1 Before Use

The NIS-Elements C is used as a function of NIS-Elements AR. It cannot be used alone. This section describes the starting/shutdown and structure of the NIS-Elements C window.

When a Galvano scanner that does not support the Fast mode is in use, replace "C2plus" written in this instruction manual with "C2." (For details, see Section 1.1.3, "Differences in Window Names Depending on Models Used."

1.1 Starting and Shutting Down the NIS-Elements C

This section describes the starting/shutdown the NIS-Elements C.

1.1.1 Starting the NIS-Elements C

Double-click the NIS-Elements AR icon.



Figure 1.1-1 NIS-Elements icon

The NIS-Elements title window appears. Then, the title window closes and NIS-Elements C starts.

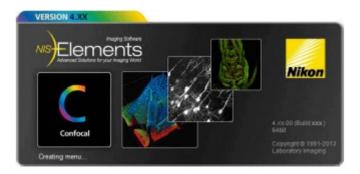


Figure 1.1-2 Title window

Then, the Driver selection window appears on the desktop. To use the regular C2 Confocal system, select "Nikon Confocal."

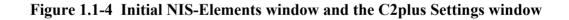
- * If only one camera is installed, the camera is automatically selected and the Driver selection window is not displayed.
- * To use the C2+TIRF system, check the [Enable Multi Camera] check box and select both Nikon Confocal and ANDOR. (see Chapter 12)

IS-Elements AR x.xx.xx (Buil	0 XXXX) 04010 - D1	IVEI SEIEULION
Nixon Confocs		-
Enable Multi Camera	OK.	Cancel

Figure 1.1-3 Driver selection window

As NIS-Elements C starts, the C2plus Settings window opens automatically as well.

likon	Caller Sellinger in
	100 CONTRACTOR CONTRAC
	Start Description Description <thdescrip< th=""> <thdescrip< th=""> Descrip<!--</td--></thdescrip<></thdescrip<>
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	Contra Co
	Interface Interface Lot Norma I 1



However, when the NIS-Elements was exited with the C2plus Settings window closed during the previous use, the window does not appear automatically at the next startup because the NIS-Elements reproduces the status at the previous exiting.

To manually display the C2plus Settings window, right-click on the gray area (without any setting window displayed) to display a menu as shown below. Select [Acquisition Controls] -> [C2plus Settings] in the menu.

-		Acquisition Controls	AVI Acquisition
Constant Sectors Cons	Analysis Controls Visualization Controls Macro Controls	Auto Capture Folder Ctrl+Alt+A Or Capture Folder Ctrl+Alt+C Or Capture Compact GLE Ctrl+Alt+C D Capture Scan Area ChiL481t+3	
	Detaint det	Left Ctrl+Alt+Num4	LINE ACCESS OF A REPORT
	Bottom Ctrl+Alt+Num2 Lavout Manager	Capture FRET Image	

Figure 1.1-5 Displaying the C2plus Settings window

* Other display methods

The C2plus Settings window can also be displayed by selecting [View] -> [Acquisition Controls] -> [C2plus Settings] from the menu bar.

ETERENEWARK IN DE ALMA CHINEN DAN ET DAN BOAR MÖNNA MON DESKA J DE REAL STORTE DAN ET DAN BOAR MÖNNA MON DESKA J DE REAL STORTE				udania •	A A & . (Str - br
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Figure 1.1-6 Displaying the C2plus Settings window

1.1.2 Shutting Down the NIS-Elements C

The layout of the NIS-Elements C is memorized when it shuts down.

1.1.3 Differences in Window Names Depending on Models Used

If a model that supports the Fast Galvano (enabling high scan speed) is in use, a name "C2plus" is displayed at the upper left of each window. For models that do not support the Fast Galvano, a name "C2" is displayed.

plus Settings x		C2 Settings x	
	۲		
Acquire	Filer and Dye	Acquire Filer and D	# 1
No Live	Detector Barner View Part Chieries Name V	🛷 Uve 🍑	Detector Detector Even Port Chiseries None V

Models that support Fast Galvan

Models that do not support Fast Galvano

Figure 1.1-7 Identification of models

Table 1.1-1 Window Names of Models The	at Support or Do Not Support Fast Galvano	
Window Names of Models That Support Window Names of Models That Do Not		
Fast Galvano	Support Fast Galvano	
C2plus Settings	C2 Settings	
C2plus Compact GUI	C2 Compact GUI	
C2plus Stimulation	C2 Stimulation	
C2plus Scan Area	C2 Scan Area	

In this instruction manual, window names of models that support Fast Galvano are written as "C2plus" hereinafter.

When a model that does not support Fast mode is in use, replace window name "C2plus" with "C2."

Differences depending on laser unit models

Indication on the excitation laser indicator and some functions that are displayed on the Optical path window vary depending on laser unit models used.

This instruction manual describes operations of the NIS-Elements C using "LU-NV" windows. (The right figure shows the LU-N4 window configuration without spectral detector.)

Avoid using combinations of LU-N4 or LU-N3 laser unit and detection mode (SD or VF) using the spectral detector as much as possible to prevent effects of excitation light.

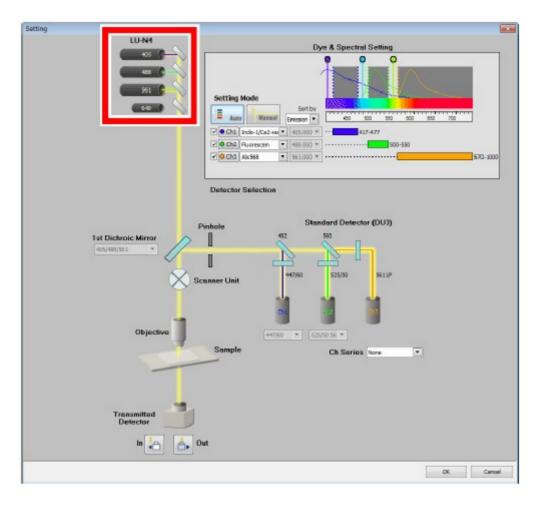


Figure 1.1-8 Distinction of laser unit

1.1.4 Setting OS

If sleep mode is specified in the power supply option of the OS, some devices may not work normally after recovery from the sleep mode. Disable the sleep mode of the OS.

- 1. Click [Start] -> [Control Panel] -> [Power Options].
- 2. Select [Change when the computer sleeps] to display the plan setup editing window.
- 3. Select [None] from the [Put the computer to sleep:] option.
- 4. Click the [Save Changes] button to confirm the settings.

1.2 Structure of C2plus Settings Window

The C2plus Settings window enables to apply various settings, including the laser, adjusting the brightness of the image, the photo activation setting, and scan resolution/speed, to use the Confocal Microscope.

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2)——	Tred Mode	ON DARE ON FITC OS Treas Red TO ON Arms from	4030 417-477 4600 900-550 281.0 570-1006
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	Photo Activative United		Ch2 PTC Leer MD.0 0 4 0
	Trigger		
5)——	Pointai Sti Li	Attens 30.6 un c- HV	
		execution: U. L.S. III Coloride	Science Pert
	ican De Scan Se		

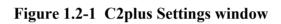


Table 1.2-1	Summary of C2plus Settings window functions
Name	Function

	Name	Function
(1)	Acquire window display/nondisplay selection	Switches display/nondisplay of the Acquire window.
(2)	Acquire window	Enables to display live images, to acquire images (see Chapter 4) or to apply the photo activation settings (see Chapter 10), to use external detector unit (see Chapter 16). The functions available with "NIS-Elements" are arranged as buttons in this area.
(3)	Filter and Dye window	Enables to select the channel series to be used and set the optical path. (See "Filter and Dye" in the chapters concerning detector modes.)
(4)	Acquisition/Photo Activation window	Acquisition window - The Acquisition window enables to set PMT brightness, laser power, and pinhole size. (See "Acquisition" in the chapters concerning detector modes.)
		Photo Activation window - The Photo Activation window enables to set the desired stimulation laser power. (see Chapter 10)
(5)	Scan setting window	Enables to set a scan method, resolution, scan speed, etc. (see Chapter 8)
(6)	Optimize button	Calculates the recommended value of resolution, zoom magnification, and Z stack step size based on the objective type and the selected excitation wavelength, and the indication/automatic application function can

be set in detail.

Combinations of Detection Modes and Functions 1.3

The following table lists settable functions in each detection mode.

Table 1.3-1 Combinations of Detection Modes and Functions **Detection Mode** Function DU3 VF SD Unidirectional Y Y Y Bidirectional Y Ν Ν **Channel Series** Y Ν Ν Multi position acquisition Available in all detection modes. (However, no photo activation experiment is available.) (Chapter 4) Fast mode (Chapter 8) Y Ν Ν HV linear correction Y Ν Ν Y Y Y Pinhole setting Photo activation (Chapter 10) Y Y Y 2Ex1Em Y Ν Ν Particular line sequential (Chapter 5) 4Ex4Em Y Ν Ν

Using external detector (Chapter 16) * Y

Y: Available N: Unavailable

Ν

* External detector units are usable only when a C2 system without a spectral detector or the Simple Si detector is in use.

Ν

When C2si (system with a spectral detector) or the Simple Si detector is in use, no external detector is usable.

* When using the Simple Si detector, see Chapter 18, "Simple Si Detector."

• Avoid using combinations of LU-N4 or LU-N3 laser unit and detection mode (SD or VF) using the spectral detector as much as possible to prevent effects of excitation light.

2 Optical Path Changeover for C2 Scan Head

This chapter describes the optical path changeover for the C2 scan head.

When using the Simple Si detector, see Chapter 18, "Simple Si Detector."

2.1 Optical Path Changeover Lever for C2 Scan Head

Confocal Microscope C2 illuminates the sample with the laser light (excitation light) transmitted from the laser unit, and detects the fluorescence from the sample by the detector unit. The detector unit to be used for the detection can be selected from the two types: the Standard Detector unit with a filter, or the Spectral Detector unit with a diffraction grating for the spectral function. To select which detector the fluorescence from the sample is transmitted to, use the optical path changeover lever on the C2 scan head.



Figure 2.1-1 Optical path changeover lever for C2 scan head

Lever position	Overview
Standard (Oblique)	Switches to Standard, and leads the fluorescence light from the pinhole to the Standard Detector unit, which has a filter.
	When this is selected, NIS-Elements C enters the "Standard Detector mode (DU3)" in which the 3 channel images at maximum are acquired by using the filter.
Spectrum (Vertical)	Switches to Spectrum, and leads the fluorescence light from the pinhole to the Spectral Detector unit, which has a diffraction grating.
	When this is selected, either of the following two detection modes can be selected for NIS-Elements C: "Spectral Detector mode (SD)" in which the 32 channel images at maximum

are acquired by the spectral function, or "Virtual Filter mode (VF)" in which 4 virtual channel images by four excitations at maximum can be acquired.

2.2 Detection Mode When Switching the Optical Path

This section describes the detection mode indication when the optical path is switched by the optical path changeover lever on the C2 scan head.

Switching operation by the lever	Mode change
Standard ↓ Spectrum	The optical path is switched to the Spectral Detector unit side, and the Spectral Detector mode (SD) is used as the detection mode of the NIS-Elements C.
	For using the Virtual Filter mode (VF), select [VF] in the Optical path window to switch the detection mode. The last settings of the detection mode are recalled.
Spectrum ↓ Standard	The optical path is switched to the Standard Detector unit side, and the Standard Detector mode (DU3) is used as the detection mode of the NIS-Elements C.
	The Standard Detector mode (DU3) can be switched to directly from either the Spectral Detector mode (SD) or the Virtual Filter mode (VF).
	The last settings of the detection mode are recalled.

Table 2.2-1 Mode indication when the optical path is switched

Page 1 of 13

3 Basic Operations

This chapter describes the basic instructions for acquiring live images in the NIS-Elements C.

Switch beforehand the optical path of the C2 scan head appropriately.

When selecting the Standard Detector mode [DU3] as the detection mode, set the optical path changeover lever on the C2 scan head to the [Standard] position.

When selecting the Spectral Detector mode [SD] or the Virtual Filter mode [VF], set the optical path changeover lever on the C2 scan head to the [Spectrum] position.

3.1 Acquiring the Live Image and Setting the Scan Area

1 Setting the Optical path

1. Display the Optical path window.

Click the [Setting] button in the Filter and Dye window.

For details of the Optical path settings, see "Filter and Dye" in the chapters concerning detection modes.

	Filer and Dyo					•	
	0.000	Detector	1.0	Oose mechanical shutter during experiment			
Setting button	· tre	Port Chiseries	None 💌	Laser	Emission		
		Chi	DAPI	408.0	417-477		
		Ch2	PITC	400.0	499-529		
		Ch3	Texas Red	543.5	552-617		
		π					

Figure 3.1-1 Filter and Dye window

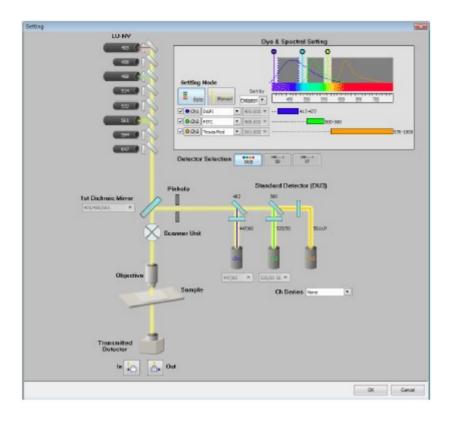


Figure 3.1-2 Optical path window

2. Select the detection mode (detector).

(This step is not needed if the Standard Detector mode [DU3] or the Spectral Detector mode [SD] is used, as they are automatically selected as the detection mode when the optical path changeover lever on the C2 scan head is switched.)

If the Virtual Filter mode [VF] is to be used, switch the optical path changeover lever on the C2 scan head to the [Spectrum] position, and then select the [VF] button in the Optical path window.

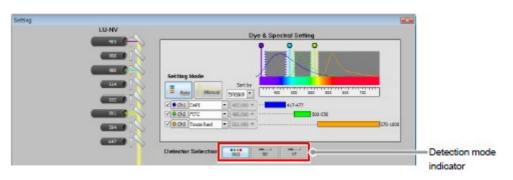


Figure 3.1-3 Displaying the Detection mode (when Standard is selected by the optical path changeover lever)

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	Beenhanner (120 *) Channals (20 *)
	5 601 (2008)-0 48 (0) 500 (0) (0)
<u>- 24</u>	Detection Selection Tele Detection mode button

Figure 3.1-4 Selecting the Detection mode (when Spectrum is selected by the optical path changeover lever)

* When using the Simple Si detector Select [CB] to use Continuous Bandpass mode; [VB] to use the Variable Bandpass mode. For details, see Chapter 18, "Simple Si Detector."

	Port1:LU-N4	Dye & Spectral Setting	
		Resolution Lata 💌 🔍 🔍 🔍 🔍	
	412 (Chervala 👥 💌	
		Dining 1	
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		47.40 T	
		261.700 • Start 200.00 + • All + End 720.00 + •	
			1000000000
		Detector Selection DLD CB V0	Detection mode
22			button

Figure 3.1-5 When using the Simple Si detector

- 3. Activate the automatic mode of Optical path setting. Click the [Auto] button.
- 4. Select the fluorescence dyes for the channels to be used. For each channel to be used, select a fluorescence dye from the pull-down menu. Once a fluorescence dye is selected, appropriate laser is automatically selected. And then the dichroic mirror recommended by the NIS-Elements is displayed.



Figure 3.1-6 Selecting mode and fluorescence dyes

5. Select the channels to be used. Check the check box for each channel to be used.

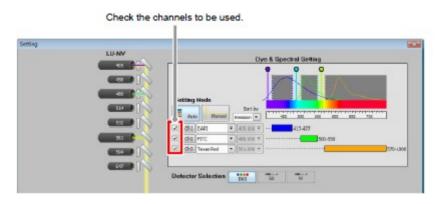


Figure 3.1-7 Selecting channels

- 6. Select the desired icon to use or disuse the transmitted detector.
- Confirm the Optical path settings. Click the [OK] button. The Optical path settings are confirmed, and the Optical path window closes.



Figure 3.1-8 Selecting the transmitted detector and confirming the Optical path settings

2 Applying Scan settings

In the Scan setting window, apply various scan settings to acquire the live image. For details of Scan settings, see Chapter 8, "Scan Setting Window."

Selects the scan method	(Unidirectional / E	idirectional).		Selects scan magnifi	cation
	Scan setting	13		Znom	•
Selects resolution.	Scan Direction	512 T	512 512 recommend	X	000
Selects scan speed.	Scan Speed Fest	1 .	Franc/sec(Pixel Dwell: 1.9 u sec)	4.803x recom	mend

Figure 3.1-9 Scan setting window

3 Acquiring the live image

Click the [Live] button.

The live image is acquired and the Live window appears.

Zplus Settings x	~					
	۲					
Armin	Filter and Dije					
2 ^e Live	Eye Port	Detector Chiseries	None (*)	Laser	ose mechanical shu Enission	tter during experimen
Find Mode		Ch1	CAPE	408.0	417-477	
2° xv	1	Ch2	FITC	488.0	499-529	
Se xx		Ch3	Texas Red	543.5	553-618	
		TD	ON A IN COL			

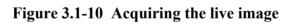




Figure 3.1-11 Live window

4 Adjusting the brightness of the live image

In the Acquisition window, adjust the brightness of the live image for each channel. For details, see "Acquisition Window" in the chapters concerning detection modes.

Acquisition / Photo Activ	ation/Bleaching(Laser::)		
Laser Power Monitor	5elect All Chan	nels H	V Linear Correction
Ch 1 DAPE	Laser 405.0	h 2 FITC	Laser 488.0
Diffset 4	▶ 40 HV ▶ 0 Offse ND 15.0 0.0		0 0 4.0 0.0
Ch 3 Texas Red	Laser 561.0		
HV 4	50 0 1.0		
Pinhole Finhole Fin	▶ 1.2 A.U. 30.0 um <- HV Offse	4000 P	
hickness of optical section : 1.28 um Optical Resolution : 0.13 um		External Port	,,

Figure 3.1-12 Acquisition window

5 Setting the scan area

Set the scan area for the acquired live image. For details of the scan area, see Chapter 9, "Navigation Mode."

1. Switch the Live window to the navigation mode. Click the [Show Scan Area] button in the Live window.

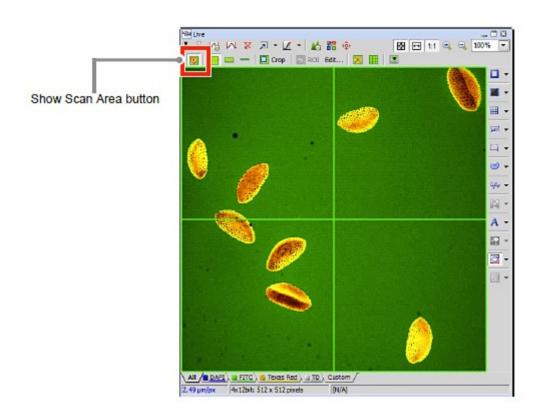


Figure 3.1-13 Switching to navigation mode

2. Select the scan area setting tool to be used. The scan area setting tools differ in their available shapes depending on the scan area selected. Scan area setting tool.

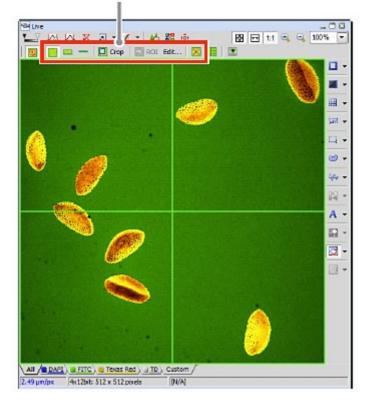


Figure 3.1-14 Selecting the scan area setting tool

 Set the scan area with the tool selected. For instructions on selecting and using scan area setting tools, see Section 9.3.2, "Scan Area Setting Tools."

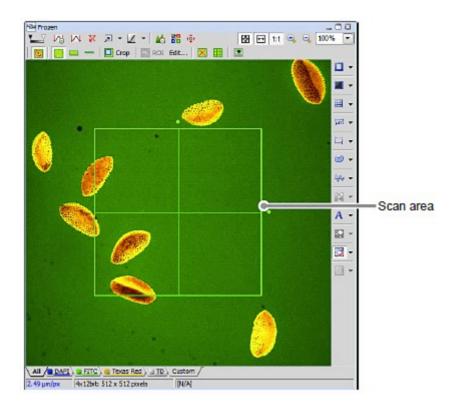


Figure 3.1-15 Setting the scan area

- 6 Acquiring the image of the set scan area
 - 1. Right click on the drawn scan area.

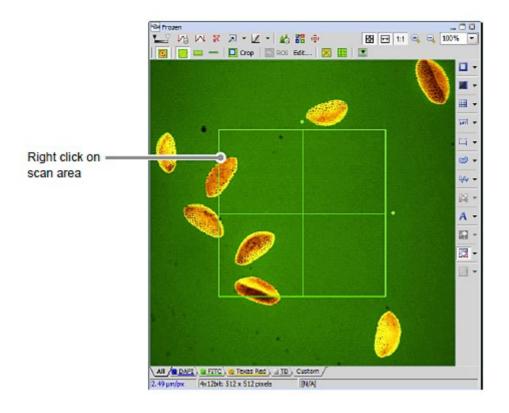


Figure 3.1-16 Acquiring the live image of scan area

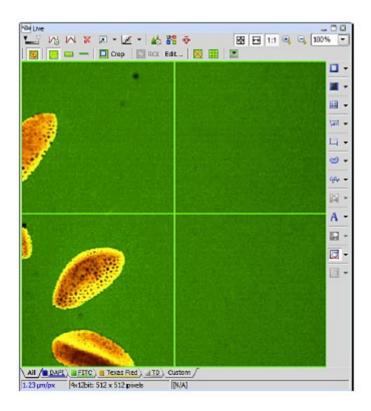


Figure 3.1-17 Live window after changing the scan area

* While working with Frozen image, the live image in the set scan area can also be acquired by clicking the [Live] button.

	(b)				
Accession	Filer and Dye				
2 ⁸ Live	000 888	Detector	819 		lose mechanical shutter during experime
2 me	Eye Port	Ch series	None 💌	Laser	Emission
Find Node		Ch1	CAPE	408.0	417-477
2 [®] XY		Ch2	FITC	488.0	499-529
No XY		Ch3	Texas Red	543.5	553-618

Figure 3.1-18 Acquiring the live image

• Laser Interlocked

Indicates the interlock status of the microscope main unit.

If the optical path of the microscope main unit is switched to the binocular system, all of the laser shutters close for safety purpose.

At this time, the [Laser Interlocked] button blinks and the confocal image acquisition cannot be executed.

When you execute the confocal image acquisition again, switch the optical path of the microscope main unit to "Confocal," and then click this [Laser Interlocked] button.

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Figure 3.1-19 Laser Interlocked indicator

• When LU4 or LU4A is in use

Note that, if an interlock occurs during image acquisition, the laser shutter operates differently as follows in LU4 and LU4A after the interlock is reset.

The laser shutter closes when an interlock occurs and opens after the interlock is reset.

LU4A:

The laser shutter closes when an interlock occurs but does not open after the interlock is reset. However, the laser shutter opens when the next image acquisition starts.

• When LU-N4 or LU-N3 is in use

When the [Clear Laser Interlock Automatically] check box in the C2plus Settings window is selected in advance, the hardware status is recognized even if an interlock occurs during image acquisition and the interlock is automatically reset.



Figure 3.1-20 Automatic release of Laser Interlocked

• When LU-NV is in use

When the [Automatic Laser Interlock release] check box in the LU-NV Configuration window is selected in advance, the hardware status is recognized even if an interlock occurs during image acquisition and the interlock is automatically reset.

	LU-NV Config	uration			and the
	Out Proc Servi Pirme	K version: 2. er version: 1. re version: 0: Nain: Vi ontrol Box: Vi	1.7.0 00).42		
	Output fibers:				
	Name	Control F	lights		Туре
	F1 1	Confoce	4	-	Single mode
	F2: 2	TIRP/ST	ORM 1	-	Multimode
	F3: 3	SIM		-	Single mode
	r4: 4	None		-	Single mode
	F51 5	TIRF/ST	ORN 1	-	Single mode
	Select Stimulation	an Output Part			
	Adapter	Output F	iber		
	TIRF/STORM	1 12		Ψ	
	Physical Laser L	ines:			
	# Lambda	Туре	Head Por	Mar	Run Time
	1; 405mm	LD	100.0	%	
	2: 458 nm	DPSS	300.0	%	
	3: 488 nm	DPS5	100.0	96	
	4: 514inni	DPSS	100.0	36	
	5: 532mm	DPSS	100.0	%	
	6: 561mm	DPSS	100.0	%	
	7: 594mm	DPSS	300.0	%	
	Bi 647 nm	DPSS	N/A	1%	
	Fiber Switching	eriment when i	and the second	dam	
		le laser power:		sec	
			30,00	200	
utomatic Laser	Interlock contro		t enterer		
nterlock release	Constant of the second		A recase		
	Laser Head Pov	-			
check box	Settings	Not Set			

Figure 3.1-21 Automatic release of Laser Interlocked

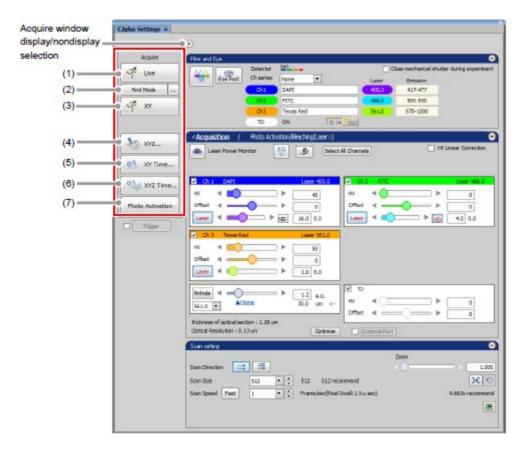
Page 1 of 28

4 Acquire Window

The Acquire window enables to display the live image, acquire the image or apply the photo activation settings.

Additionally, the functions available with NIS-Elements are arranged as buttons in this area.

4.1 Functions of Acquire Window





14010 1.1 1 1 11	
Name	Function
(1) Live button	Enables to display the live image. The Live window opens and displays the live image automatically. The live image is the real-time image that is currently observed with the microscope.
(2) Find Mode	Find ModeStarts/stops live image acquisition in Find mode. Find mode is the mode where the live image acquisition is executed by temporarily switching to the high-frame-rate setting in order to ease the detection of the observation object such as a cell.

Opens the Find mode settings window.

- For details of the Find mode, see Section 4.1.2, "Find mode."
- (3) XY button Enables acquiring the captured image of the currently displayed live image. When the captured image is acquired, the Captured window appears separately from the Live window.

The captured image is a still image that is acquired by re-scanning the scan area displayed in the current Live window.

(4) XYZ...button Opens the Capture Z-Series window.

....

Enables to acquire a three-dimensional (X-Y-Z) image. For operations of this window, refer to "NIS-Elements AR (Advanced Research) User's Guide."

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K .	Reset		138:15	20.00	atta	Set	TURP POB	
-	Bol	tam		41.00	abs	Mo	ve to TJRF	
Step: 1	.000	ļµn (⇔	0.100µm	41	Shana	Range:	40.00	1
starc (jun ju		40.00	Lau I	Relative	Positions	
		1000	1 apr	-	_	Top:	+21.85	1
2 Devices	TiPlez	S ZDRIVIE		- [<u>VB</u> P	1620 •	Bottom	-18.15	W
_							8	
Cidee a	cove setu	car curns	g Z Movement	Direction		locton to T	25	
							Advanced	>>

Figure 4.1-2 Capture Z-Series window

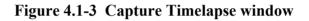
The same window can also be opened with the following procedure:

• Select [Acquire] on the menu bar and then select [Capture Z-Series] -> [Capture Automatically...] in this order.

