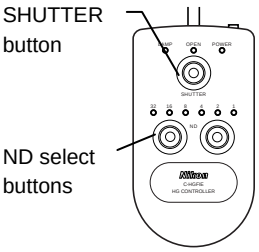
(1) Select microscopy.
When the assignment of the function buttons of the microscope main body is changed from the factory setting, select microscopy and the [A1] button.
If the desired microscopy and the [A1] button have been registered for the Optical Configuration button (hereafter called O.C button) on the NIS-Elements software beforehand, click the O.C button.

- **Optical Configuration button**
  Buttons for which the optical path has been recorded in advance
  The buttons can be customized so the number of buttons and their names vary depending on the customer's preference.

Note: To prevent fading, close the fluorescent shutter frequently. Use the ND filter to look for the sample.



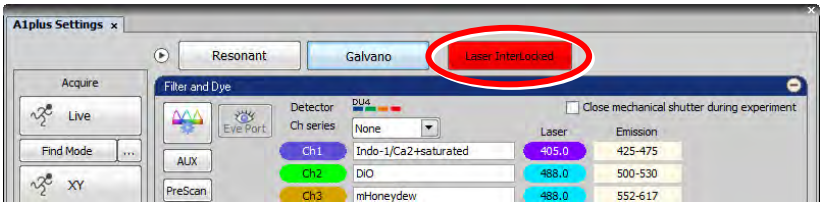




19.3 Switch the optical path to A1.

19.4 Click the [Laser InterLocked] button to reset blinking and to enable laser oscillation with the software.

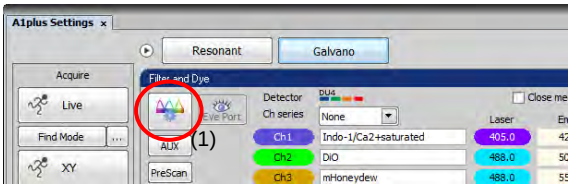
Note: If the optical path is not switched to A1, blinking cannot be reset even though the button is clicked.



19.5 Set the optical path. (Optical path setting for the confocal system required for acquiring images)

Check the settings.

(1) Click  to open the Optical path window.



(2) Click the  [SD] button to select the spectral detector.

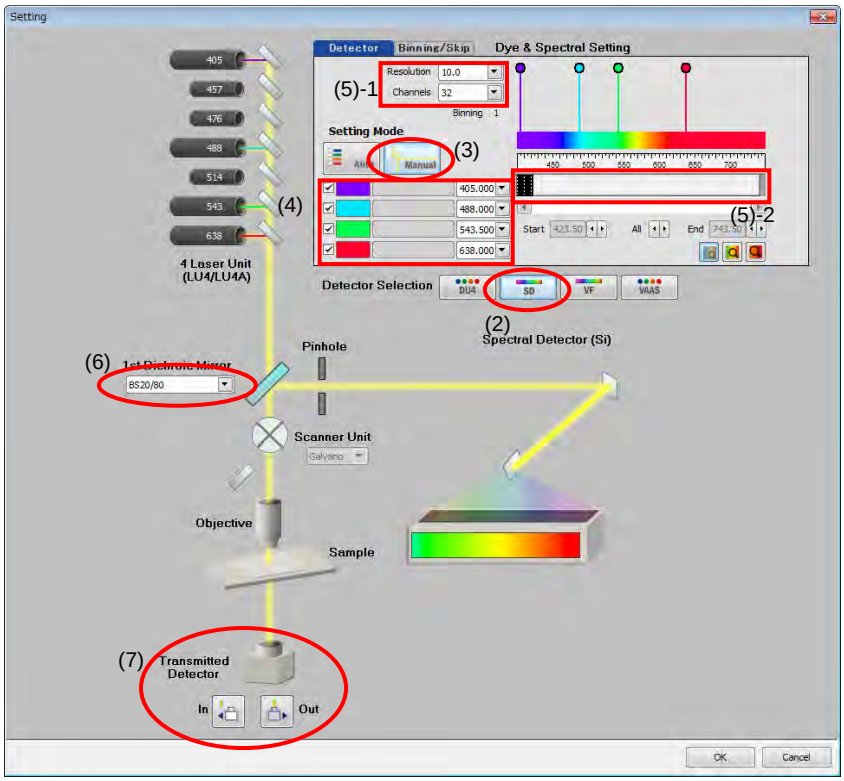
(3) Click the  [Manual] button to set the optical path in the manual mode.

(4) Check the checkboxes of the channels of lasers to be used.

(5) Select a value for [Resolution] (wavelength resolution to be used) from “2.5 nm”, “6 nm”, and “10 nm” and a value for [Channels] (number of channels to be used) from “1” to “32”. Set the wavelength band to be acquired by shifting the bar.

(6) Select “BS20/80” for [1st DM].

(7) If acquiring a transmitted image together with a spectral image, click  to bring  into the optical path.



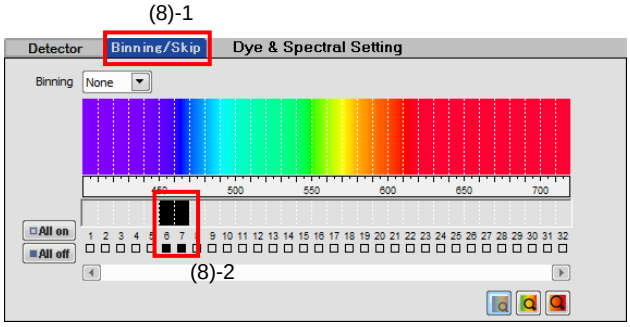
Note: Before acquiring a transmitted image, turn off the light above the microscope.

Note: Because the transmitted light detector is placed in front of transmitted light, transmitted images (differential interferences (DIC)) cannot be observed visually while putting the transmitted light detector into the optical path.
To observe transmitted images visually, remove the transmitted light detector from the optical path.

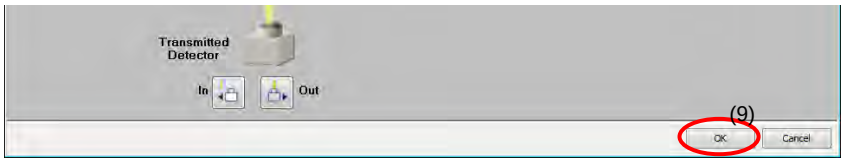
(8) For channels that directly detect laser, click channel-skip boxes 

on the Binning/Skip tab to set so that laser is not detected.

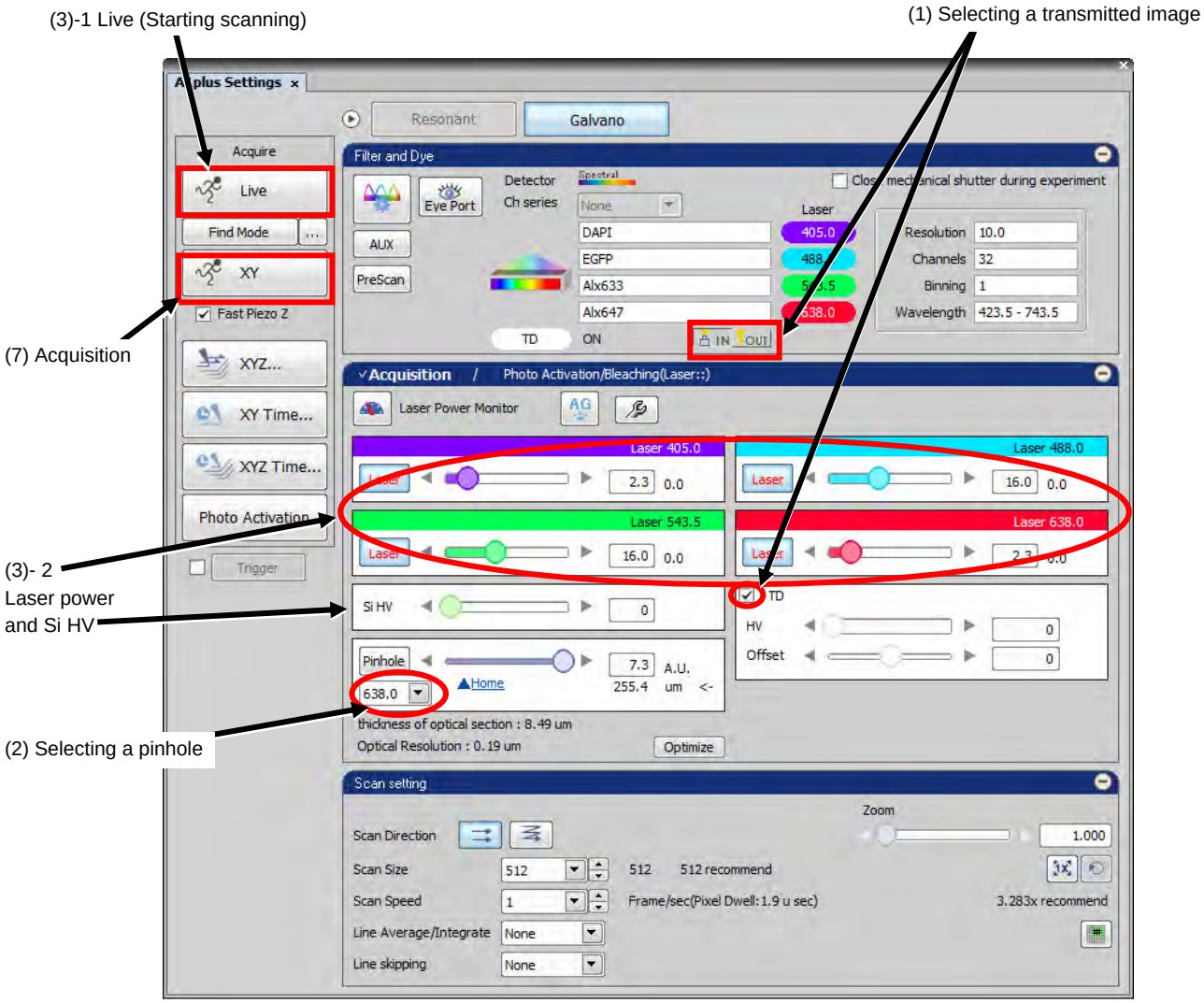
Note: Channels that catch reflected laser light are covered with a mask (plate) and channel data is not acquired. Selecting a channel-skip box facilitates subsequent spectrum analysis. The volume of data is reduced a little.



(9) Click the [OK] button to set the optical path.



19.6 Determine image acquisition conditions.



(1) If you want to acquire a transmitted image together with a spectral image, click the TD [IN] button and check the TD checkbox.

Note: Before acquiring a transmitted image, turn off the light above the microscope.

(2) Select the laser wavelength to be used from [Pinhole].
Select a pinhole size best suited for the objective with [▲Home].

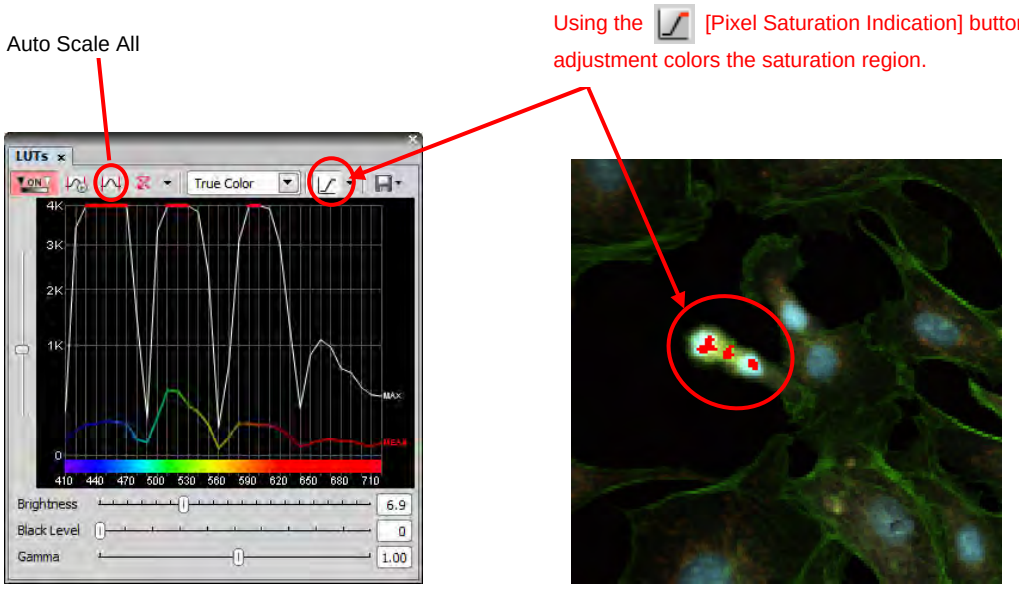
(3) Click the [Live] button and adjust [Laser] (laser power) and [Si HV] (detector sensitivity) while checking the image.

Note: The Si HV setting is common to all lasers. Make fine adjustments with the laser power setting.

Note: Using the [Pixel Saturation Indication] button in the LUTs tab during adjustment makes it easy to adjust the sensitivity.

Note: If the displayed image is dark, click the [Auto Scale All] button to adjust the contrast of the channel automatically to make the image clear.

Note: Turning on and off the [Live] button (scan ON/OFF) during adjustment minimizes fading.



Note: If the LUTs tab is not displayed, right-click the gray area of the software and select [Visualization Control] - [LUTs] from the displayed menu to call it.

(4) To use Auto Gain, a function that automatically adjusts the detector sensitivity (HV) based on the set rate of saturated pixels, click the [AG] button.

