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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 / Ti-E / PFS Motorized Stage / Piezo Z Stage / Intensilight

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

2 Startup Turn on the power to the microscope. (1) Turn on the power to the motorized stage. POWER switch

POWER switch

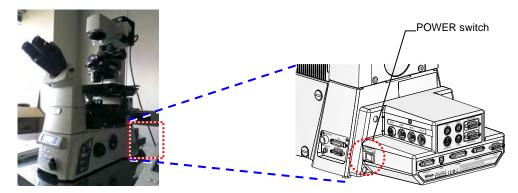
(2) Turn on the power to the piezo Z stage.

2.1

- (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).
- Brightness control knob POWER switch

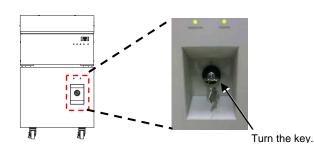
POWER switch

(5) Turn on the power to the microscope.



2.2 Turn on the power to the laser.

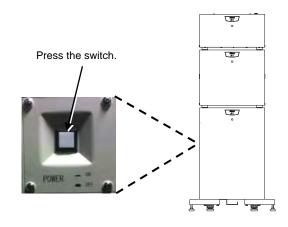
Turn the key 90 degrees clockwise from the vertical position (off).



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 NIS-Elements AR 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.

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.



"For analysis"

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

	NIS-Elements AR 4.10.00 (Build xx	xx) 64bit - Driv	er selection
(Nikon Confocal)	•
	Enable Multi Camera	ОК	Cancel

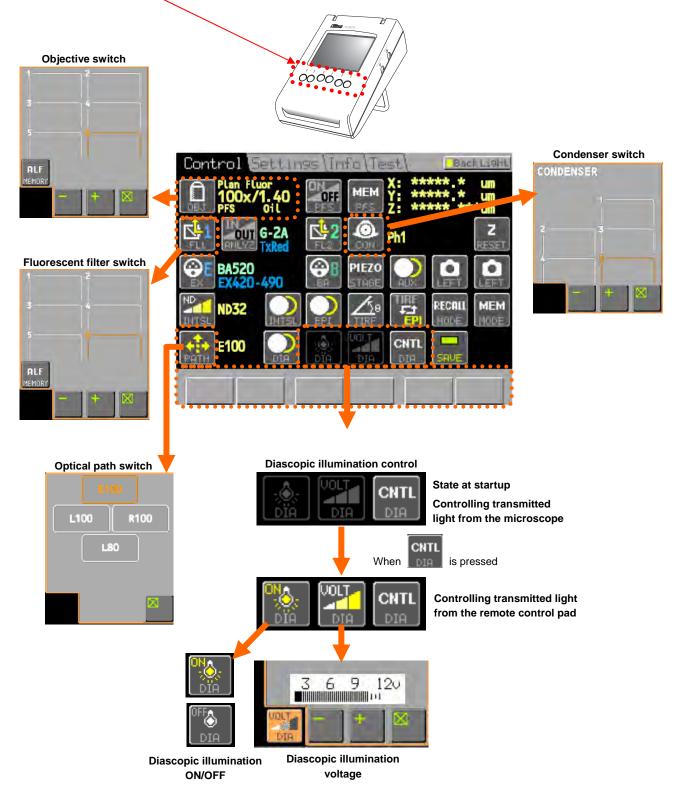
Operation of the Microscope

3.1 Ti Remote Control Pad (Ti-RCP) Operation

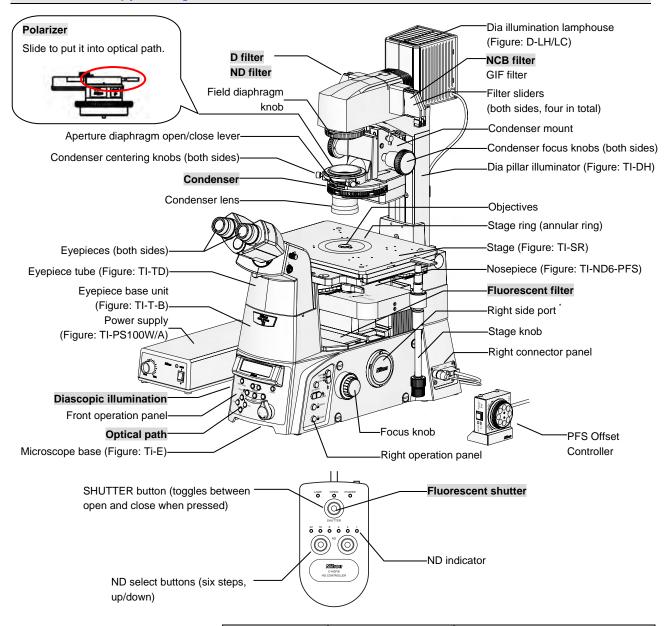
3

Pressing the icon of the motorized part you want to switch on the touch panel of Ti remote control pad displays a list of items installed in the each motorized part. Select an item you want to switch from the list.

Using function buttons enables necessary microscopy to be called at a time.



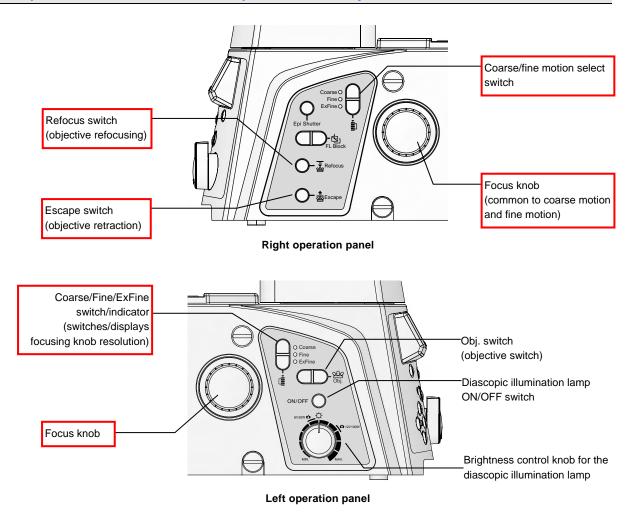
3.2 Microscopy Setting



		Diascopic microscopy (DIC)	Fluorescence microscopy	Confocal microscopy
	Optical path	E100	E100	L100
	Fluorescent filter	ANALY	DAPI, etc	Blank position
Ti control pad*	Condenser	N1 or N2	Unnecessary (No problem if provided)	Unnecessary (No problem if provided) - Necessary when acquiring a DIC image using a laser
	Diascopic illumination	On	Off	Off
HG controller	Fluorescent shutter	Off	On	Off
Microscope	Polarizer	Necessary	Unnecessary (No problem if provided)	Unnecessary (No problem if provided) - Necessary when acquiring a DIC image using a laser
	D filter NCB filter ND filter	Necessary	Unnecessary (No problem if provided)	Unnecessary - Be sure to remove it when acquiring a DIC image using a laser

*: Can be called collectively using the function button on the Ti control pad.

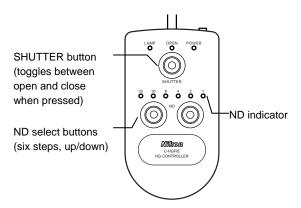
3.3 Operation Panel on the Microscope Ti-E Main Body



Other buttons on the panel can be operated from the Ti remote control pad.

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

- 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.



3.5 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.

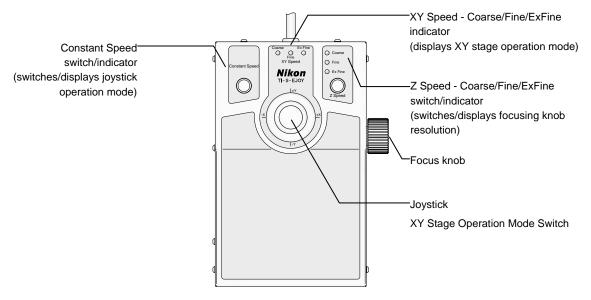
To cancel constant speed mode, press this switch again.

[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.

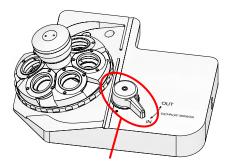
Pressing the Z speed switch changes the mode among Coarse, Fine and ExFine.



Joystick Controller

3.6 Perfect Focus System Operation (with the Front Operation Panel)

- Check that the dichroic mirror IN/OUT lever is set to IN.
- (2) Set the objective to be used in the optical path and adjust the focus with the focus knob on the microscope to focus on the sample.
- (3) Press the PFS-ON button on the front operation panel.(When the PFS starts, the PFS-ON indicator lights green.)



Dichroic mirror IN/OUT lever

(4) Focus on the position at which focus is maintained with the offset dial on the PFS offset controller and start microscopy.

Once the PFS operation is started, focusing with the focus knob on the microscope is disabled.

Registering offset amount: The focus position adjusted with the offset dial can be registered with the PFS-MEMORY button.

Recalling offset amount: Registered focus position can be called with the PFS-RECALL button.

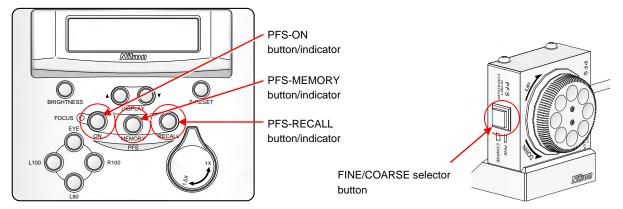


Figure Ti-E Front Operation Panel and Offset Controller

3.7 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 signal icon in the [Ch] of the A1plus setting window of the NIS-Elements.

🗹 Ch 1	Indo-1/Ca2+saturated	Laser 405.0	Ch 2 DiO Laser 488.0
н	<	40	HV 4 🚺 🕨 30
Offset	<>	0	Offset 4 - 0
Laser	<	9.0 0.0	Laser 4 4.6 0.0

- (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

Capturing Color Images (Standard Detector) —



4.10.00 64-bit

A S (1) Click the NIS-Elements AR icon to run the NIS-Elements software.

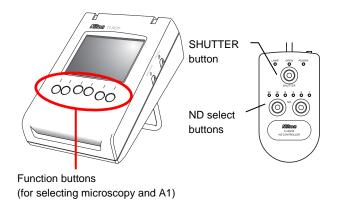
Note: When not only a confocal microscope is connected but also a camera, the Driver selection dialog box opens to select a driver.

Select "Nikon Confocal" in the Driver selection dialog box and click the [OK] button.

	NIS-Elements AR 4.10.00 (Build :	xxxx) 64bit - Driver selection
C	Nikon Confocal	

4.2 Observe the sample through the microscope.

- (1) Select the desired microscopy. Press a function button on the Ti remote control pad to select the desired microscopy.
- 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.

When the Ti remote control pad is available, press the [A1] function button to switch the optical path to A1.

If the Ti remote control pad is not available, switch the optical path to L100.

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 [Galvano].

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

Check the settings.

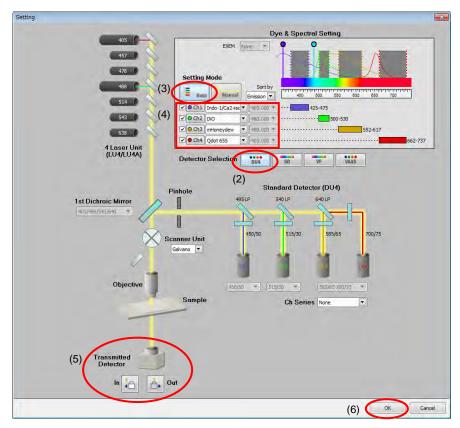
(1) Click

	 Resonant 	t	Galvano		
Acquire	Filter and Dye				
2º Live	AAA 25%	Detector	DU4	0]]	lose m
2 Live	Eve Por	t Ch series	None 💌	Laser	E
Find Mode	AUX (1)	Ch1	Indo-1/Ca2+saturated	405.0	-
v3° XY		Ch2	DIO	488.0	- 5
NO XY	PreScan	Ch3	mHoneydew	488.0	3
Fast Piezo Z		Ch4	Qdot 655	405.0	6
		TD	ON A IN O	ITU	

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

to open the Optical path window.