(5) XY Opens the Capture Timelapse window.

Time...button Enables to acquire two-dimensional (X-Y) images in time series. For operations of this window, refer to "NIS-Elements AR (Advanced Research) User's Guide."

Experiment:	elapse x		12		
-speciment.	- ACQUERTER				
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The same window can also be opened with the following procedure:

- Select [Acquire] on the menu bar and then select [Capture Timelapse] -> [Capture Automatically...] in this order.
- (6) XYZ Opens the ND Acquisition window.

Time...button Enables to acquire three-dimensional (X-Y-Z) images in time series.

For operations of this window, refer to "NIS-Elements AR (Advanced Research) User's Guide."

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Figure 4.1-4 ND Acquisition window

(7) Photo Activation button Opens the ND Stimulation window. (This button is not displayed if a threelaser unit without AOM is connected.) The photo activation observation can be set.

For operations of this window, see Chapter 10 in this instruction manual.

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The same window can also be opened with the following procedure:

• Select [Applications] on the menu bar and then select [Define/Run Sequential Stimulation...] in this order.

4.1.1 Window for Acquiring Various Images

The windows shown in (4), (5), and (6) of "Table 4.1-1 Functions of Acquire window" can also be opened with the following procedure:

• Select [Applications] on the menu bar and then select [Define/Run ND Acquisition...].

In the ND Acquisition window displayed with the above procedure, click a switching tab to select a function to use.

For operations of this window, refer to "NIS-Elements AR (Advanced Research) User's Guide."

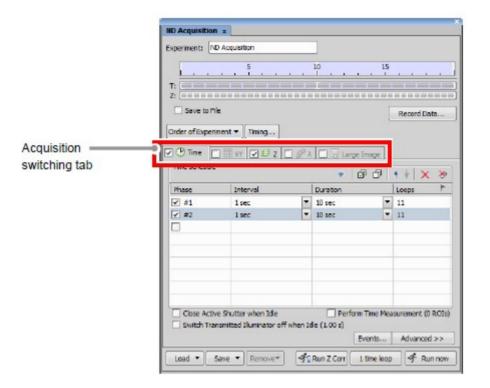


Figure 4.1-6 ND Acquisition window

4.1.1.1 Switching Z Stacks Acquisition Directions

Z stack image acquisition directions can be switched by [Bottom to Top] or [Top to Bottom].

Step: 1000 µm Close active Shutter during Z Movement View Person Relative Positions: Close active Shutter during Z Movement Direction: Image: Battom to Top Close active Shutter during Z Movement Direction: Image: Battom to Top Close active Shutter during Z Movement	Acquisition x
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2: Save to File Order of Experiment ▼ Tming P Time SBXY ■ ## Z ● # A □ a Large Dmage TDRF N/A µm N/A PTS Set TDRF Pos Move to TIRF Step: 1.000 µm ← 0.100µm 41 Steps Step: 1.000 µm ← 0.100µm 41 Steps Range: 40.00 µm Z Device: TI Piezo ZDrive Close active Shutter during Z Movement Close active Shutter during Z Movement P Content of Experiment ■ Top: +21.65 µm Bottom Close active Shutter during Z Movement P Content = Piezo ■ Pi	
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Figure 4.1-7 ND Acquisition window

4.1.1.2 Timelapse Experiment

Timestamp in Timelapse Experiment

In an experiment of timelapse, timestamp "0" is usually recorded at the beginning of the experiment (when the [Run now] button is clicked), but it is also possible to record timestamp "0" in the first frame.

For details, see "Options" in "NIS-Elements AR" Help.

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Close Acti		ff when 10		Time Mex	asurement ((0 ROIs)

Figure 4.1-8 ND Acquisition window

When Using Perform Time Measurement

If the Time Measurement is executed with the [Perform Time Measurement] check box selected, a load on processing becomes so high that it may cause the following problems:

- When the [Loop] side is set, the time for transition to the next phase may be longer than the time supposed from the frame rate.
- When the [Duration] side is set, the number of the frames may be smaller than that supposed from the frame rate.

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√ #1	1 sec	-	10 sec		11	
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	ive Shutter when Idle	off where 1d		n Time Mea	ssurement ()	I ROIs)

Figure 4.1-9 ND Acquisition window

4.1.1.3 Notes When Acquiring Images

When Executing the Large Image Function in ND Acquisition Window

The Large Image is a function to acquire a large image composed of multiple image frames and combine them to form a composite image by using the automatic algorithm, to be used when the target area is larger than the field of view (FOV) of the camera.

When this function is executed, the turning action is controlled by the stage. Therefore, it is necessary to execute the calibration before the Large Image function is executed.

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For operations of Auto calibration, refer to "NIS-Elements AR (Advanced Research) User's Guide."

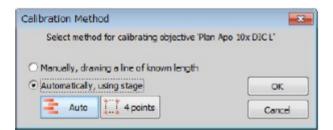


Figure 4.1-11 Auto calibration

When Executing the Scan Large Image Function While Both C2 and CCD Camera Are Used

When both C2 and CCD camera are used, if the transmitted detector (TD) is in the optical path, it blocks the light from the diascopic illumination. Therefore, the TD must be removed from the optical path before the Scan Large Image setting window is called.

* When executing the Scan Large Image function, do not use the optical configuration (O.C.) in which the TD is registered. When the TD is registered in the O.C. even if the TD is removed from the optical path, the TD automatically enters the optical path when an image is acquired.

4.1.2 Find Mode

By using the Find mode, you can acquire the live image by temporarily switching to the high-frame-rate setting in order to ease the detection of the observation object such as a cell.

4.1.2.1 Setting for Find Mode Settings Window

	_	
(1)	Change to band scan with aspect:	
	⊙ 2x ○ 4x ○ 8x	
(2)	Lower resolution:	
	⊙ 2x ○ 4x ○ 8x	
(3)	• Line skipping:	
	⊙ 2x ○ 4x ○ 8x	
4)	■ Turn OFF line&frame averaging	
(5)	Change Galvano to Resonant	
(6)	OK Cancel Apply	

Figure 4.1-12 Find mode settings window

<i>Table 4.1-2</i>	Summary of Find mode settings window functions
--------------------	--

Name Function

(1) Change to band scan Switches the band scan area by the specified ratio. with aspect:

(2)	Lower resolution:	E.g., If "2x" is selected in Scan Size 512 x 512, the band scan area is switched to 512 x 256 in the Find mode. Changes the scan size.
		E.g., If "2x" is selected in Scan Size 512 x 512, the scan size is changed to 256 x 256 in the Find mode.
(3)	Line skipping:	Unusable in the C2.
(4)	Turn OFF line & frame averaging	Changes a setting for Frame Average.
		* C2 is not equipped with the line average function.
		E.g., Even if the Frame Average is set in the normal mode, the live image is acquired by changing the setting to "None" in the Find mode.

(5)	Change Galvano to Resonant	Unusable in the C2.
(6)	OK button	Confirms the settings applied and closes the Find mode settings window.
(7)	Cancel button	Discards the settings applied and closes the Find mode settings window.
(8)	Apply button	Confirms the Find mode settings.

• If the C2plus Settings window or the C2plus Compact GUI window is closed during scan in the Find mode, no GUI menu or button can be selected and the scan cannot be stopped. In that case, press the "- (minus)" key of the ten-key to stop the scan.

4.2 Multi Position Acquisition

You can execute the experiment with multiple points within the same FOV by using the optical configuration (hereinafter referred to as O.C.) where different scan areas are respectively registered. (Photo activation experiment is not available.)

In Multi Position Acquisition, the image acquisition is executed by the ND Sequence Acquisition function of NIS-Elements AR, by using the Lambda series.

* About Lambda series:

When acquiring multiple excitation lights by emitting multiple lasers, the lasers are not emitted simultaneously but emitted in sequence. By emitting lasers in sequence, the cross talk between channels can be avoided.

4.2.1 Procedure for Multi Position Acquisition Settings

1 Register the first scan area to O.C.

- 1. Specify a scan area on the acquired image.
 - * The scan areas usable in the multi position acquisition are the square scan area and the band scan area only.

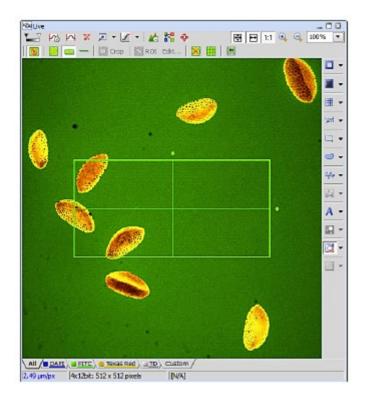


Figure 4.2-1 Specify a scan area

 Register the specified scan area to O.C. Select [Calibration] -> [New Optical Configurationc] from the menu bar to call the wizard.

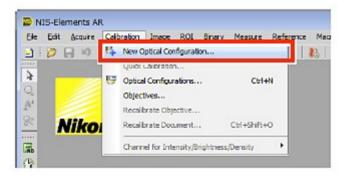


Figure 4.2-2 Call the Optical Configuration Wizard

- 3. Enter the name of O.C. to be registered.
- 4. Check the setting conditions, and then click the [Finish] button.

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Figure 4.2-3 Register the optical configuration

2 Register the second and subsequent scan areas as separate O.C., respectively

After that, repeat "Step 1" to "Step 4" of 1 to register the O.C. of each scan area to be acquired in the multi position.

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		- 620		

Figure 4.2-4 Register the optical configuration

3 Execute the multi position acquisition

Register the O.C. of each scan area for each action, and make the experiment setting, respectively.

1. Select [Applications] -> [Define/Run ND Sequence Acquisition...] from the menu bar to open the ND Sequence Acquisition window.

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	Denics for Stimulation: Ciplus P Define/Nun Secuential Stimulation	Caloum Properties
		Thration New
	H04.	Titration Calibration in Vitre

Figure 4.2-5 Call the ND Sequence Acquisition window

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	imelapse				
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1	Action	Description			
		1			

Figure 4.2-6 ND Sequence Acquisition window

2. Click the first phase and select [ND Acquisition].

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	Timelapse		
Seq.	Jence Definition		××
	Description		
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	Run Command Seg. Stimulation		
	Simult. Stimulation		
	Select Opt.Conf.		
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Figure 4.2-7 ND Sequence Acquisition window

3. Click the [Definec] button to open the experiment setting window.

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C	Timelapse		
Sec	uence Definition		+ + + X X
	Action	Description	
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	erge ND files if possible	10	

Figure 4.2-8 ND Sequence Acquisition window

- 4. Select the Lambda series tab on experiment setting window and specify the O.C. of the first scan area.
- 5. When the setting of the experiment sequence of the first scan area is completed, click the [OK] button to close the window. The ND Sequence Acquisition window is resumed.

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		er during Filter Change Ratio	The COST	
	Lie Koud Lienve		Advanced >>	
	Load * Save *	Remove*	OK (

Figure 4.2-9 Experiment setting window

- * The Lambda series is used for the multi position acquisition. However, do not set multiple O.C.s in one Lambda series for the purpose of the multi position. If O.C.s with different scan area types and/or sizes are set, displayed size will differ from the original image size, because the image size ratios must be matched within one ND image.
- 6. Click the next phase and select [ND Acquisition].
- 7. Click the [Definec] button to open the experiment setting window.

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	terge ND files if possible	T Remove T		🖋 Run Now

Figure 4.2-10 ND Sequence Acquisition window

- 8. Specify the O.C. of the second scan area.
- 9. When the setting of the experiment sequence of the second scan area is completed, click the [OK] button to close the window.

The ND Sequence Acquisition window is resumed.

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Figure 4.2-11 Experiment setting window

- 10. After that, repeat "Step 6" to "Step 9" to make the experiment setting for the O.C. of the scan area registered for acquisition within the same FOV.
- 11. Click the [Run now] button to execute the multi position acquisition.

Path: Prefix	-	NDSequence					
] Timelapse						
Sec	uence Definition —			+ + + i × 8			
	Action		Description				
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#2	ND Acquisition		Lambda(1)	Define			
V M	erge ND files if poss	ble					

Figure 4.2-12 ND Sequence Acquisition window

4.3 Function of Z Intensity Correction

When acquiring the images of a sample at Z drive positions (tomographic images) by Z stack, acquiring images with identical conditions at all Z drive positions makes some of the images to be too bright or too dark depending on the Z drive position to acquire. A solution to this problem is the Z Intensity Correction function.

To use the Z Intensity Correction function, first adjust the brightness on the live image by Z drive position you wish to acquire, and then register the optimum brightness setting values (laser power) for each Z drive position.

After that, acquire images by using the registered setting values. The brightness of the images for each Z drive position to acquire is automatically controlled, and images are acquired with the optimum brightness at all Z drive positions.

Minimum/recommended number of registrations for the Z Intensity Correction function

To use the Z Intensity Correction function, register at least two Z drive positions (Top and Bottom), and to acquire clearer images, it is recommended to register 4 or more positions (Top and Bottom plus two or more intermediate Z drive positions).

* A function to load Z drive positions from the ND Acquisition window by clicking the [From ND] button on the Z Intensity Correction window, is provided. In that case, only three points Top, Home, and Bottom are loaded. Registration of four or more Z drive positions is recommended by the Z intensity correction function, but there is no operational problem with the setting of one point less than the recommended number.

Z drive positions not registered to Z Intensity Correction

For the setting values of Z drive positions not registered to Z Intensity Correction are automatically interpolated according to the setting values of the registered Z drive position, and the interpolated setting values are used to acquire images.

To check the interpolated setting values, open the Microsoft Excel file output by using the [Export...] button on the Z Intensity Correction window.

4.3.1 Usage of Function of Z Intensity Correction

1 Setting Z Stacks position for image acquisition and Z Device

1. Call the Capture Z-Series window. For setting Z stacks instructions, see "NIS-Elements AR (Advanced Research) User's Guide."

Capture Z-Series x	×	
Experiment: ND Acquisition		
Z: [
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Top Reset Bottom	TIRF N/A µm N/A PFS Set TIRF Pos	 Z stacks Settings
Step: 1.000 µm 🗢 0.100µm 41 Steps	Range: 40.00 µm	
Bottom: 0.00 µm Top: 40.00 µm	Relative Positions:	
Z Device: TI Piezo ZDrive	Top: +21.85 µm Bottom: -18.15 µm	
	Bottom to Top Top to Bottom Advanced >>	
Load Save Remove Remove Load Corr 1	time loop	

Figure 4.3-1 Z stacks Settings

2. Select "Z Device."

Select a Z drive other than Piezo from the pull-down menu.

* When only the Z Device supplied with the microscope is installed, no Z Device selection menu is displayed in the Capture Z-Series window.

Experiment: ND Acquisition		
Z: []]]]]]]]]]]]]]]]]]]]]]]]]]]]]]]]]]]]		
Save to File	Record Data	
Order of Experiment 👻 🛛 Timing		
	N/A µm	
Reset 15.15 abs/	Set TIRF Pos	
Bottom	Move to TIRF	
0.00 abs Step: 1.000 µm ← 0.100µm 41 Step	s Range: 40.00 µm	
Bottom: 0.00 µm Top: 40.00 µm	Relative Positions:	
Z DEVICE: TI PIEZO ZDEVE	Top: +21.85 μm	
Ti 2Drive	Bottom: -18.15 µm	
Gose actine Conversion:	Bottom to Top	than Piezo.
0	Top to Bottom	
	Advanced >>	

Figure 4.3-2 Selecting the Z Device

2 Displaying the Z Intensity Correction window to read Z drive positions

 Right-click on the gray area (without any setting window displayed) to display a menu. Select [Acquisition Controls] -> [Z Intensity Correction] in the menu to open the Z Intensity Correction window.

	ŵ	Uve +	1							
	ľ				-	-				
Million	-	Acquisition Controls Analysis Controls	1.0	Art Acquisition Auto Cepture Folder	-					
Nikon		Vauakaatian Controls	-		Ctdadt+C					
		Marra Canthals	i.	Cipius Scan Area	Chinhital					
	1			Caplus Swittings						
		Let Col+Aberune	0	C2plus Stimulation						
		Right Col+Alb4Rum6		Cipture PART Image						
	-	Bolton Chi+Aterum2	9	Phone and Stuttons	CeleAber					
		Laysut Hanager	-	Capture 2.6enes						
			5	ND Acquisition History						
			4	ND Control Panel						
			8	ND Custom Acquisition						
			2	ND Multipeint Set Acquisitor	1					
			8	ND Sequence Acquisition						
				ND Stinulation						
			9	OC Panal						
			Ľ	Shading Corrections Straulation						
			2	TiPad	(01+4)-#1					
			-	Triggered Acquisition	Corneries					
			ň							

Figure 4.3-3 Displaying the Z Intensity Correction window

2. Clicking the [From ND] button in the Z Intensity Correction window reads the Top, Home, and Bottom Z drive positions.

	(fixed Correction) (offseted Correction)	
Move Z to	selected Point	* 🗙 🎘
Corr. Home	Z [µm]	Device Settings
Live Correctio	n r	Z-stack range —
Use on Li Offset Corr	ve ection Curve	To ND From ND Load Save Export

Figure 4.3-4 Z Intensity Correction window

Corr. Home	Z [µm]	Device Settings	
	30.00 (Tap)	N/A	
X	15.00 (Home)	N/A	
	0.00 (Bottom)	N/A	¢
Live Correctio		-stack range	

Figure 4.3-5	Ζ	Intensity	Correction	window

3. To read a Z drive position other than Top, Home, and Bottom, display the Z drive position you want to register and click the [Add New] button.

Dave	to File				8	Record Det	a
nder of E	periment *	Timing			_		
I	×	4	30.	00 abs		hw	
× I	Top	5.00	03 15.	.00 abs	N/A Set	PFS TIRF Pos	
-	Bottom		0.0	abs	Man	ve to TIR.F	
Step: 1	ц 000.	m 🖛 0.1	100µm 31	Steps	Range:	30.00	μm
ottom: 0	.00 u	m	Top: 30.00) µm	Relative	Positions:	
	TI ZDrive				Top:	+24.00	hw
L Device.	II 2DINE		<u> </u>		Bottom:	-6.00] µm

Figure 4.3-6 Capture Z-Series window

O Relative	Itipoint (fixed Correction C (offseted Correctio selected Point	- 33 I	Add New button
Corr. Home		Device Settings	
	30.00 (Tap)	N/A	
Ŧ	15.00 (Home)	N/A	
	0.00 (Bottom)	N/A	4

Figure 4.3-7 Z Intensity Correction window

ltipoint (fixed Correction Cur (offseted Correction C			
selected Point	* 🗙 🖗		
Z [µm]	Device Settings		
30.00 (Top)	N/A		
15.00 Alamal	n / n		
6.00 ->	(HV1; 10) (HV2; 10) (HV3; 20) (HVTD; 50) (LP1:		
0.00 (Bottom)	N/A		
n Z-s	tack range		
ve	To ND		
ection Curve	From ND Load Save Export.		
	(fixed Correction Cur (offseted Correction C selected Point Z [µm] 30.00 (Top) 15 (0 (4emo) 6.00 -> 0.00 (Bottom)		

Figure 4.3-8 Z Intensity Correction window

3 Adjust brightness at the Z drive positions and register them to Z Intensity Correction

1. Double-clicking the Top Z drive position displays the \searrow button.

Use in ND Multipoint Absolute (fixed Correction Curve) Relative (offseted Correction Curve)		1 MAR 1	
Move Z 1	to selected Point	* 🗙 ¥	
Care Home	a 7 [um]	Desites Sottlage	
	30.00 (Top)	N/A	Double-click
Ť	15.00 (Home)	N/A	
	6.00 ->	(HV1: 10) (HV2: 10) (HV3: 20) (HVTD: 50) (LP1:4	
	0.00 (Bottom)	N/A	
Live Correct	Uve	To ND From ND Load Save Export	

Figure 4.3-9 Z Intensity Correction window

	ltipoint (fixed Correction C (offseted Correction		20	orrection for: C2plu
Move Z to	selected Point			+ X 8
Corr. Home	Z [µm]	l Device Settin	gs	
	30.00 (Top)	-> I/A		
ž	15.00 (Home)	N/A		
	6.00	(HV1: 10) (H	(HV1: 10) (HV2: 10) (HV3: 20) (HVTD: 50) (LP1:	
	0.00 (Bottom)	N/A		
Live Correctio		-stack range To ND		
Offset Corr	ection Curve	From ND	Load	Save Export.

Figure 4.3-10 Z Intensity Correction window

2. Acquiring the live image of Top Z drive position. Click the [Live] button.

The live image of Top Z drive position is acquired and the Live window appears.

	۲					
Acquire	Filer and Dye					
2 ⁸ Live	[Detector	829 		lose mechanical shutt	er during experimen
2	Eve Port	Ch series	None (*)	Laser	Enission	
Find Node		Ch1	DAPL	408.0	417-477	
28 XV	<u>-</u>	Ch2	FITC	488.0	499-529	
No XY		Ch3	Texas Red	543.5	553-618	
	T	TD	ON A IN COL	1990 - 19900 - 19900 - 19900 - 1990 - 19900 - 1990 - 1990 - 1990 - 1990		

T .•	4 3 11	т.	•	• • . •
Figure	4.3-11	луе	image	acquisition

3. Adjusting the brightness of the live image. In the Acquisition window, adjust the brightness of the live image for each channel.

Laser Power Monitor	AG Select All Channels	HV Linear Correction
Ch 1 DAPI	Laser 405.0 Ch 2 FI	TC Lasser 488.0
HV 4 Diffset 4	> 40 HV 40 > 0 Offset 4 > ND 15.0 0.0 Loser 4	
Ch 3 Texas Red	Leser 561.0 ▶ 50 ▶ 0 ▶ 1.0	
inhole 4 -	→ ► 1.2 A.U. 30.0 um <- Offset ◀ o	

Figure 4.3-12 Acquisition window

4. Register the adjusted values. Click the [Add New] button in the Z Intensity Correction window. The adjusted values are registered at the Top Z drive position.

O Relative (of	xed Correction C fseted Correctio		rve)	Add New button
Move Z to sele	ected Point		+ × ~	Add New Dutton
Corr. Home Z	[µm]		Device Settings	
3	0.00 (Top)	->	N/A 🗲	
- 	.5.00 (Home)		N/A	
6	.00		(HV1: 10) (HV2: 10) (HV3: 20) (HVTD: 50) (LP1:	
0	.00 (Bottom)		N/A	

Figure 4.3-13 Z Intensity Correction window

	Itipoint (fixed Correction Cur- (offseted Correction C			
Move Z to	selected Point	* 🗙 🎘		
Corr. Home	Z [µm]	And the Antonio		
	30.00 (Top) 😔	(HV1: 20) (HV2: 20) (HV3: 50) (HVTD: 36) (LP1:		
ž	15.00 (Hame)	N/A		
	6.00	(HV1: 10) (HV2: 10) (HV3: 20) (HVTD: 50) (LP1:		
-	0.00 (Bottom)	N/A		
Live Correctio		tack range To ND		
Offset Corr	ection Curve	From ND Load Save Export		

Figure 4.3-14 Z Intensity Correction window

5. Repeat steps 1 to 4 for each Z drive position to be registered to move the multiple Z drive positions to be registered next.

2	(fixed Correction C (offseted Correctio				
Move Z to	selected Point		* 🗙 ¥		
Corr. Home	Z [µm]		Device Settings		
	30.00 (Top)		(HV1: 20) (HV2: 20) (HV3: 50) (HVTD: 36) (LP1:.		
Ť	15.00 (Home)		(HV1: 50) (HV2: 30) (HV3: 60) (HVTD: 46) (LP1:		
	6.00		(HV1; 10) (HV2; 10) (HV3; 20) (HVTD; 50) (LP1:		
	0.00 (Bottom)	->]	(HV1: 40) (HV2: 30) (HV3: 50) (HVTD: 36) (LP1:4		
Live Correctio		I-st	tack range To ND		
Offset Corr	ection Curve	F	From ND Load Save Export.		

Figure 4.3-15 Z Intensity Correction window

6. After registering all of them, click the [Run Z Corr] button in the Capture Z-Series window to execute the image acquisition.

With the registered setting values, the brightness of the image for each Z drive position is automatically controlled and images are acquired with the optimum brightness at all the Z drive

positions.

The settings registered on the Z Intensity Correction window are exportable to a file by using the [Save...] button, and an exported file is loadable by using the [Load...] button.

			Capture Z-Series x
			Experiment: ND Acquisition
			Z:
			Seve to File Record Data
7 Intensity	Correction x		Order of Experiment * Timing
UseinNDM			
Absolute	thred Correction Curv	Z Clorrection for: C2plus (e)	
O Relative	(offseted Correction O	urve)	Top N/A PPS
Nove Z to	selected Point	+ × ×	Reset Set TIRF Pog
Corr. Home	Z [µm]	Device Settings	Bottom Bottom Have to TDPF
	30.00 (Top)	0+W1: 20) 0+W2: 20) 0+W3: 50) 0+WTD: 36) 0.P1:	Dansa 30.00
-	15.00 (Home) 6.00	(HV1: 50) (HV2: 30) (HV3: 60) (HVTD: 46) (LP1:	step: 1.000 µm (~ 0.100µm [31 steps
		(HV1: 30) (HV2: 10) (HV3: 20) (HVTD: 90) (LP1: (HV1: 40) (HV2: 30) (HV3: 50) (HVTD: 36) (LP1:	Bottom: 0.00 µm Topi 30.00 µm Relative Positions;
	1		2 Device: Ti 2Drive Top: +30.00 Bottom: +0.00
Live Corrects	ion Z-et	adk range	COLUME TO A
Use on L	Station and a state	To ND	Close active Shutter during 2 Movement Direction: Bottom to Top Top to Bottom
Criset Con	rection Curve	Prom ND Load Seve Expert	Advanced
Move stage t	to item Z position on Do	uble Click.	Load * Save * Remove* Str Run Z Corr 1 true bop & Runn
			Actions Contraction Lansach

Figure 4.3-16 Image acquisition running

4.3.2 Z Intensity Correction Window

	Use in ND Mu	tipoint	2 Correction for: C2plus	
) —		(fixed Correction Cu (offseted Correction	rve)	<mark>_ (</mark>
	Move Z to	selected Point	× *	- (
	Corr. Home	Z [µm]	Device Settings	
		30.00 (Top)	(HV1: 20) (HV2: 20) (HV3: 50) (HVTD: 36) (LP1:	
_		15.00 (Home)	(HV1: 50) (HV2: 30) (HV3: 60) (HVTD: 46) (LP1:	
		6.00	(HV1: 10) (HV2: 10) (HV3: 20) (HVTD: 50) (LP1:	
		0.00 (Bottom) -	(HV1: 40) (HV2: 30) (HV3: 50) (HVTD: 36) (LP1:	
_		1		
	Live Correctio	on	stack range	_
_	Use on Li	ve	To ND	-
	Offset Corr	ection Curve	From ND Load Save Export	
			Load Save Export	
	Move stone to	item Z position on D	ouble Click	

Figure 4.3-17 Z Intensity Correction window

 Table 4.3-1
 Functions of Z Intensity Correction window

	Name	Function
(1)	Use in ND Multipoint	Absolute Interpolates registered Z drive positions when acquiring Multipoint regardless of change in Z drive positions.
		Relative Interpolates Z drive positions with offset according to change in Z drive positions when acquiring Multipoint.
(2)	Move Z to selected Point	Moves the Z drive position to the selected point.
(3)	Corr. Home	Indicates the position to be reference for correction. When [Set as Correction Home] displayed by right-clicking is selected, the selected Z drive position is the reference point for correction.
(4)	Z-Stack range	To ND Sends the Top, Home, and Bottom Z drive positions defined in the Z Intensity Correction window to the Capture Z-Series window.
		From ND Reads the Top, Home, and Bottom Z drive positions defined in the Capture Z-Series window to the Z Intensity Correction window.
(5)	Use on Live	Applies brightness adjustment at each set Z drive position to the live image.
(6)	Offset Correction Curve	Updates the Z drive position interpolation with offset according to the current Z drive position.
(7)	Add New button	Adds Z drive positions to be registered.
(8)	Remove	Emoves all registrations.
	Registrations button	Removes the selected item.
(9)	Load button	Retrieves the saved in a file.
(10)	Save button	Writes the registrations in a file and saves it.
(11)	Export button	Writes the registrations in a Microsoft Excel file. The exported file allows the user to check the interpolated values of Z drive position with setting values unregistered.