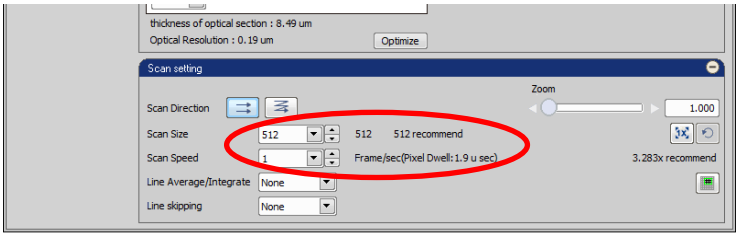
"NG" is displayed for channels that failed in Auto Gain and the HV values are returned to the previous values. Use the [Auto Gain setting] button to change the rate of saturated pixels. Set the maximum value and minimum value of the rate.

Notes:

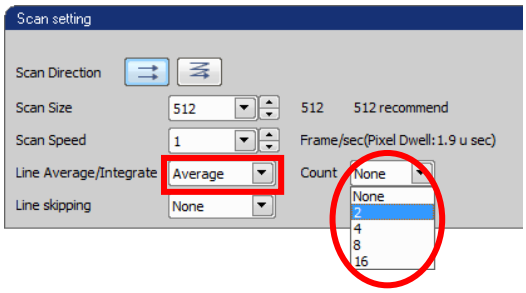
- **Auto Gain is disabled during scanning.**
- **Auto Gain is disabled when line scan is set.**
- **Do not make a manual adjustment in the Acquisition window and do not make an adjustment using the remote controller during Auto Gain.**

(5) Set the number of pixels to the necessary resolution. (e.g. 512 x 512)
If the image is dark, reduce the scan speed.

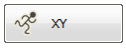
Note: Check the pixel dwell for when the resolution is changed.
Pixel dwell indicates laser application time per pixel.
The larger the value, the brighter the image that can be acquired.



(6) Apply Line Average as needed.
Average is a function to scan the same image multiple times and average it to remove noises.
Select [Line Average/Integrate] - [Average] in the Scan setting window and select an average scan frequency in [Count].



Note: Averaging reduces noise, but decreases the frame rate (number of images acquirable per second).

(7) Click the 

[XY] button to acquire an image.

19.7 Save an image.

Make the image you want to save active and select [File] - [Save As] to save it.

Note: We recommend that the image be saved in nd2 file format. Conditions such as parameters are also saved.

20 Separating Spectral Image (Unmixing)

20.1 Click to open the spectral image.

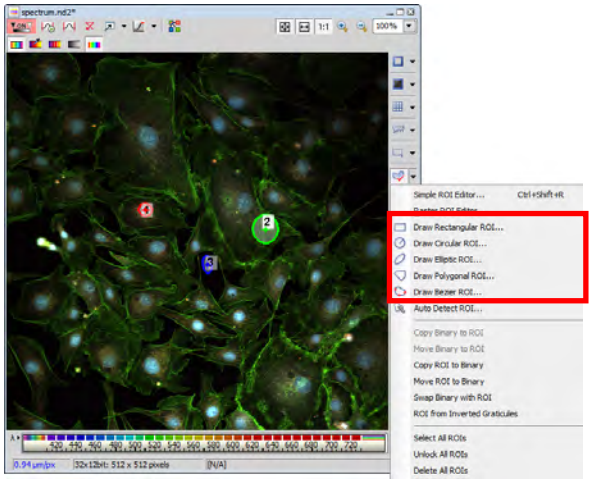
20.2 Spectral unmixing

The following three patterns can be used in combination.

1) Spectral unmixing using spectral data in ROIs

(1) Click at the side of the image frame and draw ROIs on the image using tools.

Note: Specify areas that include only shades of the same color up to the number of pigments (2 areas or more).



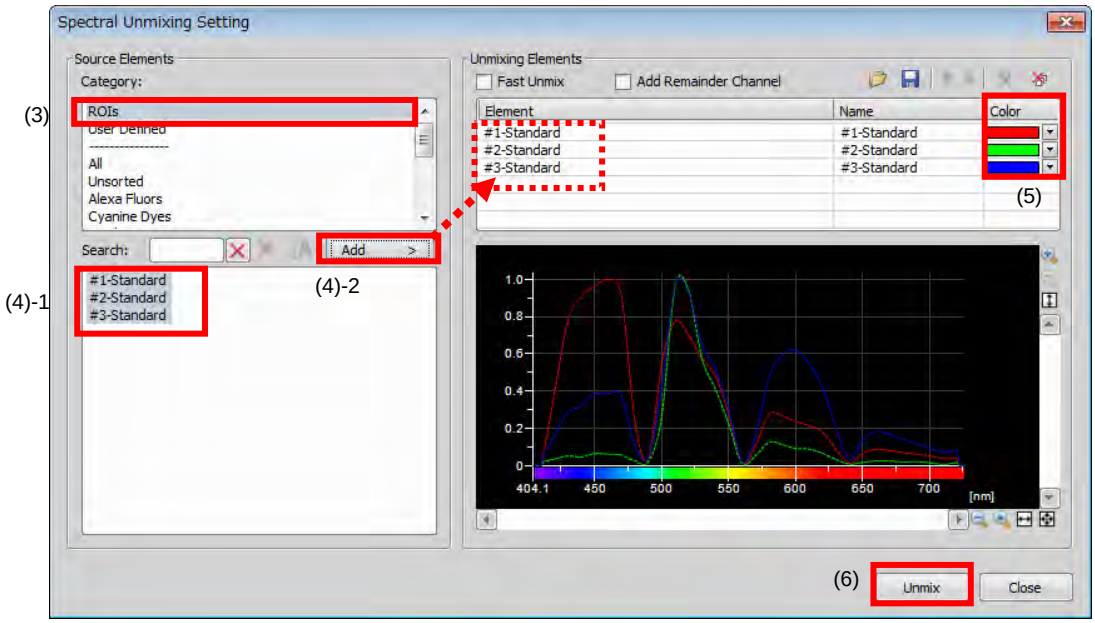
(2) Select [Image] - [Spectral Unmixing Setting] from the menu bar.

(3) Select [ROIs] from the [Category] list box.

(4) Select ROIs to be used for spectral unmixing and click the [Add] button to add them to [Unmixing Elements] (list on the right pane).

(5) Set pseudo-colors after spectral unmixing.

(6) Click the [Unmix] button to perform spectral unmixing.



2) Spectral unmixing using reference data

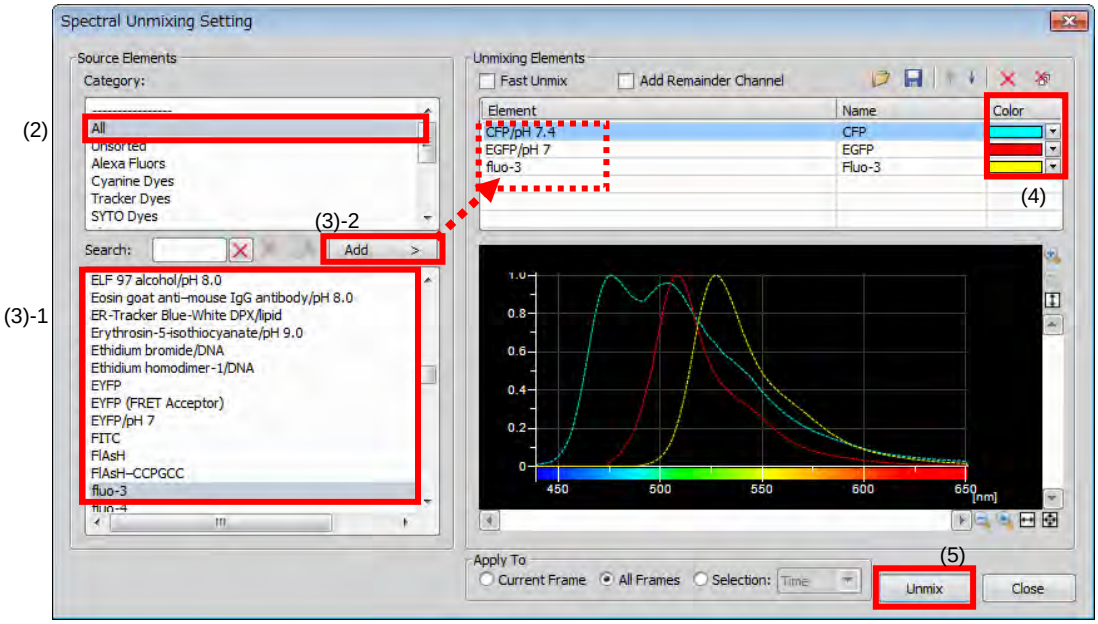
(1) Select [Image] - [Spectral Unmixing Setting] from the menu bar.

(2) Select [All] from the [Category] list box.

(3) Select reagents to be used for spectral unmixing and click the [Add] button to add them to [Unmixing Elements] (list on the right pane).

(4) Set pseudo-colors after spectral unmixing.

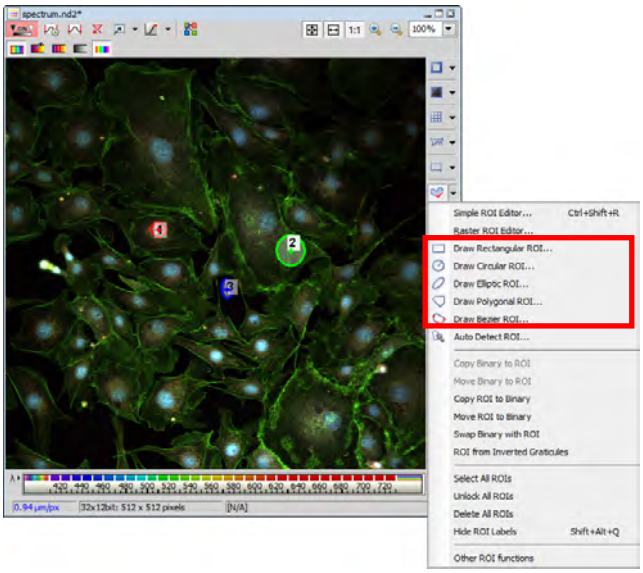
(5) Click the [Unmix] button to perform spectral unmixing.



3) Spectral unmixing by creating reference data

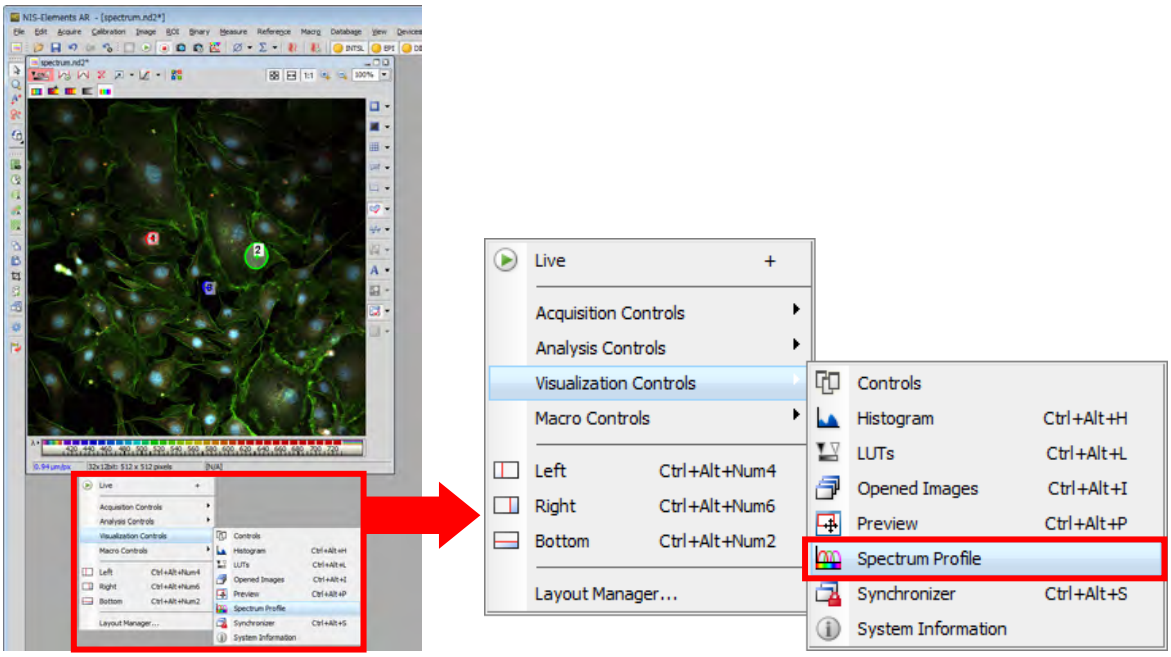
(1) Save spectral data as reference data.
Click  at the side of the image frame and draw ROIs on the image using tools.

Note: Specify simple stain areas only.

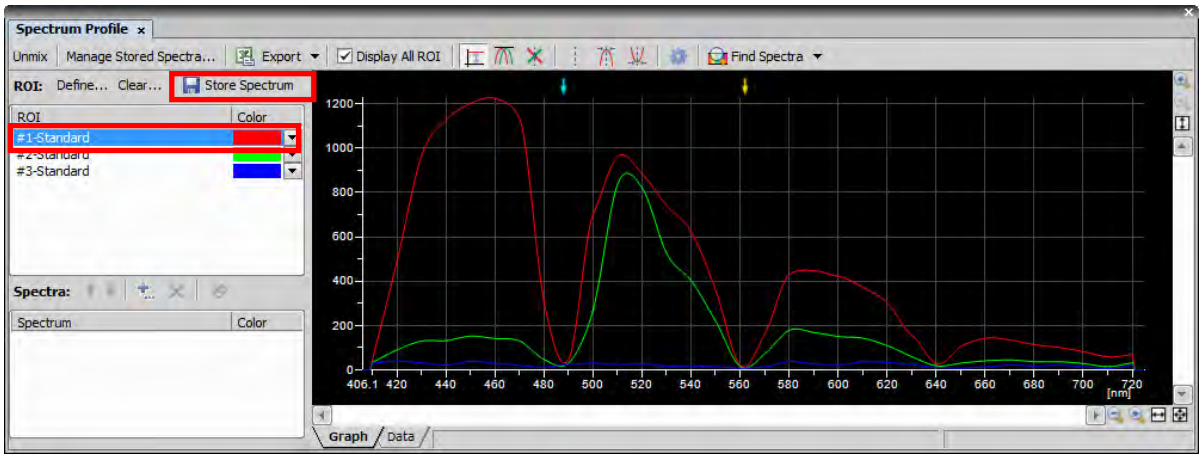


(2) The spectrum graph of the specified ROI is displayed in the Spectrum Profile window

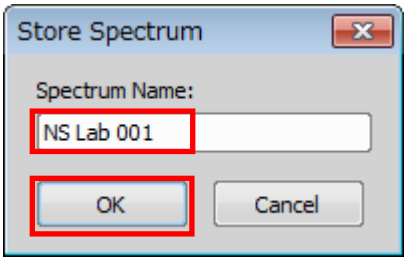
Note: If Spectrum Profile is not displayed on the software, right-click the gray area of the software and select [Visualization Controls] - [Spectrum Profile] from the displayed menu to call Spectrum Profile.