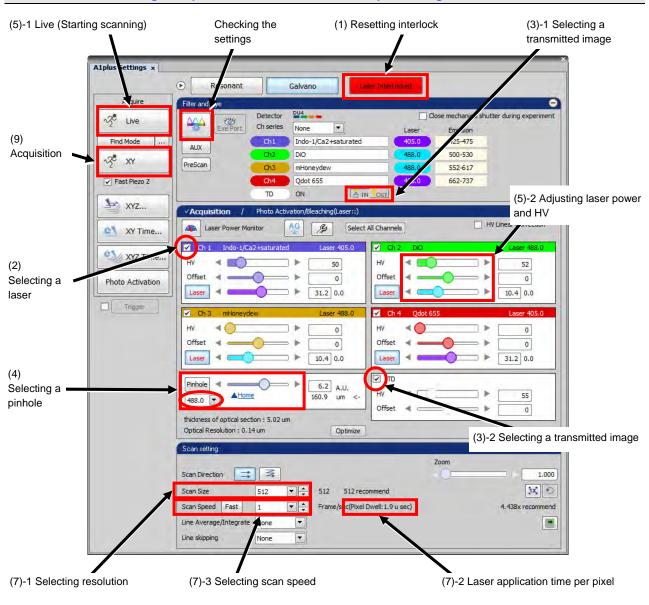
- (3) Click the E Auto [Auto] button to set the optical path in the Auto mode.
- (4) Check the checkboxes of channels to be used.
 Select a reagent name.
 Click channel and select a pseudo-color.
- (5) If acquiring a transmitted image together with a confocal image, click in to bring into the optical 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] 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.

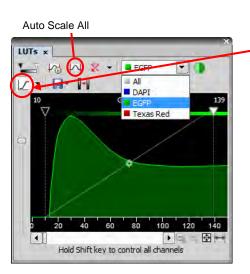
Note: Before acquiring a transmitted image, turn off the light above the microscope.

(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

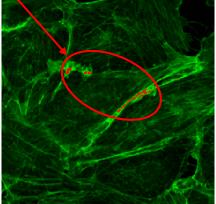
- Ch 2
 DiO
 Laser 488.0

 HV
 Image: Character and the state of the st
- Note: Use Offset "0" as the standard setting.
- Note: Using the *[Image: Second Content in C*
- Note: If the displayed image is dark, click the [1] [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.



adjustment colors the saturation region.

Using the *I* [Pixel Saturation Indication] button during



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 *S* [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.

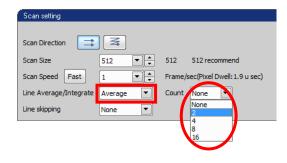
488.0 thickness of optical sect Optical Resolution : 0.06	<- Offset 4	
Scan setting		•
Scan Direction Scan Size Scan Speed Fast Line Average/Integrate Line skipping	2000 12 recommend :(Pixel Dwell: 1.9 u sec)	n 1.000 (3%) (50) 9,764x recommend

- 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 🛷 xr [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.

	Resonant		Galvano			
Acquire	Filter and Dye /	Acquisition Orde	er : [Ch1]->[Ch2,TD]->[Ch3]->[C	h4]	-	
2 ^e Live		Detector	DU4		ose mechanical shutter	during experim
of the	Eve Port	Ch series	Custom 🔽 Custom	Laser	Emission	
Find Mode	AUX	Ch1	Indo-1/Ca2+saturated	405.0	425-475	
אץ ¢ץ		Ch2	DiO	488.0	500-530	
Se XA	PreScan	Ch3	mHoneydew	488.0	552-617	
Fast Piezo Z		Ch4	Qdot 655	405.0	662-737	
		TD				

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

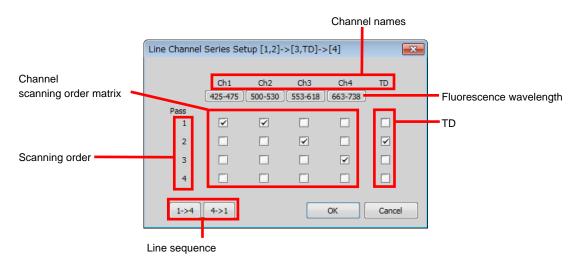
Setting the order of scanning arbitrarily:

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

Using line sequence:

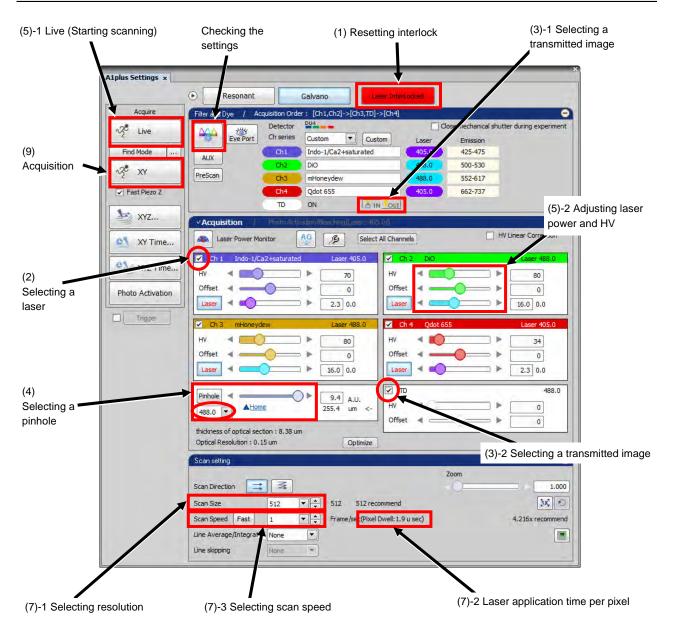
Select the [1->4] or [4->1] button.

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.)





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

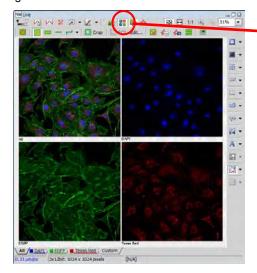
Note: If the optical path is not switched to A1, blinking cannot be reset even though the [Laser InterLocked] button is clicked.

- (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].

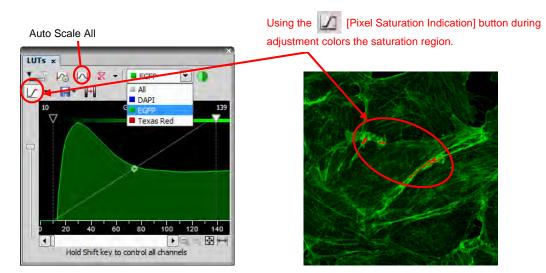
(5) Click the [Live] button and adjust [Laser] (laser power) and [HV] (detector sensitivity) while checking the image.



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

A1

- 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 [1] [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.

20

(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 *[Joint Constitution of the 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.

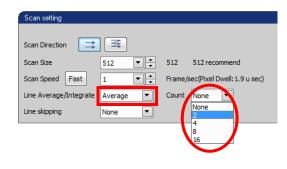
The larger the value, the brighter the image that can be acquired.



(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 🛷 xr [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.

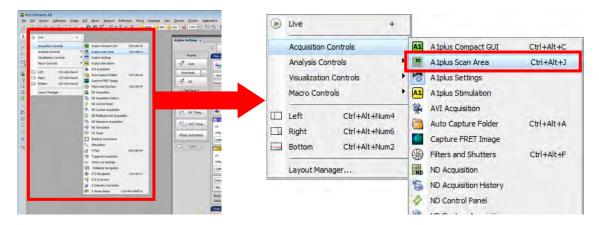
Note: We recommend that the image be saved in nd2 file format. Conditions such as parameters are also saved.

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.

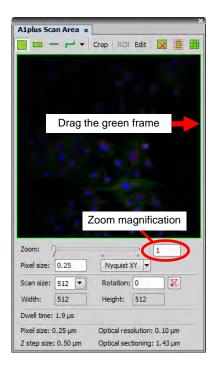


6.3 Select the zoom area.

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.

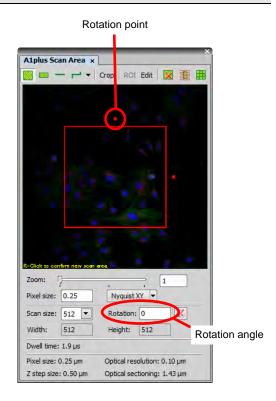
Zoom magnification is shown in [Zoom].



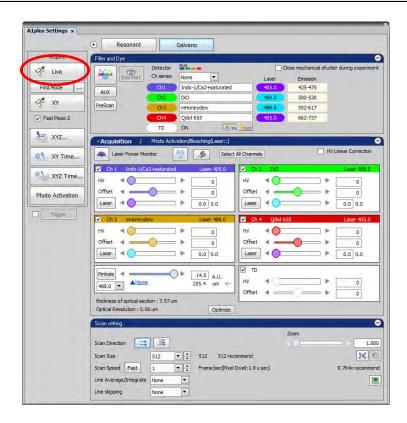
6.4 Rotate the scan area as needed.

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.



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.

Det .		Alplus Settings x		Carl and a second s	1			100 0000000
Acquester Correste 🖬 Algkie Analysis Correste * 🗶 Algkie	ican Area Ceri+Alb+J	A1044		Acquisition Controls		A1	Alplus Compact GUI	Ctrl+Alt+C
Vasuatantion Controls • 👼 Algalas Macros Controls • 🛤 Algalas Sis ant Acr	Struktor	Ansien Com		Analysis Controls			A 1plus Scan Area	Ctrl+Alt+3
Left Off-Altriduced In Arts C	pfure Filde Col+Alt+A . HR2T Snept	Find MadeAU		Visualization Controls	•	2	A 1plus Settings	
Levolt Manager		2 Failten 7		Macro Controls	+	A1	A1plus Stimulation	
	um Acquintum	C1 xY Teni			-	-	AVI Acquisition	
	lporit Set Acqueiton uence Acqueiton	C1 XY Tene		Left Ctrl+Alt+Num4		0	Auto Capture Folder	Ctrl+Alt+A
C Char	Correctore	Photo Activation		Right Ctrl+Alt+Num6		C	Capture FRET Image	
the series of series	CS1+AD+M		-	Bottom Ctrl+Alt+Num2		3	Filters and Shutters	Ctrl+Alt+F
C MAR	te havgeten	0%s		Layout Manager			ND Acquisition	
+ srithe srithe		Perte			-	ND	ND Acquisition History	
#1 Ziem	Setup CerinalityDefta2	en.				(ID)	The required the terry	

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

Optical Resolution = 0.06 um Optimore	Pediness of spitial section : 7.57 um Optical Resolution : 0.06 um Optim
Start before Zoom Scan Sine Size Size Size Scan Sine Size Size Size Size Scan Sine Size Size Size Size Size Scan Sine Size Size Size Size Size Size Line Average/Integers Nove V V V Size Size	San Diectan : ::::::::::::::::::::::::::::::::::

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].



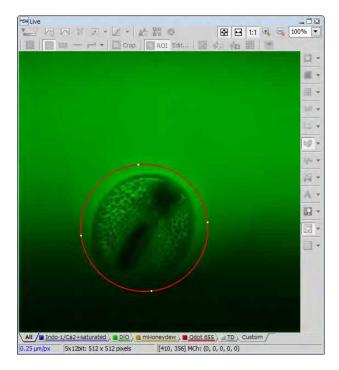


(2) Draw a ROI in the Live window using the Rectangle O Ellipse

À Pointing 🗆 Rectangle 🖉 Ellipse 🔹 🖓 Polygon 🖒 Bezier 🐼 Auto Detect 👻 🗙 Clear 🕴 Help 🛛 🔀 Finish

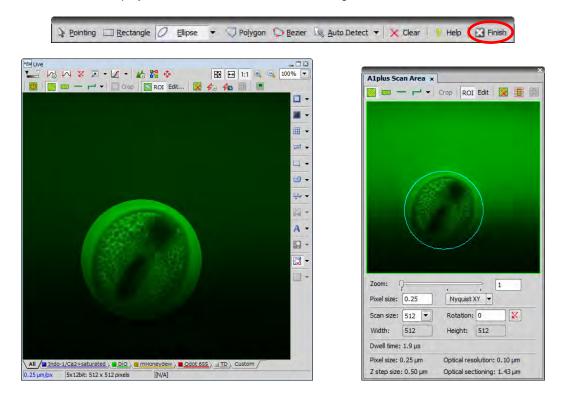
[ROI Editor].

Right-clicking commits the ROI being edited.



(3) Click the Finish [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.



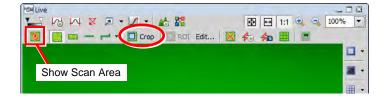
Note: If a ROI is drawn on Frozen images, click the Live button to start ROI scanning.