5 Detection Mode DU3

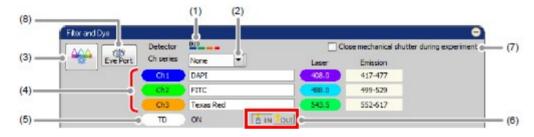
This chapter describes the settings for the Standard Detector mode (DU3).

5.1 Filter and Dye Window

This window enables to select a desired channel series and set the Optical path.

The Standard Detector mode can be used when the optical path changeover lever on the C2 scan head is set to the [Standard] position.

5.1.1 Structure of Filter and Dye Window





<i>Table 5.1-1</i>	Functions o	of Filter and	Dye window	(DU3-use)

	Name	Function
(1)	Detector	Indicates that the Standard Detector mode [DU3] is used when the optical path changeover lever on the C2 scan head is set to the [Standard] position.
(2)	Ch series	Selects whether to perform scanning by simultaneously firing all lasers for the channels in use or by firing each laser in the specified order.
		For Ch series selection, see Section 5.1.4, "Selecting the Channel Series."
		* Usable only when a four-laser unit or LU-N series laser unit is connected.
(3)	Setting button	Opens the Optical path window. To use, select the detector, the dichroic mirror, the channel, fluorescence dye for each channel, laser and others.
(4)	Status	Indicates for the settings for each channel (fluorescence dye name, laser wavelength, and wavelength band to be acquired).
(5)	TD	Indicates the status of the motorized transmitted detector.
(6)	TD IN/OUT button	Sets/removes the motorized transmitted detector in/from the optical path. (IN = Set in the optical path/ OUT = Remove from the optical

		path) As for the case where the TD IN/OUT button is not displayed, it will be displayed when the motorized transmitted detector is set in the optical path in the Optical path window.
(7)	Close mechanical shutter during experiment	If unchecked, the shutter remains open during the ND image acquisition. As the shutter is not opened/closed every image acquisition, the time for the image acquisition can be shortened.
		* During the interval period, laser power is automatically changed to the minimum but the laser cannot be shut off completely because the shutter is left open.
(8)	Eye Port button	Changes optical path to eye port. Each function on the C2plus Settings window becomes non- selectable when the Eye Port button is selected.

• Optical Configuration

Individual data items set in the Standard Detector mode (DU3) can be managed collectively with the Optical Configuration window. For storing and retrieving the [Optical Configuration] settings, see the sections concerning the optical configuration in the "NIS-Elements AR (Advanced Research) User's Guide."

5.1.2 Setting the Optical Path

Click the [Setting] button of the Filter and Dye window to display the Optical path window. The Standard Detector mode [DU3] setting screen is displayed when the optical path changeover lever on the C2 scan head is set to the [Standard] position.

There are two modes available for Optical path setting, [Auto] and [Manual]. Normally, the auto mode should be used.



Figure 5.1-2 Filter and Dye window (DU3-use)

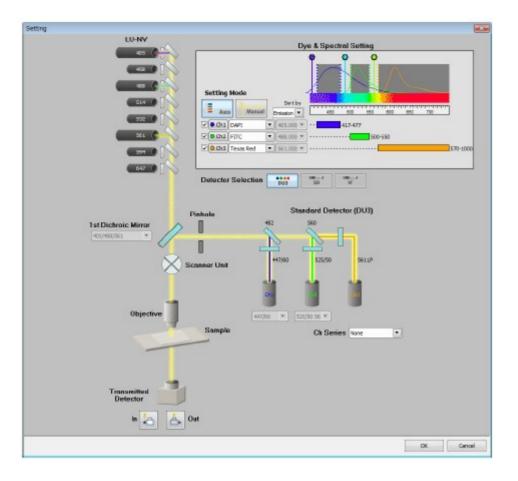


Figure 5.1-3 Optical path window (for auto mode, DU3-use)

5.1.3 Optical Path Window

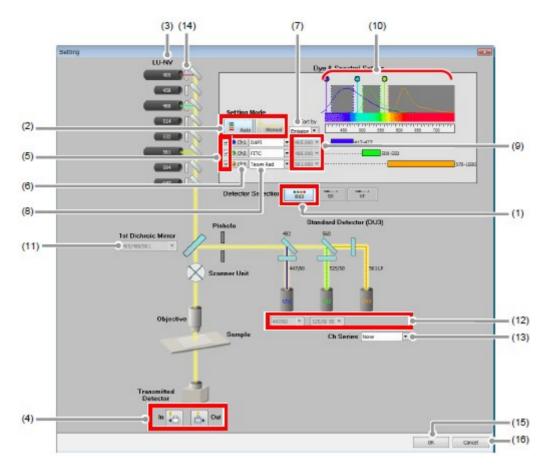


Figure 5.1-4 Optical path window (for auto mode, DU3-use)

 Table 5.1-2
 Functions of Optical path window (DU3-use)

Name

Function

	1 (antic	1 unction
(1)	Detection mode selection Indication	Standard Detector mode (DU3). Enables to acquire the 3-channel + TD images.
(2)	Mode selector	Selects the desired mode for setting the Optical path.
		Auto - Activates the auto mode. Once a fluorescence dye is selected, appropriate laser and the dichroic mirror are automatically selected.
		Manual - Activates the manual mode. Enables to set the lasers and the dichroic mirror to be used manually.
(3)	Excitation laser indicator	Displays the current setting for the laser. The currently set laser icon is displayed in a large size, and the optical path is indicated.
(4)	Motorized transmitted detector selection button	

When the motorized transmitted detector is in use, brings the transmitted detector into the Optical path, to enable the ability.



Brings the transmitted detector out of the Optical path, to disable the ability.

Enables to select the channels to be used.

Displays the Color Selection window, enables to set the desired color for each channel.

Sorts the fluorescence dye list according to the selected type.

ABC:

Displays the list in alphabetical order.

Emission:

Displays the list in the order of peak wavelength of fluorescence intensity.

Excitation:

Displays the list in excitation wavelength order.

In auto mode -Selects the fluorescence dye name to be used for each channel.

In manual mode -

Selects the in-use fluorescence dye name for each channel or enters an arbitrary channel name.

These menus are only effective while in the manual mode.

Enables to set the laser wavelength that is set with the software configuration, regardless of the setting of the Filter cube display/select.

Provides the following information:

- Wavelength band for which to acquire images (shown in color and value for each channel)
- Spectral profile of fluorescence dye
- Excitation laser for fluorescence dye
- A color band indicating the wavelengths in the entire band (400 to 750 nm)
- Scale of the wavelengths in the entire band (400 to 750 nm)

This menu is only effective while in the manual mode. Enables to manually select the 1st Dichroic mirror to be used.

These menus are only effective while in the manual mode.

The filter cube to be mounted on the detector can be selected regardless of the excitation laser.

- (5) Channel selection check box
- (6) Channel color setting button
- (7) Sorting fluorescence dye list

- (8) Fluorescence dye selection/input:
- (9) Excitation laser select
- (10) Rainbow chart

- (11) 1st Dichroic mirror select
- (12) Filter cube display/select

(13) Ch series selection	Selects whether to perform scanning by simultaneously firing all lasers for the channels in use or by firing each laser in the specified order. For Ch series selection, see Section 5.1.4, "Selecting the Channel Series."
(14) ND filter installation icon	An icon is displayed on the line of laser with ND filter installed.
	 * This icon indicates whether ND filter is installed or not, and does not indicate insertion or removal of ND filter (IN = Insert in the optical path, OUT = Remove from the optical path). Insertion or removal of ND filter is performed on the Acquisition window.
(15) OK button	Confirms the Optical path settings applied and closes the Optical path window.
(16) Cancel button	Discards the Optical path settings applied and closes the Optical path window.

• About the setting condition when the setting mode is switched

Auto mode -> Manual mode: The entire settings in the Auto mode are retained.

```
Manual mode -> Auto mode:
The fluorescence dye with the same channel name as set in the manual mode is
automatically selected.
If the same fluorescence dye name does not exist in the list, a fluorescence dye
detectable by the laser wavelength is automatically selected from the list.
```

5.1.4 Selecting the Channel Series

The [Ch series] menu enables to select whether to perform scanning by simultaneously firing all lasers for the channels to be used or by firing each laser in the specified order.

There are four options for channel series, "None," "Line 1->3," "Line 3->1," and "Custom," either of which can be selected from the pull-down menu.

- * Usable only when a four-laser unit or LU-N series laser unit is connected.
- * Before executing the line sequence by the channel series function, correct the image shift of the image in which normal bidirectional scan was performed.
- * Channel series can be applied to the image acquisition phase in the photo activation experiment sequence (but cannot be applied to the stimulation phase).

111 M	Detector	003			ose mechanical shutter during expe	eriment
Eye Port	Ch series	None 💌		Laser	Emission	
	Chi	None Line 1->3		408.0	417-477	
	Ch2	Line 3->1		488.0	499-529	
	Ch3	Custom		543.5	553-618	
	TD	ON	A IN OUT			





Figure 5.1-6 Selecting the channel series (Optical path window)

Table 5.1-3 Functions of channel series

Name	Function
None	Performs scanning by simultaneously firing all lasers for the channels to be used.
Line 1->3	Performs scanning by sequentially firing the lasers for the channels to be used (Ch1 -> Ch2 -> Ch3).
Line 3->1	Performs scanning by sequentially firing the lasers for the channels to be used (Ch3 -> Ch2 -> Ch1).
Custom	Performs scanning by firing the lasers in desired order for the channels to be used.

* When "Line 1->3" or "Line 3->1" is selected, the lasers are fired sequentially for each scan line. This scan method is called the line sequence.

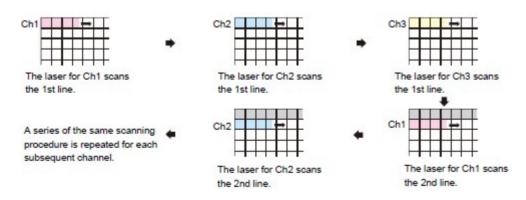


Figure 5.1-7 Scanning motion in line sequence (Line 1->3 is selected)

Selecting "Custom" from the [Ch series] menu displays the current channel scan order and the [Custom] button.

Clicking the [Custom] button opens the Line Channel Series Setup window to allow setting the scan order for each channel.



Figure 5.1-8 Channel series (Custom)



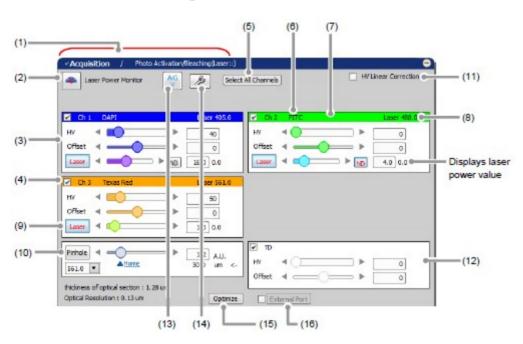
Figure 5.1-9 Line Channel Series Setup window

No. Name	Function
	Allows the user to set desired order of channels to be scanned. Each laser can also be fired simultaneously to multiple channels by a single scan.
	(Example: Firing lasers simultaneously for Ch1 and Ch2 by the first scan.)
	However, one channel cannot be set twice or more times. (Selecting two or more check boxes in the same vertical line of the matrix is prohibited.)
(2) 1->3 button	Set the channel to be scanned for each laser in order of Ch1, Ch2, and Ch3.
(3) 3->1 button	Set the channel to be scanned for each laser in order of Ch3, Ch2, and Ch1.
(4) TD check box	Allows the user to set the TD scan order if the transmitted detector (TD) is in the optical path. Be sure to set the TD scan order so that the scan order comes together with other channels because single TD scan is disabled.

	(Example: Laser is fired to Ch3 and TD by the second scan.)
(5) OK button	Confirms the settings applied and closes the window.
(6) Cancel button	Discards the settings applied and closes the window.

5.2 Acquisition Window

The Acquisition window enables to set PMT brightness (detection sensitivity), laser power, and pinhole size.



5.2.1 Structure of Acquisition Window

Figure 5.2-1 Acquisition window (DU3-use)

a certain value with the increase in the laser power

Table 5.2-1 Functions of Acquisition window (DU3-use)

	Name	Function
(1)	Acquisition/PhotoActivation window switching	Switches between the Acquisition and Photo Activation windows.
		For the Photo Activation window, see Chapter 10.
(2)	Laser power monitor button	Displays the laser power value (integer obtained after A/D conversion divided by 10) of the current channel by clicking this button.
		During the image acquisition, the laser power cannot be measured and this button is grayed out.
		* When a laser unit of the LU-N series is in use, the value displayed in the monitor does not increase over

(3) (4)	Brightness adjustment for each channel Channel selection	For each o Offset, Las the brightn	<i>et this is not a problem.</i> f the channels (Ch1 to Ch3), use the HV, ser, and ND filter IN/OUT controls to adjust tess of the live image. e channels (Ch1 to Ch3, and/or TD) to acquire
(ד)		the desired	
		1	uiring the transmitted image (TD image) only, n 5.2.1.2, "When Acquiring Transmitted Image
(5)	Select All Channels button	Selects all	channels for acquiring images.
(6)	Fluorescence dye name indication	The fluore window is	scence dye name specified in the Optical path indicated.
(7)	Channel color	Displays th window.	ne channel color specified in the Optical path
(8)	Laser wavelength indication	Displays th	ne currently selected laser wavelength.
(9)	Laser ON/OFF button	Selects wh	ether the laser is emitted or not.
		is disal	LU-NV is in use, this button is grayed out and bled while the button on the front panel of the nit is OFF or blinking.
		Laser ON status	The laser is emitted.
		Laser OFF status	The AOTF shutter closes and the laser power value becomes 0. When switched from OFF to ON, the laser power value set in the previous ON status is applied.
(10)	Pinhole	•	e pinhole size. (6 steps) e size, see Section 5.2.3, "Setting the Pinhole."
(11)	HV Linear Correction		disables HV Linear Correction. near Correction, see Section 5.2.4, "HV Linear ."
(12)	Brightness adjustment for transmitted detector		nsmitted detector, use the HV and Offset adjust the brightness of the live image.
(13)	AG button		ally adjusts the HV value (HV gain) of the elected channel to the optimum values.
(1.4)			Gain, see Section 5.2.5, "Auto Gain."
(14)	Auto Gain setting button	gain correc The windo	tio of saturation pixels used for automatic HV ction. w for range of the ratio of saturation pixels pears when this button is clicked.
		-	for ratio of saturation pixels, see "Setting for uration pixels" in the Section 5.2.5, "Auto

(15) Optimize button	Displays the XYZ Size Setup window. In the XYZ Size Setup window, the calculation method of the recommended values of the resolution, zoom magnification, and Z stack step size can be set.
	For the XYZ Size Setup window, see Section 5.2.1.1, "Recommended Value Indication/Automatic Application."
(16) External Port button	Makes the external detector unit available. For details of using the external detector unit, see Chapter 16.
	* External detector units are usable only when a C2 system without a spectral detector is in use. When C2si (system with a spectral detector) is in use, no external detector is usable.

5.2.1.1 Recommended Value Indication/Automatic Application

By the function of the recommended value indication/automatic application, the recommended values of the appropriate resolution, zoom magnification, and Z stack step size are calculated based on the objective type and the selected excitation wavelength.

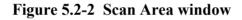
Using the calculated recommended values enables the image acquisition clearer and with less damage to the sample.

```
Recommended Value Automatic Application
```

To automatically apply the recommended values to the parameters, set the [Nyquist XY] button of the Scan Area window to ON.

Page 12 of 42

CZplus Scan Area x	
🧾 📼 — Crop ROI Edit 🔀 🧮 🛗	
Zoom: ()1	
Pixel size: 0.62 Nyquist XY	
Scan size: 512 💌 Rotation: 0 🔀	
Width: 512 Height: 512	
Width: 512 Height: 512 Dwell time: 1.9 µs	



	Scansetting		9
	Scan Direction 📑 差	Zoom	0
Indicates the	Scan Size 512 T 512 512 512 recommend Scan Speed Fast 1 T Franc/sec(Pixel Dwel: 1.9 usec)	3X A.BO3x recommen	
	Pinhole Fichae Ficha	1.2 A.U. 30.0 um <-	magnification.
	Optical Resolution : 0.13 um	Optimize	

Figure 5.2-3 Location of Recommended Value Indication

* When the laser or objective in use is changed, the recommended values are recalculated, and newly indicated and automatically applied.

Recommended Value Settings

Detailed settings of the recommended values are made in the XYZ Size Setup window that is displayed by clicking the [Optimize] button of the Acquisition window.

If the [Nyquist XY] button of the Scan Area window is ON, the recommended values are automatically

applied to the parameters.

Or if the [Nyquist XY] button is OFF, the recommended values of the scan size and zoom are indicated in the Scan setting window.

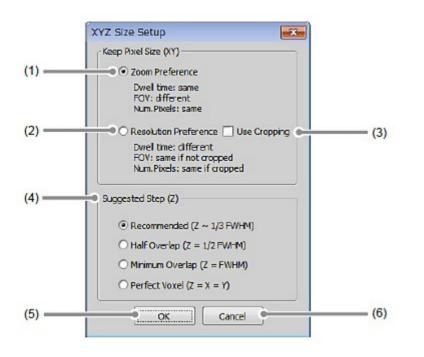




Table 5.2-2 Functions of XYZ Size Setup window

1 401	see 5.2 2 Tunenons of AT2 Size Semp Window			
	Name	Function		
(1)	Zoom Preference		XY] button is ON, keeps the scan size ommended value of the zoom.	
(2)	Resolution Preference		XY] button is ON, keeps the zoom and ended value of the scan size.	
(3)	Use Cropping	Fits the scan size in	detail by using Crop Scan.	
		Sets the Z step size	calculation method.	
		Recommend (Z~1/3 FWHM)	Approximately one third of the thickness of optical section (FWHM value).	
(4)	Suggested Step (Z)	Half Overlap (Z=1/2 FWHM)	One half of the thickness of optical section (FWHM value).	
		Minimum Overlap (Z=FWHM)	The thickness of optical section (FWHM value).	
		Perfect Voxel (Z=X=Y)	Value same as the pixel size.	
(5)	OK button	Confirms the XYZ Size Setup window.	Size Setup applied and closes the XYZ	
(6)	Cancel button	Discards the XYZ S Size Setup window.	Size Setup applied and closes the XYZ	

5.2.1.2 When Acquiring Transmitted Image Only

By using the TD channel, you can acquire the image with the TD channel only.

1. Display the Optical path window.

Select a channel for the laser power control and deselect other unnecessary channel's check box.

* Even when the multiple channels are selected in the Optical path window, the image with the TD channel only can be acquired by deselecting the unnecessary check box in the Acquisition window. In this case, the laser power value of the channel that is deselected at last is used for acquiring the TD image.

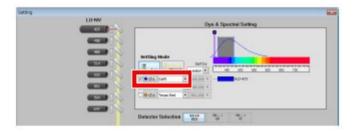


Figure 5.2-5 Optical path window (DU3-use)

2. Set the transmitted detector into the optical path and then click the [OK] button to close the Optical path window.



Figure 5.2-6 Optical path window (DU3-use)

- 3. Use the HV and Offset controls of the transmitted detector to adjust the brightness of the live image.
- 4. Use a channel that remains to "On" to control the laser power to set the laser power value.
- 5. Deselect the unnecessary check boxes other than TD.

	Acquisition / Photo Activation(StackingLearns)	Correction
Deselect this check box at last. Set the laser	Chil DAVI Lacer +05.0 Hv + - offsat + - Lacer + 0 Lacer + 0 Lacer + 0	
power value.	Pinhole Image: Constraint of the section of the sec	Adjust the transmitted detector.

Figure 5.2-7 Acquisition window (DU3-use)

6. Acquire the image.

5.2.2 Setting Image Brightness

For the live images of each channel, adjust HV, Offset, Laser and ND filter IN/OUT to obtain clear images.

Chi DAPI	Laser 405.0	Ch 2 FITC	Laser 468.0
₩ 4 1 0	40	HV 4	
Offset 4	→ ND 16.0 0.0	Offset 4	
Ch 3 Texas Red	Laser 561.0		
offset 4	→ <u>so</u> → 0		
Laser 4	▶ 1.0 0.0		
	210, 010	-	
		T TD	

Figure 5.2-8 Setting the live image brightness (DU3-use)

 Table 5.2-3 Brightness adjustment functions for the live image (DU3-use)

 Name
 Function

 (1)
 HV
 Sets the voltage to be applied to the PMT.

 Slider bar:
 Slider bar:

Slides to the right or left to set the HV value.

Arrow buttons:

		Click either arrow button to increase or decrease the HV value stepwise.
		Direct entry in HV value display field: Type the desired setting value.
(2)	Offset	Sets the BL offset value of the PMT.
		Slider bar: Slides to the right or left to set the offset value.
		Arrow buttons: Click either arrow button to increase or decrease the offset value stepwise.
		Direct entry in offset value display field: Type the desired setting value.
(3)	Laser (*)	Sets the laser power value.
		Slider bar: Slides to the right or left to set the laser power value.
		Arrow buttons: Click either arrow button to increase or decrease the laser power value stepwise.
		Direct entry in laser power value display field: Type the desired setting value.
(4)	HV (TD)	Sets the voltage to be applied to the transmitted detector.
		Slider bar: Slides to the right or left to set the HV value.
		Arrow buttons: Click either arrow button to increase or decrease the HV value stepwise.
		Direct entry in HV value display field: Type the desired setting value.
(5)	Offset (TD)	Sets the offset value of the transmitted detector.
		Slider bar: Slides to the right or left to set the offset value.
		Arrow buttons: Click either arrow button to increase or decrease the offset value stepwise.
		Direct entry in offset value display field: Type the desired setting value.

- (6) ND filter IN/OUT Inserts/removes the ND filter in/from the optical path. (IN = Insert in the optical path/ OUT = Remove from the optical path) This button is displayed only for lasers that can control insertion/removal of ND filter.
- * When LU-NV is in use, this function is grayed out and is disabled while the button on the front panel of the laser unit is OFF or blinking.

PMT Overload

If too much gain is applied to the illumination intensity, the gain is automatically shut down to protect PMT and/or transmitted detector (TD) and the following PMT Overload dialog box is displayed.

AcaCode	e 0: EventCo	de: 2: Messa	oe: Standar	dPMT(0) Overlos	ed and
	[<u> </u>	ок]	Cancel		

Figure 5.2-9 PMT Overload dialog box

5.2.3 Setting the Pinhole

Chi DAPI	Laser 405.0 🗹 Ch 2 Fi	TC Laser 48
ни 🛛 🔟	→ 40 HV 4 (
Offset 4	► 0 Offset 4	
Laser 4 -	→ ND 16.0 0.0	
Ch 3 Texas Red	Laser 561.0	
ни 🖪 🔟	► 50	
Otset 4		
Pintale 1 -		
	→ 1.2 All. 30.0 um <- HV 4 (► 0
561.0 ×		

Figure 5.2-10 Setting the Pinhole (DU3-use)

Tabl	e 5.2-4 Pinhole se	etting functions (DU3-use)
	Name	Function
(1)	Pinhole size setting	Sets a pinhole size for C2 system.
		Slider bar: Slides to the right or left to set the pinhole size. (Unit: A.U.)
		Arrow buttons: Click either arrow button to increase or decrease the pinhole size stepwise.
		Direct entry in pinhole size display field: Type the desired setting value.
(2)	Pinhole button	Displays the A.U. Calculation Settings window to calculate the pinhole size.
		(For A.U. Calculation Settings, see Section 5.2.3.1, "Calculation Settings for Pinhole Size.")
(3)	Home	Changes the pinhole to the predetermined home position. The value of the home position can be changed in the A.U. Calculation Settings window. (For A.U. Calculation Settings, see Section 5.2.3.1, "Calculation
(A)		Settings for Pinhole Size.")
(4)	Pinhole size thickness of	Indicates pinhole size of C2 system. (Unit: µm)
(5)	optical section	Indicates the FWHM (full width at half maximum) of z airy disk.
(6)	Optical Resolution	The actual size of 1 pixel square calculated from the optical information (for objectives and scan parameters) and the size acquired from an image.
(7)	Reference excitation wavelength for the pinhole size calculation	Selects the excitation wavelength as the reference of the automatic calculation of the pinhole size from the laser wavelengths, or enter it manually in the A.U. Calculation Settings window. (For A.U. Calculation Settings, see Section 5.2.3.1, "Calculation Settings for Pinhole Size.")

5.2.3.1 Calculation Settings for Pinhole Size

This section describes the setting window for calculating the pinhole size.

Click the [Pinhole] button in Acquisition window, the A.U. Calculation Settings window appears. (Usually, the [Recommend] is selected to enable automatic calculation. [Recommend] calculates the A.U. value by using the Nikon-recommended EM and NA values.)

	1.0 AU Pinhole Size=1.22*EM(NA*Nag.[um]	
	Recormend	
	EMsemission wavelength(jum)	
	Selected Laser wavelength + 40[nm]	
	Selected Laser wavelength 488.0 nm	
	O Manual 400.0 nm	
-	NA:Numerical Aperture	
	O NA_abj(NA of objective lens)	
	Min(NA_obj, NA_sample)	
	"Ary" Home Position 1.2 A.U.	
	• Keep A.U.	
	OK Cancel	

Figure 5.2-11 A.U. Calculation Settings window

 Table 5.2-5
 A.U. Calculation Settings window

	Name	Function
(1)	Select calculation method	Recommend - Sets parameters automatically. (Nikon recommended)
		User Setting - Allows the user to manually set parameters.
wavelength[µm] C th er T		Selected Laser wavelength - Calculates parameters by using the laser wavelength selected in the pinhole combo box of the Acquisition window as the emission wavelength (EM value). The wavelength displayed in the combo box is to be the laser wavelength set in the Optical Setting window.
		Manual - Allows the user to manually set parameters. (The parameter is calculated with the input value as the emission wavelength (EM value).) Enter the value directly from the keyboard.
(3)	NA: Numerical	Sets refractive index of the objective.
	Aperture	NA_obj(NA of objective lens) - Regardless of whether or not the objective NA value exceeds the refractive index of the sample, executes calculation by using the objective NA as the calculation parameter.
		Min(NA_obj, NA_sample) - When the objective NA value does not exceed the refractive index of the sample, executes calculation by using the objective NA as the calculation parameter. When the objective NA value exceeds the refractive index of the sample, executes calculation by using the sample refractive index.
(4)	"Airy" Home Position	Sets a home position of pinhole. Enter the value directly from the keyboard.

The pinhole size can be selected from six types in C2.

		herefore, if the entered value does not match any of the types, the size that is larger than and the closest to the entered value is set as the home position.
(5)	Keep A.U. check box	When checked, the pinhole size is fixed by the A.U. when the selected wavelength or objective is changed. (However changes by the um.) When unchecked, the pinhole size is fixed by the um. (However
		changes by the A.U.)
		The pinhole size can be selected from six types in C2. Therefore, if the to-be-fixed A.U. value does not match any of the types, the size that is larger than and the closest to the A.U. value is selected.
(6)	OK button	Confirms the A.U. Calculation Settings applied and closes the A.U. Calculation Settings window.
(7)	Cancel button	Discards the A.U. Calculation Settings applied and closes the A.U. Calculation Settings window.