(3) Select the spectrum graph of a ROI which you want to save, and then click Store Spectrum.



(4) Enter a reagent name in the [Spectrum Name] box, and then click the [OK] button to save the spectral data.



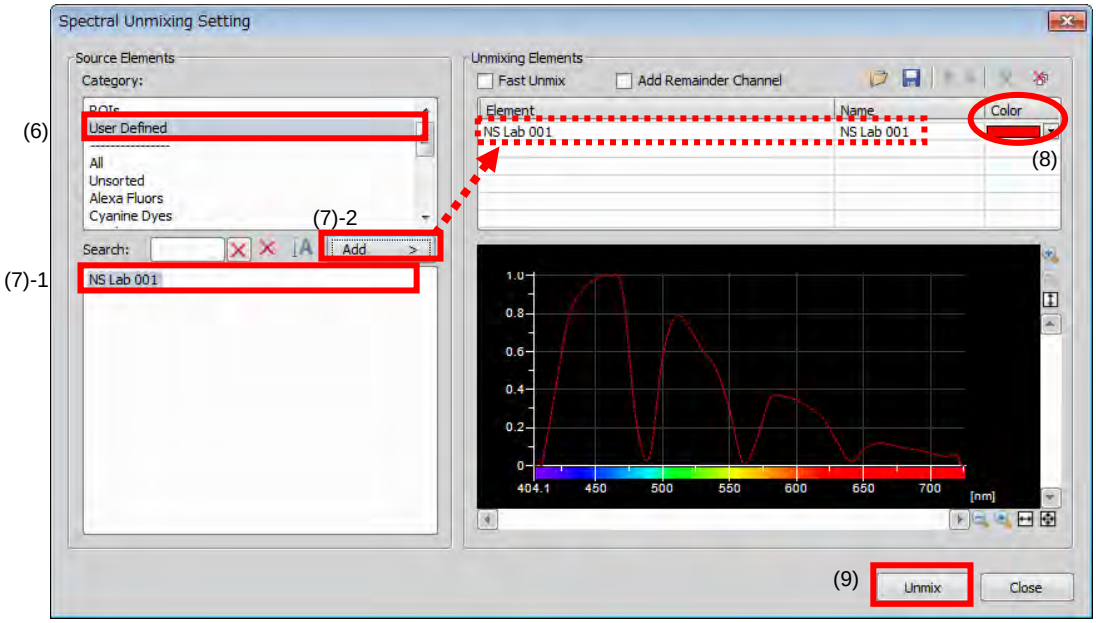
(5) Select [Image] - [Spectral Unmixing Setting] from the menu bar.

(6) Select [User Defined] from the [Category] list box.

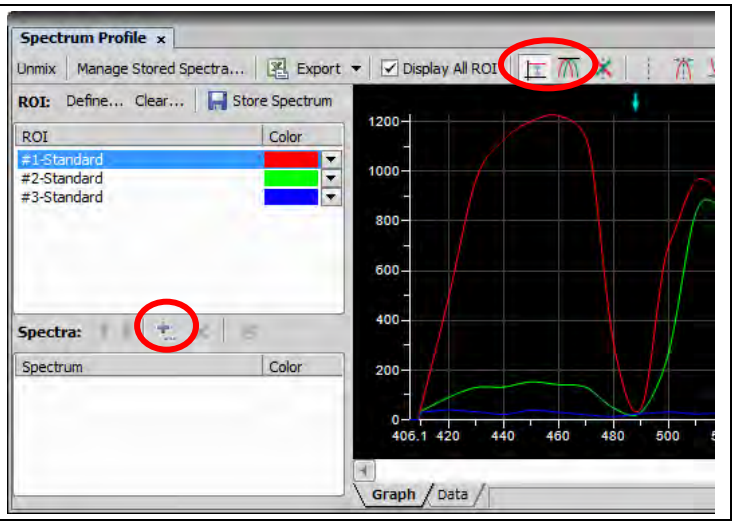
(7) Select reagents (spectral data saved in step 4) to be used for spectral unmixing and click the [Add] button to add them to [Unmixing Elements] (list on the right pane).

(8) Set pseudo-colors after spectral unmixing.

(9) Click the [Unmix] button to perform spectral unmixing.



How to use Spectrum Profile

Displays the Y axis of graph as relative values. Displays the Y axis of graph as brightness values. Inserts a spectrum graph originated by the fluorescence manufacturer as reference of analysis.	

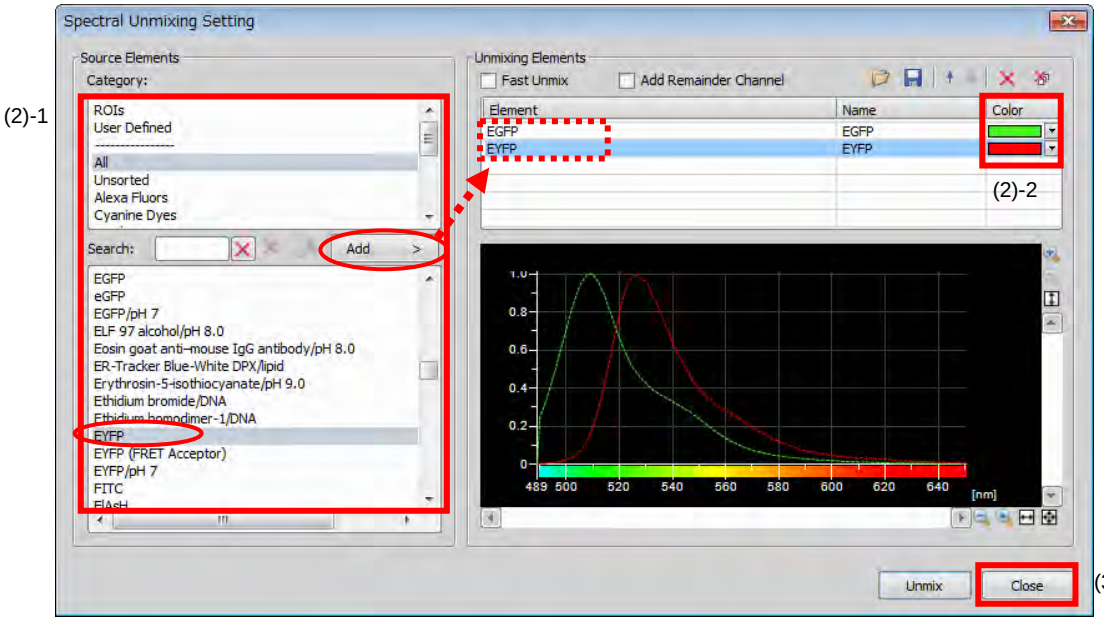
21 Live Unmixing (Spectral Unmixing for Live Image)

21.1 Set the reference data to be used for spectral unmixing.

(1) Select [Image] - [Spectral Unmixing] from the menu bar to open the Spectral Unmixing Setting window.

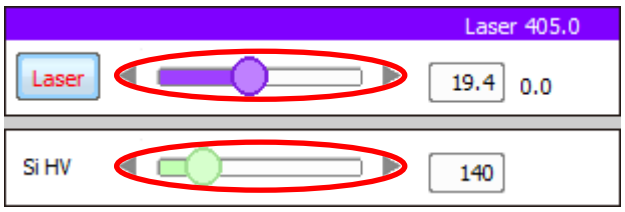
(2) Set a spectrum to be used for unmixing from [ROIs]/[Users Defined]/[All] data of [Category] by following the procedure for “Separating Spectral Image”.

(3) Click the [Cancel] or [Close] button to close the Spectral Unmixing Setting window.



21.2 Perform spectral unmixing for live image.

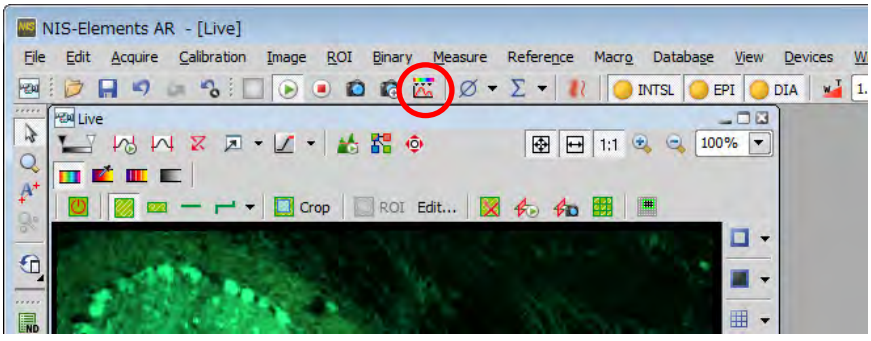
(1) Click the [Live] button and adjust [Laser] (laser power) and [Si HV] (detector sensitivity) while checking the image.

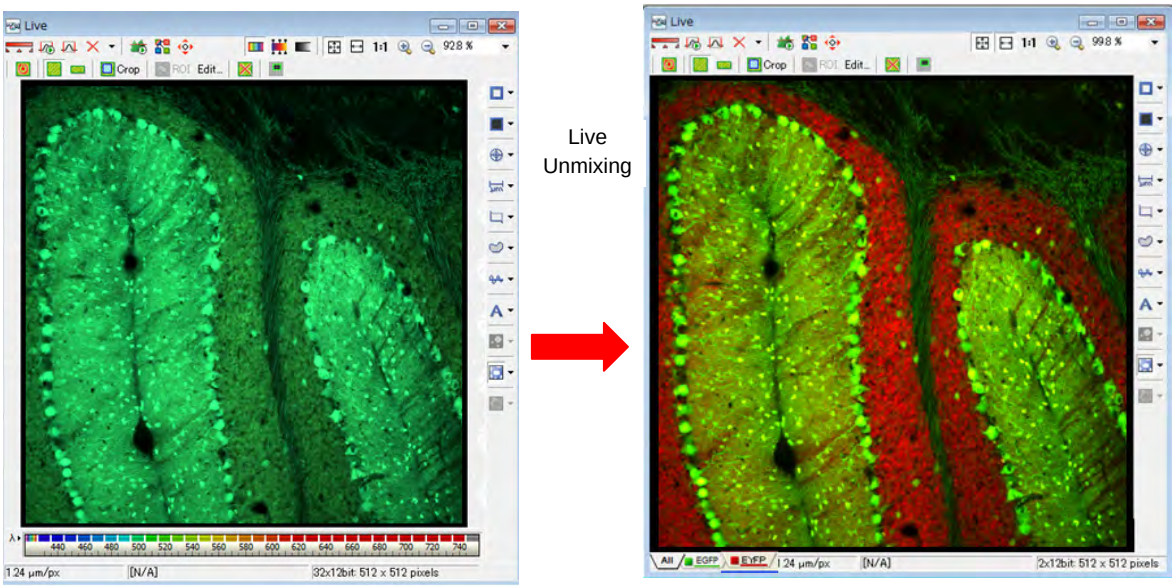


Note: The Si HV setting is common to all lasers. Make fine adjustments with the laser power setting.

(2) Click the [Live Unmixing] button to switch the Live image to the Unmix Live image.

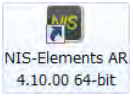
(3) Click the [Live Unmixing] button again to return to the Live image.




Live
Unmixing

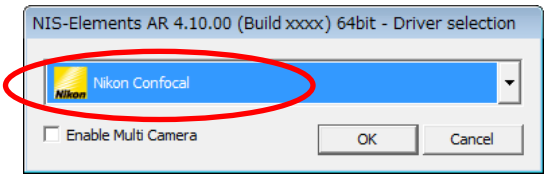
22 Capturing Virtual Filter Images (Spectral Detector)

22.1 Run the NIS-Elements software.

(1) Click the 

icon to run the NIS-Elements software.

Note: When not only a confocal microscope is connected but also a camera, the Driver selection dialog box opens to select a driver.
Select "Nikon Confocal" in the Driver selection dialog box and click the [OK] button.

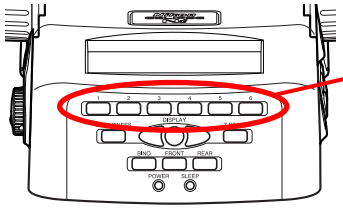


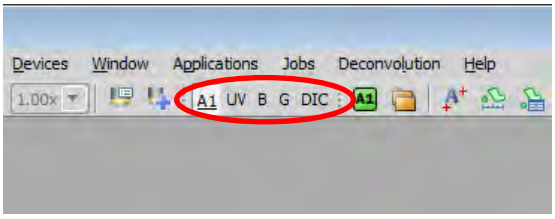
22.2 Observe the sample through the microscope.