A1

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.



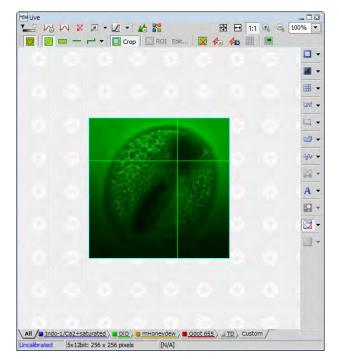


(2) In the Scan Area window, drag the light blue frame to reduce its size.

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.



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.

- Click the xrz. [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.)

- (2) Click the **I** [Defined top & bottom] button.
- (3) Click the Reset [Reset] button.

	×
	Capture Z-Series ×
	Experiment: ND Acquisition
	Z: []
	Save to File Record Data
	Order of Experiment 💌
(2)	
(3)	Reset
	Step: 2.850 µm 🗢 2.850µm 19 Steps Range: 50.00 µm
	Bottom: -20.00 µm Top: 30.00 µm Relative Positions:
	Top: +29.98 um
	Z Device: Nikon A1 Piezo Z Drive ▼ Piezo ▼ Bottom: -20.02 µm
	Close active Shutter during Z Movement Use HW sequencer
	Advanced >>
	Load Save Remove Remo



(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** [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** [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.

1	A1plus Settings x		×
			Capture Z-Series x
			Experiment: ND Acquisition
	Acquire		Z:
(4), (5)	NZ Live		Save to File Record Data
	Find Mode		Order of Experiment
	√2° XY		
	Fast Piezo Z	(4)	Top (5,03 (4), (5) Note
	₩ XYZ	(5)	-20,00 abs
	XY Time		Step: 2.850 µm 年 2.850µm 19 Steps Range: 50.00 µm
			Bottom: -20.00 µm Top: 30.00 µm Relative Positions:
	XYZ Time		Z Device: Nikon A1 Piezo Z Drive ▼
	Photo Activation		Close active Shutter during Z Movement Use HW sequencer
	Trigger		Advanced >>
			Load Save Remove Remo

- (6) Determine [Step].
- (7) Select "Ti 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.

	Capture Z-Series x
	Experiment: ND Acquisition
(8)	Zt Record Data Order of Experiment
	Top 30.00 abs K Reset 0.03 65 5.00 abs Bottom -20.00 abs
(6)	Step: 2.850 µ (= 0.500µm) 19 Steps Range: 50.00 µm
	Bottom: -20.00 μm Top: 30.00 μm Relative Positions: Top: +29.98 μm
(7)	Z Device: Ti ZDrive Bottom: -20.02 um
	Close active Shutter during Z Movement Use HW sequencer Advanced >> Load Save Remove Remo

8.3 Acquire Z series images.

 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.



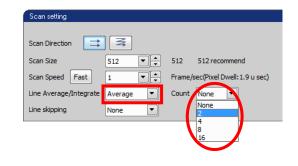
(2) Apply Line Average as needed.

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 <u>Run now</u> [Run now] button to acquire Z series images.



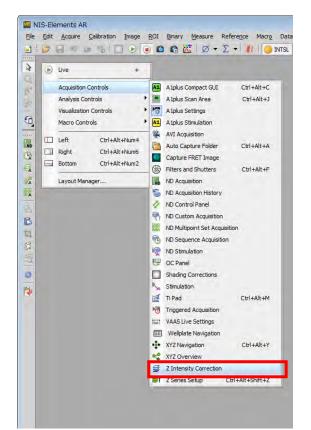
Capture Z-Series x	
Experiment: ND Acquisition	
Z:	
Save to File	Record Data
Order of Experiment 💌	
Image: Second state 30.00 abs Top 0.03 ds Image: Second state 0.03 ds	
Step: 2.850 µm 🗢 0.500µm 19 Steps	Range: 50.00 µm
Bottom: -20.00 µm Top: 30.00 µm	Relative Positions:
Z Device: Ti ZDrive	Top: +29.98 μm
	Bottom: -20.02 µm
Close active Shutter during Z Movement Use HW seque	encer
	Advanced >>
Load Save Remove Remove Run Z Corr	ime loop 🧳 Run now

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.



Use in ND Mu	(fixed Correction Cu	rve)	
O Relative	(offseted Correction	Curve)	
Move Z to	selected Point		+ 🗙
Corr. Home	Z [µm]	Device Settings	
Live Correcti	on (Z-	stack range —	
Use on Li	ve	To ND	
Offset Corr	ection Curve	From ND	Y
)[Load	Save Export

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.)

Z Intensity (orrection x									
Use in ND Mu	ltipoint									
Absolute	(fixed Correction Curv	ve)								
Relative (offseted Correction Curve)										
Move Z to selected Point										
Corr. Home	Z [µm]	Device Settings								
	30.00 (Top)	N/A								
1 -	4.35 (Home) ->] N/A 🗲								
	-21.30 (Bottom)	N/A								
Use on Li	Live Correction Z-stack range Use on Live To ND Offset Correction Curve From ND Load Save Export									
Move stage to	item Z position on Do	ouble Click.								

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

- 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.

Z Intensity Correction × Use in ND Multipoint Absolute (fixed Correction Curve) Relative (offseted Correction Curve)									
Move Z to	selected Point	(1)) 🔹 🗙 🗞						
Corr. Home	Z [µm]		Device Settings						
	30.00 (Top)		N/A						
X	4.35 (Home)	->	N/A 🔶						
	-21.30 (Bottom))	N/A						
Use on Li	Live Correction (2) Use on Live (2) Offset Correction Curve From ND Load Save Export Move stage to item Z position on Double Click.								

- (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].

XY Time	A Laser Power Monitor AG B Select	All Channels	HV Linear Correction
🖉 XYZ Time	✓ Ch 1 Indo-1/Ca2+saturated Lastr 405.0 HV < ○ ▶ 0	<mark>⊮ 0h2 DO</mark> HV ≪ ()	Laser-495.0
oto Activation	Offset 4	Offset 4	► 0 ► 0.0]0.0
Trigger	Chi 3 métameyelese Lacer 488,0	Ch 4 Qubit 655	Laser 405.0
	HY 4 ○ ► 0 Offset 4 ○ ► 0 Lase 4 ○ ► 0.0 0.0	HV <	
(3	Pinhais 4 Image: Pinhais 14.5 A.U. #88.0 * Attaine 255.4 um <-		

Z Intensity (Correction ×						
Use in ND Mu	ltipoint						
Absolute	(fixed Correction	Curve]					
O Relative	(offseted Correct	ion Cur	/e)				
Move Z to	selected Point				+	x	≽
Corr. Home	Z [µm]	(4) _{I p}	evice Settings				
	30.00 (Top)	->	IV1: 70) (HV2	80) (HV3: 8	0) (HV4: 34)) (HVTI	D#
×.	4.35 (Home)		/A				
	-21.30 (Bottom)	N	/A				
							_
Live Correction	n	Z-stac	k range				
🖌 Use on Li	ve	T	ND				
Offset Corr	ection Curve	Fre	m ND	Load	Save	Expo	vrt
<u> </u>				Loau		Texh	
Move stage to	item Z position o	n Doub	e Click.				

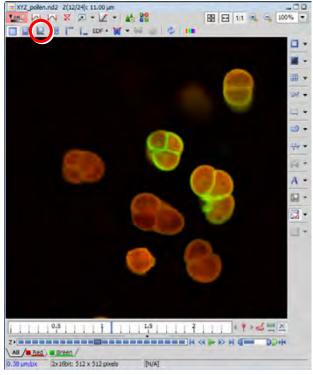
(5) After you finish all registrations, click the [Run Z Corr] button in the Capture Z-Series dialog box to start image acquisition.

Capture Z-Series x	
Experiment: ND Acquisition	
Z: [
Save to File	Record Data
Order of Experiment 🔹	
30.00 abs	
Reset	
-20.00 abs	F2 00
Step: 2.850 µm 🗢 0.500µm 19 Steps	Range: 50.00 µm
Bottom: -20.00 µm Top: 30.00 µm	Relative Positions:
Z Device: Ti ZDrive	Top: +29.98 µm
	Bottom: -20.02 µm
Close active Shutter during Z Movement Use HW seque	ncer
\sim	Advanced >>
Load 🔻 Save 👻 Remove 🐨 🛠 Run Z Corr 1 tir	ne loop 🛛 🛷 Run now

10 Creating Three-Dimensional Image

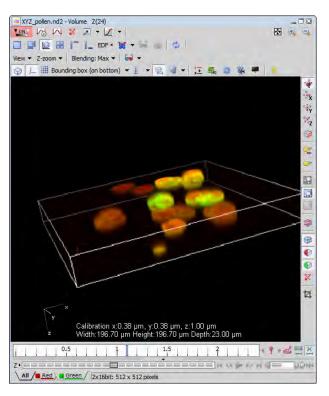
10.1 Create a three-dimensional image.

- (1) Click D 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.

- 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.

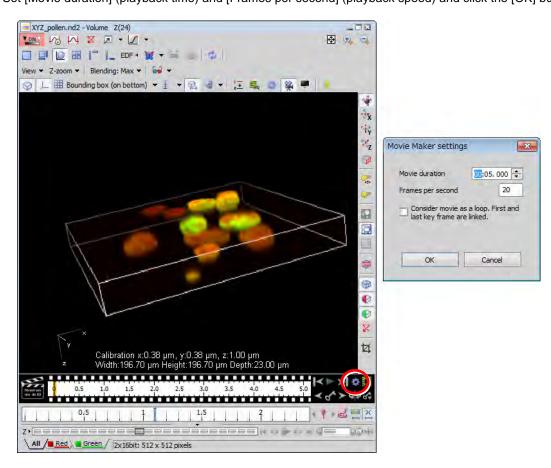
(1) Turn on the 🙀 [Show Movie Maker] button.



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

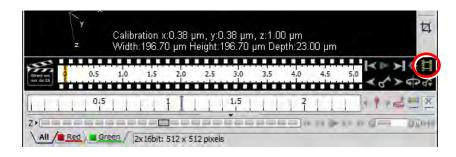
	z		Calib Widt	ration h:196.	х:0.38 70 µm	3 μm i Heiį	, y:0. ght:1	.38 μn 96.70	n, z:1 μm D	OO μ epth:	m 23.0	0 µm				
		0.5	1.0	1.5	2.0		2.5	3.0	3,5	4 4 11	.0	4,5	5,0	× ×	1	0
111	11	015	11		11	1	11	1.5			2	11	1	1	10	11

(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.

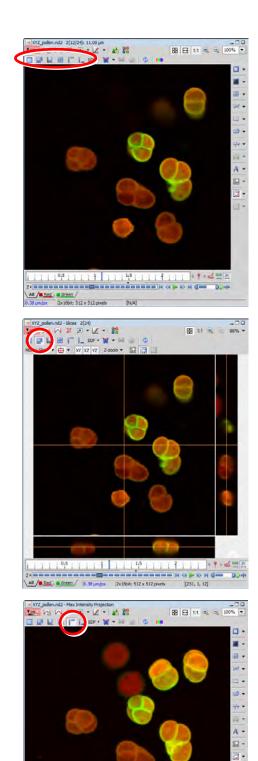
Save in:	NIS-Eleme	ents 🔹	• 🕝 🤣 📁 🖽 •		
(En)	Name		Date modified	Туре	
Recent Places	NIS-Elen	nents	7/2/2012 2:05 PM 7/2/2012 2:05 PM	File folder File folder	
Network					
Network	<	111			Saut
Network	File name:	TH PPP_2 - Movie AVI Image File Format (* avi)	(5)	•	<u>S</u> ave Cancel

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

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

Creating a Slice View Image _____ and a Projection Image

- 11.1 Create a slice view image or a projection image.
 - (1) Click D to open the Z series image.
 - (2) Click the image frame to create an image.



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THE R OWNER WHERE THE R.

[Show Slices View] button:

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

Clicking ightarrow 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.

A COLOR

2x16bit: 512 x 512 pix

All Bed Green

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.

 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.

	Capture Timelapse × Experiment: ND Acquisition T: Save to File			×	
		iles¥NIS-Elements¥Images¥	Browse. Record Data		
	Order of Experiment	•	Ø Ø X	*	
(2)	Phase Interve	al Duration In sec	Loops ▼ 11	r	
	Close Active Shutter with Switch Transmitted Illur	ninator off when Idle	rm Time Measurement (0 R	ncer p > p	ime measurement can be erformed while acquiring me series images.