5.2.4 HV Linear Correction

When HV changes, Gain changes as shown in the graph captioned "Without HV Linear Correction."

As HV increases, the gain variation (the variation of image brightness) is gradual initially, and it becomes steep beyond a certain point.

The gain variation can be automatically corrected to be linear with HV variation by the function called "HV Linear Correction." With this correction, gain varies at the same rate as the HV adjustment.

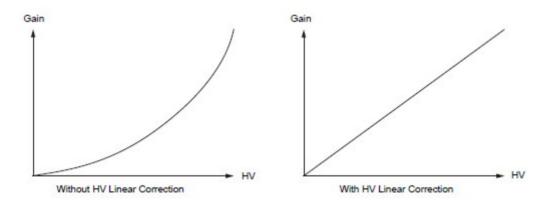


Figure 5.2-12 Gain vs. HV

To enable HV Linear Correction, check the [HV Linear Correction] check box.

Acquisition / Photo Activation/6	leaching(Laser::)		
Laser Power Monitor	🏂 Select	All Channels	HV Linear Correction
Chi DAPI	Laser 405.0	Ch 2 FTTC	Laser 498.0
HV 4 🔵 🕨 🕨	0	HV 4	►
Criset 4	0	Offset 4	▶ ► 0 ► ► ► 4.0 0.0
Ch 3 Texas Red	Laser 561.0		
1/ 4 🜔 🕨 🕨	0		
Xfiset 4 — 🕨 🕨	0		
laser 4 🕡 🕨 🕨	1.0 0.0		
inhole 4 🛋 🕨 🕨	11	σ №	
561.0 ·	1.2 A.U. 30.0 um <-	HV 4	
idmess of optical section : 1, 28 um		Under 4	
ptical Resolution : 0.13 um	Optimize	External Port	

Figure 5.2-13 HV Linerar Correction

- When HV Linear Correction is enabled or disabled, HV is reset to 0 V once.
- If the Offset slider bar is moved, the accurate correction is not performed.

5.2.5 Auto Gain

Auto Gain is a function to automatically correct the value of HV gain to set the optimum image brightness.

Automatic HV gain correction is performed within the predetermined range of the ratio of saturation pixels.

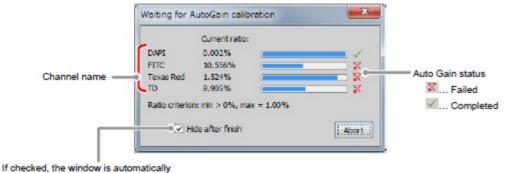
Automatic HV gain correction is performed only when channels are selected. For a TD, automatic adjustment is performed when it is selected.

After execution of Auto Gain, in the window indicating the progress of Auto Gain, the correction values actually used (ratio of saturation pixels) are displayed by channel. For a channel on which Auto Gain failed, "x" is indicated and the HV value returns to its original value.

- When setting the line scan, Auto Gain cannot be executed.
- During execution of Auto Gain, do not perform manual adjustments in the Acquisition window.

	Auto Gain button	Auto Gain setting	button
		Select All Channels	H/ Linear Correction
Auto Gain does not		Laser 4000 40 0 0 0 1 1 1 1 1 1 1 1 1 1 1 1 1	Later 428.0
	H/ 4 Cfluet 4 P	50 0 1.0 0.0	
		1.2 A.U. 0.0 um <-	

Figure 5.2-14 Execution of Auto Gain (DU3-use)



closed when Auto Gain is completed.

Figure 5.2-15 Auto Gain progress

Setting for ratio of saturation pixels

Set the maximum and minimum value for the ratio of saturation pixels used for automatic HV gain correction.

Click the [Auto Gain Setting] button to display the Auto gain setup window. Set the maximum and minimum value for the ratio of saturation pixels in the Auto gain setup window.



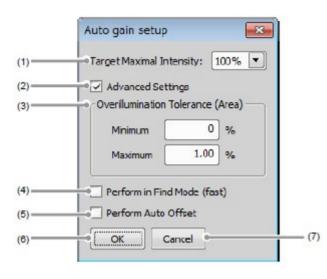
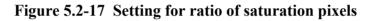


Figure 5.2-16 Displaying the Auto gain setup window



<i>Table 5.2-6</i>	Setting for ratio	of saturation	pixels
100000.20	Setting joi i anto	0) Server erron	pinens

		J
	Name	Function
(1)	Target Maximal Intensity	Specifies the application ratio of the setting of the ratio of saturation pixels.
	1110011010	Sets the percentage (%) of the maximum value to be applied.
(2)	Advanced Settings	If checked, advanced settings of the ratio of saturation pixels are enabled.
(3)	Overillumination	Minimum Sets the minimum value for ratio of saturation pixels.
	Tolerance (Area)	Maximum Sets the maximum value for ratio of saturation pixels.
(4)	Perform in Find Mode (fast)	If checked, execution in the Find mode is enabled.
(5)	Perform Auto Offset	If checked, the offset value is set automatically.
(6)	OK button	Confirms the settings of Auto gain setup applied and closes the Auto gain setup window.
(7)	Cancel button	Discards the settings of Auto gain setup applied and closes the Auto gain setup window.

5.3 Experiments by Using Lambda Series

By using the Lambda series as the function of the NIS-Elements AR, the multiwavelength excitation and emission experiments such as 2Ex 1Em (2 excitations 1 emission experiment) and 4Ex 4Em (4 excitations 4 emissions experiment) can be executed.

Multiwavelength excitation and emission experiment means an experiment made when using a

fluorescence dye whose fluorescence intensity varies with the wavelength of excitation lasers. The excitation lasers are changed, but the wavelength of acquired fluorescence is identical.

* About Lambda series:

When acquiring multiple excitation lights by emitting multiple lasers, the lasers are not emitted simultaneously but emitted in sequence. By emitting lasers in sequence, the cross talk between channels can be avoided.

5.3.1 2Ex 1Em Acquisition

This section describes the settings to make the 2 excitations 1 emission experiment.

5.3.1.1 Procedure for 2Ex 1Em Settings

In the 2 excitations 1 emission experiment, the 2 excitation wavelengths are to be respectively registered to the optical configuration (hereinafter referred to as O.C.), and the O.C. is used by the Lambda series.

1 Switching the optical path of the C2 scan head

Switch the optical path changeover lever on the C2 scan head to the [Standard] position.



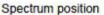




Figure 5.3-1 Switching the optical path of the C2 scan head

2 Setting the optical path of the 1st excitation wavelength to be registered to O.C.

Click the [Setting] button in the Filter and Dye window.
 For details of the Optical path settings, see Section 5.1, "Filter and Dye Window."

	000	dity Detector	1.0	0	ase mechanical shutter of	turing experiment
Setting button		e Port Chiseries	None 💌	Laser	Emission	
		Ch1	DAPI	405.0	417-477	
		Chiz	FITC	488.0	499-529	
		Ch3	Texas Red	543.5	552-617	
		π	ON A IN	pur		

Figure 5.3-2 Filter and Dye window

2. Activate the Manual mode of Optical path setting. Click the [Manual] button.

Setting		1
LU-NV	Dye & Spectral Setting	-92
	• • •	
	Setting Wode	
	Satby	
	Auto Manual Poctator 400 500 550 600 550 700	
	2 Chi OAPE • 405.000 • ·· 417-477	
Si 🕒	C 0 Ch2 7TTC * 485.000 *	
	2 0 Chil Texas Red 951.000	301

Figure 5.3-3 Selecting the Manual mode

3. Select a channel to be excited. (e.g. Ch1) Change the channel name and channel color freely.

	Sele	ects channel	
Setting		Dye & Spectrel Setting	
		Sarby Anno Manual Ecolarite • 2 tithi Code • 405.000 • 2 tithi Code • 405.000 • 700 ± 41,7477 • 40.000 • 700 ± 41,7477 • 40.000 • 700 ± 41,7477	

Figure 5.3-4 Selecting the channel

4. Select the 1st excitation wavelength. (e.g. 488 nm) Select the wavelength for excitation lasers from the pull-down menu.

		Selects 1st e	xcitation wavelength
Cetting		Dy	& Spectral Setting
		Setting Mode	
		Auto Manual Sortby Poctator*	450 500 500 500 500 700
	24	Chi Truck Red Sil 800 *	

Figure 5.3-5 Selecting 1st excitation wavelength

5. Click the [OK] button to confirm the Optical path settings.



Figure 5.3-6 Optical path settings

 In the Acquisition window, adjust a PMT for the excitation lasers. Set the HV value, the Offset value, and the Laser power value. For details of the acquisition settings, see Section 5.2, "Acquisition Window."

Laser Power Monitor	AG Select All Channels	HV Linear Correction
Ch1 EX1	Laser 485.0	
v 🛛 🚺		
	ND 15.0 D.0	
inhole 4 -	⇒ ▶ 1.3 A.U.	
88.0	30.0 um <-	

Figure 5.3-7 Acquisition window

3 Registering the set optical path as an O.C.

1. Select [Calibration] -> [New Optical Configuration] from the menu bar to open the Wizard.





2. Enter the name of O.C. to be registered, and then click the [Finish] button.

be registered.				Carvera - Hikor Ciplus	
	Causera setting:				
		Centers Restures:	Property Values: Detector = "4" Opsia@kadcal = "1" PratDMbrakx = "3" ChannetberesNude = "0" Tbbi = "4" TbChannetCalor = "15777215"	Î	
	Channel astaps	Name Dia Sensor 4400 [pm] > Calor	T		
	🕢 Microscope setting:	Active Shutter :	<u>*</u>		
		Nicon Ti Smulator, PfilerChanger (Turnet)) Nicon Ti Smulator, PfilerChanger (Turnet)) Nicon Ti Smulator, PfilerChanger (Turnet) Nicon Ti Smulator, PfilerChanger (Socialiton,) Nicon Ti Smulator, Substater (CA) Nicon Ti Smulator, Substater (Substater (S	Moreorep: T. Moreorep: New Concept: T. Moreorep: T. Moreore: T. Moreorep: T. Moreorep: T. Moreorep: T. Moreorep: T. Moreorep:	urwt2]: 1 (444 mssou Wheel) E attation Wheel Copened pened pened pened pened	
		Tri Tana		the second se	

Figure 5.3-9 Registration of optical configuration

- 4 Setting the optical path of the 2nd excitation wavelength to be registered to O.C.
 - Click the [Setting] button in the Filter and Dye window. For details of the Optical path settings, see Section 5.1, "Filter and Dye Window."



Figure 5.3-10 Filter and Dye window

2. Check that the Optical path setting is set to Manual mode. If it is not set to Manual mode, click the [Manual] button.

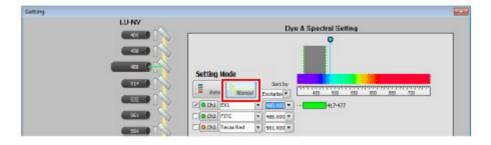


Figure 5.3-11 Selecting the Manual mode

3. Select the same channel as selected in the setting of the 1st excitation wavelength. (e.g. Ch1) Change the channel name and channel color freely.

etting		
	LU-NV	Dye & Spectral Setting
		•
		ietting Mode
		Auto Manual Factator
	.	Auto Manual Pochato + 42 00 00 00 00 70
	21	
		Cha Texas Red + 551,000 +

Figure 5.3-12 Selecting the channel

4. Select the 2nd excitation wavelength. (e.g. 543 nm) Select the wavelength for excitation lasers from the pull-down menu.

	Selects 2nd excitation wavelength			
Setting	LU-NV			
		re & Spectral Setting	٦.	
		Setting Mode		
	50 (X)	Auto Manual Exclusion 450 500 500 500 500 500 700 Co Co TC V 450 Co TC V TC TC		
		Chil Texas Red + 551.000 +		

Figure 5.3-13 Selecting 2nd excitation wavelength

5. Click the [OK] button to confirm the Optical path settings.



Figure 5.3-14 Optical path settings

 In the Acquisition window, adjust a PMT for the excitation lasers. Set the HV value, the Offset value, and the Laser power value. For details of the acquisition settings, see Section 5.2, "Acquisition Window."

Acquisition / Photo Acquisition / Photo Laser Power Monitor	Activation/Bleaching(Laser::)	HV Linear Correction
HV 4 Contraction of the second	Laser 561.0 40 0 16.0 0.0	
Finhale 561.0 thickness of optical section : 1. Optical Resolution : 0.13 um	▶ 1.2 A.U. 30.0 um <- 28 um Optimze	Port

Figure 5.3-15 Acquisition window

- 5 Registering the optical path of the 2nd excitation wavelength as the second O.C.
 - 1. Select [Calibration] -> [New Optical Configuration] from the menu bar to open the Wizard.



Figure 5.3-16 To display the Optical Configuration Wizard

2. Enter the name of O.C. to be registered, and then click the [Finish] button.

be registered.				Canwy - Nikor Cliplus	
	🗹 Cansela settingi	Carnera features:			
		R scan area settings R Scan Area Position	Property Values: Detector = "4" Optical/weakCatr = "1" PhratDiffication = "1" Diffication = "3" TBUn = "3" TBUn = "4" TBUn = "4"		
	Charved actups			*	
		Neme: D2	•]		
	Microscope setting:				
		Active Shutter :	8		
		Lised devices:			
		Tokin T Soulitor, FRechange (Livit) Tokin T Soulitor, FRechange (Livit) Tokin T Soulitor, FRechange (Install) Tokin T Soulitor, FRechange (Install) Tokin T Soulitor, Solida (TT) Tokin T Soulitor, Solida (TT)	 Meconcept: TMicroscope Neor TSimulator, FloreChargeri Neor TSimulator, FloreChargeri Neor TSimulator, FloreChargeri Neor TSimulator, FloreChargeri Neor TSimulator, FloreChargeri Neor TSimulator, Shutar(VA) Neor TSimulator, Shutar(VA) Neor TSimulator, Shutar(VA) Neor TSimulator, Shutar(VA) 	Terret2: 1 (ANA Emission Wheel) 2 Excitation Wheel (Copened Spered Copened Copened Chall Re	
		 Niton Ti Sinulator, Silaminotor (Siluminotor 1912) LightStath e dartmed in Camera Light Path Analyzer Conversion 	⊡ shaw on toakar	-	
		TIP's offset	Contrast C		

Figure 5.3-17 Registration of optical configuration

6 Execute the Lambda series

1. Select [Applications] -> [Define/Run ND Acquisition...] from the menu bar to open the ND Acquisition window.

1.00x		
9	Define,Run ND Acquisition Ctri+Alt+N	Ratio, Ca2+, FRET Ratio View
	Define,Run ND Multipoint Set Acquisition	Ratio Properties
	Device for Stmuleton: C2plus	Caldum Properties
	Define,Run Sequential Stinulation	Titration Vev

Figure 5.3-18 To display the ND Acquisition window

2. Select and check the [Lambda] tab among the tabs displayed in the ND Acquisition window.

	Lambda Series ta	b	
ND Acquisition ×	iton]	
Order of Experiment •			Record Data
Setup			++ × %]
Optical Conf.	Name	Comp. Color	Focus Offset

Figure 5.3-19 ND Acquisition window

- 3. In the first column of the [Optical Conf.], select the O.C. to which the optical path of the 1st excitation wavelength has been registered.
- 4. In the second column of the [Optical Conf.], select the O.C. to which the optical path of the 2nd excitation wavelength has been registered.

Select the O.C. of	1st excitation	wavelength
--------------------	----------------	------------

100	ion x		2	
Experiment:	ND Acquisition	1]	
λ: [
Save	o File			Record Data
Order of E)	reriment • Ti	ming		
7.00			🗖 🖓 Large Image	
Setup	ILI 885 XY IL			
			* 🗗 🗗	+ + × >
Optical O	ıf.	Name	Comp. Color	Focus Offset
ZEx1E	m_1	▼ EX1		1
ZEX18	m_2	▼ EX2		0
	_			
2				
Close	ctive Shutter du	ring Filter Change	100	
Use R	io Define Rat	io Use FR	Define FRET	
				Advanced >>
				Advanced >>
		temover 50	Run Z Corr 1 time loop	of Run now

Select the O.C. of 2nd excitation wavelength

Figure 5.3-20 ND Acquisition window

5. Click the [Run now] button to acquire the image.

A: [Save to File Order of Experiment ▼] Timing γ [□ =] z [✔ ♂ λ		Record Data
Setup		+ 00	+ + <mark>x</mark>
Optical Conf.	Name EX1	Comp. Color	Focus Offset
ZEX1Em_1 ZEX1Em_2	• EXI		0
	er during Filter Change Ratio	T Define PRET)	Advanced >>

Figure 5.3-21 ND Acquisition window

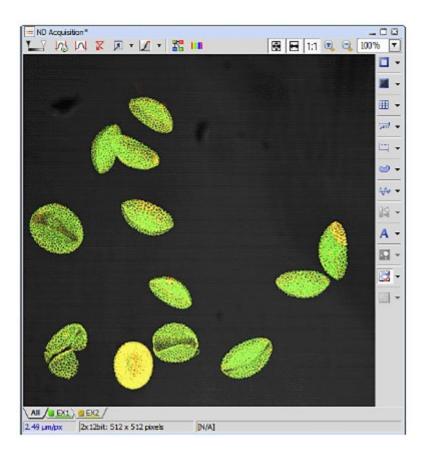


Figure 5.3-22 Acquired image

5.3.2 4Ex 4Em Acquisition

This section describes the settings to make the 4 excitations 4 emissions experiment.

To execute the 4 excitations 4 emissions experiment, a special filter cube for "DAPI/CY5 Dual" needs to be used.

5.3.2.1 Procedure for 4Ex 4Em Settings

In the 4 excitations 4 emission experiment, the 4 excitation wavelengths are to be separately registered to the 2 optical configurations (hereinafter referred to as O.C.), and the O.C. is used by the Lambda series.

(e.g., if the 4 channels of DAPI, FITC, TRITC, and Cy5 are to be acquired, the lasers of 405, 488, 543, and 640 are to be used.)

1 Switching the optical path of the C2 scan head

Switch the optical path changeover lever on the C2 scan head to the [Standard] position.



Figure 5.3-23 Switching the optical path of the C2 scan head

2 Setting the optical path of the Ch1 and Ch3 to be registered to O.C.

 Click the [Setting] button in the Filter and Dye window. For details of the Optical path settings, see Section 5.1, "Filter and Dye Window."

	Filer and	Dje					•
101 I I I I I I I I I I I I I I I I I I	000	105	Detector	1.0	_0	ase mechanical sha	tter during experiment
Setting button	-	EvePort	Ch series	None 💌	Laser	Emission	
			Chl	DAPI	403.0	417-477	
			Ch2	FITC	488.0	499-529	
			Ch3	Texas Red	543.5	552-617	
			π	ON AIN_D	ul		

Figure 5.3-24 Filter and Dye window

2. Activate the Manual mode of Optical path setting. Click the [Manual] button.

Setting					
	LU-NV		Dye & Spectral Se	tting	
	46		• •	9	
				Δ	
(Setting	Mode			
	314		Satby		
	53 C		tato * 450 500	660 600 680 700	1
		hand 1	15.000 417-47	2	
			95.000	308-550	
	204 D 20 Ch1	Texas Red ¥ 55	st. 800 •		578-3006

Figure 5.3-25 Selecting the Manual mode

3. Select the two channels to be excited. (Select Ch1 and Ch3) Change the channel name and channel color freely.



Figure 5.3-26 Selecting the channel

4. Assign an excitation wavelength to each of the selected channels. (e.g. 408 nm and 543 nm) Select the wavelength for excitation lasers from the pull-down menu.

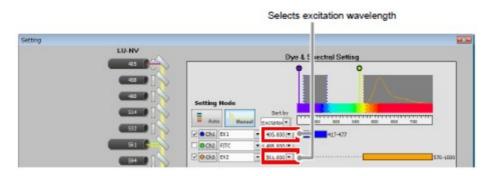


Figure 5.3-27 Selecting 1st, 2nd, and 3rd excitation wavelength

5. Click the [OK] button to confirm the Optical path settings.

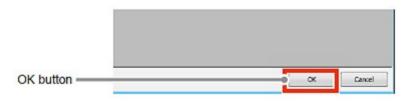


Figure 5.3-28 Optical path settings

6. In the Acquisition window, adjust a PMT for the excitation lasers. Set the HV value, the Offset value, and the Laser power value.

Ch 1 Ext Laser 405.0 Ch 3 EX2 HV 40 HV 10 0 <th>Laser 561.0</th>	Laser 561.0
	▶ 1.0 0.0

For details of the acquisition settings, see Section 5.2, "Acquisition Window."

Figure 5.3-29 Acquisition window

- **3** Registering the optical path of the Ch1 and Ch3 as an O.C.
 - 1. Select [Calibration] -> [New Optical Configuration] from the menu bar to open the Wizard.

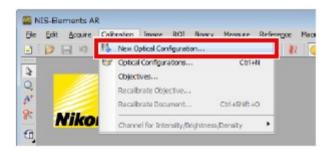


Figure 5.3-30 To display the Optical Configuration Wizard

2. Enter the name of O.C. to be registered, and then click the [Finish] button.

		Caruwa - Hikor Ciplus
😥 Canaeca cettings	Carrena features:	k
	Con Area bettings	Poperty Walanc Detector = "1" Cprictories = "1" "Intelligence = "1" Traditioner = "1" TbCharrestCoole = "10"77215"
Channel astup:		
(E) promotion analysis	Nome Drisolari (m) Color D11 447.0 0 D12 585.0 0	
😒 Microscope setting :		
	Active Shutter :	*
	Silven T Smaletz, Piercharger (Turrit) Silven T Smaletz, Piercharger (Turrit) Silven T Smaletz, Piercharger (Turrit) Silven T Smaletz, Piercharger (States), Silven T Smaletz, Piercharger (States), Silven T Smaletz, Shater (VTA) Silven T Smaletz, Shater (Sk) Silven T Smaletz, Shater (Sk) Silven T Smaletz, Shater (Sk)	
	Niton Ti Simulator, Staninstor (Stuninator 191) Licht Faith is clinited in Canana Licht Path	⊡ Shav on toabar
	Egnister is binned by Alexie Egnister is binned by Alexie Endorser Pris Offset These	Connect A

Figure 5.3-31 Registration of optical configuration

4 Setting the optical path of the Ch1 and Ch2 to be registered to O.C.

1. Click the [Setting] button in the Filter and Dye window. For details of the Optical path settings, see Section 5.1, "Filter and Dye Window."

	File and Dye						
	000	285	Detector	10	Close mechanical shutter during experiment		
Setting button		Eve Port	Ch series	None 💌	Leser	Emission	
			Ch1	EXI	408.0	417-477	
			Ch 3	EX2	548.5	552-617	
			TD	OFF			

Figure 5.3-32 Filter and Dye window

2. Check that the Optical path setting is set to Manual mode. If it is not set to Manual mode, click the [Manual] button.

Setting		
	LU-NV	Dye & Spectral Setting
	45	• •
		Sotting Hada
	S14	E Batby
	532	
		🗹 👁 Chu 🛤 🔍 405.000 💌 🔜 413-477
	Sei 🕒 🔨	Ch2 FITC • 488.000 •
	54	Cha Diz + 561.000 +

Figure 5.3-33 Selecting the Manual mode

3. Select the two channels to be excited. (Select Ch1 and Ch2)

ting		
	LU-NV	Dye & Spectral Setting
		• •
		Setting Hode
	314	
		Auto Hanaal Exctato -
	N	Chi EX1 • 647.000 • 447.477
	- Si - 🔪	2 D Ch2 EX3 * 488.000 * 900-190
		Chal EV2 • 501.000 •

Figure 5.3-34 Selecting the channel

- 4. Select the excitation wavelength. (e.g. 637 nm and 488 nm) Select the wavelength for excitation lasers from the pull-down menu.
 - * Set beforehand the special filter cube for "DAPI/CY5 Dual" in the 1st FL Cube.

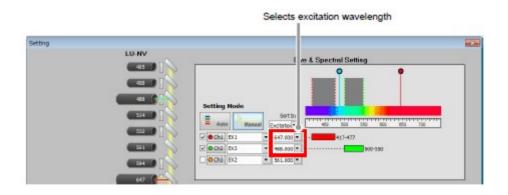


Figure 5.3-35 Selecting 4th excitation wavelength

5. Click the [OK] button to confirm the Optical path settings.

Objective	447/60 💌 525/50 56 💌		
Sample	Ch Series	None P	1
Transmitted Detector			
In 📩 Out			
			OK Cancel

Figure 5.3-36 Optical path settings

 In the Acquisition window, adjust a PMT for the excitation lasers. Set the HV value, the Offset value, and the Laser power value. For details of the acquisition settings, see Section 5.2, "Acquisition Window."

Acquisition / Photo Activation/Blea Laser Power Monitor	B Select	All Channels	HV Linear Correction
Ch 1 EX1 HV Ch 1 EX1 Offset Character Characte	Leser 647.0 40 5 16.0 0.0	HV Ch 2 EX3	Lever 483.0 + 40 + 0 + 10 + 10
Pinhale Home 3 647.0 Home 3 thickness of optical section : 1.34 um Optical Resolution : 0.15 um	1.0 A.U. 0.0 um <-	External Port	

Figure 5.3-37 Acquisition window

- 5 Registering the set optical path of the Ch1 and Ch2 as the 2nd O.C.
 - 1. Select [Calibration] -> [New Optical Configuration] from the menu bar to open the Wizard.

File Edit Acquire	Calibration Image ROI Binary	v Measure Reference M
DID B 9	New Optical Configuration	12
A 0 *	Optical Configurations Objectives Recalibrate Objective	CRIHN
	Recalibrate Document	Ctrl+Shift+O
Niko	Channel for Intensity/Brightne	ss/Density

Figure 5.3-38 To display the Optical Configuration Wizard

e registered.				Carvers - Nikoe Cliptus
	Camera aettings	Carrena featurea		*
		I Scan Area Reting: I Scan Area Position	Poperty Value: Detector = "4" Opsiel/modeCtrl = "1" ProEPPortex = "1" Drannelis/settode = "0" Totr = "1" Totr = "1"	
	Channel setup:			+
		Name Emission [rm] Color EV4 ±07.0 EV3 EV4.0		
	Microecope petting:	Active Glutter :		
		Used devices	-	
		Silon T. Saudetz, Filter Charger (Larrett) Silon T. Saudetz, Saudet (STR3) Silon T. Saudetz, Sauter (SU) Silon T. Saudetz, Sauter (SU)	 Nikor Ti Smulator, Shuttar (1913); S Nikor Ti Smulator, Shuttar (1911); S Nikor Ti Smulator, Shuttar (1911); S Nikor Ti Smulator, Shuttar (1918); S Nikor Ti Smulator, Shuttar (1918); 	rvet2) i z (douk amano Vitwell: * ditation futhreel Diparted erred award web-0142-Re
		Netion 11 Smuliter, Bannister (Burnineter-891) Lightfach a christeilin Camera Light Path Parkner Dondeman Pro Offact Toma	Carveers	:

2. Enter the name of O.C. to be registered, and then click the [Finish] button.

Figure 5.3-39 Registration of optical configuration

6 Execute the Lambda series

1. Select [Applications] -> [Define/Run ND Acquisition...] from the menu bar to open the ND Acquisition window.



Figure 5.3-40 To display the ND Acquisition window

2. Select and check the [Lambda] tab among the tabs displayed in the ND Acquisition window.

	Lambda Series	tab	
ND Acquisition ×	ton]	×
λ: Save to File Order of Experiment •		2	Record Data
		Large Image	++ × >)
Optical Conf.	Name	Comp. Color	

Figure 5.3-41 ND Acquisition window

- 3. In the first column of the [Optical Conf.], select the O.C. to which the optical path of the Ch1 and Ch3 have been registered.
- 4. In the second column of the [Optical Conf.], select the O.C. to which the optical path of the Ch1 and Ch2 have been registered.