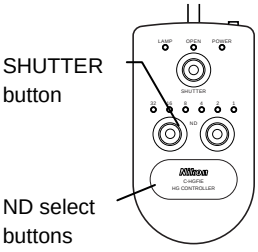
(1) Select microscopy.
When the assignment of the function buttons of the microscope main body is changed from the factory setting, select microscopy and the [A1] button.
If the desired microscopy and the [A1] button have been registered for the Optical Configuration button (hereafter called O.C button) on the NIS-Elements software beforehand, click the O.C button.

- **Optical Configuration button**
  Buttons for which the optical path has been recorded in advance
  The buttons can be customized so the number of buttons and their names vary depending on the customer's preference.

Note: To prevent fading, close the fluorescent shutter frequently. Use the ND filter to look for the sample.



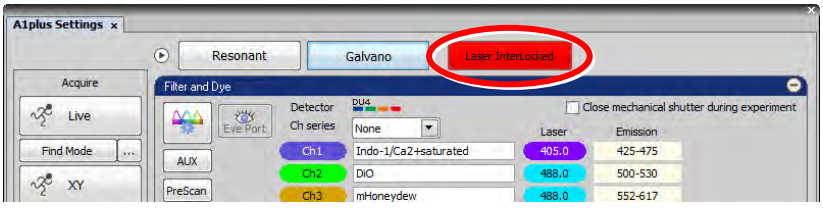




22.3 Switch the optical path to A1.

22.4 Click the [Laser InterLocked] button to reset blinking and to enable laser oscillation with the software.

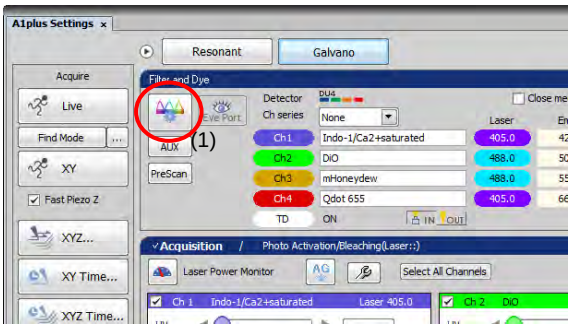
Note: If the optical path is not switched to A1, blinking cannot be reset even though the button is clicked.



22.5 Set the optical path. (Optical path setting for the confocal system required for acquiring images)

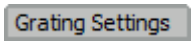
Check the settings.

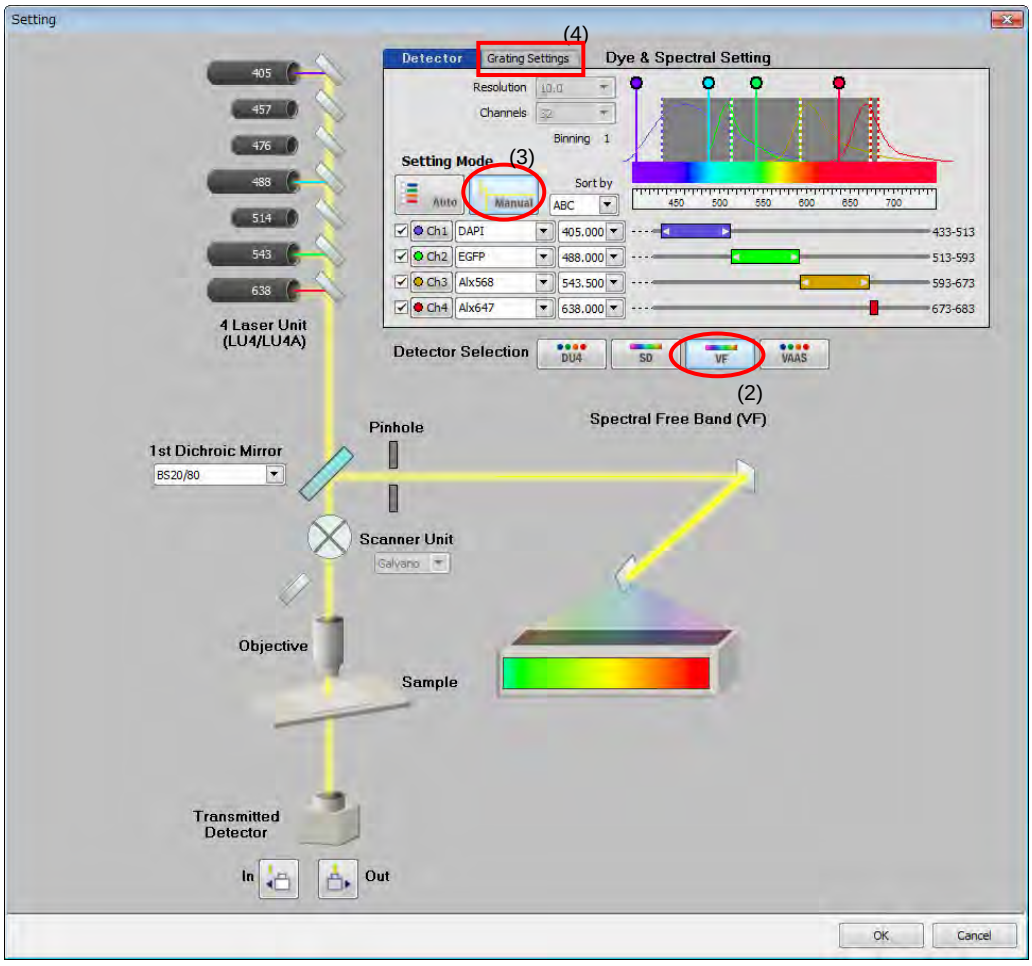
(1) Click  to open the Optical path window.



(2) Click the  [VF] button to use the spectral detector in the virtual filter mode.

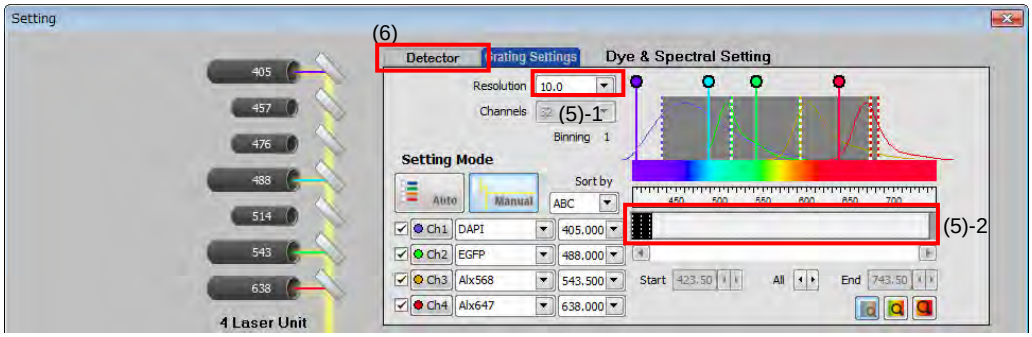
(3) Click the  [Manual] button to set the optical path in the manual mode.

(4) Click the  [Grating Settings] tab.



(5) Select a value for [Resolution] (wavelength resolution to be used) from “2.5 nm”, “6 nm”, and “10 nm”. Set the wavelength band to be acquired by shifting the bar.

(6) Click the **Detector** [Detector] tab and make other settings.



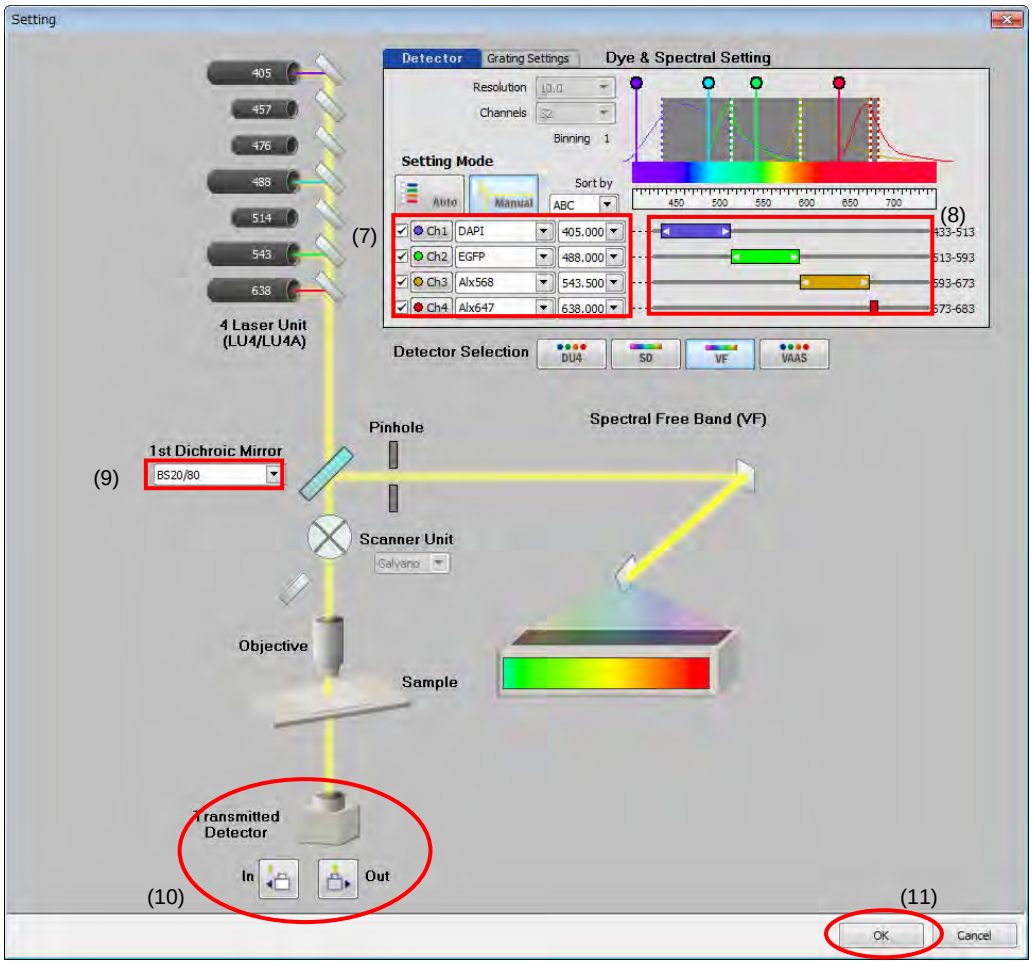
(7) Select the laser to be used.

(8) Set the channel detecting range by extending/reducing the bar.

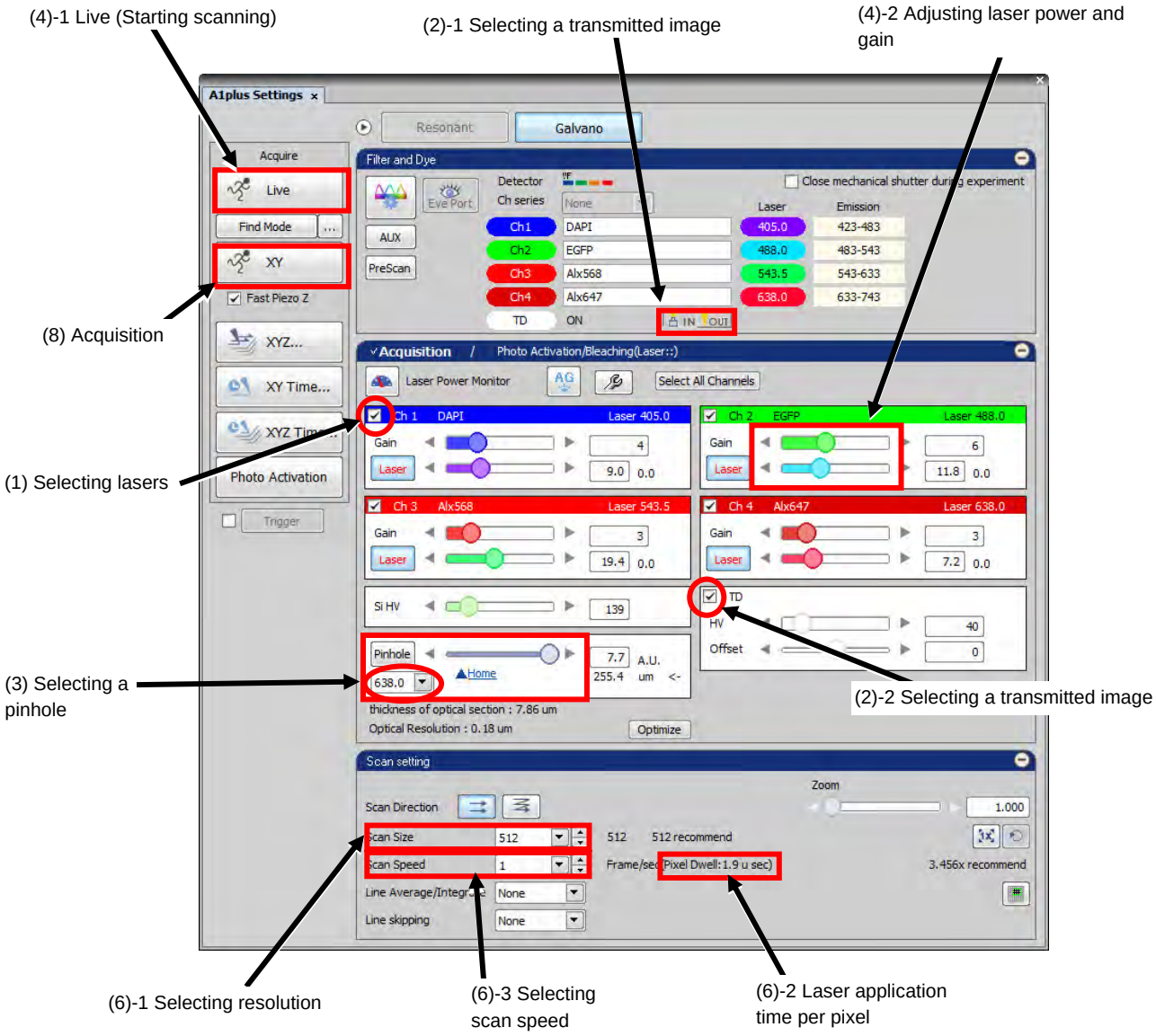
(9) Select combination of lasers to be used for [1st DM].