12.3 Acquire time series images.

 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.

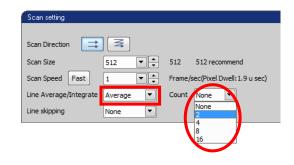


(2) Apply Line Average as needed.

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.



Capture Tir	nelapse x		X					
Experiment:	ND Acquisition							
т: 📖								
Save to	o File							
Path:	C:¥Program Files¥NIS-Elements¥Images¥ Browse							
Filename:	test.nd2 Record Data							
Order of Experiment ▼ Time schedule								
Phase	Interval	Duration	Loops					
✓ #1	1 sec	▼ 10 sec	▼ 11					
Close Active Shutter when Idle Perform Time Measurement (0 ROTs)								
	tive Snutter when Idle ransmitted Illuminator of		rm Time Measurement (0 ROIs)					
			Events Advanced >>					
Load 🔻	Save Remove	Run Z Corr	1 time loop 🛛 🥠 Run now					



13.1 Click 💋 to open the time series image.

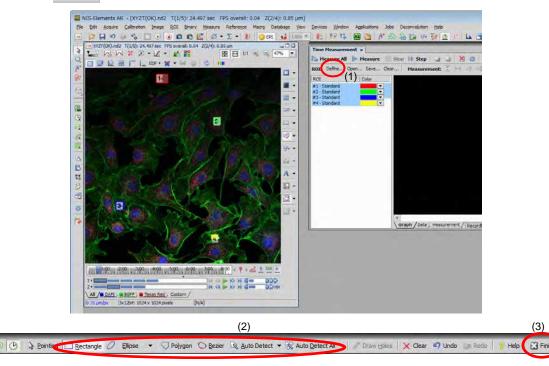
13.2 Display [Time Measurement].

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.

			۲	Live +			
				Acquisition Controls	•		
NIS-Elements AR + (XYZT(0K) nd2 T(1/5): 24.497 sec. FPS overal: 0.04 Z(2/4): 0.85 un	1			Analysis Controls	18	3D Object Measurement	
Ett grave Çakiraton (naçe 501 Bravy Hamura Reference Maco Debitage ()				visualization controls	· A		Ctrl+Alt+ Ctrl+Alt+F
【】 (A) (A) (A) · (A) · (A) · (A) (A) (A) · (A) (A) (A) (A) · (A) · (A)	(e) tive + Acquistor Controls	•		Left Ctrl+Alt+Num4 Right Ctrl+Alt+Num6 Bottom Ctrl+Alt+Num2	8	Automated Measurement Results Binary Layers Binary Toolbar Colocalization	Ctrl+F Ctrl+Alt+ Ctrl+Alt+Shift+
	Analysis Controls Visualization Controls	A* Averations and Nessurements Cb1+AR+E		Layout Manager		EDF Z-Profile	Ctrl+Alt+
	Marro Controls	Automated Massurement Coll+AR+F5 Automated Massurement Results Coll+F5	L		100	Intensity Profile	Shift+F
	Right Col+Alt+Rum6 fottom Col+Alt+Rum2	Brary Layers Cbl+AB+8 Snary Toobar Cbl+AB+9bB+Y Colocalization			1	Object Catalog	Ctrl+Alt+
	Layout Manager	La SEF 2 Profile Ch1+40+2			Σ	Object Classifier Object Count	Ctrl+Alt+
		Uve De Bur Object Catalog Chi +48 + 6					Carrier
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NEL Set 1 The Markhadde State State		Time Measurement Collsaid +1 Time Series Draph			****	, time series graph	
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0.31 um/thr The Liber, 1024 x 1024 presis		Geli-At-Ki			16	Volume Measurement	Ctrl+Alt+

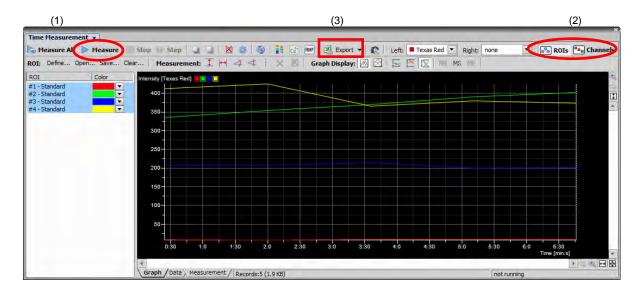
13.3 Set a ROI (Region of Interest) on the image.

- (1) Click the Define... [Define] button to open the Define ROI window.
- (2) Set a ROI on the image.
- (3) Click the SFinish [Finish] button to finish the setting.



13.4 Perform time measurement.

- Measure [Measure] button to draw a graph. (1) Click the
- (2) Use either of ROIs **Channels** measurement modes.
 - Multi ROIs: Displays change with time of multiple ROIs. (Only a single channel can be selected.)
 - Displays change with time of multiple channels ("ALL" or "Custom"). Multi Dyes: (Only a single ROI can be selected.)
- and select "Export Data", and then click the (Export 🔣 Export [Export] button to save (3) Click 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.



13.5 How to Use Time Measurement

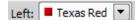


Select the ROI to be displayed on the graph.

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

Select the channel to be displayed on the graph.

measured.

Brightness difference between two lines can

be measured.

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.

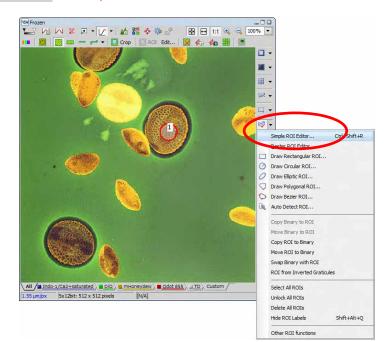
Note: When using Ch series, photo activation cannot be set. Select [None] in Ch series.

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

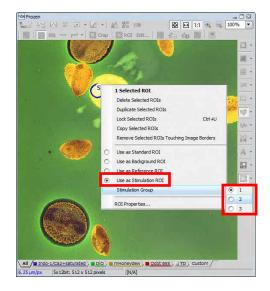
Elipse - 🖓 Pointing 🗆 Bectangle 🖉 Elipse - 🖓 Polygon 🛇 Bezier 🛞 Auto Detect - 🛞 Auto Detect All - 🔸 Stim. Point - 🖍 Draw Holes 🗙 Clear 🦃 Undo 🕼 Redo 🔋 Help 🔀 Finish tool bar.

Note: Using 📌 Stim. Point 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 SFinish [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).

(3)	(2)	(1)	(4)	
XYZ	Acquisition / 🗸	Photo Activation/) leaching	(Laser::405.0/488.0/488/0/405.0/)	2
C XY Time			HV Linear Correction	
XYZ Time		lect Stimulation Area) 🛛 🔽 All stimu	lation area set to same Manual Shift	
	405.0 🔺 🔶	.00	☑ 458.0 ◀ ◯ ▶ 0.0	
Photo Activation	488.0 <	▶ 0.0	☑ 405.0 ◀ 🔵 💽 🕨 0.0	
Trigger	Stimulation Scan settin	g		
	(5) Scan Spe	eed 1 Sec / Fran	ne (Pixel dwell: 0.0 u sec)	
				í

14.4 Set time series for photo activation imaging.

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



A1

(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.

	ND Stimu	lation ×				_		_		,
	Experiment	t: ND Stin	nulation							
		0.5	†	1.5		2 2.5		3	3.	5
3)	🚽 Save									
	Path: C:¥Program Files¥NIS-Elements¥Images¥ Browse									
	Filename: test.nd2 Record Data									
	Time schedule (A 1plus Galvano / A 1plus Galvano)									
	Phase	Group	Acq/Stim	ROIs	;	Interval		Duration		Loops
2)	#1		Waiting	•		No acquisi	•	1 sec	•	0
Í	#2		Acquisi	•		1 sec	•	1 sec	•	2
	#3		Stimul	▼ S1	-	No delay		0 sec	•	2
	#4		Acquisi	•		No delay	•	2 sec	•	2
	Perfor	m Time Me	asurement (0	ROIs, 1	l stim	. /bleaching RC	Is)			
	Close	Active Shu	tter when Idl	e [Us	e HW sequenci	er			
4)	Apply	y Stimulatio	n Settings							
				_				Adv	/an	ced >>
			-						ž	
	Load 🔻	Save	Remove	2		1	ume	e loop	2	Run now

15 Image Display Function

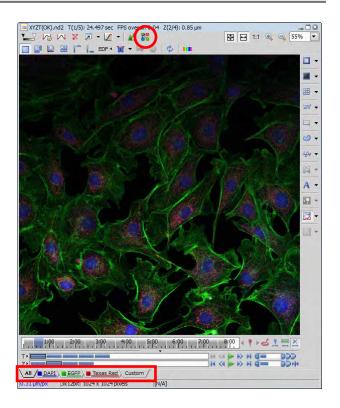
15.1 Channel display switch

(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 Reclicking this button displays all channels at the same time.
 Reclicking this button restores the previous display.

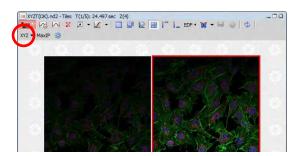


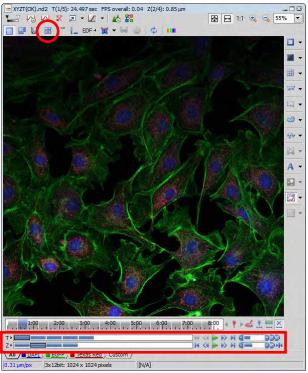
15.2 Multidimensional image display switch

(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 is to play back the image. Reclicking this button stops playback. Use is to adjust the playback speed.
 Use is for real-time playback (at the speed of actual image acquisition) and use is for fastest playback.
- (3) Clicking the [Show Tiled View] button
 displays series images in tiling mode.
 Use XYZ to select series images for tiling.



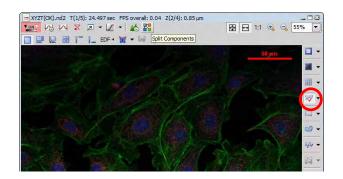


15.3 **Inserting a scale**

(1) Clicking the Imi [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].

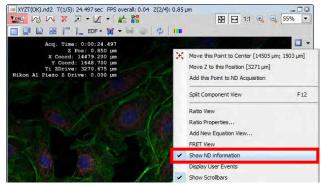
In addition, the scale bar and text color and size can be changed.



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.

	Label Properties	
You can select information to be inserted.	Available ND information Acon Time Z Pos X Coord Y Coord T Z Drive Nkon A1 Piezo Z Drive Format options	
	Connet cipulots Full format (hh:mm:ss.ms) Auto format Show in Millseconds ▼ Digits in ms: 3 ▼ Show only significant digits OK 年やセル 施用(A)	

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 D 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

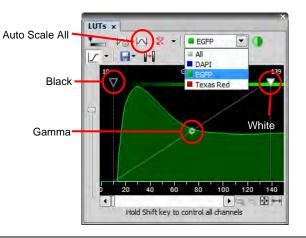
The control state of the microscope at the time of acquisition can be read from the acquired image.

- (1) Click D 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.

- 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)

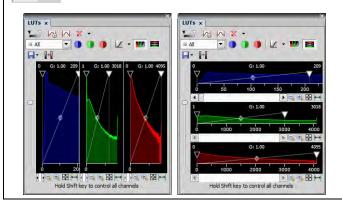
Display of the LUTs varies with the number of channels.

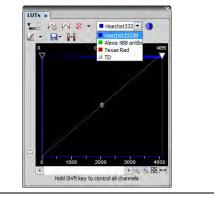
<u>Up to three channels</u>: All channels are displayed simultaneously.

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.

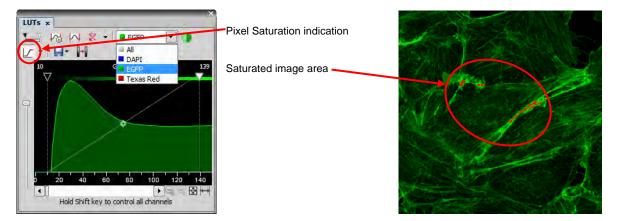




(3) Clicking the 🔀 [Reset LUTs] button returns the display without contrast adjustment.