ND Acquisition x				
Experiment: ND Acq.	isibon			
λ: []				
Save SFile			Record Data.	
Order of Exeriment	• Timing			
		Large Image	1	
	XT LL SS Z LL G *	· [_] 08 Large Image		
Setup		* 🗗 🗗	+ + ×	ø
Optical Cr vf.	Name	Comp. Color	Focus Offset	
✓ 4Ex4Em_1	• EX1		Ŧ	
	EX2			E
✓ 4Ex48m_2	▼ EX4		0	
	EK3			
				٠
Close stive Shur	tter during Filter Change	•		
Use Ri jo Defi	ne Ratio 🔲 Use Fi	RET Define PRET		
			Advanced >:	

Select the O.C. of Ch1 and Ch2

5. Click the [Run now] button to acquire the image.

Save to File				Record Data	
Setup	or 🗖			++ ×	*
Optical Conf.	_	Name	Comp. Color	Focus Offset	-
✓ 4Ex46m_1		EX1 EX2		-	
✓ 4Ex46m_2	-	EX4		0	1
		EK3			۲
Close active Shut	ter durin	g Filter Change			



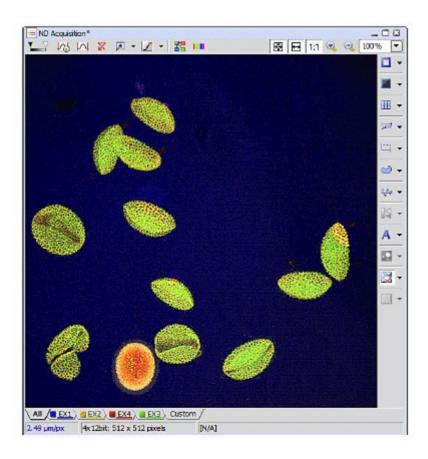


Figure 5.3-44 Acquired image

6 Detection Mode SD

This chapter describes the settings for the Spectral Detector mode (SD).

6.1 Filter and Dye Window

This window enables to set the Optical path.

The Spectral Detector mode can be used when the optical path changeover lever on the C2 scan head is set to the [Spectrum] position and the Spectral Detector (SD) is selected as the detection mode in the Optical path window.

6.1.1 Structure of Filter and Dye Window

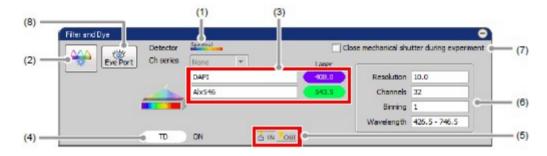


Figure 6.1-1 Filter and Dye window (SD-use)

 Table 6.1-1
 Functions of Filter and Dye window (SD-use)

	Name	Function
(1)	Detector	Indicates that the Spectral Detector mode [SD] is used when the optical path changeover lever on the C2 scan head is set to the [Spectrum] position and the Spectral Detector (SD) is selected as the detection mode in the Optical path window.
(2)	Setting button	Opens the Optical path window. To use, select the detector, the dichroic mirror, the excitation laser, fluorescence dye for each excitation laser and others.
(3)	Status	Indicates for the settings for each excitation laser (fluorescence dye name, laser wavelength, and wavelength band to be acquired).
(4)	TD	Indicates the status of the motorized transmitted detector.
(5)	TD IN/OUT button	Sets/removes the motorized transmitted detector in/from the optical path. (IN = Set in the optical path/ OUT = Remove from the optical path) As for the case where the TD IN/OUT button is not displayed, it
		will be displayed when the motorized transmitted detector is set in the optical path in the Optical path window.

(6)	Spectral Detector setting information	Displays the information set on the Spectral Detector.
(7)	Close mechanical shutter during experiment	If unchecked, the shutter remains open during the ND image acquisition. As the shutter is not opened/closed every image acquisition, the time for the image acquisition can be shortened.
		* During the interval period, laser power is automatically changed to the minimum but the laser cannot be shut off completely because the shutter is left open.
(8)	Eye Port button	Changes optical path to eye port. Each function on the C2plus Settings window becomes non- selectable when the Eye Port button is selected.

• Optical Configuration

Individual data items set in the Spectral Detector mode (SD) can be managed collectively with the Optical Configuration window. For storing and retrieving the [Optical Configuration] settings, see the sections concerning the optical configuration in the "NIS-Elements AR (Advanced Research) User's Guide."

6.1.2 Setting the Optical Path

Click the [Setting] button of the Filter and Dye window to display the Optical path window. The Spectral Detector mode [SD] setting screen is displayed when the optical path changeover lever on the C2 scan head is set to the [Spectrum] position and the Spectral Detector (SD) is selected as the detection mode in the Optical path window.

There are two modes available for Optical path setting, [Auto] and [Manual]. Normally, the auto mode should be used.

	AAA	1440	Detector	Spearal Clos		ose mechanical shi	e mechanical shutter during experiment	
Setting button -	÷**	Eve Port	Ch series	None	*	Laser		
				DAP1		408.0	Resolution	10.0
			- Alexandre	Alx546		543.5	Channels	32
				J			Binning	1
							Wavelength	426.5 - 746.5

Figure 6.1-2 Filter and Dye window

Setting			at.in
LU-NV	Detector Binning/Ship Dy	ve & Spectral Setting	
405	Resolution 10.0 *	• •	
	Channels 32 *	In h	
455	Birring 1	V V	
514	Setting Mode		
	Anto Manual Excitato	450 500 550 600	650 700
532	Cryes DAPS]	
901	☑ Dye2 Ab/546 ▼ 561,100 *		
294		Start 403.90 + All	End 743.50 + +
	Detector Selection 003	SD VF	
Par	hole Spe	ctral Detector (Si)	
Tst Dichroic Mirror			
RS 20,954 *	C		
×			
🚫 Sce	nner Unit		
		<u>//</u>	
Objective			
Copicano -			
	S-ample		
_			
Transmitted Detector			
in 🛵 🔥 Out			
			OK Carcal

Figure 6.1-3 Optical path window (for auto mode)

6.1.3 Optical Path Window

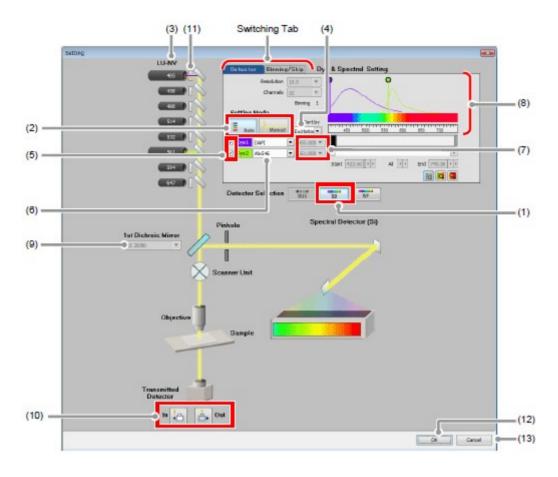


Figure 6.1-4 Optical path window (for auto mode, SD-use)

<i>Table 6.1-2</i>	Functions	of Optical	path window	(SD-use)
10000 0.1 2	1 1110110115	of optioni	pain minuon	(SD USC)

Nomo

Function

	Name	runction
(1)	Detection mode selection button	Enabled to select the Spectral Detector mode (SD). Enables to acquire the 32-channel + TD spectral images simultaneously.
(2)	Mode selector	Selects the desired mode for setting the Optical path.
		Auto - Activates the auto mode. Once the fluorescence dye to be used is selected, the appropriate laser and the dichroic mirror, and the acquired wavelength range and resolution are automatically selected. Up to 2 lasers can be selected.
		Manual - Activates the manual mode. Enables to set all of the laser, the dichroic mirror, acquired wavelength range, and resolution to be used manually. Up to 4 lasers can be selected.
(3)	Excitation laser indicator	Displays the current setting for the laser. The currently set laser icon is displayed in a large size,

		and the optical path is indicated.
(4)	Sorting fluorescence dye list	This menu is only effective while in the auto mode.
		Sorts the fluorescence dye list according to the selected type.
		ABC : Displays the list in alphabetical order.
		Emission : Displays the list in the order of peak wavelength of fluorescence intensity.
		Excitation : Displays the list in excitation wavelength order.
(6)	Fluorescence dye selection/input:	This menu is only effective while in the auto mode. Selects the in-use fluorescence dye name for each channel or enters an arbitrary channel name.
(7)	Excitation laser wavelength select	These menus are only effective while in the manual mode. Enables to set the laser wavelength that is set with the software configuration, regardless of the setting of the Filter cube display/select.
(8)	Rainbow chart	Provides the following information:
		 Wavelength band for which to acquire images (shown in color and value for each excitation laser) Spectral profile of fluorescence dye Excitation laser for fluorescence dye A color band indicating the wavelengths in the entire band (400 to 750 nm) Scale of the wavelengths in the entire band (400 to 750 nm)
(9)	1st Dichroic mirror select	This menu is only effective while in the manual mode. Enables to manually select the 1st Dichroic mirror to be used.
、 <i>,</i>	Motorized transmitted detector selection button	When the motorized transmitted detector is in use, brings the transmitted detector into the Optical path, to enable the ability. Brings the transmitted detector out of the Optical path, to disable the ability.
(11)	ND filter installation icon	An icon is displayed on the line of laser with ND filter

An icon is displayed on the line of laser with ND filter installed.

* This icon indicates whether ND filter is installed or

	not, and does not indicate insertion or removal of ND
	filter (IN = Insert in the optical path, $OUT =$
	Remove from the optical path).
	Insertion or removal of ND filter is performed on the Acquisition window.
(12) OK button	Confirms the Optical path settings applied and closes the Optical path window.
(13) Cancel button	Discards the Optical path settings applied and closes the Optical path window.

• About switching between SD and VF

SD -> **VF**:

The last settings in the Virtual Filter mode (VF) are recalled.

VF -> SD:

The last settings in the Spectral Detector mode (SD) are recalled.

• About the setting condition when the setting mode is switched

Auto mode -> Manual mode:

The entire settings in the Auto mode are retained.

Manual mode -> Auto mode:

The last settings in the Auto mode are recalled.

6.1.4 Optical Path Window Switching Tab

The tab for switching between [Detector] and [Binning/Skip] is displayed on the right top of the Optical path window.

6.1.4.1 Detector Tab

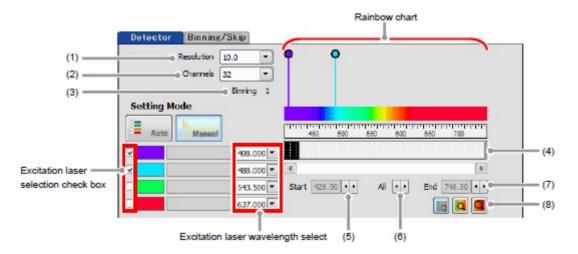


Figure 6.1-5 Optical path window (for manual mode, Detector tab)

<i>Table 6.1-3</i>	Functions	of Detector tab
--------------------	-----------	-----------------

	Name	Function
(1)	Resolution	Selects a wavelength resolution. (Enabled in the manual mode only.)
		Selectable from 2.5, 5, or 10 nm.
(2)	Channels	Selects the number of channels (number of PMTs). (Enabled in the manual mode only.) Up to 32 channels can be selected in the wavelength range of 400 nm to 750 nm.
(3)	Binning	Displays the number of channel binning currently set.
(4)	Wavelength range setting bar	Sets a wavelength range in a wavelength range from 400 to 750 nm. (Enabled in the manual mode only.) Sets a range by shifting the wavelength range setting bar to the right or left or by enlarging or reducing it. (Linked with the above setting of the number of channels.)
		* A part of the wavelength range may be displayed in black depending on the setting conditions. In the wavelength range displayed in black, no wavelength range can be set.
(5)	Start	Displays the start wavelength of the wavelength range currently selected. Enabled to enlarge or reduce the range of the short wavelength in units of wavelength resolution with the right or left button in the manual mode.
(6)	All	In the currently selected wavelength range, enables shifting to the right or left in units of 0.25 nm without changing the width of the wavelength. (Enabled in the manual mode only.)
(7)	End	Displays the end wavelength of the wavelength range currently selected. In the manual mode, the range of the long wavelength in units of wavelength resolution can be enlarged or reduced using the right and left buttons.
(8)	Enlarge button	Enlarges the rainbow chart. The display is switched in three levels.

• Restriction on the detection wavelength range for the long wavelength

To prevent the incidence of the second-order light of excitation light to the detector, there are restrictions on the settings of the detection wavelength range for the long wavelength, as shown below.

1. If there is a possibility of the incidence of the second-order light of excitation light to the detection wavelength range, the wavelength resolution of the diffraction grating is increased (the detection range is narrowed) to prevent the incidence of the second-order light of excitation light to the detection wavelength range.

(The wavelength resolution of the diffraction grating automatically transits from 10.0 nm to 5.00 nm, and then to 2.50 nm.)

2. When the wavelength resolution is 2.50 nm, the detection wavelength range is limited so as not to move to the wavelength longer than the wavelength of second-order light.

6.1.4.2 Binning/Skip Tab

With the inter-channel binning, the dark image can be brightened. (Enabled in the manual mode only.) Further, channels within the set wavelength range can be arbitrarily skipped. Since masked channel data is not acquired, the data volume can be reduced.

Set this tab after the setting of the [Detector] tab is confirmed.

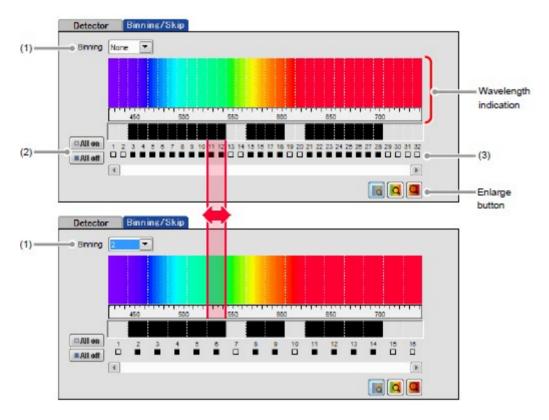


Figure 6.1-6 Optical path window (for manual mode, Binning/Skip tab)

Table 6.1-4 Functions of Binning/Skip tab	<i>Table 6.1-4</i>	Functions	of Binning	/Skip tab
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	Name	Function
(1)	Binning	Sets the number of channels to be combined into one channel.
		Two to four channels can be set.
		When Binning is set, the number of channels set with the
		[Detector] tab is automatically re-set to the closest number of channels that can be divided by the binning value.
(2)	PMT All on/off button	All on - Resets all PMT skips that have been set.
		All off -

		Leaves one channel and skips all of other PMTs.
(3)	PMT skip selection check	Sets skip in each channel. If this box is clicked, i (black) is
	box	displayed and skip is set.
		Channel data with skip set is not acquired during scan.

* If the setting of the [Detector] tab is changed, the setting with the [Binning/Skip] tab is cancelled.

6.2 Acquisition Window

The Acquisition window enables to set PMT brightness (detection sensitivity), laser power, and pinhole size.

(3) (4) (1) (2) -B 100 Laser Power Monito on di 561.0 (5) -16 0 0.0 0 9.0 0.0 Later 4 Þ Displays laser power value TD (6) SHV -16 0 (8) offset 0 (7) 1E 4 A.U. Pinhole 561.0 × um thickness of optical section : 0.90 um Optical Resolution 10.01 um Optimins (10) (11) (12) (9)

6.2.1 Structure of Acquisition Window

Figure 6.2-1 Acquisition window (SD-use)

<i>Table 6.2-1</i>	Functions	of Acquisition	window (SD-use)
--------------------	-----------	----------------	-----------------

1 0000				
	Name	Function		
(1)	Acquisition/Photo Activation window switching	Switches between the Acquisition and Photo Activation windows.		
		For the Photo Activation window, see Chapter 10.		
(2)	Laser power monitor button	Displays the laser power value (integer obtained after A/D conversion divided by 10) of the current excitation laser by clicking this button. During the image acquisition, the laser power cannot be measured and this button is grayed out.		
(3)	Excitation laser color	Displays the excitation laser color specified in the Optical path window.		
(4)	Laser wavelength	The currently selected laser wavelength is indicated.		

indication

(5) Laser ON/OFF button Selects whether the laser is emitted or not.

* When LU-NV is in use, this button is grayed out and is disabled while the button on the front panel of the laser unit is OFF or blinking.

Laser	
ON status	s

The laser is emitted.

LaserThe AOTF shutter closes and the laser power value
becomes 0.OFF statusWhen switched from OFF to ON, the laser power
value set in the previous ON status is applied.Adjusts HV of the Spectral Detector.

- (6) Si HV Adjusts HV of the Spectral Det(7) Pinhole Adjusts the pinhole size.
- For pinhole size, see Section 6.2.3, "Setting the Pinhole."
 Brightness adjustment for transmitted detector, use the HV and Offset controls to adjust the brightness of the live image.
- (9) TD channel selection
 (10) AG button
 Enables to acquire TD images by checking the check box.
 Automatically adjusts the Si HV value (Si HV gain) of the currently selected excitation laser to the optimum values.
- (11) Auto Gain setting button
 (12) Optimize button
 (12) Optimize button
 (12) Optimize button
 (13) For Auto Gain, see Section 6.2.4, "Auto Gain."
 (14) Displays the XYZ Size Setup window. In the XYZ Size Setup window, the calculation method of the recommended values of the resolution, zoom magnification, and Z stack step size can be set.

For the XYZ Size Setup window, see Section 6.2.1.1, "Recommended Value Indication/Automatic Application."

6.2.1.1 Recommended Value Indication/Automatic Application

By the function of the recommended value indication/automatic application, the recommended values of the appropriate resolution, zoom magnification, and Z stack step size are calculated based on the objective type and the selected excitation wavelength.

Using the calculated recommended values enables the image acquisition clearer and with less damage to the sample.

To automatically apply the recommended values to the parameters, set the [Nyquist XY] button of the Scan Area window to ON.

C2plus Scan Area x
📴 🚥 — Crop R.OJ Edit 🔀 🇮
Zoom: () 1
Pixel size: 0.62 Nyquist XY
Scan size: 512 Rotation: 0
Width: 512 Height: 512
Dwell time: 1.9 µs
Pixel size: 0.62 µm Optical resolution: 0.1
Z step size: 0.42 µm Optical sectioning: 1.2

Figure 6.2-2 Scan Area window

	Scan setting					•	
	Scan Direction	11 12			Zoom	► 1.000	
Indicates the	Scan Size Scan Speed	512 • •				38 🕤 4.563x recommen	 Indicates the recommended
resolution.							value of the scan
		Pinhole 561.0	Atlans	⇒ ► 18.4 30.0] A.U. um <-		magnification.
		and the second sec	foptical section : 0.90 olution : 0.01 um	lum	Optimize		

Figure 6.2-3 Location of Recommended Value Indication

* When the laser or objective in use is changed, the recommended values are recalculated, and newly indicated and automatically applied.

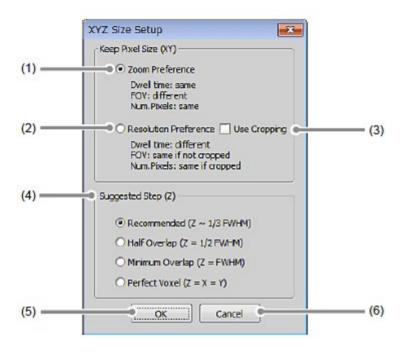
Recommended Value Settings

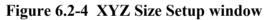
Detailed settings of the recommended values are made in the XYZ Size Setup window that is displayed

by clicking the [Optimize] button of the Acquisition window.

If the [Nyquist XY] button of the Scan Area window is ON, the recommended values are automatically applied to the parameters.

Or if the [Nyquist XY] button is OFF, the recommended values of the scan size and zoom are indicated in the Scan setting window.





<i>Table 6.2-2</i>	Functions	of XYZ Size Setup window
--------------------	-----------	--------------------------

1 401	c 0.2 2 1 unclions of $M12$			
	Name	Function		
(1)	Zoom Preference		XY] button is ON, keeps the scan size ommended value of the zoom.	
(2)	Resolution Preference	When the [Nyquist XY] button is ON, keeps the zoom and applied the recommended value of the scan size.		
(3)	Use Cropping	Fits the scan size in	detail by using Crop Scan.	
		Sets the Z step size	calculation method.	
		Recommend (Z~1/3 FWHM)	Approximately one third of the thickness of optical section (FWHM value).	
(4)	Suggested Step (Z)	Half Overlap (Z=1/2 FWHM)	One half of the thickness of optical section (FWHM value).	
		Minimum Overlap (Z=FWHM)	The thickness of optical section (FWHM value).	
		Perfect Voxel (Z=X=Y)	Value same as the pixel size.	
(5)	OK button	Confirms the XYZ Size Setup window.	Size Setup applied and closes the XYZ	
(6)	Cancel button	Discards the XYZ S Size Setup window.	Size Setup applied and closes the XYZ	

6.2.2 Setting Image Brightness

For each excitation laser, adjust HV, Offset, Laser, and ND filter IN/OUT to obtain clear images.

Laser Power Monitor	
Laser 405.0	Laser 561.0
0.0 0.1 00	
 манк ч Останка	
Pinhale 4	



Tabi	le 6.2-3	Brightness adjustment functions for the live image (SD-use)
	Name	Function
(1)	Laser ('	Sets the laser power value.
		Slider bar: Slides to the right or left to set the laser power value.
		Arrow buttons: Click either arrow button to increase or decrease the laser power value stepwise.
		Direct entry in laser power value display field: Type the desired setting value.
(2)	HV	Sets the voltage to be applied to the transmitted detector.
		Slider bar: Slides to the right or left to set the HV value.
		Arrow buttons: Click either arrow button to increase or decrease the HV value stepwise.
		Direct entry in HV value display field: Type the desired setting value.
(3)	Offset	Sets the offset value of the transmitted detector.
		Slider bar: Slides to the right or left to set the offset value.

Arrow buttons:

Click either arrow button to increase or decrease the offset value stepwise.

Direct entry in offset value display field:

Type the desired setting value.

(4) Si HV

Adjusts HV of the Spectral Detector. (Applied to all excitation lasers.)

Slider bar:

Slides to the right or left to set the Si HV value.

Arrow buttons:

Click either arrow button to increase or decrease the Si HV value stepwise.

Direct entry in Si HV value display field:

Type the desired setting value.

- (5) ND filter IN/OUT Inserts/removes the ND filter in/from the optical path. (IN = Insert in the optical path/ OUT = Remove from the optical path)
 This button is displayed only for lasers that can control insertion/removal of ND filter.
- * When LU-NV is in use, this function is grayed out and is disabled while the button on the front panel of the laser unit is OFF or blinking.

PMT Overload

If too much gain is applied to the illumination intensity, the gain is automatically shut down to protect PMT and/or transmitted detector (TD), and then following PMT Overload dialog box is displayed.

ialog	de: 0; EventCode: 2; M	laassaat Saacha mD	MT Outriand
Action	Je: V; Evenuoue: 2; P	lessage: Speculume	MI Overkau
	OK	Cancel	

Figure 6.2-6 PMT Overload dialog box

	Later Power Monitor	
	Laser 405.0	Laser 561.0
	Laser ◄ ━━━━ ► №0 16.0 0.0	9.0 0.0
_		
		0
_	561.0 V 410me 30 0 um <-	
	Fridmess of optical ction : 0.90 um Optical Resrutton : 0.01 um Optimize	

Figure 6.2-7 Setting the Pinhole (SD-use)

Table 6.2-4 Pinhole setting functions (SD-u

	Name	Function
(1)	Pinhole size setting	Sets a pinhole size for C2 system.
	8	Slider bar: Slides to the right or left to set the pinhole size. (Unit: A.U.)
		Arrow buttons:
		Click either arrow button to increase or decrease the pinhole size stepwise.
		Direct entry in pinhole size display field: Type the desired setting value.
(2)	Pinhole button	Displays the A.U. Calculation Settings window to calculate the pinhole size.
		(For A.U. Calculation Settings, see Section 6.2.3.1, "Calculation Settings for Pinhole Size.")
(3)	Home	Changes the pinhole to the predetermined home position.The value of the home position can be changed in the A.U.Calculation Settings window.(For A.U. Calculation Settings, see Section 6.2.3.1, "Calculation Settings for Pinhole Size.")
(4)	Pinhole size	Indicates pinhole size of C2 system. (Unit: μm)
(1)	thickness of optical section	Indicates the FWHM (full width at half maximum) of z airy disk.
(6)	Optical Resolution	The actual size of 1 pixel square calculated from the optical information (for objectives and scan parameters) and the size acquired from an image.
(7)	Reference excitation wavelength for the pinhole size	Selects the excitation wavelength as the reference of the automatic calculation of the pinhole size from the laser wavelengths, or enter it manually in the A.U. Calculation Settings window. (For A.U. Calculation Settings, see Section 6.2.3.1, "Calculation Settings for

Page 16 of 53

calculation Pinhole Size.")

6.2.3.1 Calculation Settings for Pinhole Size

This section describes the setting window for calculating the pinhole size.

Click the [Pinhole] button in Acquisition window, the A.U. Calculation Settings window appears. (Usually, the [Recommend] is selected to enable automatic calculation. [Recommend] calculates the A.U. value by using the Nikon-recommended EM and NA values.)

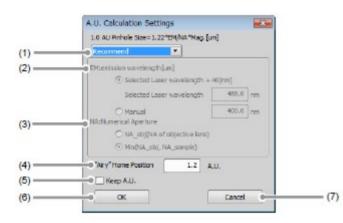


Figure 6.2-8 A.U. Calculation Settings window

<i>Table 6.2-5</i>	A.U.	Calculation	Settings window
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	Name	Function
(1)	Select calculation method	Recommend - Sets parameters automatically. (Nikon recommended)
		User Setting - Allows the user to manually set parameters.
(2)	EM:emission wavelength[µm]	 Selected Laser wavelength - Calculates parameters by using the laser wavelength selected in the pinhole combo box of the Acquisition window as the emission wavelength (EM value). The wavelength displayed in the combo box is to be the laser wavelength set in the Optical Setting window.
		Manual - Allows the user to manually set parameters. (The parameter is calculated with the input value as the emission wavelength (EM value).) Enter the value directly from the keyboard.
(3)	NA:Numerical Aperture	Sets refractive index of the objective.
		NA_obj(NA of objective lens) - Regardless of whether or not the objective NA value exceeds the refractive index of the sample, executes calculation by using

		the objective NA as the calculation parameter. Min(NA_obj, NA_sample) - When the objective NA value does not exceed the refractive index of the sample, executes calculation by using the objective NA as the calculation parameter. When the objective NA value exceeds the refractive index of the sample, executes calculation by using the sample refractive index.
(4)	"Airy" Home Position	Sets a home position of pinhole. Enter the value directly from the keyboard.
		The pinhole size can be selected from six types in C2. Therefore, if the entered value does not match any of the types, the size that is larger than and the closest to the entered value is set as the home position.
(5)	Keep A.U. check box	When checked, the pinhole size is fixed by the A.U. when the selected wavelength or objective is changed. (However changes by the um.) When unchecked, the pinhole size is fixed by the um. (However changes by the A.U.)
		The pinhole size can be selected from six types in C2. Therefore, if the to-be-fixed A.U. value does not match any of the types, the size that is larger than and the closest to the A.U. value is selected.
(6)	OK button	Confirms the A.U. Calculation Settings applied and closes the A.U. Calculation Settings window.
(7)	Cancel button	Discards the A.U. Calculation Settings applied and closes the A.U. Calculation Settings window.

6.2.4 Auto Gain

Auto Gain is a function to automatically correct the value of Si HV gain to set the optimum image brightness.

Automatic Si HV gain correction is performed within the predetermined range of the ratio of saturation pixels.

Automatic Si HV gain correction is performed only Si HV. For a TD, automatic adjustment is performed when it is selected.