(10) If acquiring a transmitted image together with a fluorescent image, click In to bring into the optical path.

(11) Click the [OK] button to set the optical path.



22.6 Determine image acquisition conditions.



(1) Select the laser and channel to be used.

(2) If you want to acquire a transmitted image together with a spectral image, click the TD [IN] button and check the TD checkbox.

Note: Before acquiring a transmitted image, turn off the light above the microscope.

(3) Select the laser wavelength to be used from the pull-down menu of [Pinhole].
Select a pinhole size best suited for the objective with [▲Home].

(4) Click the [Live] button and adjust [Laser] (laser power), [Si HV] (detector sensitivity), and [Gain] while checking the image.

Note: The Si HV setting is common to all lasers. Make fine adjustments with the laser power setting.

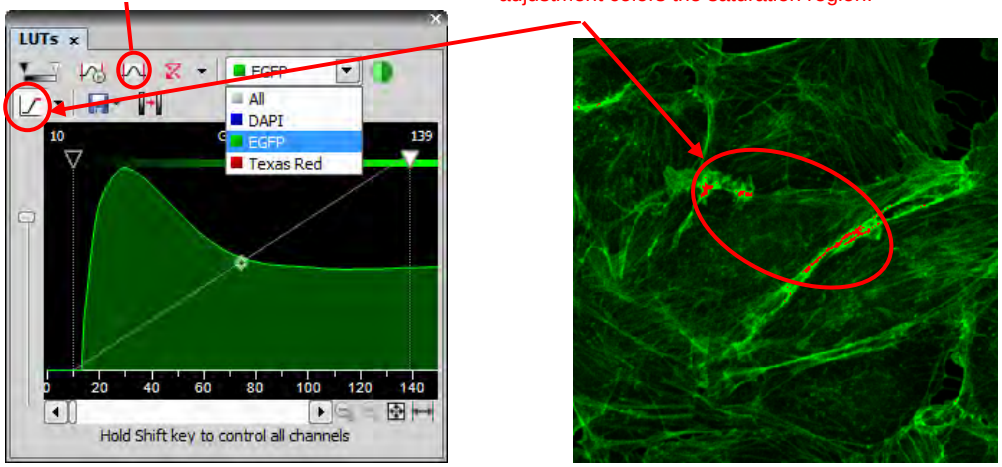
Note: Using the [Pixel Saturation Indication] button in the LUTs tab during adjustment makes it easy to adjust the sensitivity.

Note: If the displayed image is dark, click the [Auto Scale All] button to adjust the contrast of the channel automatically to make the image clear.

Note: Turning on and off the [Live] button (scan ON/OFF) during adjustment minimizes fading.

Auto Scale All

Using the [Pixel Saturation Indication] button during adjustment colors the saturation region.



Note: If the LUTs tab is not displayed, right-click the gray area of the software and select [Visualization Control] - [LUTs] from the displayed menu to call it.

(5) To use Auto Gain, a function that automatically adjusts the detector sensitivity (HV) based on the set rate of saturated pixels, click the [AG] button.

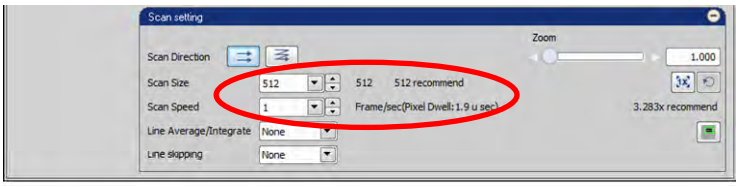
"NG" is displayed for channels that failed in Auto Gain and the HV values are returned to the previous values. Use the [Auto Gain setting] button to change the rate of saturated pixels. Set the maximum value and minimum value of the rate.

Notes:

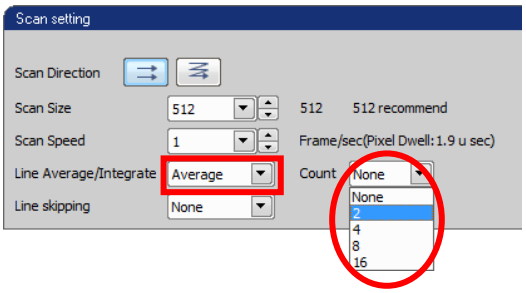
- **Auto Gain is disabled during scanning.**
- **Auto Gain is disabled when line scan is set.**
- **Do not make a manual adjustment in the Acquisition window and do not make an adjustment using the remote controller during Auto Gain.**

(6) Set the number of pixels to the necessary resolution. (e.g. 512 x 512)
If the image is dark, reduce the scan speed.

Note: Check the pixel dwell for when the resolution is changed.
Pixel dwell indicates laser application time per pixel.
The larger the value, the brighter the image that can be acquired.



(7) Apply Line Average as needed.
Average is a function to scan the same image multiple times and average it to remove noises.
Select [Line Average/Integrate] - [Average] in the Scan setting window and select an average scan frequency in [Count].



Note: Averaging reduces noise, but decreases the frame rate (number of images acquirable per second).

(8) Click the XY [XY] button to acquire an image.

22.7 Save an image.

Make the image you want to save active and select [File] - [Save As] to save it.

Note: We recommend that the image be saved in nd2 file format. Conditions such as parameters are also saved.

Motorized Stage

This edition does not include some functions depending on model and option settings.

23 Capturing Multipoint Time Series Images

23.1 Perform Steps 4.1 to 4.7 in Chapter 4, “Capturing Color Images” to determine image acquisition conditions.

23.2 Set image acquisition points.

(1) Click the XYZ Time... [XYZ Time...] button to open the ND Acquisition dialog box.

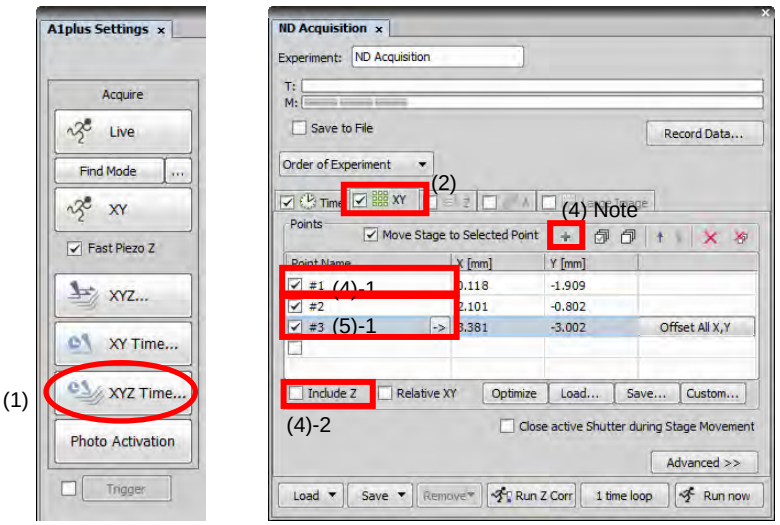
(2) Select the [XY] tab.

(3) Move the point to the image acquisition point by using the joystick on the motorized stage while checking the live image.

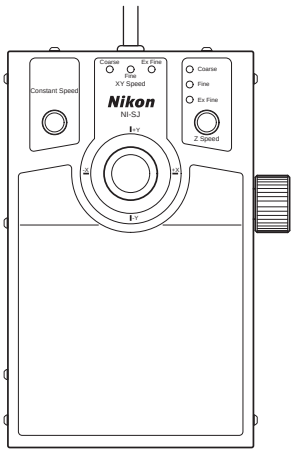
(4) Check the checkboxes in the [Point Name] column and register position information.
Checking the [Include Z] checkbox also registers the Z position.
The Z position of the microscope can be registered.

Note: If the checkboxes in the [Point Name] column are not displayed, click .

(5) Repeat Steps (3) to (4) to register points as needed.



(3), (5)-2 Joystick Controller



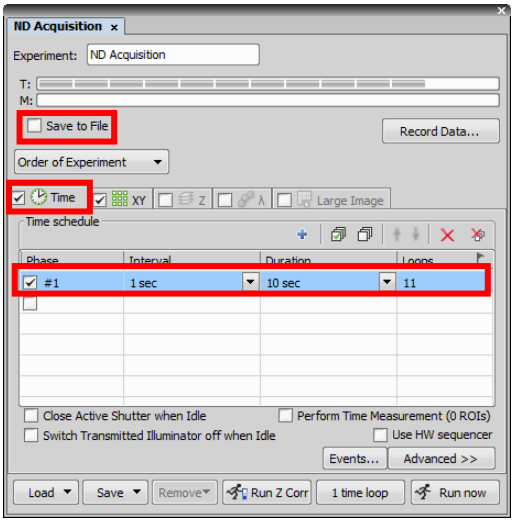
(6) Check the [Time] tab.

(7) Determine [Interval] (time interval) and [Duration] (duration time).

(8) Check the [Save to File] checkbox to acquire images while saving them.

Note: Images are saved in nd2 file format.

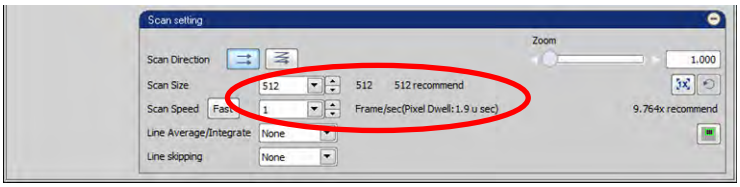
Note: We recommend acquiring time series images while saving them.



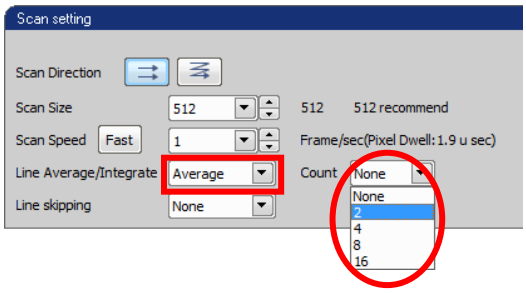
23.3 Acquire a multipoint time series image.

(1) Set the number of pixels to the necessary resolution. (e.g. 512 x 512)

Note: Check the pixel dwell for when the resolution is changed.
Pixel dwell indicates laser application time per pixel.
The larger the value, the brighter the image that can be acquired.

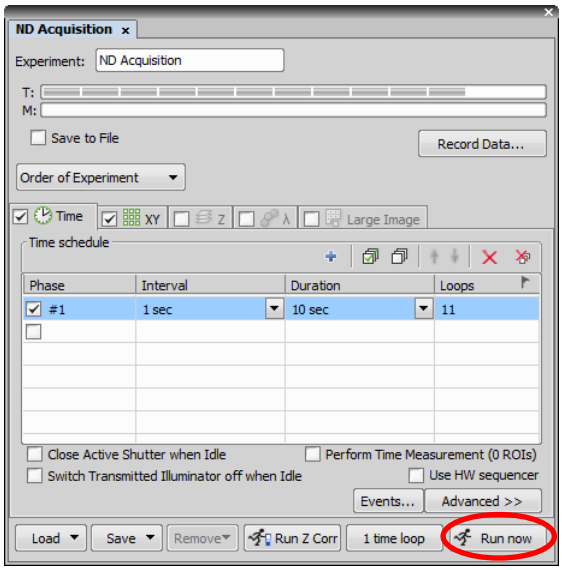


(2) Apply Line Average as needed.
Average is a function to scan the same image multiple times and average it to remove noises.
Select [Line Average/Integrate] - [Average] in the Scan setting window and select an average scan frequency in [Count].



Note: Averaging reduces noise, but decreases the frame rate (number of images acquirable per second).

(3) Click the Run now [Run now] button to acquire a multipoint time series image.



24 Capturing Large Images

24.1 Perform Steps 4.1 to 4.7 in Chapter 4, “Capturing Color Images” to determine image acquisition conditions.

24.2 To set Z series, set Z stack beforehand.

(1) Click the [XYZ Time...] button to open the ND Acquisition dialog box, and then click the Z tab.

(2) Click the [Defined top & bottom] button.

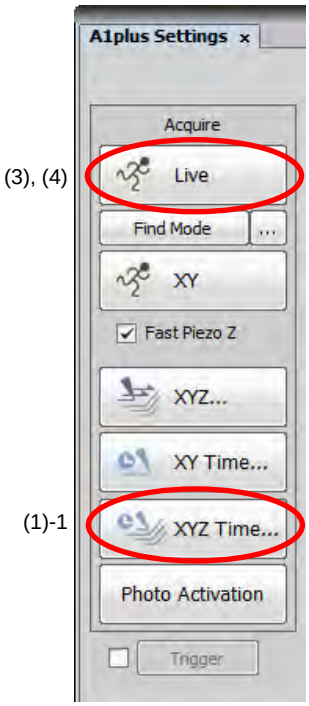
(3) Click the [Live] button and move the focus knob (fine motion mode) of the microscope while checking the image, and then click the [Top] button to determine the top position.