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

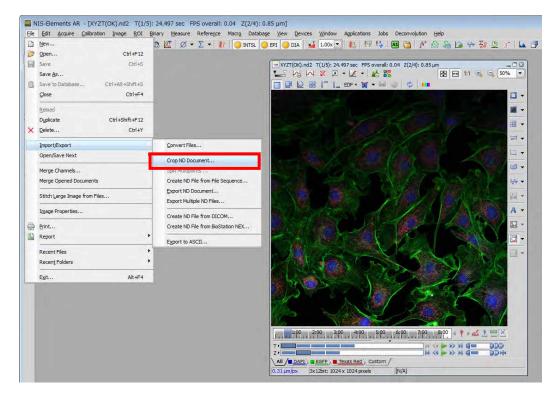
Note: Turning on and off the [Live] button (scan ON/OFF) during adjustment minimizes fading.



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 *b* to open the nd2 file.
- (2) Select [File] [Import/Export] [Crop ND Document] from the menu bar.



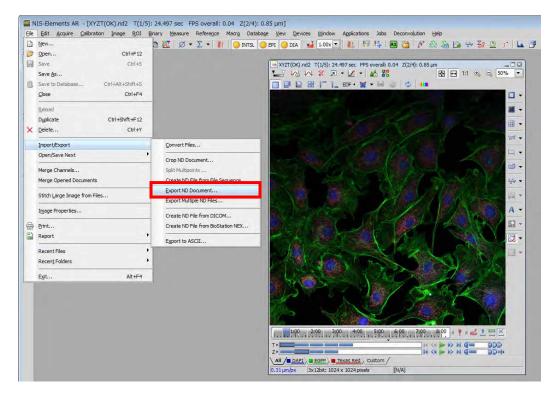
- (3) Specify the dimension and range of files you want to crop.
- (4) Click the [Crop] button to create a new nd2 file.

	Multidimensional Crop	×
	Image Selection Left: 0 Top: 0 Units: µm ▼ Select All Width: 318.48 Height: 318.48	Crop
Time axis Only arbitrary time axis	Time dimension	
range is selectable.	Step: 1	
Z axis Only arbitrary Z axis range is selectable.	Z series dimension From frame: 1 To frame: 4 Z low: 3269.80 μm Z high: 3272.35 μm Step: 1	
Wavelength Only arbitrary channels are selectable.	Wavelength dimension DAPI () EGFP () Texas Red ()	

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 *b* 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.

	ND Export		—
Save destination	File folder: C:¥Users¥Sim_Test¥Desktop¥N	IIS-Elements Browse	Export
File format	File type: Tagged Image Format 💌	Channels	Cancel
File name	File prefix: [xyzt(ok)]	O Color image for each channel	
	Index order: t 💌 z 💌	$ \overline{\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!$	
	Apply LUTs	TIF Compatibility Options	
	Insert Overlays	 Keep bit depth 	
		O Scale 12 bit to 16 bit	
		Scale 12 bit to 8 bit	

(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.

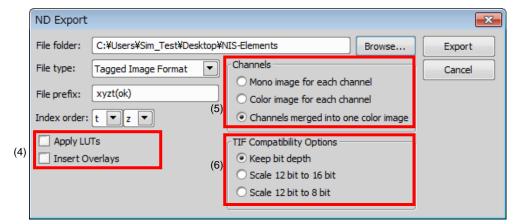
[Mono image]:A single channel is saved in monochrome.[Color image]:A single channel is saved in color.[Channels merged into color image]:Overlapping images are saved.

(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 channelMerged: Overlapping imagesLight yellow: Output in color

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

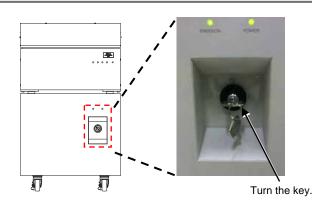
- (1) Right-click on a time lapse image and select [Display ND Information] [Acq.Time] from the displayed menu to display acquisition time on the image.
- (2) Click 1:1 at the side of the image frame to set the image display size to 100%.
- (3) Select [Edit] [Create Full View Snapshot] from the menu bar to capture the screen.
- (4) Perform Steps (1) to (3) in Chapter 17, "Exporting ND2 Files" to export images as a TIF file.



18.1 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).



18.3 Turn off the power to the controller.

18.4 Turn off the power to the microscope.

- (1) Turn off the power to the microscope main body.
- (2) Turn off the power to the mercury lamp (for visual fluorescence microscopy).
- (3) Turn off the power to the halogen lamp (for visual diascopic microscopy).
- (4) Turn off the power to the piezo Z stage.
- (5) Turn off the power to the motorized stage.

18.5 Shut down the PC.

Spectrum Imaging

A1 / Ti-E / PFS Motorized Stage / Piezo Z Stage / Intensilight

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

Capturing Spectral Images (Spectral Detector) —

19.1 Run the NIS-Elements software.

4.10.00 64-bit

A S (1) Click the NIS-Elements AR icon to run the NIS-Elements software.

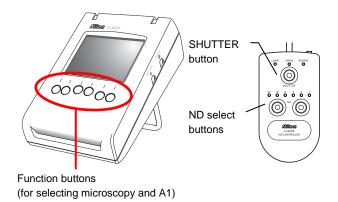
Note: When not only a confocal microscope is connected but also a camera, the Driver selection dialog box opens to select a driver.

Select "Nikon Confocal" in the Driver selection dialog box and click the [OK] button.



19.2 Observe the sample through the microscope.

- (1) Select the desired microscopy. Press a function button on the Ti remote control pad to select the desired microscopy.
- 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.

When the Ti remote control pad is available, press the [A1] function button to switch the optical path to A1.

If the Ti remote control pad is not available, switch the optical path to L100.

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.

		Resonant		Galvano	r InterLocked)	
Acquire	Filter	and Dye					
2º Live		A 244	Detector	DU4		lose mechanical shu	tter during experime
2		Eve Port	Ch series	None	Laser	Emission	
Find Mode		N I	Ch1	Indo-1/Ca2+saturated	405.0	425-475	
X XY		<u> </u>	Ch2	DiO	488.0	500-530	
No XY	PreS	can	Ch3	mHoneydew	488.0	552-617	

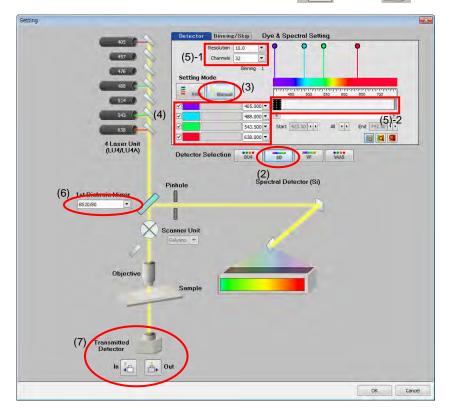
19.5 Set the optical path. (Optical path setting for the confocal system required for acquiring images)

Check the settings.

(1) Click to open the Optical path window.

۲	Resonant		Galvano		
Acquire	ter and Dye				
2º Live	111 224	Detector	DU4		ose m
2	Eve Port	Ch series	None 💌	Laser	E
Find Mode	AUX (1)	Ch1	Indo-1/Ca2+saturated	405.0	4
	HUA I	Ch2	DiO	488.0	5
No XY	reScan	Ch3	mHoneydew	488.0	5

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

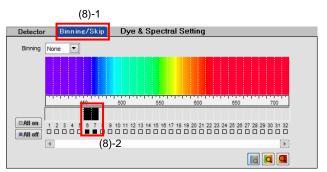


Note: Before acquiring a transmitted image, turn off the light above the microscope.

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.

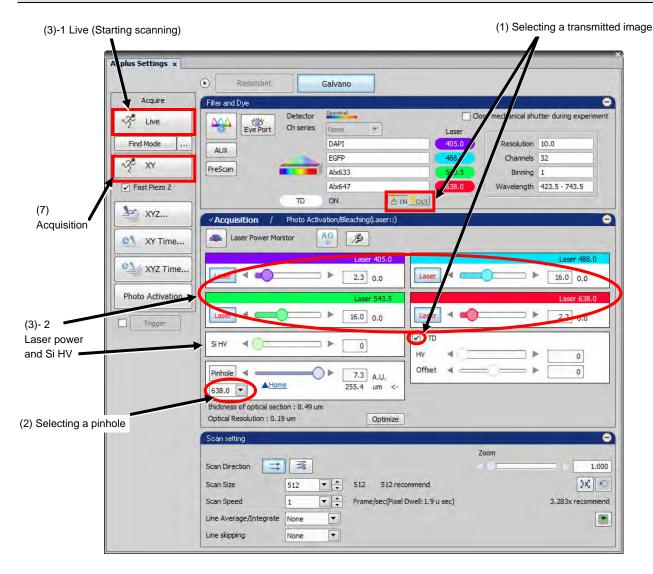
- (8) For channels that directly detect laser, click channel-skip boxes
 a model
 b model
 channel-skip boxes
 channe
- Note: Channels that catch reflected laser light are covered with a mask (plate) and channel data is not acquired. Selecting a channel-skip box facilitates subsequent spectrum analysis. The volume of data is reduced a little.



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



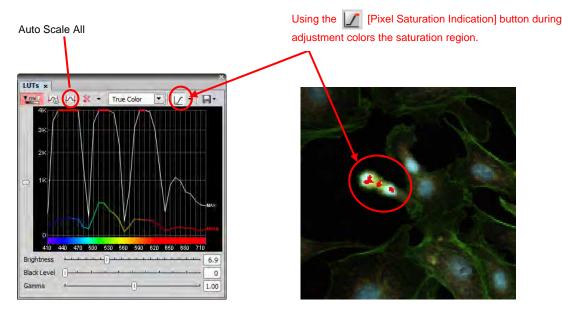
19.6 Determine image acquisition conditions.



 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 *Set* [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.

thickness of optical sect Optical Resolution : 0.1		Optimize		
Scan setting				Θ
Scan Direction Scan Size Scan Speed Line Average/Integrate Line skipping	512 • 1 • None •	512 512 recommend Frame/sec(Pixel Dwell: 1.9 u se	Zoom	1.000 3.283x recommend

Scan setting

Scan Direction

Scan Size

Scan Speed

Line skipping

Line Average/Integ

3

512

Average

None

1

• • 512

◄

Co

-

512 recommend

Frame/sec(Pixel Dwell: 1.9 u sec)

(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 🛷 xr [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) -

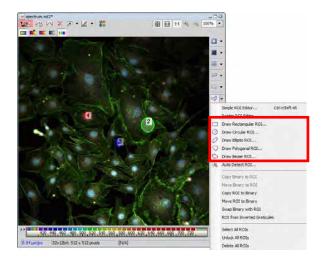
20.1 Click *b* to open the spectral image.

20.2 Spectral unmixing

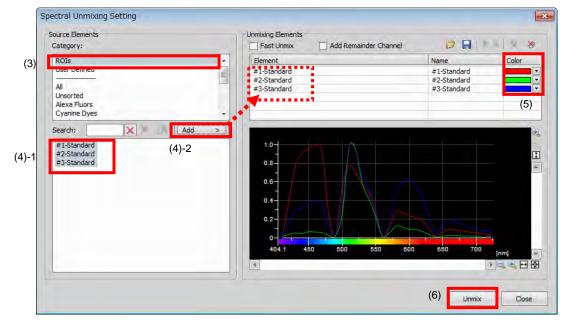
The following three patterns can be used in combination.

1) Spectral unmixing using spectral data in ROIs

- 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).

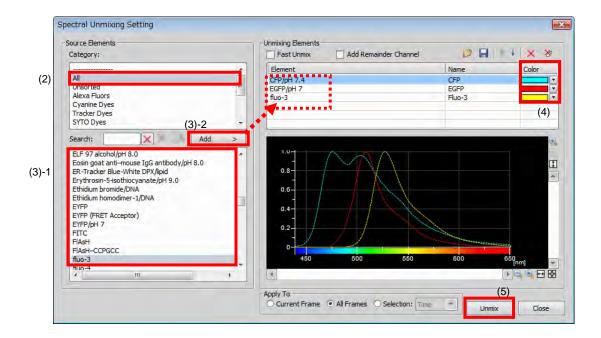


- (2) Select [Image] [Spectral Unmixing Setting] from the menu bar.
- (3) Select [ROIs] from the [Category] list box.
- (4) Select ROIs to be used for spectral unmixing and click the [Add] button to add them to [Unmixing Elements] (list on the right pane).
- (5) Set pseudo-colors after spectral unmixing.
- (6) Click the [Unmix] button to perform spectral unmixing.