After execution of Auto Gain, in the window indicating the progress of Auto Gain, the correction values actually used (ratio of saturation pixels) are displayed.

If Auto Gain failed, "x" is indicated and the Si HV value returns to its original value.

- When setting the line scan, Auto Gain cannot be executed.
- During execution of Auto Gain, do not perform manual adjustments in the Acquisition window.

Acquisition / Photo Active on/Blead to(Lesers)	
Laser +05.0	Laser 561.0
Image Image	

Figure 6.2-9 Execution of Auto Gain (SD-use)

	Waiting for AutoGain calibration	×	
	Current ratio: Spectral 2.475% Transmitted 99.998% Ratio criterion: min > 0%, max = 1.00%	N N N N N N N N N N N N N N N N N N N	Auto Gain status Failed Completed
If checked, the window is auto	-	<u>i.Abort.i</u>	

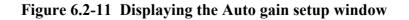
Figure 6.2-10 Auto Gain progress

Setting for ratio of saturation pixels

Set the maximum and minimum value for the ratio of saturation pixels used for automatic Si HV gain correction.

Click the [Auto Gain Setting] button to display the Auto gain setup window. Set the maximum and minimum value for the ratio of saturation pixels in the Auto gain setup window.

✓Acquisition / Photo Activation/Eleaching(Laser::)	
A Laser Power Monitor	
Laser 405.0	Laser 561.0
Laser 4 - ND 16.0 0.0	Laser 4 - 9.0 0.0



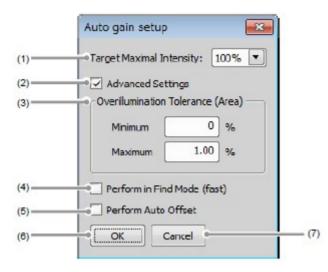




Table 6.2-6 Setting for ratio of saturation pixels

	Name	Function
(1)	Target Maximal Intensity	Specifies the application ratio of the setting of the ratio of saturation pixels. Sets the percentage (%) of the maximum value to be applied.
(2)	Advanced Settings	If checked, advanced settings of the ratio of saturation pixels are enabled.
(3)	Overillumination Tolerance (Area)	MinimumSets the minimum value for ratio of saturation pixels.MaximumSets the maximum value for ratio of saturation pixels.
(4)	Perform in Find Mode (fast)	If checked, execution in the Find mode is enabled.
(5)	Perform Auto Offset	If checked, the offset value is set automatically.
(6)	OK button	Confirms the settings of Auto gain setup applied and closes the Auto gain setup window.
(7)	Cancel button	Discards the settings of Auto gain setup applied and closes the Auto gain setup window.

6.3 Various Views (Spectral Detector-use)

This section describes various spectral views.

6.3.1 Channel View Setting

6.3.1.1 Channel Mixed View

From multiple channels acquired with the Spectral Detector (SD), selected channels are mixed and

displayed.

1. Open the Live window.

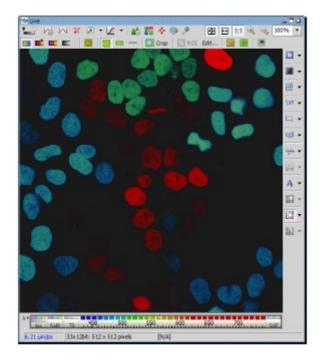


Figure 6.3-1 Live window

2. Select desired channels.

While pressing the [Ctrl] key, click desired channels.

To select a range, select the channel as the start point first, then while pressing the [Shift] key, click the channel as the end point.

For selection of channels in multiple ranges, see Section 6.3.1.4, "Multi-Range Channel View."

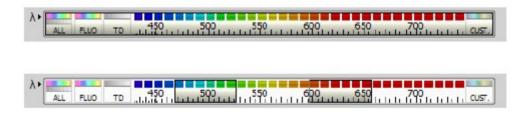


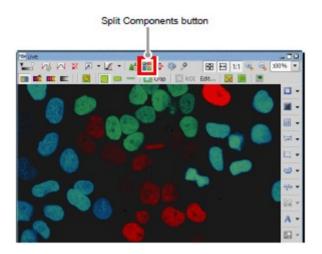
Figure 6.3-2 Channel view bar

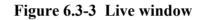
6.3.1.2 Split Channel View

Selected channels are split into respective channels and displayed.

1. Click the [Split Components] button.

"All image" mixing all channels, respective channel images, "TD image," "Ratio image," "Custom image" are displayed.





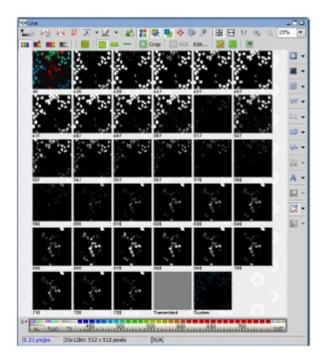


Figure 6.3-4 Split channel view

- * For switching from Split channel view to Channel mixed view, click the [Split Components] button again.
- 2. Right-click on the [Custom] button and a menu appears. Select [Properties...] on the menu. The Custom window appears to allow you to change the channels for the Custom View.

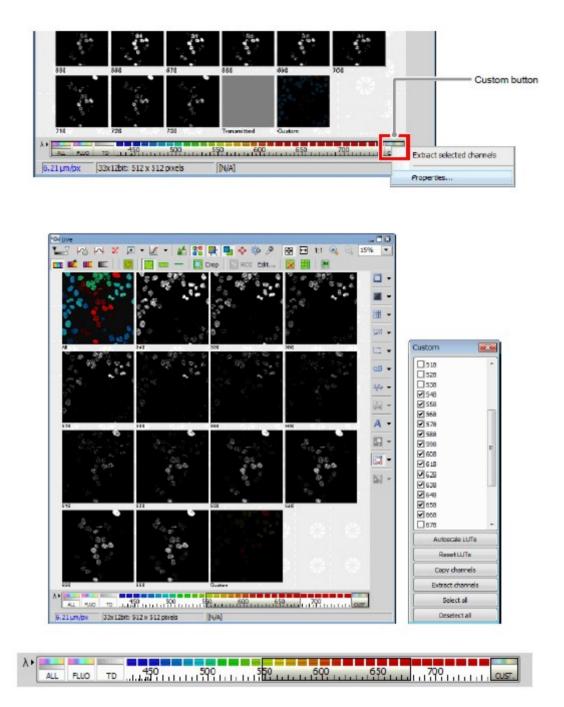


Figure 6.3-5 Split channel view (Custom image)

6.3.1.3 Ratio Image View

The Ratio image view is displayed.

Right-click on the window to display a menu. Selecting [Ratio View] from the menu changes the window to the Ratio image.

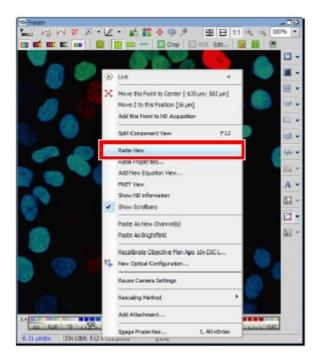


Figure 6.3-6 Displaying the Ratio image view

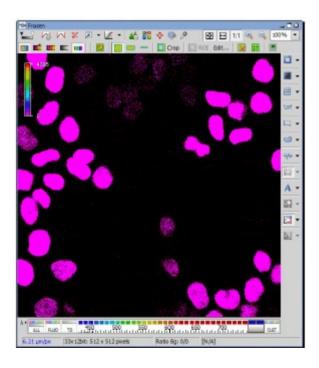


Figure 6.3-7 Ratio image view

6.3.1.4 Multi-Range Channel View

Mouse operation for displaying multi-range channels is as follows:

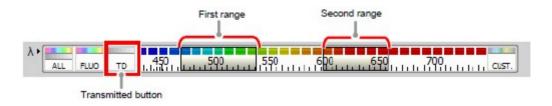


Figure 6.3-8 Multi-range channel view

- 1. Set and display the First range. Click the channel at the left end of the First range.
- 2. While pressing the [Shift] key, click the channel at the right end of the First range.
- 3. Select the Second range. While pressing the [Ctrl] key, click the channel at the left end of the Second range.
- 4. While pressing the [Ctrl] + [Shift] key, click the channel at the left end of the Second range.
- 5. Click the [Transmitted] button.

While pressing the [Ctrl] key, click the [Transmitted] button. Then, the TD image and the images of the selected channels are mixed and displayed.

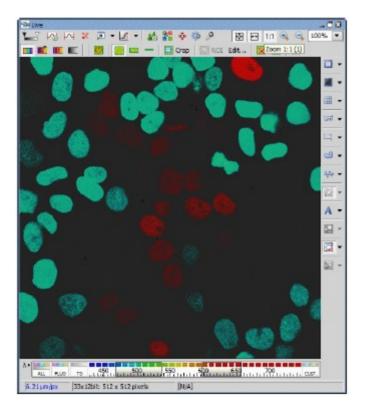


Figure 6.3-9 Channel mixed view

6.3.2 Color Mode Setting

6.3.2.1 Color Mode

The color mode switching method and channel color assignment are shown below.

Select the desired color mode from three modes; True Color, Custom Color, Grouped Color and Gray Scale and switch the display.

To set the color mode, be sure to turn "ON" the [Treat as Spectral] button. (If it is turned "OFF," spectral information hidden.)

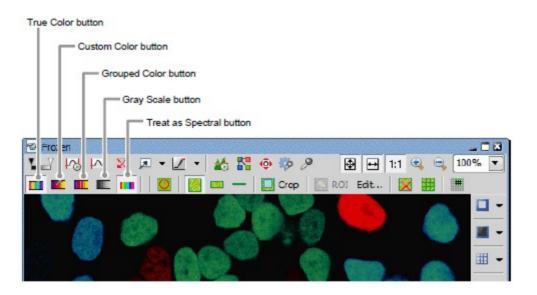


Figure 6.3-10 Frozen window

* Settings of Custom Color, Grouped Color, Gray Scale To configure detailed settings, use the LUTs window. To Displaying the LUTs window is shown below.

Click the [Show LUTs window] button or right-click on the gray area (without any setting window displayed) to display a menu as shown below. Select [Visualization Controls] -> [LUTs] in the menu to open the LUTs window.

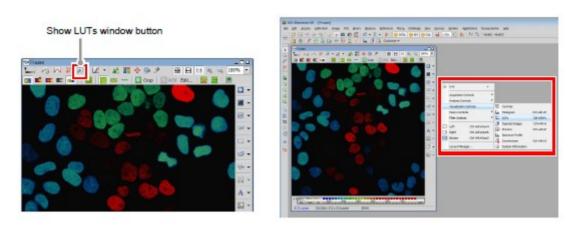


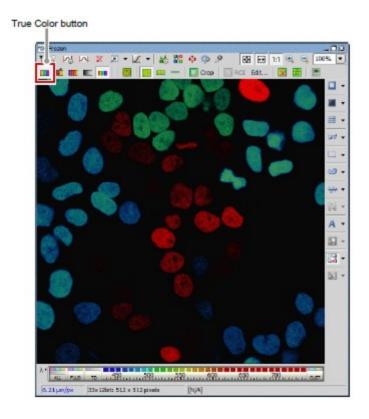
Figure 6.3-11 Displaying the LUTs window

Displaying the True Color Image

Images of all channel data are displayed using the wavelength colors corresponding to the wavelength range provided during data acquisition.

Colors that are approximately same as those viewed by bare eyes are displayed.

Click the [True Color] button to display the True color image.



Displaying the Custom Color Image

Custom Colors are assigned to respective channel data and images are displayed using multiple channel data.

Custom Color assignment uses the LUTs window.

Click the [Custom Color] button to display the Custom color image.

Custom Color button

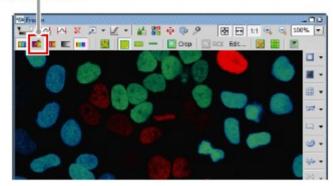


Figure 6.3-13 Custom Color button

Custom Color Setting

Click on the [Reference color] button, then opens the Select New Color window. For the Select New Color window, see Section 6.3.2.2, "Select New Color Window."

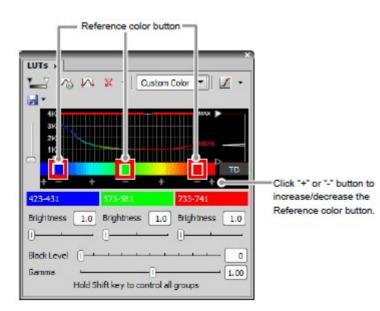


Figure 6.3-14 Custom Color setting window

* In Custom Color mode, channels between [Reference color] buttons are color-interpolated and displayed.

Displaying the Grouped Color Image

With image acquired using the Spectral Detector, channels in a specified range can be grouped and colors can be assigned by group.

Grouped Color assignment uses the LUTs window.

Click the [Grouped Color] button to display the Grouped color image.

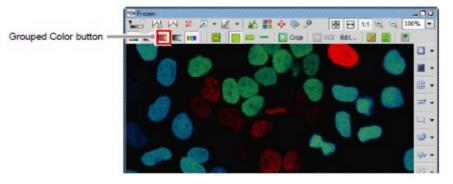


Figure 6.3-15 Grouped Color button

Grouped Color Setting

Click on the [Reference color] button, then opens the Select New Color window. For the Select New Color window, see Section 6.3.2.2, "Select New Color Window."

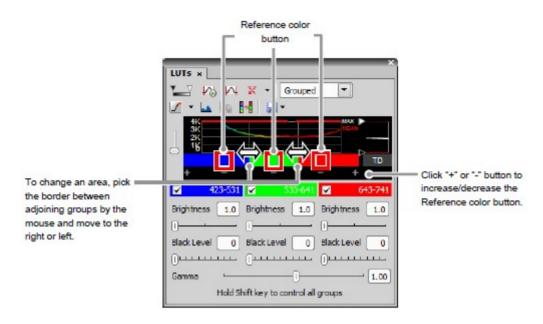


Figure 6.3-16 Grouped Color setting window

- * In Grouped Color mode, the area is split by the number set in [Reference color] button. Channels in each area are all displayed with the same color.
- * In the channel bar of image window, can change an area too. Click the group to change, then pick the border between adjoining groups by the mouse and move to the right or left.

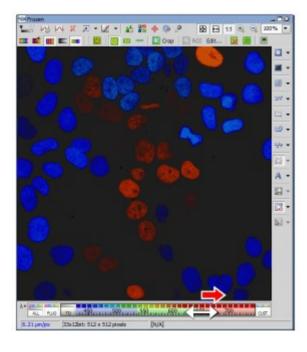


Figure 6.3-17 Grouped Color mode

Displaying the Gray Scale Image

Each channel is displayed with Gray Scale (Monochrome 256 gradations). Gray Scale assignment uses the LUTs window.

Click the [Gray Scale] button to display the Gray Scale image.



Figure 6.3-18 Gray Scale button

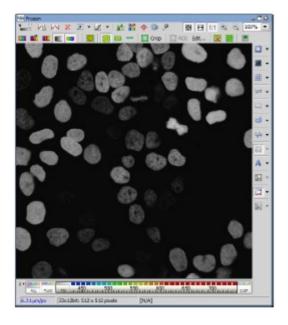


Figure 6.3-19 Gray Scale image

Gray Scale Setting

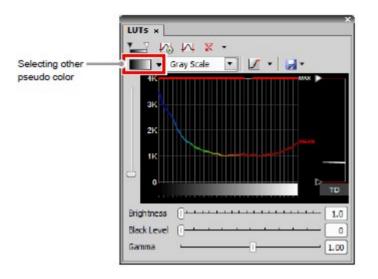


Figure 6.3-20 Gray Scale setting window

* The pseudo color menu also allows changing the displayed color settings.

6.3.2.2 Select New Color Window

In this window, colors to be assigned to channels are selected.

Click the [Reference color] button on Custom Color or Grouped Color settings to display this window.

1. In the Select New Color window, select the desired tab from three [Color palette] tabs.



Figure 6.3-21 Select new color window (Palette)

E[Palette]

Select a color from red, green, blue, yellow, purple, cyan, and white. Colors; yellow, purple, and cyan, support color weakness.

2. Select the color to be assigned.

In the [Hue] and [Wavelength] tabs, a numeric value can be directly entered or the bar displayed in the color range can be moved to the right or left for selection.

Palette Hue	Wavelength	
Huc:	170	
Sample:		
	[токт] с	ancel



E[Hue]

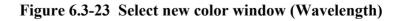
A hue is set.

A hue in a range of 0 to 240 can be set.

A numeric value can be directly entered or the bar displayed in the color range can be moved to the right or left for selection.

3. The selected color is displayed in [Sample].

alette Hue	Wavelength		
Wavelength:	400nr	¦ → ,)	650nm
Sample:			
	-1		



E[Wavelength]

A color is set using a wavelength in the wavelength range.

A wavelength is specified with a numeric value or bar to select a wavelength color.

6.3.3 Spectrum Profile

Brightness of the ROI area specified in the spectral image can be decomposed and displayed for each 32 channels.

6.3.3.1 Displaying the Spectrum Profile

1. Specify the ROI area in the spectral image. (If two or more ROI areas are selected, graphs are displayed for the colors of the ROI selected areas on the profile graph.)

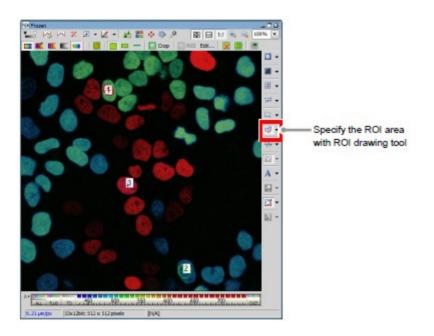


Figure 6.3-24 Specify the ROI area (Spectral image)

As shown below, right-click on the gray area (without any setting window displayed) to display a menu.
 Select [Visualization Controls] -> [Spectrum Profile] in the menu to open Spectrum Profile.

denotes that the set for the provided by the provided the set of	۲	Live +			
		Acquisition Controls Analysis Controls	•		
Same and Same and Same and Same		Visualization Controls	6	Controls	
The second secon		Macro Controls Filter Analysis		LUTs	Ctrl+Alt+H Ctrl+Alt+L
		Left Ctrl+Alt+Num4		Opened Images Preview	Ctrl+Alt+] Ctrl+Alt+P
		Right Ctrl+Alt+Num6 Bottom Ctrl+Alt+Num2	92	Spectrum Profile	
		Boltom Cortextenumz	10	Synchronizer	Ctrl+Alt+S
		Layout Manager	1	System Information	

Figure 6.3-25 Displaying the Spectrum Profile

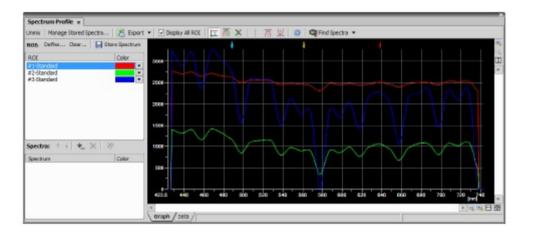


Figure 6.3-26 Spectrum Profile (all ROI areas are displayed)

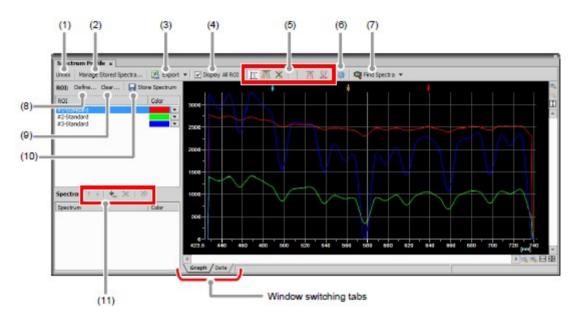
3. To display the ROI on the graph, remove the check mark from [Display All ROI] and select the desired ROI from the ROI list.

ROI area selection	Information of all ROI areas is displayed.
Spectrum Profile × Unnis Manage Store Rote Define Gas - Spectrum Rote Color	t ▼Depart # ROL II T T X 71 ½ ⊕ I C Find Spectra ▼
en Anne dent	
Spectrae + + + × & Spectrum Color	
	4255 440 460 480 500 500 545 660 686 695 696 696 790 700 100 100 100 100 100 100 100 100 10

Figure 6.3-27 Spectrum Profile (displayed for each ROI area)

X-axis: 32-channel spectral colors displayed.

Y-axis: ROI brightness value or background brightness value displayed.



6.3.3.2 Spectrum Profile Setting

Figure 6.3-28 Spectrum Profile

* If any fluorescence dye is added in [Add spectra] the ideal line of fluorescence dye reaction is displayed on the graph and can be used as an indicator about whether the fluorescence dye is correctly reacting.

Table 6.3-1 Summary of Spectrum Profile functions

	Name	Function
(1)	Unmix	Separates the wavelength information of a spectral image and displays an Unmixing image. For Unmix, see Section 6.3.4, "Spectral Unmixing Setting."
(2)	Manage Stored Spectra	Displays the item registered in Stored.
(3)	Export	Exports numeric data to Microsoft Excel.
(4)	Display All ROI	Displays all of the active ROIs.
(5)	Vertical Scale Absolute	Ŧ

Enlarges the window assuming that the brightness minimum to maximum displayed in the graph as 100%.

Vertical Scale Normalized



Displays the brightness of each ROI in the Y-axis direction

		as a relative value to 100%. (Normalizing correction)
	Scale to cursor	×
		Calculates the aberration of the curve so that the cross point between bar graphs will be Y:1 and displays a relative graph.
	Free cursor	
		Displays a cursor that can be moved to any position. When the cursor is picked with the mouse, brightness of the pixel at the cursor position can be checked as information.
	Cursor to maximum	7Å
		Moves the cursor to the maximum value of the specified ROIfs brightness.
	Cursor to minimum	₩.
		Moves the cursor to the minimum value (0 or larger) of the specified ROIfs brightness.
(6)	Options	*
		Opens the Options window for Spectrum Profile.
(7)	Find Spectra	Automatically detects spectra. Specifies the number of classifications (2 to 4) for spectra to automatically separate the wavelength or use "Auto Search" for separation without specifying the number of classifications.
(8)	Define	Opens the Simple ROI Editor tool.
(9)	Clear	Clears the ROI area specified in the image. (Before clearing, a confirmation message is displayed.)
(10)	Store Spectrum	Stores the user-defined spectrum (wavelength information). The spectrum defined here is also added to the fluorescence dye selection list in the Optical path window.
(11)	Move Up	Ť
		Brings the selected spectra to one line above.
	Move down	+
		Brings the selected spectra to one line below.
	Add spectra	*
		Adds a spectrum as an indicator.
	Remove spectra	-
	-	Removes a spectrum as an indicator.

6.3.3.3 Registering Spectrum and the Usage

The spectrum (wavelength information) defined in the Spectrum Profile window by the user is available as fluorescence dye on the Optical path window.

1. Click the [Store Spectrum] button to display the Store Spectrum window.

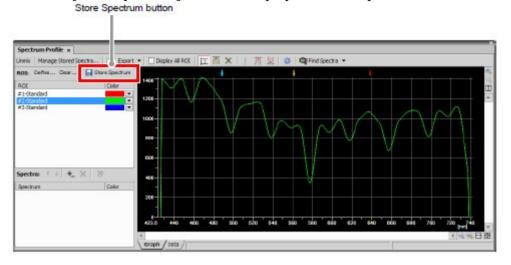


Figure 6.3-29 Spectrum Profile

2. Enter a fluorescence dye name in the [Spectrum Name:] field and click the [OK] button.

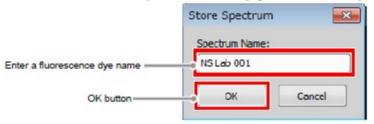


Figure 6.3-30 Store Spectrum

3. Display the Optical path window.

	Filter and Dye	Detector	Spannel	Dida	se mechanical shi	utter during experimen
Setting button	Eve Port	Ch series	None *	Laser		
			DAPI	408.0	Resolution	10.0
		and the second	Alx546	543.5	Channels	32
					Binning	1
					Wavelength	426.5 - 746.5
		π			ų	



4. Select the registered fluorescence dye name from the fluorescence dye selection menu of the selected channel.

LU-NV	Detector Binnins/Skip Dye & Spectral Setting	x
	Resolution 5.0 * Channels 32 *	
445	Binning 1 Setting Mode Sort by Encision FUTURE PERFECTOR PERFECTOR PERFECTOR PERFECTOR Auto Soc Soc Soc Soc Soc Soc Soc Soc Soc So	
532	Image: Comparison of the company of the com	
	Anglex UtraRed peroxidation product(pH 7.5 End 574.25 + E	
	Detector Research (19:14-0. Odde: 305 Infangerine DiA (Reid UrsoTracker Red(MeOH Rhodamine Red X Coffrage of own red-ownswidt 7.5	

The window to select the excitation laser wavelength to be used appears.

Figure 6.3-32 Optical path window (for auto mode)

5. Select the excitation laser wavelength to be used for this fluorescence dye and click the [OK] button.

Dialog	×
Dye Name	NS Lab 001
Excitation	
	405.000 457.900
OK	476.500 488.000
	514.500
	561.000 638.000

Figure 6.3-33 Selecting excitation laser

The fluorescence dye name and the excitation laser wavelength of the selected channel are updated.

	Resolution 10.0 * • • •
	Chennels 32 * Binning 1
514	Sort by Enission
	The Deep Tel 405,000 T
551	Pyw2 NSI Lab 001 - 4.000 - 4
	Start 423.50 + AI + End 243.50 +

Figure 6.3-34 Optical path window (for auto mode)

* <u>When changing the excitation laser wavelength defined for each fluorescence dye</u> If you click the fluorescence dye name while pressing the [Ctrl] key with the fluorescence dye (for which you want to change the excitation laser wavelength) set for the channel, the excitation laser selection window (described in step 5) appears. Confirm that the displayed fluorescence dye name is that for which you want to change the excitation laser wavelength, and then re-set the excitation laser wavelength.

6.3.4 Spectral Unmixing Setting

Separate the wavelength information of a spectral image and display an Unmixing image. If wavelengths overlap (because multiple fluorescence dyes are in use) and differences are hard to identify, wavelength information can be separated and displayed.

6.3.4.1 Displaying the Spectral Unmixing Setting

1. Specify the wavelength to be separated in the spectral image or on the Frozen window using the ROI area.

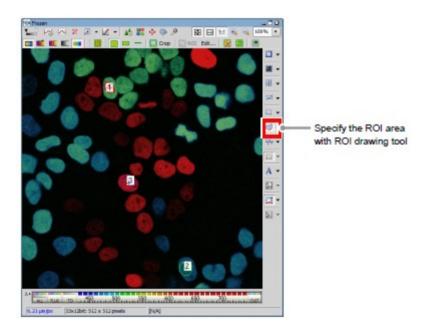
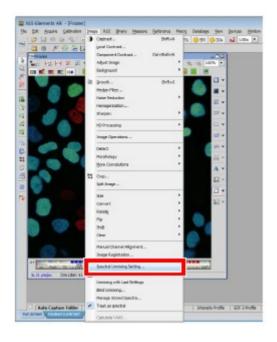
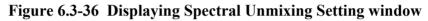


Figure 6.3-35 Specifying the ROI area (Spectral image)

2. Open the Spectral Unmixing Setting window.

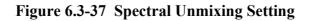
Select [Imagen-> [Spectral Unmixing Setting...non the menu bar.





3. If [ROIs] is selected from [Category:] in [Source Elements], [Elements:] displays the elements (ROIs) of the target to be separated.

ctral Unming Setting			
surce Elemen s Sategory:	Unniking Elements		× 8
RDIS All Unsorted	Element	Name	Color
Alexa Fluors Cyanine Dyse Rearch: XXIA Add	-		
#1-Gtendard #2-Standard #3-Stendard	0.6		
	0.6		
	0 0 00 00 00 00 00 00 00 00 00 00 00 00	50 700 750 800 _{[7}	
	<u>(4)</u>	1	



Page 41 of 53

4. Using the [Add] button, add the elements of the target to be separated from [Elements:] in [Source Elements] to [Unmixing Elements].