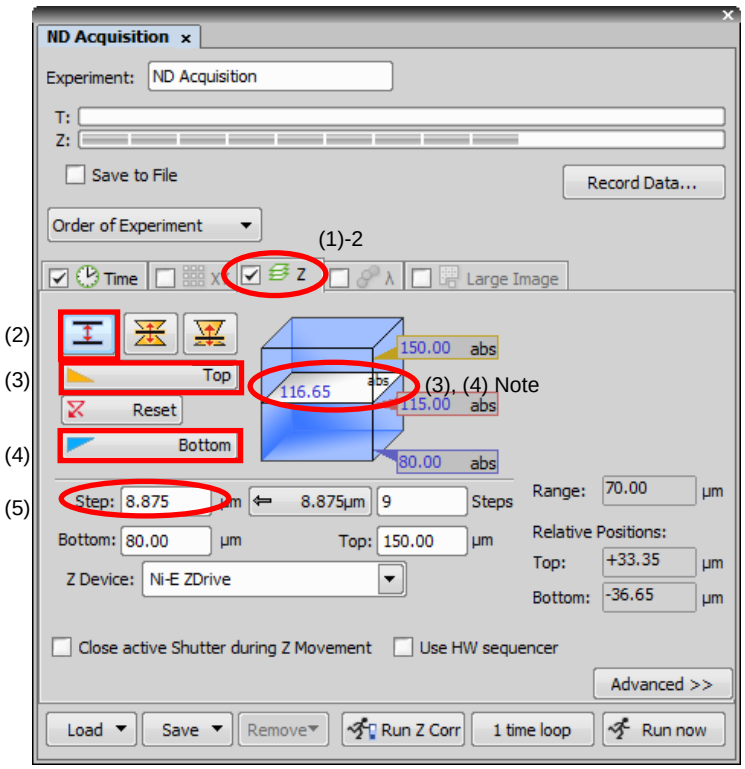
Note: Move the focus knob in the direction where the value of the plane in the cube increases.

(4) Click the [Live] button and move the focus knob (fine motion mode) of the microscope while checking the image. Click the [Bottom] button to determine the bottom position.

Note: Move the focus knob in the direction where the value of the plane in the cube decreases.

(5) Determine [Step].

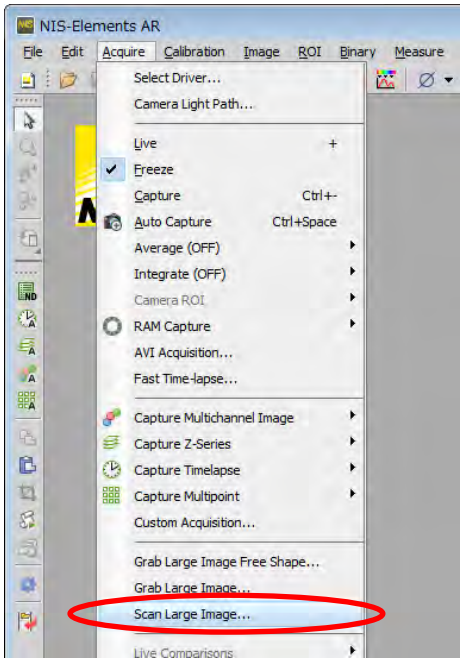




(6) Close the ND Acquisition dialog box.

24.3 Determine the large image acquisition area.

(1) Select [Acquire] - [Scan Large Image] from the menu bar to open the Scan Large Image window.



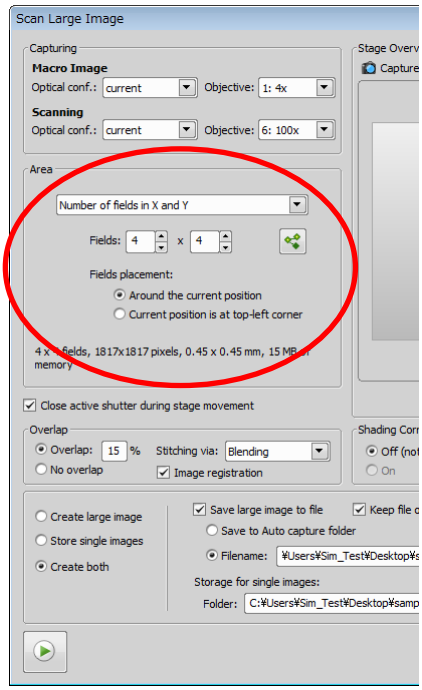
(2) Set the scan range.

<When selecting “Number of fields in X and Y”>
Select the number of lines of the scan range.
Set the position on the large image at which the current field of view is to be set in [Fields Placement].

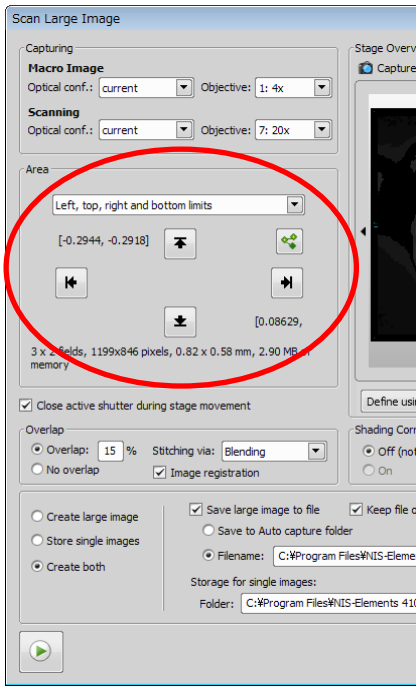
Around the current position: Scanning is performed around the current position.

Current position is at top-left corner: Scanning is performed with the current position set at the top left corner.

<When selecting “Left, top, right and bottom limits”>
Select the top, bottom, right, and left margins.



Selecting “Number of fields in X and Y”



Selecting “Left, top, right and bottom limits”

24.4 Perform the advanced settings of large images.

(1) Set the Z series options.

- None: Z series is not used.
- Z Series: The values set in the Z tab of the ND Acquisition dialog box are reflected. (See 24.2)
- Max IP: Creates the max intensity projection.
- EDF: Creates an extended depth of focus (EDF) image.
- Z-drive: Selects a Z device to be used.
- Order: Selects a combination with Z.

(2) To close the shutter during stage movement, check the [Close active shutter during stage movement] checkbox.

(3) Set image overlap correction.

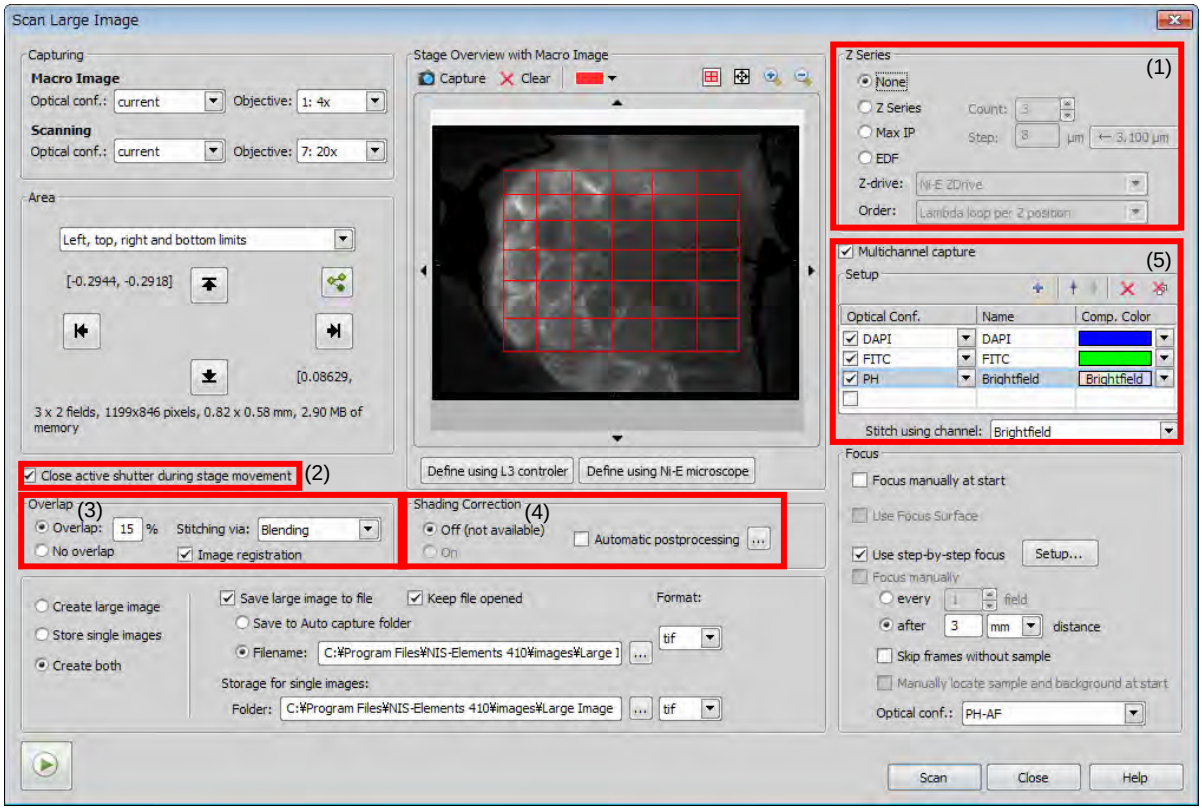
- Overlap: Sets the percentage of image overlap correction.
- No overlap: Stitches images without image overlap correction.
- Stitching via: Selects how the images are stitched.
- Image registration: Enables image overlap correction (position correction) when stitching images.

(4) Select whether or not to use shading correction.

- Automatic postprocessing: Enables automatic post-processing of acquired images.

(5) To acquire multicolor images, set the following options.

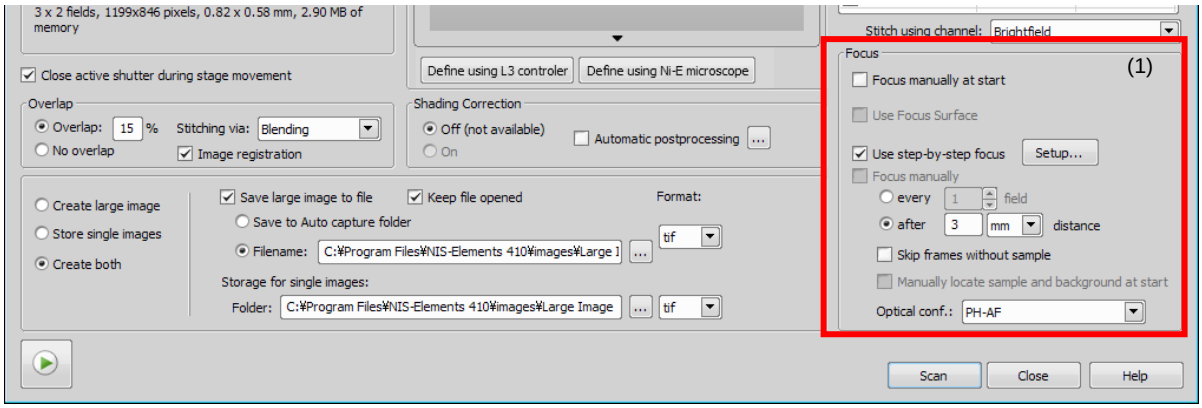
- Multichannel capture: Checking this checkbox acquires multicolor images.
- Optical Conf.: Selects the optical configuration to be used.
- Stitch using channel: Selects which wavelength of acquired λ to be used as a guideline when stitching images.



24.5 Specify the way of focusing

(1) Specify the way of focusing in the Focus area.

- Focus manually at start: Adjusts the focus manually at the time of start.
- Use Focus Surface: Performs automatic focusing using the Focus Surface function during scanning of a large image.
  This option is enabled when three or more points are registered in the Focus Surface tab of the XYZ Overview window.
- Use step-by-step focus: Set the number of images after which the focus is readjusted.
- Focus manually: Adjusts display magnification so that the display area can fit the macro screen.



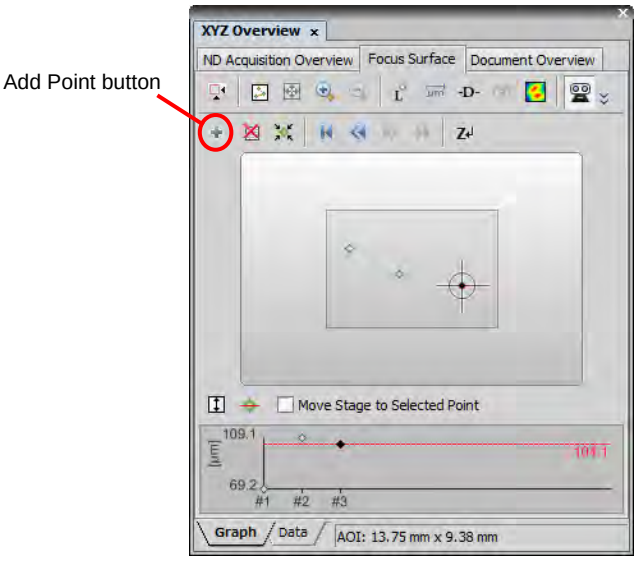
Note: To use [Use Focus Surface] for focusing, set the Focus Surface in accordance with the following procedure.

1) Select [Devices] - [Focus Surface Setup] from the menu bar to open the Focus Surface tab of the XYZ Overview window. (Display the XYZ Overview window before opening the Scan Large Image window.)

2) Move the XY stage and vertical Z to display a focus plane to be registered.

3) Click the [Add Point] button.

4) Repeat Steps (1) to (3) to register three or more focus planes.



24.6 Perform the save settings and acquire images.

(1) Select the save method.

- Create large image: Creates a large image.
- Store single image: Saves images acquired one by one.
- Create both: Saves both of the above two types of images.

(2) Select the save destination folder and file format.

Check necessary items.

Save large image to file: Saves large images to a file.
Keep file opened: Opens the saved file.

Specify the save destination folder. (This option is enabled when the [Save large image to file] checkbox is checked.)

Save to Auto capture folder: Saves large images to the auto capture folder.

Filename: Specify the save destination folder and file name of large images.
(By default, the file name is set to "Large Image". To alter the file name, change the "Large Image" part.)