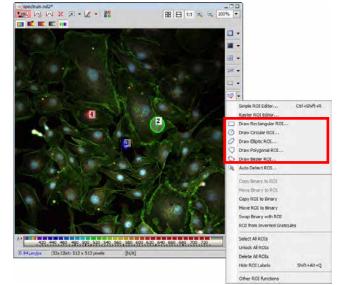
2) Spectral unmixing using reference data

- (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.

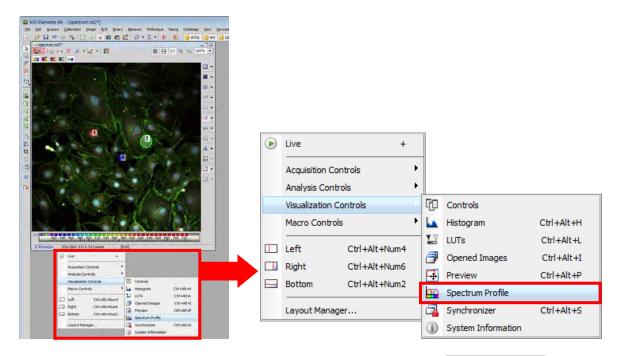


3) Spectral unmixing by creating reference data

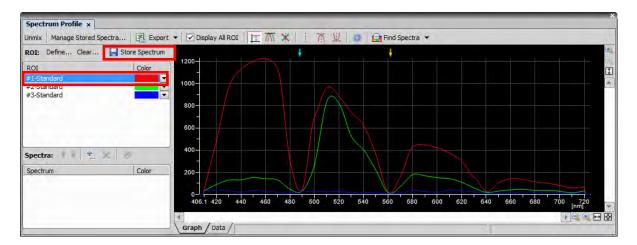
Note: Specify simple stain areas only.



- (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.



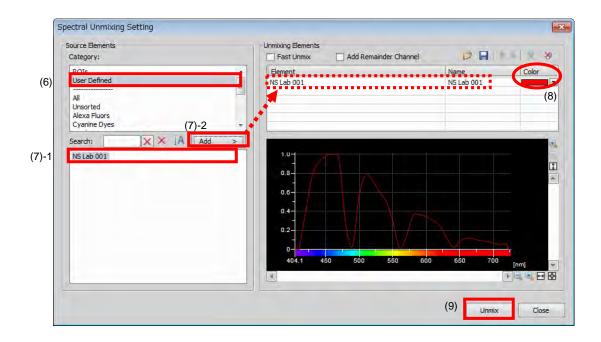
(3) Select the spectrum graph of a ROI which you want to save, and then click 📮 Store Spectrum.



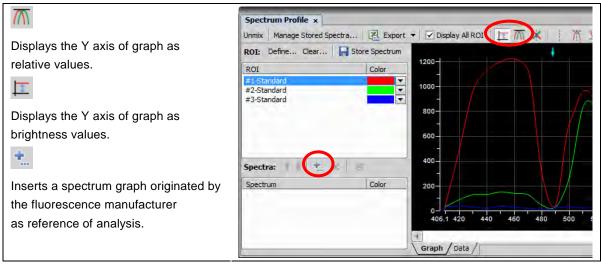
(4) Enter a reagent name in the [Spectrum Name] box, and then click the [OK] button to save the spectral data.

Store Spectrun	n 💌
Spectrum Name:	
NS Lab 001	
ОК	Cancel

- (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



21 Live Unmixing (Spectral Unmixing for Live Image) —

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

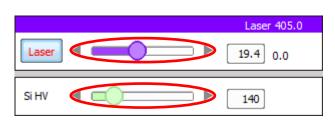
- (1) Select [Image] [Spectral Unmixing] from the menu bar to open the Spectral Unmixing Setting window.
- (2) Set a spectrum to be used for unmixing from [ROIs]/[Users Defined]/[All] data of [Category] by following the procedure for "Separating Spectral Image".
- (3) Click the [Cancel] or [Close] button to close the Spectral Unmixing Setting window.

Source Elements Category:	Unmixing Elements	nel 🕞 🔒	+ x *
ROIs User Defined	EGFP	Name EGFP EYFP	Color
All Unsorted Alexa Fluors Cyanine Dyes		2.07	(2)-2
Search: Add >			
EGFP eGFP EGFP/pH 7 ELF 97 alcohol/pH 8.0 Eosin goat anti-mouse IgG antibody/pH 8.0 ER-Tracker Blue-White DPX/lipid Erythrosin-5-isothiccyanate/pH 9.0 Ethiclium bronodimer-1/DNA Ethiclium bronodimer-1/DNA			
EYFP EYFP (FRET Acceptor) EYFP/pH 7 FITC Elast	0- 489 500 520 540 560	580 600 620 644	[nm]
K III F			(F) 🖻 🖬 🖬

21.2 Perform spectral unmixing for live image.

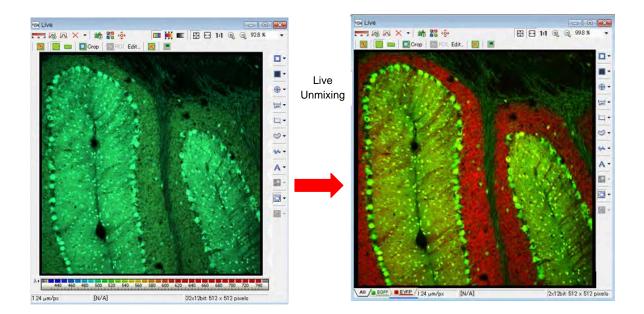
Click the [Live] button and adjust
 [Laser] (laser power) and [Si HV] (detector sensitivity) while checking the image.

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.

	ments AR - [Live]					
<u>Eile Edit</u>	Acquire Calibration			Reference Macr	o Databa <u>s</u> e <u>V</u> iew	Devices 1
		🔲 💽 🖲 🗋	@ 🖾 🖉 🔻	Σ - 11) INTSL 🔵 EPI 🤘	DIA 🖌 🎽
		• 🗹 • 🛛 👪 🖁	ê ()	🔁 🖽 1:1	• • 100% •	
₽* [®]		▼ Crop	ROI Edit 🔀	to to 🖪		
0			No. 1			



Capturing Virtual Filter Images. (Spectral Detector)

Run the NIS-Elements software. 22.1

4.10.00 64-bit

A'S (1) Click the NIS-Elements AR icon to run the NIS-Elements software.

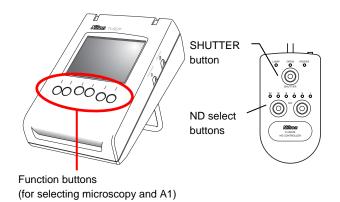
Note: When not only a confocal microscope is connected but also a camera, the Driver selection dialog box opens to select a driver.

Select "Nikon Confocal" in the Driver selection dialog box and click the [OK] button.



22.2 Observe the sample through the microscope.

- (1) Select the desired microscopy. Press a function button on the Ti remote control pad to select the desired microscopy.
- 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.

When the Ti remote control pad is available, press the [A1] function button to switch the optical path to A1.

If the Ti remote control pad is not available, switch the optical path to L100.

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.

	•	Resonant		Galvano Laser Inte	erLocked		
Acquire	Filter an	id Dye				and the second second	
2º Live			Detector	DU4	E	Close mechanical shutt	er during experin
of the		Eve Port	Ch series	None 🔻	Laser	Emission	
Find Mode		Ĩ	Ch1	Indo-1/Ca2+saturated	405.0	425-475	
XY XY			Ch2	DiO	488.0	500-530	
N. YI	PreSca	n	Ch3	mHoneydew	488.0	552-617	
Fast Piezo Z			Ch4	Qdot 655	405.0	662-737	
			TD				

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

Check the settings.

(1)	Click
(1)	CIICK

to open the Optical path window.

Acquire	Filter and Dye				
2 Live	AAA 2000	Detector	DU4		ose m
2	Eve Po	rt Ch series	None 💌	Laser	E
Find Mode	AUX (1)	Ch1	Indo-1/Ca2+saturated	405.0	
α [®] xγ		Ch2	DIO	488.0	
ye xy	PreScan	Ch3	mHoneydew	488.0	3
Fast Piezo Z		Ch4	Qdot 655	405.0	
		TD	ON AIN O	UT	
¥ XYZ	Acquisition	/ Photo Activ	vation/Bleaching(Laser::)		
C1 XY Time	Laser Powe		AG Select All C	Concerned in	

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

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

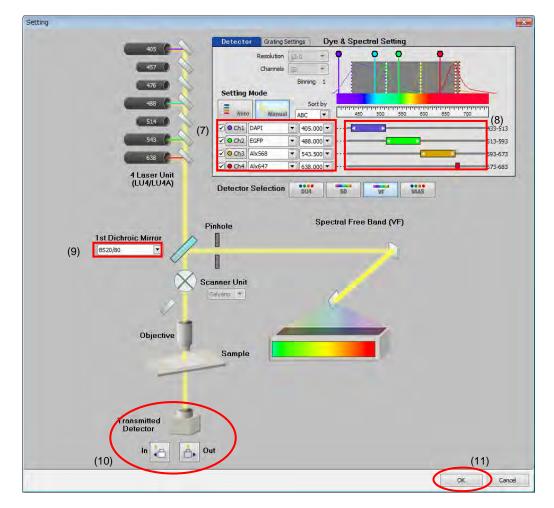
(4) Click the Grating Settings [Grating Settings] tab.

Setting	(4)
	Detector Grating Settings Dyc & Spectral Setting
405	Resolution 10.0 + • • • •
457	
476	Binning 1
	Setting Mode (3)
488	Sort by Auto ABC 450 500 550 800 850 700
514	Auto Manual ABC 460 500 500 600 690 700 Chi DAPI 405,000 -433-513
543	← Ch2 EGFP ▼ 488.000 ▼ ···· ← 513-593
638	✓ Ch3 Alx568 ▼ 543.500 ▼ ····
	✓ Ch4 Alx647 ▼ 638.000 ▼ ···· 673-683
4 Laser L (LU4/LU4	
	(A) Detector Selection DU4 SO VF VIAS
	(2)
	Pinhole Spectral Free Band (VF)
1st Dichroic Mirror	
BS20/80	
	Scanner Unit
	Galvano 🐨
Object	ive
	Sample
-	
Transmitted	
Detector	
in 🕌	A, Out
	OK. Cancel

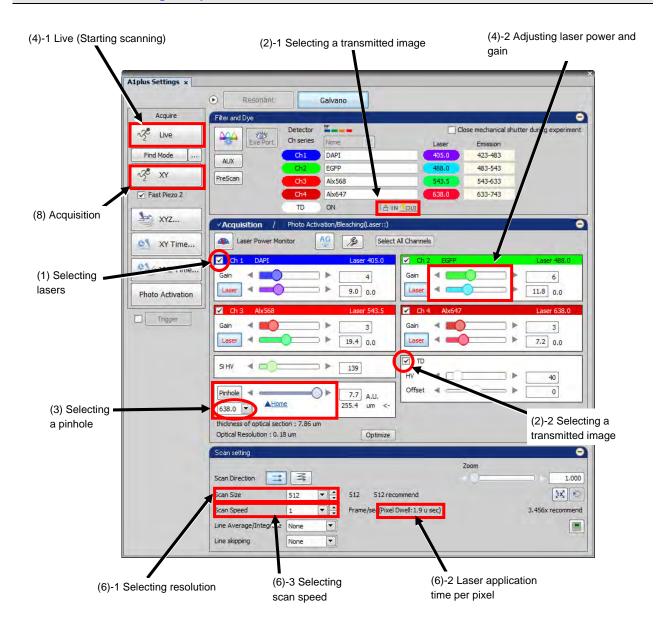
- (5) Select a value for [Resolution] (wavelength resolution to be used) from "2.5 nm", "6 nm", and "10 nm". Set the wavelength band to be acquired by shifting the bar.
- (6) Click the **Detector** [Detector] tab and make other settings.

	(6) Detector frating Settings Dye & Spectral Setting
405	Resolution 10.0 V O O O
	Channels ⇒ (5)-1 Binning 1 Sottine Mode
476	Setting Mode
458	Auto Manual ABC
514	✓ ● Ch1 DAPI ▼ 405.000 ▼
543	Ch2 EGFP 488.000 (
	Ch3 Alx568 V 543.500 V Start 423.50 V All V End 743.50 V

- (7) Select the laser to be used.
- (8) Set the channel detecting range by extending/reducing the **A** 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.