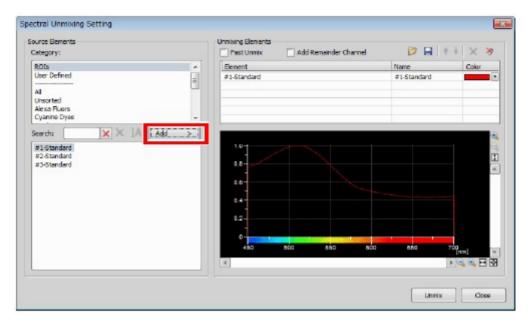


Figure 6.3-38 Spectral Unmixing Setting

5. Click the [Unmix] button to open the unmixed image window separately from the Frozen window.

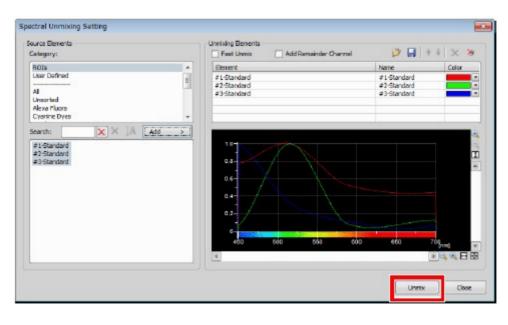


Figure 6.3-39 Spectral Unmixing Setting

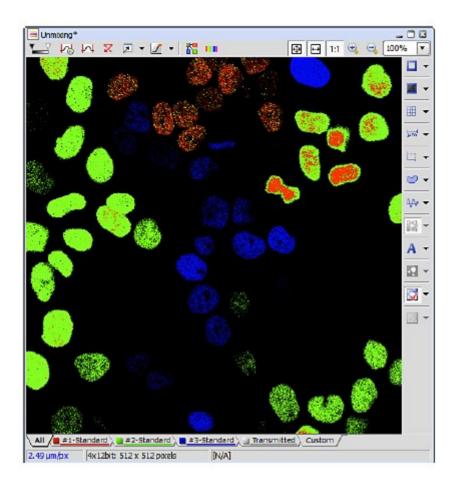


Figure 6.3-40 Spectral Unmixing view

- * In addition to specifying using the ROI area, wavelength information can be separated by specifying the fluorescence dye in use. However, noise provided upon image acquisition may appear.
- * If the image as a result of normal Unmix looks ambiguous, performing Unmix with the [Fast Unmix] check box selected may lead to a good result.

* Specifying the background color of ROI. As shown below, specify the ROI area in the part to be designated as the background color. Right-click the mouse on the created ROI area to display a menu. From the menu, select [Use as Background ROI].

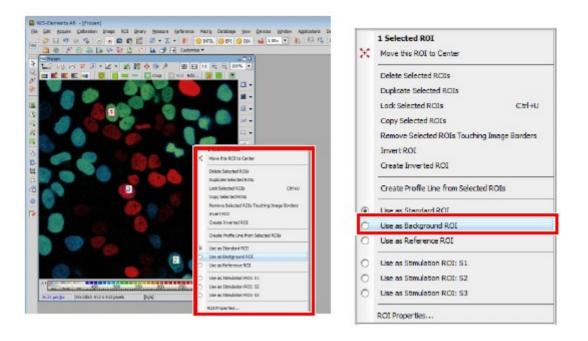


Figure 6.3-41 Changing the setting of the ROI area

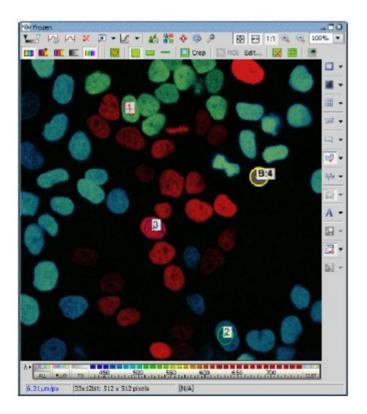
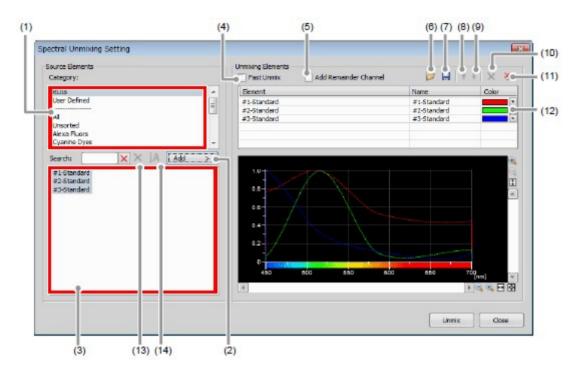
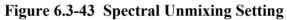


Figure 6.3-42 Spectral Unmixing view





<i>Table 6.3-2</i>	Summary of Sp	ectral Unmixing	Setting functions
10000 0.0 2	Summer y of Sp	con an chination	Setting Junetions

Tabl	le 6.3-2 Summary of Spectr	al Unmixing Setting functions
	Name	Function
(1)	Category:	Displays the category of the ROI, user-registered wavelength information, fluorescence dye, etc.
(2)	Add>	Selects the elements of the target to be separated from [Elements:] and adds to [Unmixing Elements].
(3)	Elements:	Selects the elements of the target to be separated.
(4)	Fast Unmix	If check is turned "ON," the calculation algorithm is simplified and higher-speed separation is performed compared with normal Unmix.
		* Since Live Unmixing uses high-speed calculations unlike normal Unmix, Fast Unmix is not applied even if this check box is selected.
(5)	Add Remainder Channel	This function enables calculation of remainder data in the Unmxing calculation. When selected, the remainder data is shown as an image in the Unmixing calculation result. When deselected, the remainder data is not shown.
(6)	Open	
(7)	Save	Retrieves the setting information saved in an XML file.
		Writes the setting information in an YML file and saves it

Writes the setting information in an XML file and saves it.

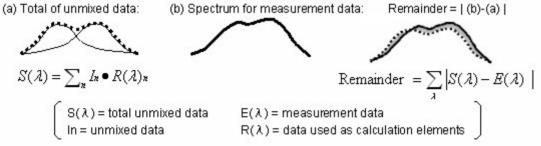
Up	Ť
	Brings the selected Element to one line above.
(9) Move the Element one lin down	e 🔰
	Brings the selected Element to one line below.
(10) Remove the Element	\times
	Removes the selected Element.
(11) Remove all	×
	Removes all Elements.
(12) Color	The graph color and post-Unmix image can be set to any color.
(13) Remove Spectra	Enabled only when [User Defined] or [From Blind Unmixing] is selected for [Category:] area. Removes the items selected in [Elements:] area.
(14) Rename Spectrum	Enabled only when [User Defined] or [From Blind Unmixing] is selected for [Category:] area. Changes the names of items selected in [Elements:] area.

Note:

[Remainder data]

The Remainder data is used as a quality standard for the data produced by the Unmix calculation.

The Remainder data is represented as an absolute value for the total of differences between measurement data (b) and the total of Unmixed data (a).



This data is added as one channel data to Unmixed data.

6.3.5 Live Unmixing

Live observation is available in the state where spectral images are separated for each wavelength.

6.3.5.1 Displaying the Live Unmixing

1. Specify the wavelength to be separated in the spectral image using the ROI area.

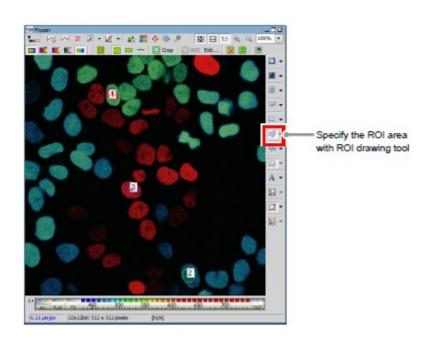


Figure 6.3-44 Specify the ROI area (Spectral image)

 Open the Spectral Unmixing Setting window. Select [Imagen-> [Spectral Unmixing Setting...] on the menu bar.

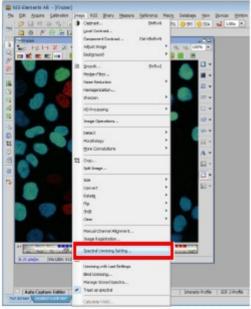


Figure 6.3-45 Displaying the Spectral Unmixing Setting window

3. If [ROIs] is selected from [Category:] in [Source Elements], [Elements:] displays the elements (ROIs) of the target to be separated.

kurce Eleme is lategory:	Unnixing Elements Past Unnix Add Remainder Channel	🕫 🗄 🕴 🗙 🗞
RDIs A Construction of Dennes All Inserted Alexa Fluors Cyanine Dyses	Eenent	Name Color
earch: X X IA Add > #1-6tandard #3-Standard #3-Standard		
	400 450 500 250 650 650	700 720 800 _(nm) -



4. Using the [Add] button, add the elements of the target to be separated from [Elements:] in [Source Elements] to [Unmixing Elements].

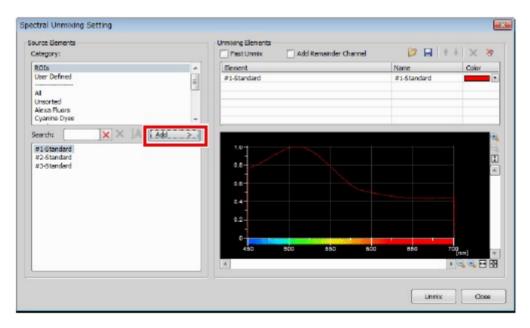


Figure 6.3-47 Spectral Unmixing Setting

5. Click the [Close] button to confirm the wavelength you want to separate.

ategory:	Unitiving Benents	🎾 🖬 🕂 4	× *
	Element	Name	Color
iser Defined 1 Unacried Hear Fluors	#1-Standard #2-Standard #3-Standard	#1-Standard #2-Standard #3-Standard	
earch: X A Add 2 #1-Standard #3-Standard #3-Standard	38 0.4 0.4 0.2		छ । हा <mark>म</mark> ्र
	**************************************	, in the second s	

(If you click the [Unmix] button instead, normal Unmix image starts to be captured.)

Figure 6.3-48 Spectral Unmixing Setting

* Fast Unmix

Since Live Unmixing uses high-speed calculations unlike normal Unmix, Fast Unmix is not applied even if the [Fast Unmix] check box is selected.

- * In addition to specifying using the ROI area, wavelength information can be separated by specifying the fluorescence dye in use. However, noise provided upon image acquisition may appear.
- 6. Click the [Live Unmixing] button on the horizontal toolbar. If the wavelength to be separated is not specified, the message of "Invalid unmixing definition no unmixing elements defined." appears.

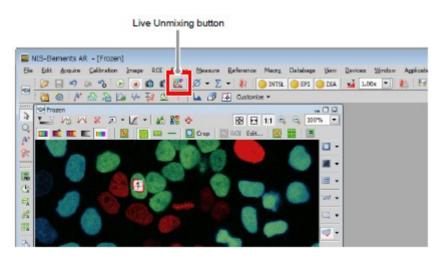
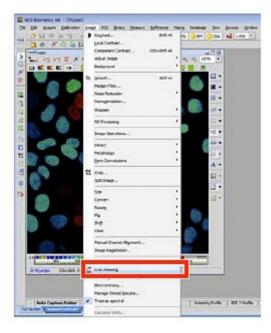
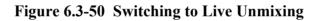


Figure 6.3-49 Live Unmixing

* Alternative method of switching to Live Unmixing As shown in the figure on the right, select [Image] -> [Live Unmixing] on the menu bar.





7. Click the [Live] button, the live image is switched to the Unmix live image.

	•				
Acquire	Filter and Dye				
e Live	0.00	Detector	Spectral	🗌 Ge	se mechanical shutter during exper
2 Dec	EvePart	Ch series	None *	Løser	
Find Node	•		DAPI	408.0	Resolution 10.0
			Abx546	543.5	Channels 32
2° XY		-			Sinning 1

Figure 6.3-51 Acquiring the Unmix live image

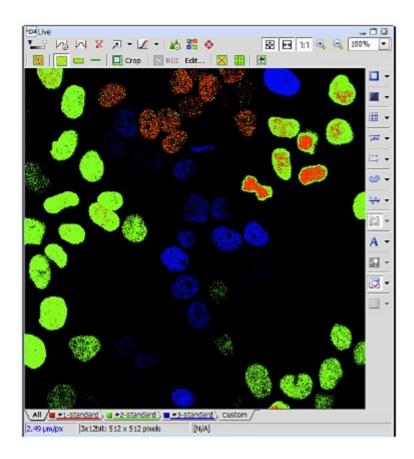


Figure 6.3-52 Live Unmixing

* Click the [Live Unmixing] button or select [Image] -> [Live Unmixing] on the menu bar again to return to the regular live image.

6.3.6 Blind Unmix

Automatically search for typical spectra and display an Unmix image separated by the spectral wavelength information.

If wavelengths overlap (because multiple fluorescence dyes are in use) and differences are hard to identify, wavelength information can be separated and displayed.

Blind Unmix allows automatic separation by specifying the number of classifications or separation without specifying the number of classifications by using [Auto Search.]

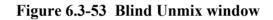
6.3.6.1 Displaying the Blind Unmix image

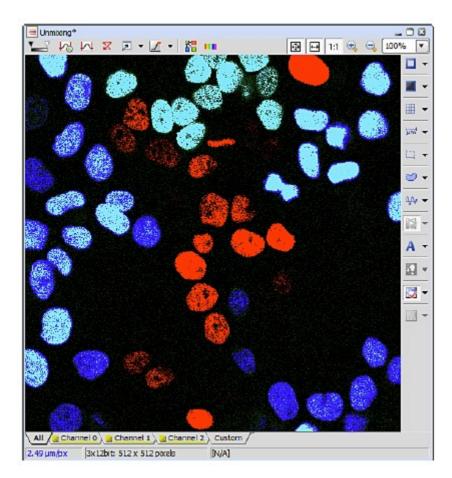
- 1. Open the Blind Unmix window while the acquired spectral image is displayed. Select [Image] -> [Blind Unmixing...] on the menu bar.
- 2. To specify the number of classifications, select one from [2] to [4] radio button in the Number of Classifications pane.

Select [Auto Search] radio button when not specifying the number of classifications.

3. Click the [Find] button to execute the Blind Unmix. On completion of Blind Unmix, an image window opens for the image unmixed with the detected spectra.

Blind Unmixing	
Number of Classifications	
 2 3 4 Auto Search 	
Background 0	
Remainder Find	





6.3.6.2 Setting for Blind Unmix window

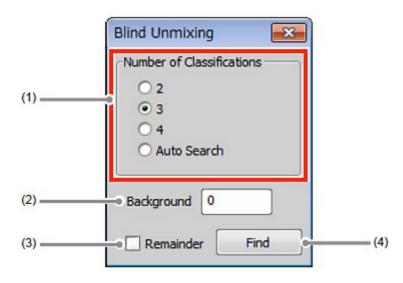




Table 6.3-3	Summary of Blind	Unmix window	functions
-------------	------------------	--------------	-----------

	Name	Function
(1)	Number of Classifications	Allows you to select the number of classifications for automatic separation of spectral wavelength information.
		Number of Classifications [2] to [4]: Automatically separate the spectral wavelength information by the specified number of classifications.
		Auto Search: Automatically separate the spectral wavelength information without specifying the number of classifications.
(2)	Background	Allows you to set the threshold for elimination of the background offset noise. 0 to 4095 is specifiable.
		For a 16-bit spectral image, 0 to 65535 is specifiable. (Specifying the maximum value causes all to be regarded as background offset noise and no spectral wavelength information to be detected.)
(3)	Remainder	This function enables calculation of remainder data in the Unmixing calculation. When selected, the remainder data is shown as an image in
		the Unmixing calculation result. When deselected, the remainder data is not shown.
(4)	Find	Starts automatic detection of the spectral wavelength information.

6. Detection Mode SD

Page 53 of 53

7 Detection Mode VF

This chapter describes the settings for the Virtual Filter mode (VF).

The Virtual Filter is a function that provides up to four binning groups for up to 32 channels spectral data and adjusts brightness of each group.

7.1 Filter and Dye Window

This window enables to set the Optical path.

The Virtual Filter detection mode can be used when the optical path changeover lever on the C2 scan head is set to the [Spectrum] position and the Virtual Filter (VF) is selected as the detection mode in the Optical path window.

7.1.1 Structure of Filter and Dye Window



Figure 7.1-1 Filter and Dye window (VF-use)

Table 7.1-1 Functions of Filter and Dye window (VF-use)

	Name	Function
(1)	Detector	Indicates that the Virtual Filter detection mode [VF] is used when the optical path changeover lever on the C2 scan head is set to the [Spectrum] position and the Virtual Filter (VF) is selected as the detection mode in the Optical path window.
(2)	Setting button	Opens the Optical path window. To use, select the detector, the dichroic mirror, the channel, fluorescence dye for each channel, laser and others.
(3)	Status	Indicates for the settings for each channel (fluorescence dye name, laser wavelength, and wavelength band to be acquired).
(4)	TD	Indicates the status of the motorized transmitted detector.
(5)	TD IN/OUT button	Sets/removes the motorized transmitted detector in/from the optical path. (IN = Set in the optical path/ OUT = Remove from the optical path)

		As for the case where the TD IN/OUT button is not displayed, it will be displayed when the motorized transmitted detector is set in the optical path in the Optical path window.
(6)	Close mechanical shutter during experiment	If unchecked, the shutter remains open during the ND image acquisition. As the shutter is not opened/closed every image acquisition, the
		time for the image acquisition can be shortened.
		* During the interval period, laser power is automatically changed to the minimum but the laser cannot be shut off completely because the shutter is left open.
(7)	Eye Port button	Changes optical path to eye port. Each function on the C2plus Settings window becomes non- selectable when the Eye Port button is selected.

• Optical Configuration

Individual data items set in the Virtual Filter mode (VF) can be managed collectively with the Optical Configuration window. For storing and retrieving the [Optical Configuration] settings, see the sections concerning the optical configuration in the "NIS-Elements AR (Advanced Research) User's Guide."

• Time-lapse image acquisition using the Virtual Filter (VF) mode

Detector setting change takes time because the spectral detector is used in the Virtual Filter (VF) mode. For this reason, the frame time may be longer than the estimated time.

7.1.2 Setting the Optical Path

Click the [Setting] button of the Filter and Dye window to display the Optical path window. The Virtual Filter detection mode [VF] setting screen is displayed when the optical path changeover lever on the C2 scan head is set to the [Spectrum] position and the Virtual Filter (VF) is selected as the detection mode in the Optical path window.

There are two modes available for Optical path setting, [Auto] and [Manual]. Normally, the auto mode should be used.

	Film and Da					
Catting button	0.00	rite Deteck	- Inter		ose mechanical shut	tter during experiment.
Setting button	100	Eve Part Chiserie	S None *	Later	Envisaion	
		Oti	DAPE	405.0	405-485	
		Ch2	РЛС	488.0	485-545	
		(ins	mineydev	468.0	949-999	
		014	Alexa Ruor 680 R-shycoerythric	561.0	\$\$\$.725	
		TD				

Figure 7.1-2 Filter and Dye window (VF-use)

Setting	and an and a second
LU-NV	Detrotor Dye & Spectral Setting
435	Resolution (20.0 * • • • • •
	Damela 22 *
	bring 1
44 6	Setting Node
534 0	Auto Sart by Preparation Provide Provi
532	
201	12 Och DAP: • 05-65
24	2 0 012 FTC • (48.000 *
	2 0 03 Henrykev • 481.000 *
	2 ChR Alexa Ruor 68 • 961.000 • -
	Detector Selection
Pir	holo Spectral Free Bond (VF)
1st Dichroic Mirror	
85 22,80 ×	
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Sca.	ener Unit
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Objective	
Callective	
	Sample Sample
Transmitted	
Detector	
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	OK Geneel

Figure 7.1-3 Optical path window (for auto mode, VF-use)

## 7.1.3 Optical Path Window

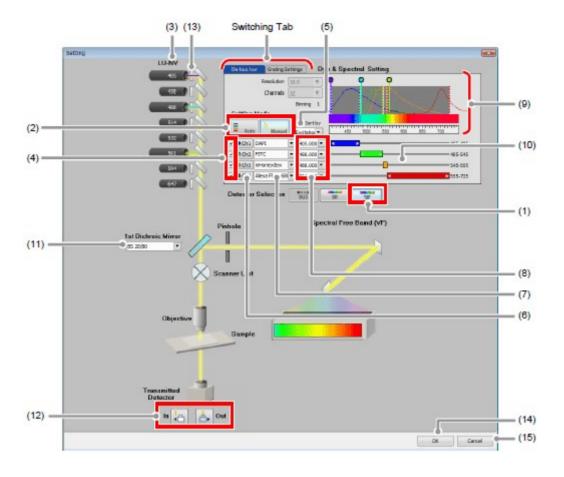


Figure 7.1-4 Optical path window (for manual mode, VF-use)

 Table 7.1-2
 Functions of Optical path window (VF-use)

	Name	Function
(1)	Detection mode selection button	Enabled to select the Virtual Filter mode (VF). Binning is performed for the spectral data of the concurrent 32 channels to group it into up to four groups, enabling acquisition of an image of light of a specified wavelength range.
(2)	Mode selector	Selects the desired mode for setting the Optical path.
		Auto - Activates the auto mode. Once the fluorescence dye to be used is selected, the appropriate laser, the dichroic mirror, and the wavelength range acquired from the virtual channel are automatically selected.
		Manual - Activates the manual mode. Enables to set all of the laser, the dichroic mirror, and the wavelength range acquired from the virtual channel to be used manually.
(3)	Excitation laser indicator	Displays the current setting for the laser.

		The currently set laser icon is displayed in a large size, and the optical path is indicated.
(4)	Channel selection check box	Enables to select the channels to be used. (Up to 4 channel.)
(6)	Sorting fluorescence dye list	Sorts the fluorescence dye list according to the selected type.
		<b>ABC</b> : Displays the list in alphabetical order.
		<b>Emission</b> : Displays the list in the order of peak wavelength of fluorescence intensity.
		<b>Excitation</b> : Displays the list in excitation wavelength order.
(6)	Channel color setting button	Displays the Color Selection window, enables to set the desired color for each channel.
(7)	Fluorescence dye selection/input:	<b>In auto mode -</b> Selects the fluorescence dye name to be used for each channel.
		<b>In manual mode -</b> Selects the in-use fluorescence dye name for each channel or enters an arbitrary channel name.
(8)	Excitation laser select	These menus are only effective while in the manual mode. Enables to set the laser wavelength that is set with the software configuration, regardless of the setting of the Filter cube display/select.
(9)	Rainbow chart	Provides the following information:
		<ul> <li>Wavelength band for which to acquire images (shown in color and value for each channel)</li> <li>Spectral profile of fluorescence dye</li> <li>Excitation laser for fluorescence dye</li> <li>A color band indicating the wavelengths in the entire band (400 to 750 nm)</li> <li>Scale of the wavelengths in the entire band (400 to 750 nm)</li> </ul>
(10)	Acquisition range for each virtual channel slider bar	Specifies the laser wavelength range to be acquired for each virtual channel.
		* When shifting the slider bar in Auto mode, the Mode selector changes to manual mode.
(11)	1st Dichroic mirror select	This menu is only effective while in the manual mode. Enables to manually select the 1st Dichroic mirror to be used.
(12)	Motorized transmitted detector	

selection button	When the motorized transmitted detector is in use, brings the transmitted detector into the Optical path, to enable the ability.
	Brings the transmitted detector out of the Optical path, to disable the ability.
(13) ND filter installation icon	An icon is displayed on the line of laser with ND filter installed.
	<ul> <li>* This icon indicates whether ND filter is installed or not, and does not indicate insertion or removal of ND filter (IN = Insert in the optical path, OUT = Remove from the optical path).</li> <li>Insertion or removal of ND filter is performed on the Acquisition window.</li> </ul>
(14) OK button	Confirms the Optical path settings applied and closes the Optical path window.
(15) Cancel button	Discards the Optical path settings applied and closes the Optical path window.

• About switching between SD and VF

**SD** -> **VF**:

The last settings in the Virtual Filter mode (VF) are recalled.

**VF -> SD:** 

The last settings in the Spectral Detector mode (SD) are recalled.

• About the setting condition when the setting mode is switched

Auto mode -> Manual mode: The entire settings in the Auto mode are retained.

Manual mode -> Auto mode:

The fluorescence dye with the same channel name as set in the manual mode is automatically selected.

If the same fluorescence dye name does not exist in the list, a fluorescence dye detectable by the laser wavelength is automatically selected from the list.

In the Auto mode, the resolution and the number of channels are automatically set so as to accommodate the wavelength range to detect all of the selected fluorescence dyes.



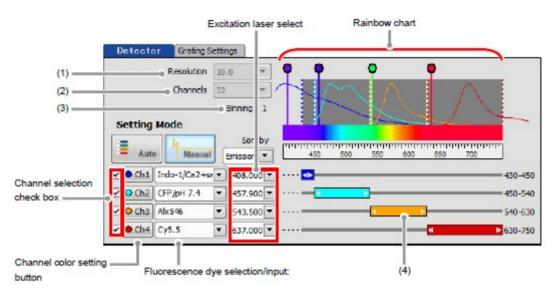


Figure 7.1-5 Optical path window (Detector tab)

#### Table 7.1-3 Functions of Detector tab

	Name	Function
(1)	Resolution	Displays the wavelength resolution currently set.
(2)	Channels	Displays the number of channels (number of PMTs) currently set.
(3)	Binning	The number of channel binning is fixed to 1.
(4)	Acquisition range for each virtual channel slider bar	Specifies the laser wavelength range to be acquired for each virtual channel.
		The wavelength range can be overlapped between channels. The settable range is the grating range (the gray zone indicated in the rainbow chart).

#### 7.1.4.2 Grating Settings Tab

The [Grating Settings] tab is displayed only when the manual mode is selected at setting mode. Set the range for grating and set the wavelength range for the channels selected within the range.

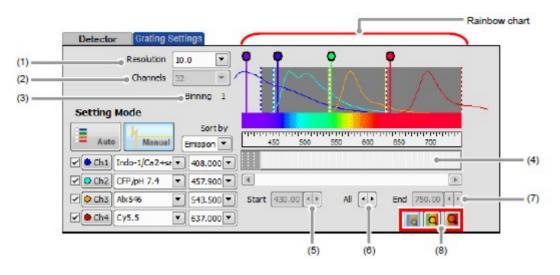


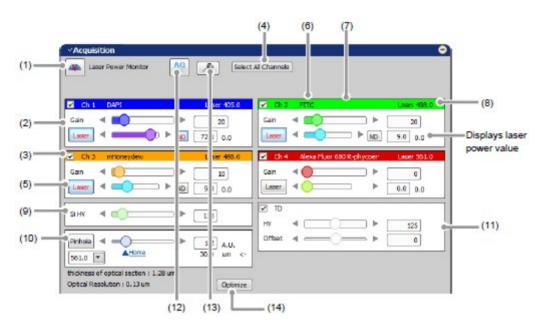
Figure 7.1-6 Optical path window (Grating Settings tab)

	Name	Function
(1)	Resolution	Selects a wavelength resolution. Selectable from 2.5, 5, or 10 nm.
(2)	Channels	The number of channel is fixed to 32.
(3)	Binning	The number of channel binning is fixed to 1.
(4)	Grating range setting bar	Sets a wavelength range in a wavelength range from 400 nm to 750 nm. The range depends on the grating resolution. It is shiftable horizontally but the width of the bar cannot be
		reduced.
(5)	Start	Displays the start wavelength of the Grating range currently selected. The right and left buttons cannot be used when the Virtual Filter mode is selected.
(6)	All	In the currently selected wavelength range, enables shifting to the right or left in units of 0.25 nm without changing the width of the wavelength.
(7)	End	Displays the end wavelength of the Grating range currently selected. The right and left buttons cannot be used when the Virtual Filter mode is selected.
(8)	Enlarge button	Enlarges the rainbow chart. The display is switched in three levels.