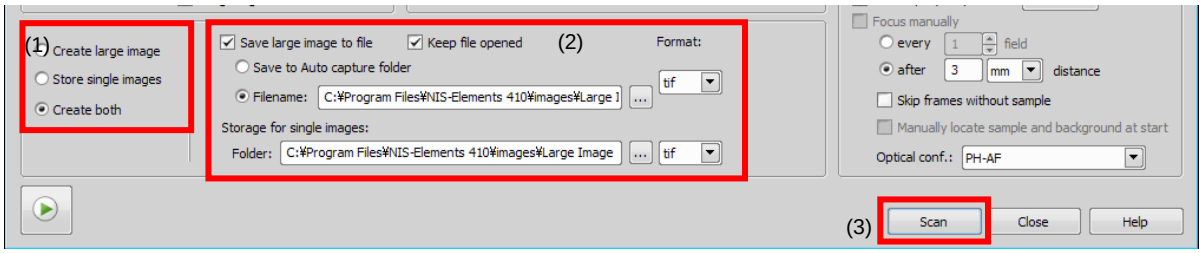
Format: Selects the file format of large images from nd2 and tif.

<When selecting [Store single image] as the save method>

Folder: Select a folder to which images acquired one by one are saved.

Format: Select a file format of images acquired one by one.
The file format can be selected from tiff, jp2, png, bmp, and jpg.

(3) Click Scan to acquire a large image.



High-Speed Imaging

A1 / Ni-E /
Motorized Stage / Piezo Z Stage / Intensilight

This edition may have unavailable functions depending on model in use and option settings.

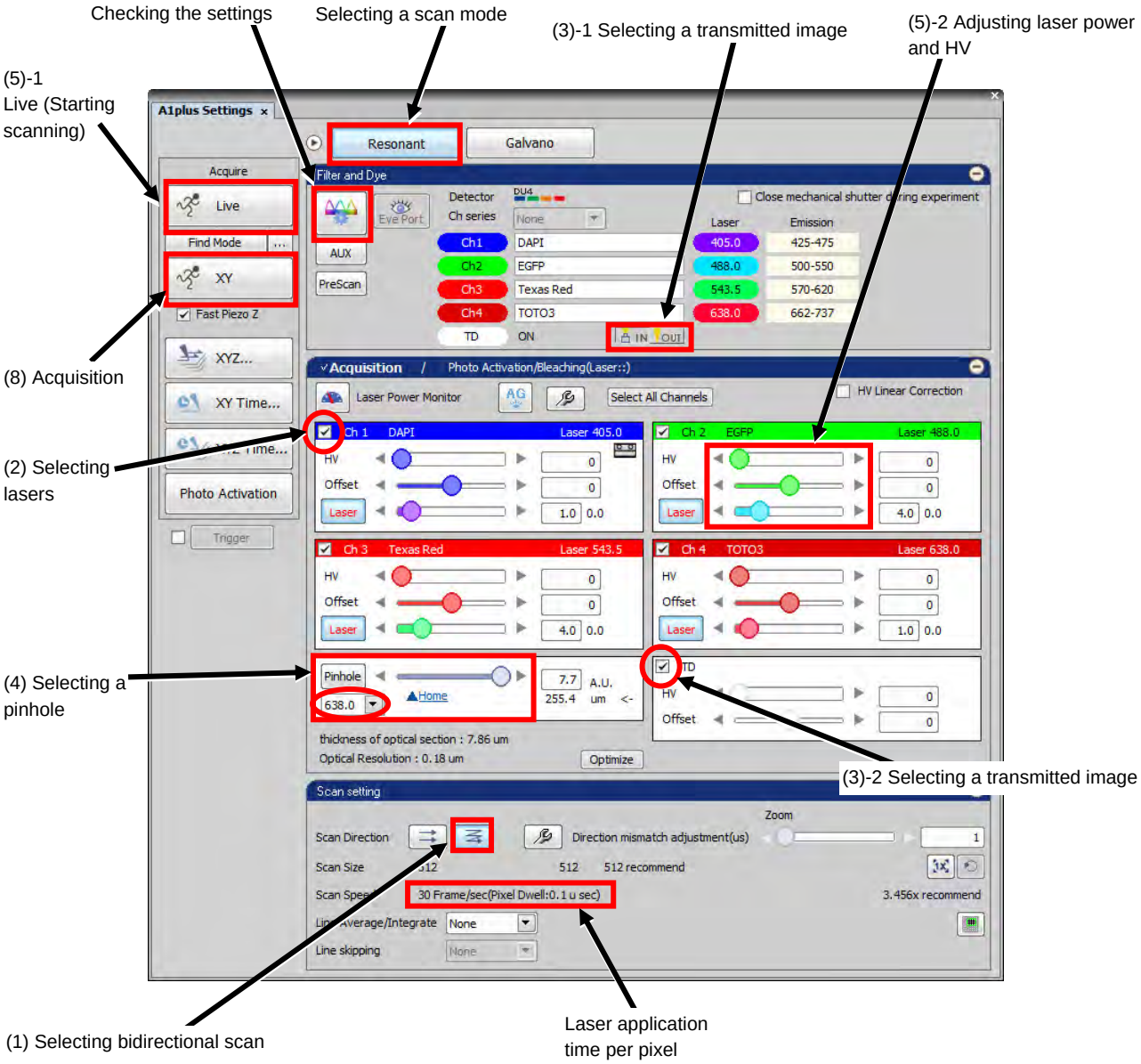
25 Capturing High-Speed Images (Resonant Scanner)

25.1 Perform Steps 4.1 to 4.6 in Chapter 4, “Capturing Color Images”.

25.2 Select a scan mode.

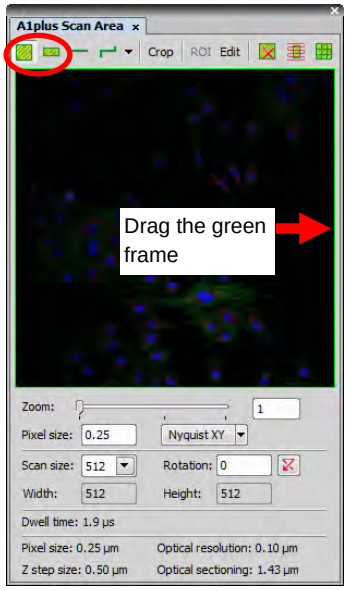
Select [Resonant] [Resonant].

25.3 Determine image acquisition conditions.

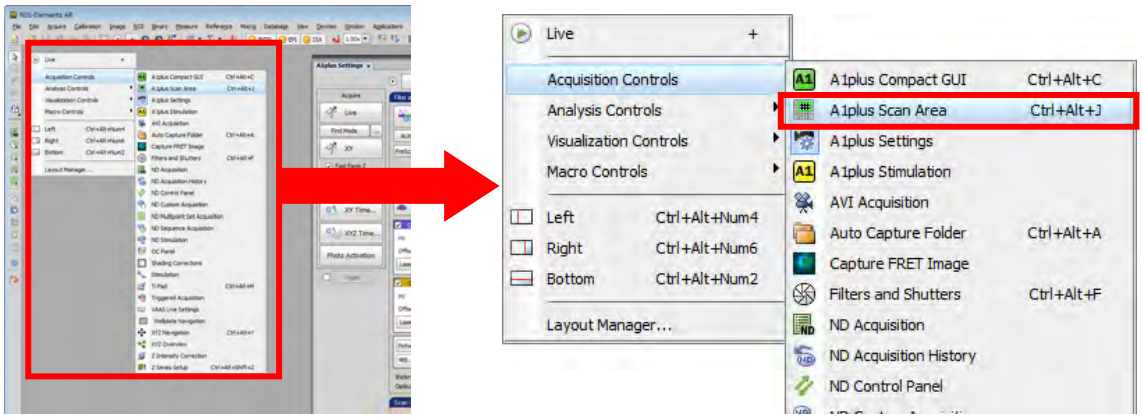


(1) Click to select bidirectional scan (scan speed: typical 30 frames/second).

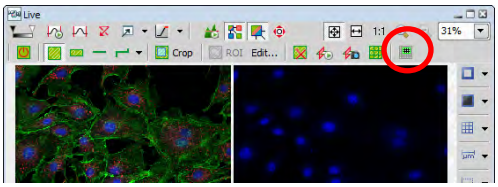
Note: If you need higher-speed scan, reduce the scan area.
Click in the Scan Area window and drag the green frame to reduce the scan area.

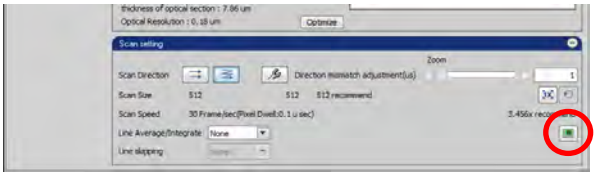


Note: If [Scan Area] is not displayed on the software, right-click the gray area of the software and select [Acquisition Controls] - [A1plus Scan Area] from the displayed menu to call it.



* This window also opens by clicking the button shown below that is displayed in the Live window or A1plus Settings window.





Scan Area Setting	X Resolution	Y Resolution	Scan Speed and Unit	
Square scan area	512	512	30	Frame/sec
Band scan area (X:Y = 1:1/2)	512	256	60	Frame/sec
Band scan area (X:Y = 1:1/4)	512	128	120	Frame/sec
Band scan area (X:Y = 1:1/8)	512	64	230	Frame/sec
Band scan area (X:Y = 1:1/16)	512	32	420	Frame/sec
Line scan	512	1	7634	Line/sec

Note: In the case of a band scan area (512 × 32) 420 fps, images in the upper four lines are not available.

(2) Select the laser and channel to be used.

(3) If you want to acquire a transmitted image together with a confocal image, click the TD [IN] button and check the TD checkbox.

Note: Before acquiring a transmitted image, turn off the light above the microscope.

(4) Select the laser wavelength to be used from [Pinhole].
Select a pinhole size best suited for the objective with [▲Home].

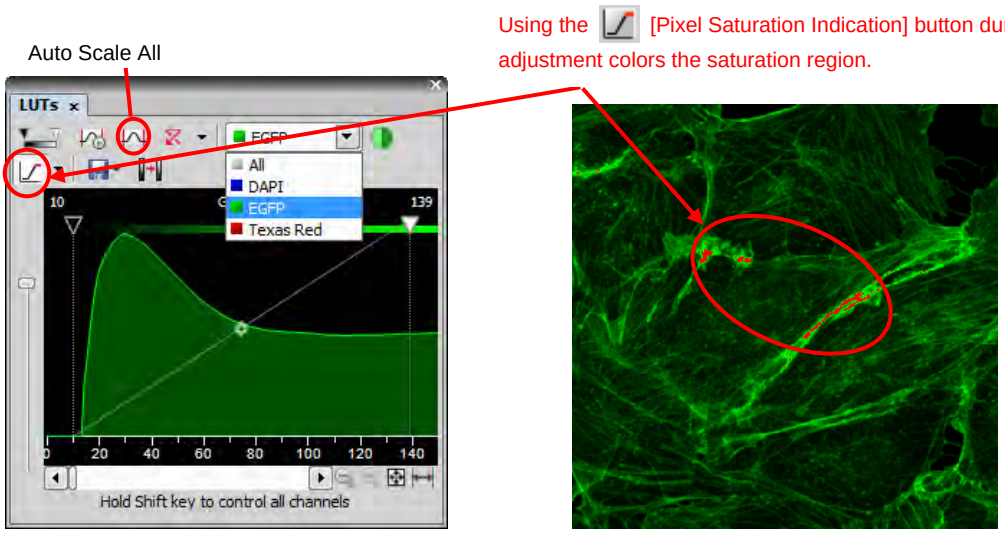
(5) Click the [Live] button and adjust [Laser] (laser power) and [HV] (detector sensitivity) while checking the image.

Note: Use Offset "0" as the standard setting.

Note: Using the [Pixel Saturation Indication] button in the LUTs tab during adjustment makes it easy to adjust the sensitivity.

Note: If the displayed image is dark, click the [Auto Scale All] button to adjust the contrast of the channel automatically to make the image clear.

Note: Turning on and off the [Live] button (scan ON/OFF) during adjustment minimizes fading.



Note: If the LUTs tab is not displayed, right-click the gray area of the software and select [Visualization Control] - [LUTs] from the displayed menu to call it.

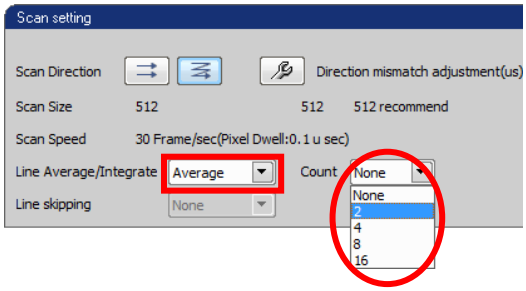
(6) To use Auto Gain, a function that automatically adjusts the detector sensitivity (HV) based on the set rate of saturated pixels, click the [AG] button.

"NG" is displayed for channels that failed in Auto Gain and the HV values are returned to the previous values. Use the [Auto Gain setting] button to change the rate of saturated pixels. Set the maximum value and minimum value of the rate.

Notes:

- **Auto Gain is disabled during scanning.**
- **Auto Gain is disabled during 2Ex1Em or 1Ex2Emx2 line sequence.**
- **Auto Gain is disabled when line scan is set.**
- **Do not make a manual adjustment in the Acquisition window and do not make an adjustment using the remote controller during Auto Gain.**

(7) Apply Line Average as needed.
Average is a function to scan the same image multiple times and average it to remove noises.
Select [Line Average/Integrate] - [Average] in the Scan setting window and select an average scan frequency in [Count].



Note: Averaging reduces noise, but decreases the frame rate (number of images acquirable per second).

(8) Click the XY [XY] button to acquire an image.

25.4 Save an image.

Make the image you want to save active and select [File] - [Save As] to save it.

Note: We recommend that the image be saved in nd2 file format. Conditions such as parameters are also saved.