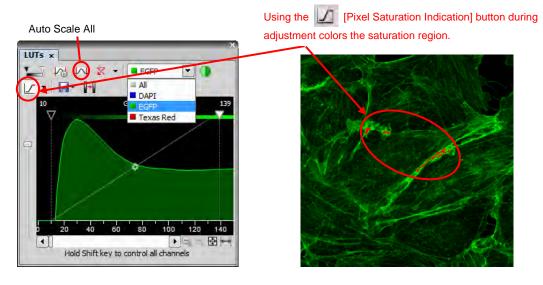


- (1) Select the laser and channel to be used.
- (2) If you want to acquire a transmitted image together with a spectral image, click the TD [IN] button and check the TD checkbox.

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]. A1

- (4) Click the <u>set ine</u> [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.



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 *Set* [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.

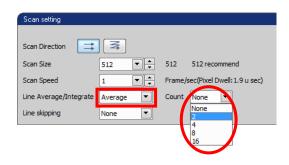
- (6) Set the number of pixels to the necessary resolution. (e.g. 512 x 512) If the image is dark, reduce the scan speed.
- Note: Check the pixel dwell for when the resolution is changed. Pixel dwell indicates laser application time per pixel. The larger the value, the brighter the image that can be acquired.

Scan setting		-
Scan Direction Scan Size Scan Speed Line Average/Integrate Line skipping	S12 • • S12 recommend 1 • • Frame/sec(Pixel Dwel: 1.9 u set) None • •	1.000 (x) (2) 3.283x recommend

 (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 🛷 xr [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.

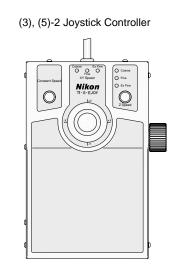
23.2 Set image acquisition points.

- (1) Click the MYZ 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 or the offset value (tracking focus position) of the perfect focus system 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.

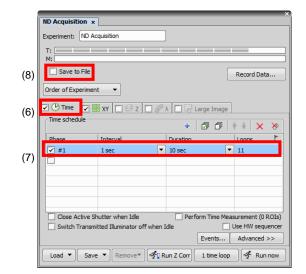
1plus Settings ×	ND Acquisition ×
	Experiment: ND Acquisition
Acquire	T:
3ª Live	Save to File Record Data
Find Mode	Order of Experiment 🔹
NO XY	
Fast Piezo Z	Move Stage to Selected Point 🕂 🗇 🕆 🗴 🗞
	Point Name X [mm] Y [mm]
1 XYZ	#1 (A)_1 .118 .1.909
- X12	✓ #2 2.101 -0.802
1	✓ #3 (5)-1 -> 3.381 -3.002 Offset All X,Y
XY Time	
23 XYZ Time	Include Z Relative XY Optimize Load Save Custom
	(4)-2 Close active Shutter during Stage Movement
Photo Activation	
	Advanced >>
Trigger	Load V Save V Remove Run Z Corr 1 time loop Run now



- (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.



Scan setting

Scan Direction

Scan Size

(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].

	Scan Speed Fast		Frame/sec(Pixel Dv
ge] in	Line Average/Integrate	Average 💌	Count None
	Line skipping	None 💌	None 2
			4 8
			16
eases			

3 3

512

T 512

512 recommend

ell: 1.9 u sec)

- 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.

ND Acquisition ×					×
Experiment: ND Ac	quisition]			
T:					
Save to File				Record Data	٦
Order of Experimen	t 🔻				
Time 🔽	🗄 XY 🔲 🖅 🗖 🔗	λ 📄 🗄 La	arge Image]	
Time schedule		+	7 1	+ + X &	,
Phase	Interval	Duration		Loops 🕨	
✓ #1	1 sec 💌	10 sec	•	11	
Close Active St	utter when Idle	Perfo	rm Time Me:	asurement (0 ROIs	-
	tted Illuminator off when			Use HW sequence	
		(Events	Advanced >>	
Load	Remove	Run Z Corr	1 time loop	🛷 Run now)

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 MYZ Time...] button to open the ND Acquisition dialog box, and then click the Z tab.
- (2) Click the **I** [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** [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** [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.

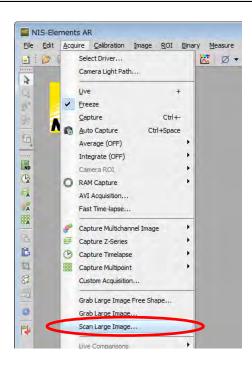
(5) Determine [Step].

		ND Acquisition x
		Experiment: ND Acquisition
	A1plus Settings ×	T: [] Z: []
	Acquire	Save to File Record Data
(3), (4)	Not Live	Order of Experiment (1)-2
	Find Mode	Image Image
	NZ [®] XY	(2) I X X 30.00 abs
	Fast Piezo Z	(3) Top (3), (4) Note (3), (4)
	₩ xyz	(4) Bottom -20.00 abs
	N Time	(5) Step: 2.850 m ← 2.925µm 19 Steps Range: 50.00 µm
	XY Time	Bottom: -20.00 μm Top: 30.00 μm Relative Positions: Top: +25.70 μm
(1)-1	XYZ Time	Z Device: Ti ZDrive Bottom: -24.30 µm
	Photo Activation	Close active Shutter during Z Movement Use HW sequencer
		Advanced >>
	Trigger	Load 🔻 Save 🔻 Remove 🛪 🖓 Run Z Corr 1 time loop 🚿 Run now

(6) Close the ND Acquisition dialog box.

24.3 Determine the large image acquisition area.

 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.

(
Scan Large Image	
Capturing	Stage Overv
Macro Image	🚺 Capture
Optical conf.: current Objective: 1: 4x	
Scanning	
Optical conf.: current Objective: 6: 100x	
Area	
Number of fields in X and Y	
Fields: 4 🗙 x 4 🔭	
Fields placement:	
Around the current position	
O Current position is at top-left corner	
A sti fields, 1817x1817 pixels, 0.45 x 0.45 mm, 15 MB defined with the state movement Close active shutter during stage movement	
Overlap	Shading Corr
	-
	Off (not On
No overlap Image registration	Oun
Create large image	
Store single images Filename: ¥Users¥Sim Te	et¥Decktop¥r
Create both	cst+Desktop+s
Storage for single images:	
Folder: C:¥Users¥Sim_Test¥E	Desktop¥samp

Scan Large Image Capturing ge Ove Macro Im 🚺 Captur Optical conf.: current ▼ Objective: 1: 4x -Scanning Optical conf.: current ▼ Objective: 7: 20x • Area Left, top, right and bottom limits ¥ [-0.2944, -0.2918] Ŧ **\$** ₩ H ± [0.08629 ds, 1199x846 pixels, 0.82 x 0.58 mm, 2.90 MB Define us Close active shutter during stage movement Shading Cor Overlap: 15 % Stitching via: Blending • Off (no OOn O No overlap ✓ Image registration Save large image to file Keep file O Create large image Save to Auto capture folder O Store single images Filename: C:¥Program Files¥NIS-Elen Create both Storage for single images: Folder: C:¥Program Files¥NIS-Elements 41

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.

Optical conf.: current Objective: 1: 4x Scanning Objective: Optical conf.: current Objective: 7: 20x Area [Left, top, right and bottom limits [-0.2944, -0.2918] Image: Contract of the second s	O Max IP O EDF Z-drive: Mi-E 2Dr	Step: 3	()))))))))))))
ptical conf.: current Objective: 1: 4x canning ptical conf.: current Objective: 7: 20x rea Left, top, right and bottom limits [-0.2944, -0.2918] Context Objective: Objective: 0: 0: 0: 0: 0: 0: 0: 0: 0: 0: 0: 0: 0:	Z Series Max IP EDF Z-drive: MI-E 2Dr Order: Lambda Multichannel captur	Step: 3	µm (← 3,100)
ea Left, top, right and bottom limits [-0.2944, -0.2918]	Max IP EDF Z-drive: <u>MI-E ZDn</u> Order: Lambda Multichannel captur	Step: 3	µm (← 3,100)
ea Left, top, right and bottom limits [-0.2944, -0.2918] H	O EDF Z-drive: NI-E ZDn Order: Lambda Multichannel captur	ive loop per 2 positio	*
Left, top, right and bottom limits	Order: Lambda Multichannel captur	loop per 2 positio	
Left, top, right and bottom limits [-0.2944, -0.2918] (+) (+) (+) (+) (+) (+) (+) (+	Order: Lambda Multichannel captur	loop per 2 positio	
[-0.2944, -0.2918] T Se	Multichannel captur		-
[-0.2944, -0.2918] T Se	and the second second second	re	
	etup		(
		+	+ + ×
	ptical Conf.	Name	Comp. Color
	DAPI	DAPI	
	FITC	TITE	
± [0.08629,	PH	Brightfield	Brightfield
x 2 fields, 1199x846 pixels, 0.82 x 0.58 mm, 2.90 MB of	1		
mory 🗸 🗸 🗸	Stitch using chann	iel: Brightfield	
(2) Define using L3 controlor Define using Ni E microscope	ocus		
	Focus manually a	at start	
rlap (3) Shading Correction (4)	Use Focus Surfa	ce	
Overlap: 15 % Stitching via: Blending Off (not available) Automatic postprocessing	-		
	Use step-by-step	p focus Setu;	p
Save lange to the Very the anomal Formati	O every	field	
Create large image	after 3		istance
Store single images			Stance
Create both	Skip frames		
Storage for single images:	Manually loc	ate sample and b	
Folder: C:¥Program Files¥NIS-Elements 410¥images¥Large Image tif 💌	Optical conf.:	PH-AF	•

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.

3 x 2 fields, 1199x846 pixels, 0.82 x 0.58 mm, 2.90 MB of memory	-	Stitch using channel: Brightfield
Close active shutter during stage movement	Define using L3 controler Define using Ni-E microscope	Focus Focus manually at start (1)
Overlap Overlap: 15 % Stitching via: Blending Voerlap Image registration	Shading Correction Off (not available) On Automatic postprocessing	Use Focus Surface
Create large image Store single images Create both Create bo	focus manually every 1 + field field after 3 mm distance Skip frames without sample Manually locate sample and background at start Optical conf.: PH-AF	
		Scan Close Help

- 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.

Add Point button	XVZ Overview XVZ Overview ND Acquisition Overview Focus Surface Document Overview X X X In X X X Z ⁴
	* * +
	I → Move Stage to Selected Point 59.2 41 #2 #3 Graph / Data / AOI: 13.75 mm x 9.38 mm

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.

(1) Create large image ○ Store single images ⊙ Create both	✓ Save large image to file ✓ Keep file opened (2) Format: ○ Save to Auto capture folder ● If Image: 100 minute If Image: 100 minute ● Filename: C:¥Program Files¥NIS-Elements 410¥images¥Large I If Image: 100 minute If Image: 100 minute Storage for single images: Folder: C:¥Program Files¥NIS-Elements 410¥images¥Large Image If Image: 100 minute	Focus manually every \$\frac{1}{\overline{\phi}} field \$\circ\$ after \$\$ distance \$
		(3) Scan Close Help

High-Speed Imaging

A1 / Ti-E / PFS 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)

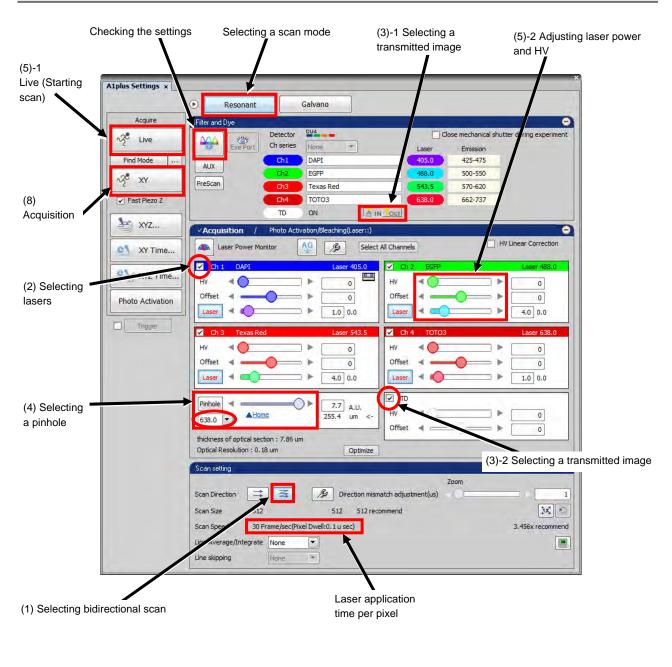
25.1 Perform Steps 4.1 to 4.6 in Chapter 4, "Capturing Color Images".