26 Capturing High-Speed ZT Series Images (Resonant Scanner)

26.1 Perform Steps 25.1 to 25.3 in Chapter 25, “Capturing High-Speed Images.”

26.2 Open the ND Acquisition dialog box.

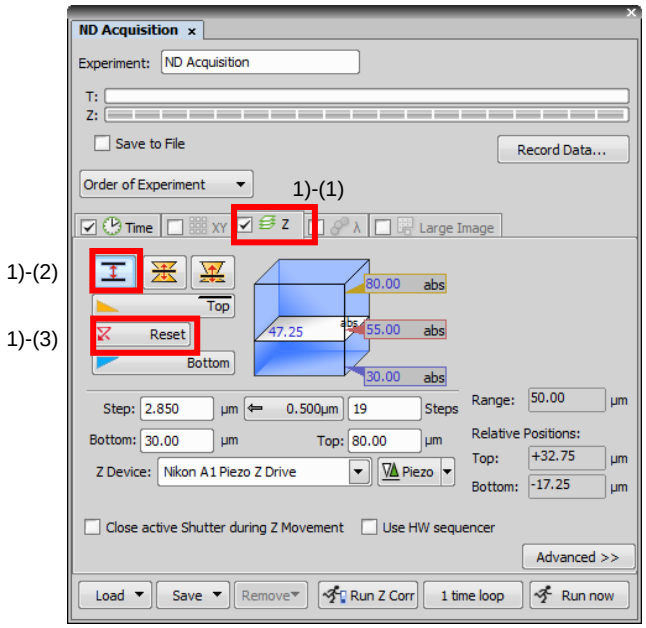
(1) Click the XYZ Time... [XYZ Time...] button to open the ND Acquisition dialog box.

26.3 Determine the Z acquisition range of the ZT series.

(1) Click the Z tab.

(2) Click the [Defined top & bottom] button.

(3) Click the Reset [Reset] button.



(4) Click the [Live] button and move the focus knob (fine motion mode) of the microscope while checking the image, and then click the [Top] button to determine the top position.

Note: Move the focus knob in the direction where the value of the plane in the cube increases.

(5) Click the [Live] button and move the focus knob (fine motion mode) of the microscope while checking the image. Click the [Bottom] button to determine the bottom position.

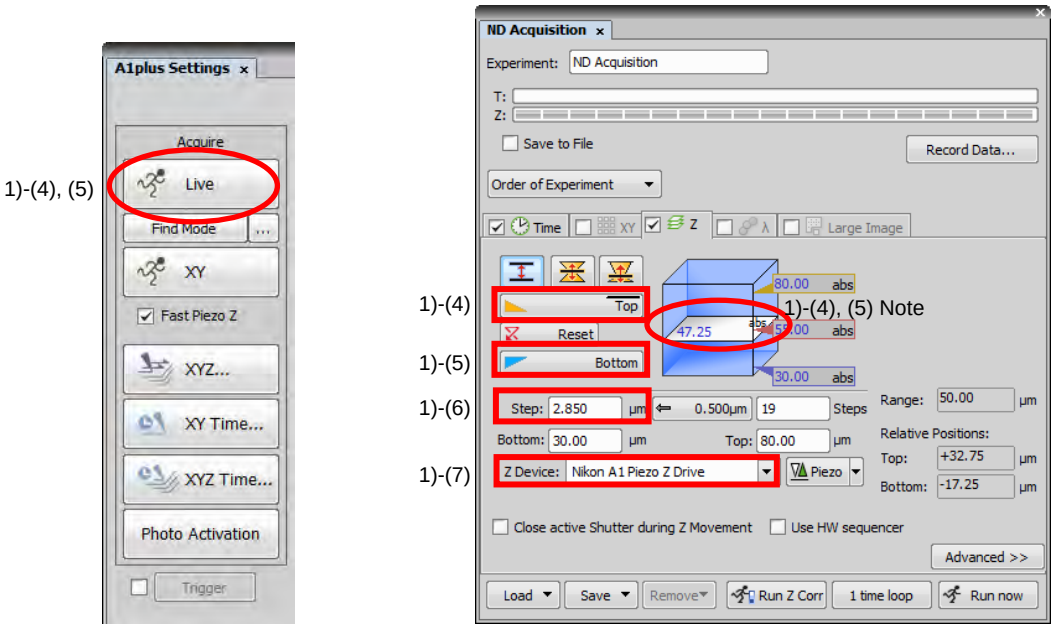
Note: Move the focus knob in the direction where the value of the plane in the cube decreases.

(6) Determine [Step].

(7) Specify [A1 Piezo Z Drive] for [Z Device].

Note: The drive range of Piezo ZDrive is 100 µm (at ordinary temperature).
Set the total range to 100 µm or less.

Note: The drive range of Piezo ZDrive is approx. 50 µm at 37 degrees C.



26.4 Set the ZT series time settings.

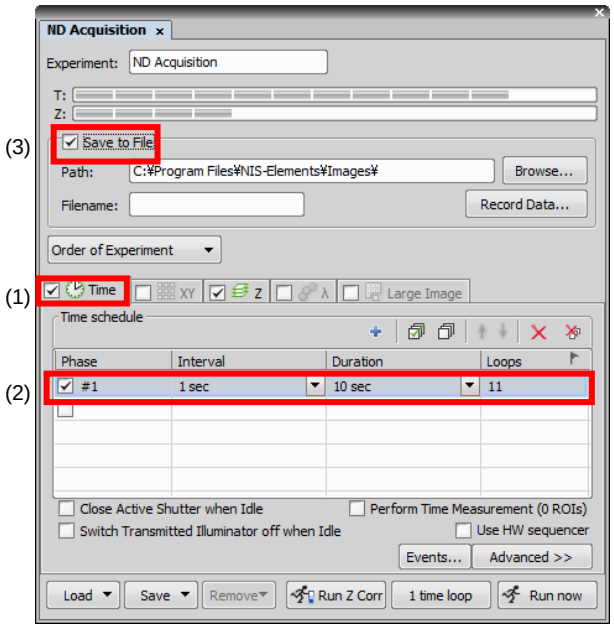
(1) Click the Time tab.

(2) Determine [Interval] (time interval) and [Duration] (duration time).

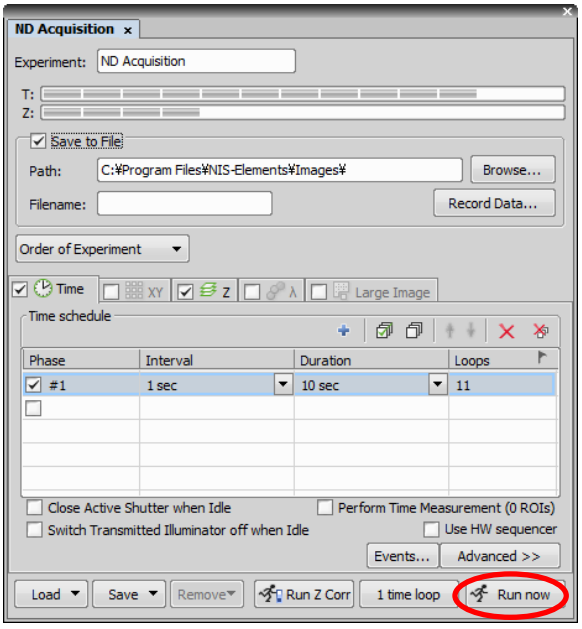
(3) Check the [Save to File] checkbox to acquire images while saving them.

Note: Images are saved in nd2 file format.

Note: We recommend acquiring time series images while saving them.



26.5 Click the [Run now] button to acquire ZT series images.



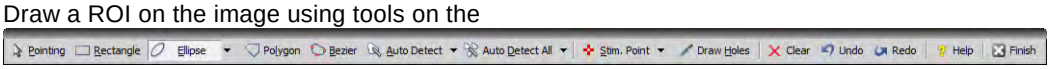
27 Capturing Simultaneous Photo Activation Imaging (Resonant & Galvano Scanner)

27.1 Perform Steps 25.1 to 25.3 in Chapter 25, “Capturing High-Speed Images.”

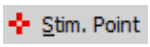
27.2 Set the area where photo activation is to be performed.

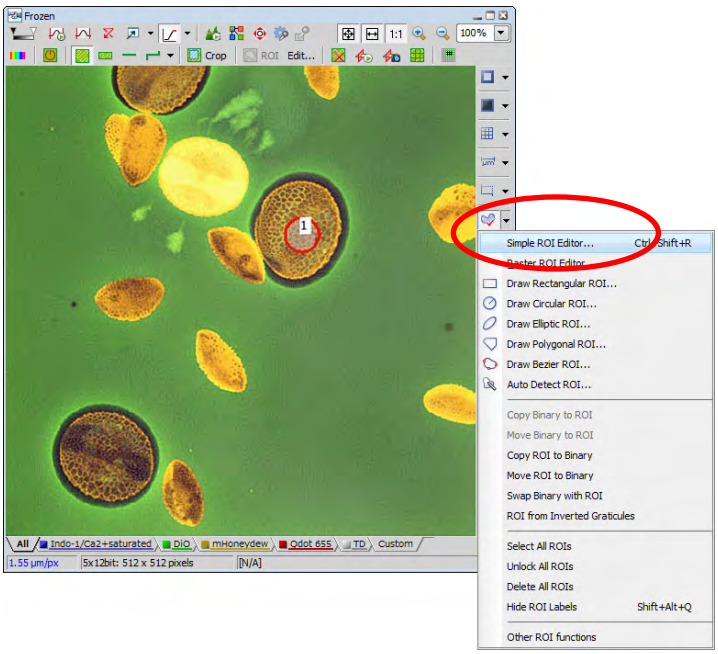
(1) Click  at the side of the image frame and select “Simple ROI Editor”.

Draw a ROI on the image using tools on the



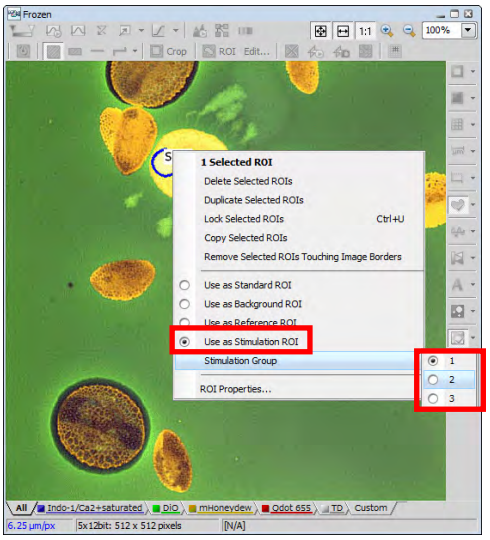
tool bar.

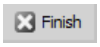
Note: Using  **allows point activation.**



(2) Right-click on the ROI and select [Use as Stimulation ROI] from the displayed menu, and then select [Stimulation Group].

Note: ROIs can be divided into up to three groups. A group can contain two or more ROIs.
Different activation conditions can be set by grouping ROIs.



(3) Click the  [Finish] button of “Simple ROI Editor” to finish the setting.

27.3 Set the laser light for activation.

(1) Click [Photo Activation] to switch the setting window.

(2) Click Tab 1 (Stimulation Group 1 setting).

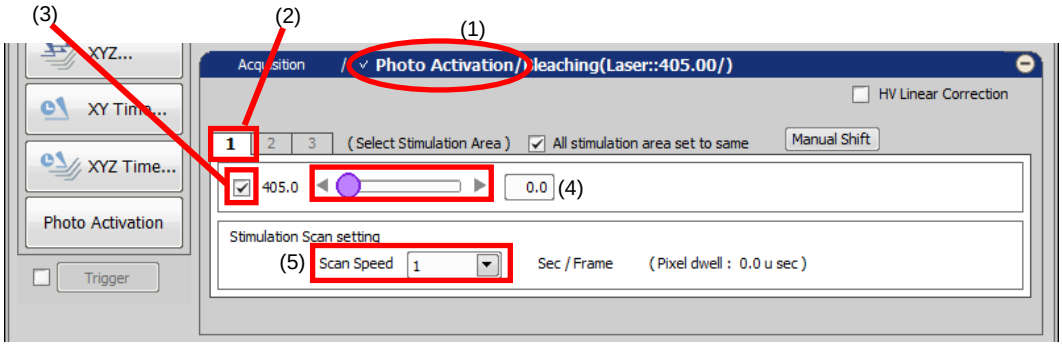
(3) Select lasers used for activation.

Note: During simultaneous photo activation observation, 405 nm and 488 nm lasers can be used. To use 488 nm lasers, however, the filter for 488 nm simultaneous activation needs to be installed.

(4) Move the laser bar to select the laser power for activation.

(5) Select [Scan Speed] for activation.

Note: Consider that Scan Speed is the time required for a single activation.
When "1 Sec/Frame" is selected, the time for a single activation is one second.



(6) When there are Stimulation Groups 2 and 3, repeat Steps (2) to (5).

27.4 Set time series for photo activation simultaneous imaging.

(1) Click the [Photo Activation] button to open the ND Stimulation dialog box.

(2) Set the photo activation time settings.

Wait: Set the waiting time until activation starts.

Interval: Set the time interval of photo activation.

ROIs: Set stimulation groups used for activation.

Duration: Set the duration time of photo activation.
When [Loops] is set, duration is automatically determined.

Loops: Set the number of photo activation execution times.

(3) Set the time series time settings.

Duration: Set the duration time of image acquisition.
When [Loops] is set, duration is automatically determined.

Loops: Set the number of image acquisition execution times.

(4) Check the [Save to File] checkbox to acquire images while saving them.

Note: Images are saved in nd2 file format.

Note: We recommend acquiring time series images while saving them.

(5) Click Apply Stimulation Settings to read the settings for photo activation simultaneous imaging.

(6) Click the Run now [Run now] button to start photo activation simultaneous imaging.