25.2 Select a scan mode.



Resonant [Resonant].

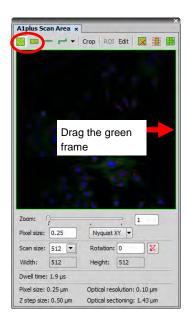
25.3 Determine image acquisition conditions.



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

Note: If you need higher-speed scan, reduce

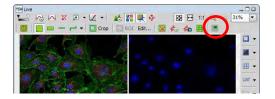
the scan area. Click is in the Scan Area window and drag the green frame to reduce the scan area.



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.

		-	Live +				
Acquester Comrole 😸 Alpha Compact GUS Coll+Allt+C Analyse Control 🕴 Alpha Scan Area Coll+Allt+C	Alpin Settings x		Acquisition Controls		A1	A 1plus Compact GUI	Ctrl+Alt+C
Visualization Controls • 🧖 Alpha Settings Herro Controls • 🍓 Alpha Setulation	Algen Car	-	Analysis Controls	•	#	A 1plus Scan Area	Ctrl+Alt+J
Left Cel-Altivised & Attacement Right Cel-Altivised & Auto Cesture Filder Cel-Altivia. Browner Cel-Altivised & Cel-Altivia.	Pred Made		Visualization Controls	1	8	A 1plus Settings	
Levul Manage 10 Arguinters	2 Failure 2		Macro Controls	+	A1	A 1 plus Stimulation	
 ND Control Famil ND Control Assimts 	C1_XYTEN	_		-	-	AVI Acquisition	
ND Multiport Set Acquaiton ND Sequence Acquaiton RD Sequence Acquaiton	01_ XY2 Tane		Left Ctrl+Alt+Num4		0	Auto Capture Folder	Ctrl+Alt+A
19 CC Panil Disbig Constans Simulators	Phota Activation		Right Ctrl+Alt+Num6		C	Capture FRET Image	
If the Cat-At-At			Bottom Ctrl+Alt+Num2		8	Filters and Shutters	Ctrl+Alt+F
til MAS Uve fartings ☐ Indijante Navigation ∲ 3172 Navigation Chicabitvi	Offic		Layout Manager		ND	ND Acquisition	
172 Overview 2 2 Merselly Comertilien	Perin 495			-	50	ND Acquisition History	
Il Zóres inter Criviti vOrti 42	Picker Openie				4	ND Control Panel	
	Scat				NIN		

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



*

Scan setting				_	
-				Zoom	
Scan Direction	: 3	A D	recton milmatch adjustment(us)		
Scan Sun 5	12	512	S12 recovered		30
Scan Speed 3	0 Frame/sec(P	tvel Dwell:0.1 u i	sec)		3.456x recor
Liné Average/Integra	té None	Ŧ			(
Une slapping		1.0			

Scan Area Setting	X Resolution	Y Resolution	Scan Speed and Un	
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.

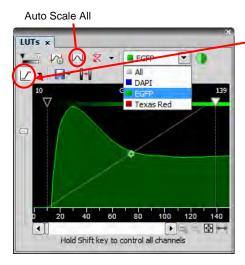
- (2) Select the laser and channel to be used.
- (3) If you want to acquire a transmitted image together with a confocal image, click the TD [IN] button and check the TD checkbox.

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

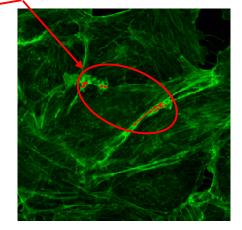
- (4) Select the laser wavelength to be used from [Pinhole].
 Select a pinhole size best suited for the objective with [▲ Home].
- (5) Click the [Live] Live] [Live] button and adjust [Laser] (laser power) and [HV] (detector sensitivity) while checking the image.

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

- Note: Using the *[I]* [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.



Using the [2] [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 *Set* [Auto Gain setting] button to change the rate of saturated pixels. Set the maximum value and minimum value of the rate.

Notes:

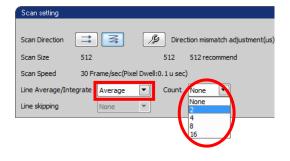
- Auto Gain is disabled during scanning.
- Auto Gain is disabled during 2Ex1Em or 1Ex2Emx2 line sequence.
- Auto Gain is disabled when line scan is set.
- Do not make a manual adjustment in the Acquisition window and do not make an adjustment using the remote controller during Auto Gain.

(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 🛷 xr [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.

 Click the XYZ Time...] button to open the ND Acquisition dialog box.



26.3 Determine the Z acquisition range of the ZT series.

1) Without perfect focus system

(1) Click the Z tab.

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

(3) Click the Reset [Reset] button.

	× *
	ND Acquisition ×
	Experiment: ND Acquisition
n.	T:
	Save to File Record Data
	Order of Experiment T)-(1)
	Time 🔲 🕅 Xλ 🗹 😂 Ζ 🔤 🧬 λ 📋 🖓 Large Image
1)-(2)	王 <u>恶 栗</u>
	Тор
1)-(3)	Reset 47.25 45 55.00 abs
	Bottom 30.00 abs
	Step: 2.850 µm 🗢 0.500µm 19 Steps Range: 50.00 µm
	Bottom: 30.00 µm Top: 80.00 µm Relative Positions:
	Z Device: Nikon A1 Piezo Z Drive
	Bottom: -17.25 µm
	Close active Shutter during Z Movement Use HW sequencer
	Advanced >>
	Load ▼ Save ▼ Remove▼ Save ▼ Run z Corr 1 time loop

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

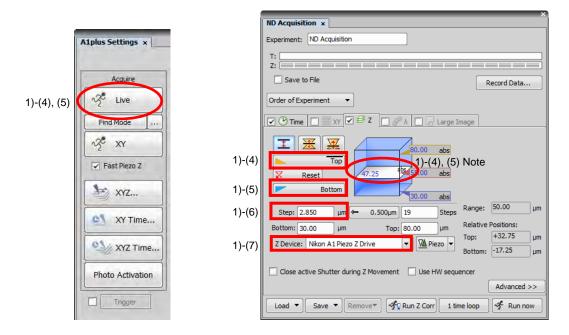
Note: Move the focus knob in the direction where the value of the plane in the cube increases.

(5) Click the [Live] button and move the focus knob (fine motion mode) of the microscope while checking the image. Click the **Bottom** [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 μ m (at ordinary temperature). Set the total range to 100 μ m or less.

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



Description about how to set the Z acquisition range of the ZT series without the perfect focus system is finished.

Go to 26.4.

2) With perfect focus system

(1) Turn on the perfect focus. Use the offset dial to move the focus.

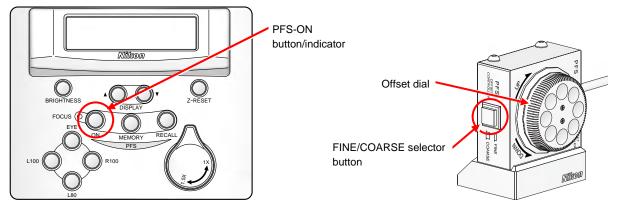


Figure Ti-E Front Operation Panel and Offset Controller

- (2) Click the Z tab.
- (3) Click the 🔀 [Asymmetric mode defined by range] button.
- (4) Turn the offset dial in the direction where the value in the cube decreases and determine the position of the sample bottom (near the objective).
- (5) Click 🕂 Relative
- (6) Enter "0" in [Below].
- (7) Enter a value of the sample thickness in [Above].
- (8) Determine [Step].
- (9) Specify [A1 Piezo Z Drive] for [Z Device].

	ND Acquisition x
	Experiment: ND Acquisition
	T:
	Z:
	Record Data
	Order of Experiment 2)-(2)
	🗹 🕑 Time 🔲 🏭 XΥ 🗹 🖅 🔤 🖉 λ 🔲 记 Large Image
2)-(3)	
2)-(5)	Relative Home Bottom
2)-(8)	Step: 2.850 µm (= 0.500µm 19 Steps Range: N/A µm
2)-(6)	Below: -25.00 µm Above: +25.00 µm
2)-(9)	Z Device: Nikon A1 Piezo Z Drive V MA µm Bottom: N/A µm N/A µm
_, (0)	Close active Shutter during Z Movement Use HW sequencer
	Advanced >>
	Load Save Remove Remove Kave Corr 1 time loop

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.

	ND Acquisition ×	K
	Experiment: ND Acquisition	
(2)	Z: Save to File	
(3)	Path: C:¥Program Files¥NIS-Elements¥Images¥ Browse	
	Filename: Record Data	
	Order of Experiment	
(1)	Ime Image Ime Image Ime Schedule	
	+ 0 0 + × ×	
	Phase Interval Duration Loops	l
(2)	✓ #1 1 sec ▼ 10 sec ▼ 11	I
		I
		I
	Close Active Shutter when Idle Perform Time Measurement (0 ROIs)	I
	Switch Transmitted Illuminator off when Idle Use HW sequencer	I
	Events Advanced >>	
	Load 🔹 Save 🔹 Remove Run z Corr 1 time loop	

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

ND Acquisition ×]		×			
Experiment: ND Ac	quisition					
Z: Save to File						
	ogram Files¥NIS-Elements	¥Images¥	Browse			
Filename:		ſ	Record Data			
Order of Experimen	t					
	XY 🗹 € Z 🗌 🔗	λ 📄 🗄 Large Image				
Time schedule	+ @ @ X X					
Phase	Interval	Duration	Loops 🕨			
✓ #1	1 sec 💌	10 sec 🔹	11			
Close Active Shutter when Idle Perform Time Measurement (0 ROIs)						
Switch Transmitted Illuminator off when Idle						
		Events	Advanced >>			
Load	Remove	Run Z Corr 1 time loop	Run now			

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."

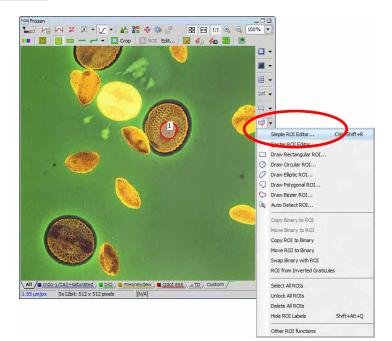
27.2 Set the area where photo activation is to be performed.

(1) Click 🥑 🗸 at the side of the image frame and select "Simple ROI Editor".

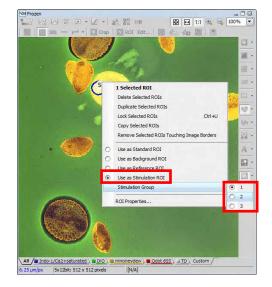
Draw a ROI on the image using tools on the

A Bointing Rectangle O Ellipse - O Polygon O Bezier & Auto Detect + 🗞 Auto Detect All + 🔸 Stim. Point - 🖍 Draw Holes 🗙 Clear P Undo (a Redo 🕴 Help 🖸 Frish

Note: Using 💠 Stim. Point 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 **K** Finish [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.

	(3)	(2) (1)
	XYZ	Acquisition / Photo Activation/) leaching(Laser::405.00/)
	C1 XY Time	HV Linear Correction
		1 2 3 (Select Stimulation Area) ✓ All stimulation area set to same Manual Shift
	SYZ Time	405.0 4 05.0 (4)
	Photo Activation	Stimulation Scan setting
		(5) Scan Speed 1 Sec / Frame (Pixel dwell : 0.0 u sec)

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



A1

(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.

	ND Stimulat	ion ×						×
	Experiment:	ND Stimulation						
(4)	Save to	Save to File						
	Path: C:¥Program Files¥NIS-Elements¥Images¥ Browse							
	Filename:	1.nd2				Rec	ord Data	
		ule (A 1plus Resonant / A	1plus (Galvano) —				
	Acquisition:	on: Interval		Duration			Loops	
(3)		No delay		30 sec		•	920	
	Stimulation/Bleaching:							
	Wait	Interval	Durat	tion	Loops		ROIs	
2)	10 sec 💌	No delay 💌	1 mse	ec i	• 15		S1	-
	Perform Time Measurement (0 ROIs, 2 stim./bleaching ROIs)							
	Galvano S	hutter						
5)	Apply St	imulation Settings	Enable lasers for acquisition					
	Load 🔻	Save		(1 time loo	p 🤇	🖗 Run no	W