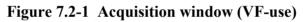
* If the grating range is changed in the Grating Settings tab, return to the Detector tab and reset the acquisition range for each virtual channel.

## 7.2 Acquisition Window

The Acquisition window enables to set PMT brightness (detection sensitivity), laser power, and pinhole size.



#### 7.2.1 Structure of Acquisition Window



Tahle 7 2-1	Functions of Acquisition window (VF-use	0)
1 <i>uble</i> 7.2-1	Tunctions of Acquisition window (VT-use	<i>ב</i> )

	5	1
	Name	Function
(1)	Laser power monitor button	Displays the laser power value (integer obtained after A/D conversion divided by 10) of the current channel by clicking this button. During the image acquisition, the laser power cannot be measured and this button is grayed out.
(2)	Brightness adjustment for each channel	For each of the virtual channels, use the Gain Laser and ND filter IN/OUT controls to adjust the brightness of the live image.
(3)	Channel selection	Selects the virtual channels (Ch1 to Ch4, and/or TD) to acquire the desired images. Do this by adding a check mark. When acquiring the transmitted image (TD image) only, see
		Section 7.2.1.2, "When Acquiring Transmitted Image Only."
(4)	Select All Channels button	Selects all channels for acquiring images.
(5)	Laser ON/OFF button	Selects whether the laser is emitted or not.
		* When LU-NV is in use, this button is grayed out and is disabled while the button on the front panel of the laser unit is OFF or blinking.

1	Laser
c	N status

Lase

The laser is emitted.

_	The AOTF shutter closes and the laser power value	
er	becomes 0.	
	When gwitched from OFE to ON the loger newson	

OFF status When switched from OFF to ON, the laser power value set in the previous ON status is applied.

(6) Fluorescence dye The fluorescence dye name specified in the Optical path name indication window is indicated. (7) Channel color Displays the channel color specified in the Optical path window. (8) Laser wavelength The currently selected laser wavelength is indicated. indication (9) Si HV Adjusts HV of the Spectral detector. (10) Pinhole Adjusts the pinhole size. For pinhole size, see Section 7.2.3, "Setting the Pinhole." (11) Brightness adjustment For the transmitted detector, use the HV and Offset controls to for transmitted adjust the brightness of the live image. detector (12) AG button Automatically adjusts the Si HV value (Si HV gain) of the currently selected channel to the optimum values. For Auto Gain, see Section 7.2.4, "Auto Gain."

 (13) Auto Gain setting button
 Sets the ratio of saturation pixels used for automatic Si HV gain correction. The window for range of the ratio of saturation pixels settings appears when this button is clicked.

For setting for ratio of saturation pixels, see "Setting for ratio of saturation pixels" in the Section 7.2.4, "Auto Gain."
(14) Optimize button Displays the XYZ Size Setup window.

In the XYZ Size Setup window, the calculation method of the recommended values of the resolution, zoom magnification, and Z stack step size can be set.

For the XYZ Size Setup window, see Section 7.2.1.1, "Recommended Value Indication/Automatic Application."

#### 7.2.1.1 Recommended Value Indication/Automatic Application

By the function of the recommended value indication/automatic application, the recommended values of the appropriate resolution, zoom magnification, and Z stack step size are calculated based on the objective type and the selected excitation wavelength.

Using the calculated recommended values enables the image acquisition clearer and with less damage to the sample.

**Recommended Value Automatic Application** 

To automatically apply the recommended values to the parameters, set the [Nyquist XY] button of the Scan Area window to ON.

C2plus Scan Area 🗴	
🧕 🚥 — Crop R.C.I Edit 🔀 🚟 🖽	
Zoom: 0	Numiet VV In the
Pixel size: 0.62 Nyquist XY	
Scan size: 512 💌 Rotation: 0 🔀	
Width: 512 Height: 512	
Dwel time: 1.9 µs	
Pixel size: 0.62 µm Optical resolution: 0.13 µm	
Z step size: 0.42 µm Optical sectioning: 1.26 µm	

Figure 7.2-2 Scan Area window

	Scan setting	*		Zoom	• .000	
Indicates the recommended value of the resolution.	Scan Size Scan Speed	512 • -	512 S12 recommend Frame/sec/Porel Dwell 14.8 u	sec)	3X C	Indicates the recommended value of the scan
		and the second se	bolical section : 1.28 um ubon : 0.13 um	1.2 A.U, 30.0 um <-		magnification.

#### Figure 7.2-3 Location of Recommended Value Indication

* When the laser or objective in use is changed, the recommended values are recalculated, and newly indicated and automatically applied.

**Recommended Value Settings** 

Detailed settings of the recommended values are made in the XYZ Size Setup window that is displayed by clicking the [Optimize] button of the Acquisition window.

If the [Nyquist XY] button of the Scan Area window is ON, the recommended values are automatically applied to the parameters.

Or if the [Nyquist XY] button is OFF, the recommended values of the scan size and zoom are indicated in the Scan setting window.

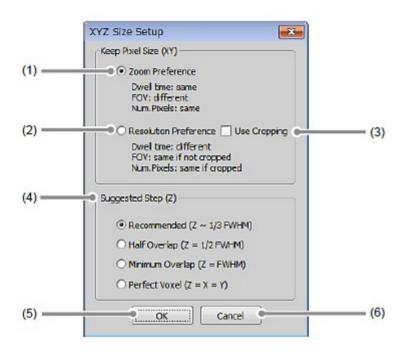


Figure 7.2-4 XYZ Size Setup window

Table 7.2-2 Functions of XYZ Size Setup window

	Name	Function	
(1)	Zoom Preference		XY] button is ON, keeps the scan size ommended value of the zoom.
(2)	Resolution Preference		XY] button is ON, keeps the zoom and nended value of the scan size.
(3)	Use Cropping	Fits the scan size in	detail by using Crop Scan.
		Sets the Z step size	calculation method.
		Recommend (Z ^{-1/3} FWHM)	Approximately one third of the thickness of optical section (FWHM value).
(4)	Suggested Step (Z)	Half Overlap (Z=1/2 FWHM)	One half of the thickness of optical section (FWHM value).
		Minimum Overlap (Z=FWHM)	The thickness of optical section (FWHM value).
		Perfect Voxel (Z=X=Y)	Value same as the pixel size.
(5)	OK button	Confirms the XYZ	Size Setup applied and closes the XYZ

		Size Setup window.
(6)	Cancel button	Discards the XYZ Size Setup applied and closes the XYZ Size Setup window.

#### 7.2.1.2 When Acquiring Transmitted Image Only

By using the TD channel, you can acquire the image with the TD channel only.

- 1. Display the Optical path window. Select a channel for the laser power control and deselect other unnecessary channel's check box.
  - * Even when the multiple channels are selected in the Optical path window, the image with the TD channel only can be acquired by deselecting the unnecessary check box in the Acquisition window. In this case, the laser power value of the channel that is deselected at last is used for acquiring the TD image.



Figure 7.2-5 Optical path window (VF-use)

2. Set the transmitted detector into the optical path and then click the [OK] button to close the Optical path window.



Figure 7.2-6 Optical path window (VF-use)

- 3. Use the HV and Offset controls of the transmitted detector to adjust the brightness of the live image.
- 4. Use a channel that remains to "On" to control the laser power to set the laser power value.
- 5. Deselect the unnecessary check boxes other than TD.

	Acquisition  tase Power Norman  M  Select Al Channels	D
Deselect this check = box at last. Set the laser power = value.	Ch1         Loss         All           Cdn         Image: All         Image: All         Image: All           Care         Image: All         Image: All         Image: All         Image: All           Prinde         Image: All         Image: All         Image: All         Image: All         Image: All           Prinde         Image: All         Image: All	Adjust the transmitted detector.

Figure 7.2-7 Acquisition window (VF-use)

6. Acquire the image.

#### 7.2.2 Setting Image Brightness

For the live images of each Virtual channel, adjust Gain, Laser, ND filter IN/OUT, Si HV, HV (TD), and Offset (TD) to obtain clear images.

Sain	Gain 4 20
	Laser 4 - ND 9.0 0.0
Ch 3 mtioneydew Laser 488.0	Ch 4 Alexa Pluor 080 R-phycoler Laser 351.0
San 4 000 b 10 Leee 4 - 00 6.0 0.0	Sain ◀ ●
ahv ∢ đ	

Figure 7.2-8 Setting the live image brightness (VF-use)

<i>Table 7.2-3</i>	Brightness	adjustment	functions for	or the live	image (VF-use)
	0		/ /		0 (

	Name	Function
(1)	Gain	Sets the PMT Gain.

## **Slider bar:** Slides to the right or left to set the gain value.

		Arrow buttons: Click either arrow button to increase or decrease the gain value stepwise.
		<b>Direct entry in gain value display field:</b> Type the desired setting value.
(2)	Laser (*)	Sets the laser power value.
		Slider bar: Slides to the right or left to set the laser power value.
		Arrow buttons: Click either arrow button to increase or decrease the laser power value stepwise.
		<b>Direct entry in laser power value display field:</b> Type the desired setting value.
(3)	Si HV	Adjusts HV of the Spectral detector. (Applied to all Virtual channel groups.)
		Slider bar: Slides to the right or left to set the Si HV value.
		Arrow buttons: Click either arrow button to increase or decrease the Si HV value stepwise.
		<b>Direct entry in Si HV value display field:</b> Type the desired setting value.
(4)	HV	Sets the voltage to be applied to the transmitted detector.
		Slider bar: Slides to the right or left to set the HV value.
		Arrow buttons: Click either arrow button to increase or decrease the HV value stepwise.
		<b>Direct entry in HV value display field:</b> Type the desired setting value.
(5)	Offset	Sets the offset value of the transmitted detector.
		Slider bar: Slides to the right or left to set the offset value.
		Arrow buttons: Click either arrow button to increase or decrease the offset value stepwise.

#### Direct entry in offset value display field:

Type the desired setting value.

 (6) ND filter IN/OUT Inserts/removes the ND filter in/from the optical path. (IN = Insert in button (*)
 the optical path/ OUT = Remove from the optical path) This button is displayed only for lasers that can control insertion/removal of ND filter.

**PMT Overload** 

If too much gain is applied to the illumination intensity, the gain is automatically shut down to protect PMT and/or transmitted detector (TD), and then following PMT Overload dialog box is displayed.

Dialog		×
Ao	cqCode: 0; EventCode: 2; Nessage: SpectrumPMT O	verload
	[DK] Cancel	

Figure 7.2-9 PMT Overload dialog box

#### 7.2.3 Setting the Pinhole

Laser Power Monitor	M 🔊 (	Select Al Channels	
Ch 1 DAPI	Laser 405	0 Ch 2 FCTC	Laser 488
Gain 4	► 20	can 4	► 20
	0 1 10.0		■ ND 9.0 0.0
Gain 4	Laser 430	Gan 4	0 R-phycoer Laser 561
Laser 4	□ ▶ ND 9.0 0.0	Locer 4	□ ▶ 0.0 0.0
9HV 4 (C)	> ▶ [133]	01 V	
			125
Pinhole	===> ► [1,2] ∧ u 30.0_ un		• 0
561.0			
<ul> <li>thickness of optical sec in : 1</li> <li>Optical Resolution : 0.1 un</li> </ul>	Contraction of the second	nize	

^{*} When LU-NV is in use, this function is grayed out and is disabled while the button on the front panel of the laser unit is OFF or blinking.

#### Figure 7.2-10 Setting the Pinhole (VF-use)

Table 7.2-4	Pinhole setting functions	(VF-use)
-------------	---------------------------	----------

	Name	Function
(1)	Pinhole size setting	Sets a pinhole size for C2 system.
	C	Slider bar:
		Slides to the right or left to set the pinhole size. (Unit: A.U.)
		Arrow buttons:
		Click either arrow button to increase or decrease the pinhole size stepwise.
		<b>Direct entry in pinhole size display field:</b> Type the desired setting value.
(2)	Pinhole button	Displays the A.U. Calculation Settings window to calculate the pinhole size.
		(For A.U. Calculation Settings, see Section 7.2.3.1, "Calculation Settings for Pinhole Size.")
(3)	Home	Changes the pinhole to the predetermined home position. The value of the home position can be changed in the A.U. Calculation Settings window.
		(For A.U. Calculation Settings, see Section 7.2.3.1, "Calculation Settings for Pinhole Size.")
(4)	Pinhole size	Indicates pinhole size of C2 system. (Unit: µm)
(5)	thickness of optical section	Indicates the FWHM (full width at half maximum) of z airy disk.
(6)	Optical Resolution	The actual size of 1 pixel square calculated from the optical information (for objectives and scan parameters) and the size acquired from an image.
(7)	Reference excitation wavelength for the pinhole size calculation	Selects the excitation wavelength as the reference of the automatic calculation of the pinhole size from the laser wavelengths, or enter it manually in the A.U. Calculation Settings window. (For A.U. Calculation Settings, see Section 7.2.3.1, "Calculation Settings for Pinhole Size.")

#### 7.2.3.1 Calculation Settings for Pinhole Size

This section describes the setting window for calculating the pinhole size.

Click the [Pinhole] button in Acquisition window, the A.U. Calculation Settings window appears. (Usually, the [Recommend] is selected to enable automatic calculation. [Recommend] calculates the A.U. value by using the Nikon-recommended EM and NA values.)

	A.U. Colculation Settings	
) —	Recormend	
) (	EM:enission wavelength[.un]     ③ Selected Laser wavelength + 40[nm]	
	Selected Laser wavelength 488.0 nm	
3) ———	Manual 400.0 nm     NA:Numerical Aperture     NA_obj(NA of objective lans)     ⊙ Min(NA_ob), NA_semple)	
)	● "Ary" Home Position 1.2 A.U.	
5)	OK Cancel	(7)

## Figure 7.2-11 A.U. Calculation Settings window

Table 7.2-5         A.U. Calculation Settings window	ЭW
------------------------------------------------------	----

	Name	Function
(1)	Select calculation method	<b>Recommend -</b> Sets parameters automatically. (Nikon recommended)
		<b>User Setting -</b> Allows the user to manually set parameters.
(2)	EM:emission wavelength[µm]	Selected Laser wavelength - Calculates parameters by using the laser wavelength selected in the pinhole combo box of the Acquisition window as the emission wavelength (EM value). The wavelength displayed in the combo box is to be the laser wavelength set in the Optical Setting window.
		Manual - Allows the user to manually set parameters. (The parameter is calculated with the input value as the emission wavelength (EM value).) Enter the value directly from the keyboard.
(3)	NA:Numerical	Sets refractive index of the objective.
	Aperture	<b>NA_obj(NA of objective lens)</b> - Regardless of whether or not the objective NA value exceeds the refractive index of the sample, executes calculation by using the objective NA as the calculation parameter.
(4)	"Airy" Home Position	<b>Min(NA_obj, NA_sample)</b> - When the objective NA value does not exceed the refractive index of the sample, executes calculation by using the objective NA as the calculation parameter. When the objective NA value exceeds the refractive index of the sample, executes calculation by using the sample refractive index.
(4)	"Airy" Home Position	Sets a home position of pinhole. Enter the value directly from the keyboard.
		The pinhole size can be selected from six types in C2. Therefore, if the entered value does not match any of the types,

		e size that is larger than and the closest to the entered value is set as the home position.
(5)	Keep A.U. check box	When checked, the pinhole size is fixed by the A.U. when the selected wavelength or objective is changed. (However changes by the um.) When unchecked, the pinhole size is fixed by the um. (However changes by the A.U.)
		The pinhole size can be selected from six types in C2. Therefore, if the to-be-fixed A.U. value does not match any of the types, the size that is larger than and the closest to the A.U. value is selected.
(6)	OK button	Confirms the A.U. Calculation Settings applied and closes the A.U. Calculation Settings window.
(7)	Cancel button	Discards the A.U. Calculation Settings applied and closes the A.U. Calculation Settings window.

#### 7.2.4 Auto Gain

Auto Gain is a function to automatically correct the value of Si HV gain to set the optimum image brightness.

Automatic Si HV gain correction is performed within the predetermined range of the ratio of saturation pixels.

Automatic Si HV gain correction is performed only Si HV. For a TD, automatic adjustment is performed when it is selected.

After execution of Auto Gain, in the window indicating the progress of Auto Gain, the correction values actually used (ratio of saturation pixels) are displayed. If Auto Gain failed, "x" is indicated and the Si HV value returns to its original value.

- When setting the line scan, Auto Gain cannot be executed.
- During execution of Auto Gain, do not perform manual adjustments in the Acquisition window.

Channela
Ch 2 FETC Laser 483.0
Cain 4 20
Ch 4 Alexa Huor 680 R-phyccer Laser 551.0
Gain ◀ ● 0.0 0.0
2 TD HV ◀ ▶125
offset 4 b

Figure 7.2-12 Execution of Auto Gain (VF-use)

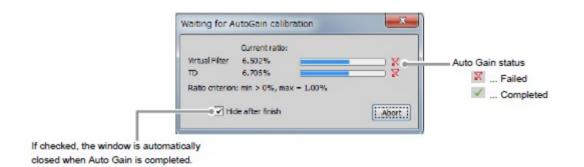


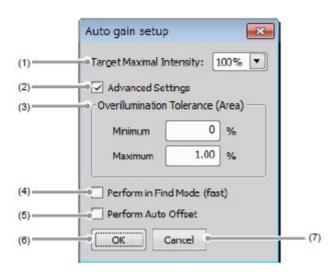
Figure 7.2-13 Auto Gain progress

Setting for ratio of saturation pixels

Set the maximum and minimum value for the ratio of saturation pixels used for automatic Si HV gain correction.

Click the [Auto Gain Setting] button to display the Auto gain setup window. Set the maximum and minimum value for the ratio of saturation pixels in the Auto gain setup window.

Laser Power Monitor	AG B Select	All Channels	
-			
Ch 1 DAPI	Laser 405.0	Ch 2 FITC	Laser 488.0



#### Figure 7.2-14 Displaying the Auto gain setup window



i delle i 2 o selling jei i dille of salla allon palets	<i>Table 7.2-6</i>	Setting for rat	io of saturation	pixels
---------------------------------------------------------	--------------------	-----------------	------------------	--------

	Name	Function
(1)	Target Maximal Intensity	Specifies the application ratio of the setting of the ratio of saturation pixels.
		Sets the percentage (%) of the maximum value to be applied.
(2)	Advanced Settings	If checked, advanced settings of the ratio of saturation pixels are enabled.
(3)	Overillumination	Minimum Sets the minimum value for ratio of saturation pixels.
	Tolerance (Area)	Maximum Sets the maximum value for ratio of saturation pixels.
(4)	Perform in Find Mode (fast)	If checked, execution in the Find mode is enabled.
(5)	Perform Auto Offset	If checked, the offset value is set automatically.
(6)	OK button	Confirms the settings of Auto gain setup applied and closes the Auto gain setup window.
(7)	Cancel button	Discards the settings of Auto gain setup applied and closes the Auto gain setup window.

## 7.3 Various Views (Virtual Filter mode-use)

This section describes various Virtual Filter mode (VF) views.

#### 7.3.1.1 Channel Mixed View

Images acquired in the Virtual Filter mode (VF) are displayed in the method suitable to the purpose.

#### All image

The [All] tab is selected, all the virtual channels are mixed to display.

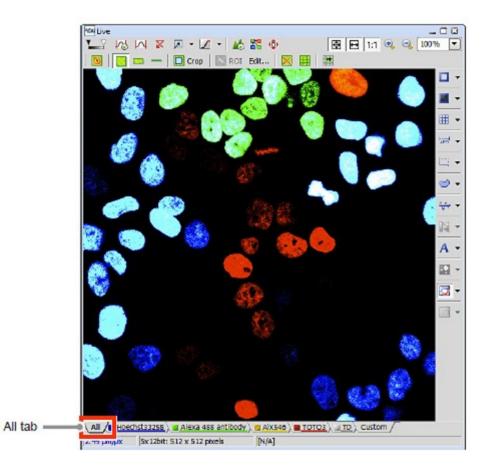


Figure 7.3-1 All image

Each channel image

To display the image of each virtual channel, select the tab corresponding to the channel.

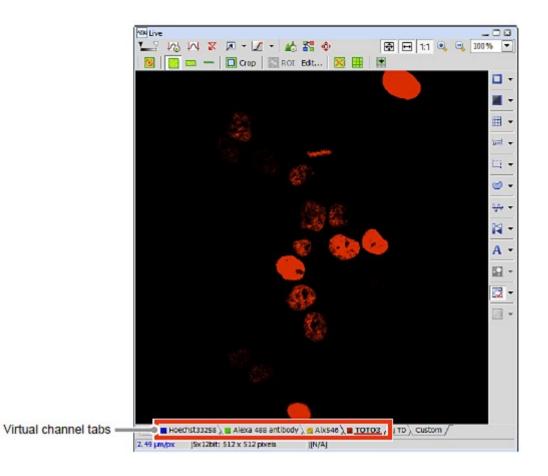


Figure 7.3-2 Each channel image

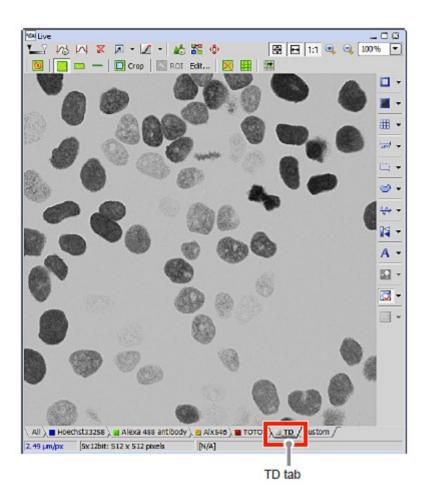


Figure 7.3-3 TD image

#### **Custom image**

Custom image displays a mixed image of selected multiple channels. To change channels to be mixed, re-select channels.

Right-click on the [Custom] tab and a menu appears. Select [Properties...] on the menu. The Custom window appears to allow you to change the channels for the Custom View.

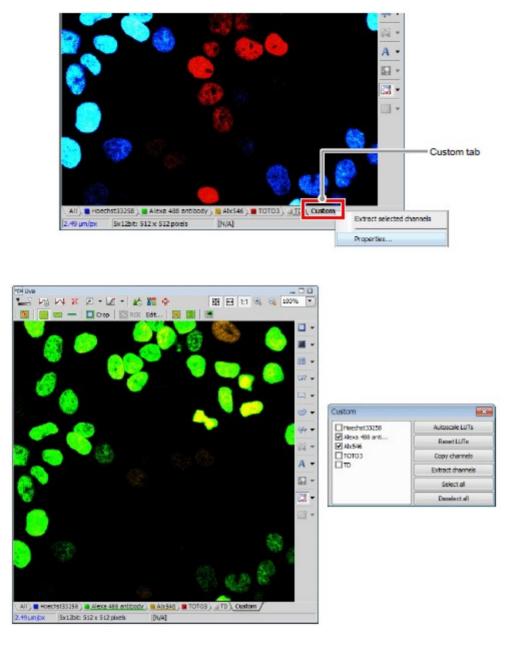


Figure 7.3-4 Selecting channels (Custom image)

**Ratio Image** 

The Ratio image view is displayed.

Right-click on the window to display a menu. Selecting [Ratio View] from the menu changes the window to the Ratio image.

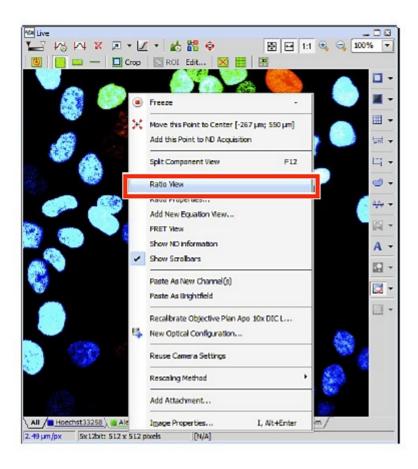


Figure 7.3-5 Displaying the Ratio image view

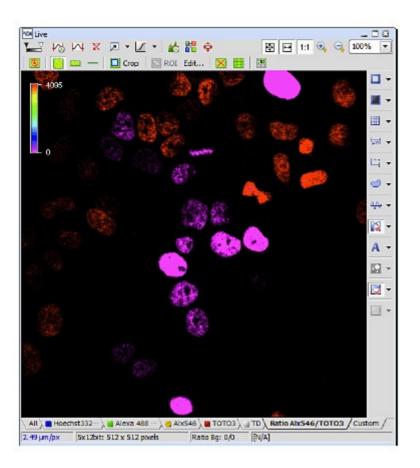


Figure 7.3-6 Ratio image

* You can change the combination of channels to be displayed in the Ratio View. Right-click on the window and a menu appears. Select [Ratio Properties...] on the menu. The Ratio Properties window appears to allow you to change the channels for the Ratio View.

hannels —		
Numerator:		44
enominator:	Alx546 - Background: 29	98
atio Range — Min: 0	Max: 14.419 Autor	

#### 7.3.1.2 Split Channel View

Virtual channels are split into respective channels and displayed.

Click the [Split Components] button.

"All image" mixing all channels, respective channel images, "TD image," "Ratio image," "Custom image" are displayed.

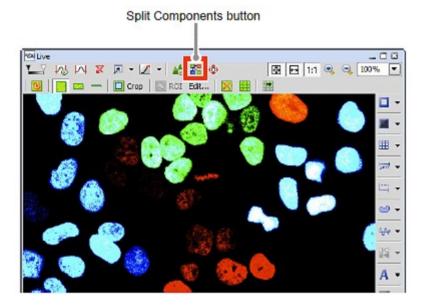


Figure 7.3-8 Frozen window

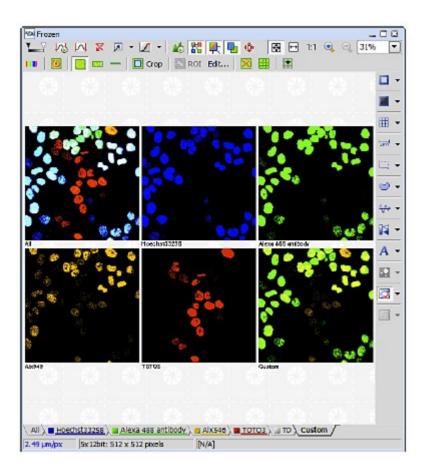


Figure 7.3-9 Split channel view

* For switching from Split channel view to Channel mixed view, click the [Split Components] button again.

## 8 Scan Setting Window

This window enables to set scanning conditions, such as resolution, scan speed, and magnification. The setting items in the Scan setting window vary, for example depending on the scan area.

In the Standard Detector mode [DU3], imaging is possible with 2048 x 2048 pixels at maximum. In the Spectral Detector mode [SD] and Virtual Filter mode [VF], imaging is possible with 1024 x 1024 pixels at maximum.

## 8.1 Structure of Scan Setting Window



Figure 8.1-1 Scan setting window

Table 8.1-1 Summary of functions in Scan setting window

Name	Function
(1) Scan setting	Sets conditions for scanning. For details of scan settings, see Section 8.3, "Scan Settings."
	<b>Scan Direction:</b> Selects Unidirectional or Bidirectional scan.
	Scan Size: Selects a resolution. (Unit=pixel)
	<b>Fast button:</b> Turning on this button makes the scan speed high. When a Fast Galvano compatible model (C2plus) that supports high scan speed is in use, the scan speed is further increased by turning on the [Fast] button and set the scan magnification to 8X or higher.
	* For speeding-up of the scan speed, see Section 8.3.1, "Fast Mode."
	Selecting Scan Speed:

Selects a scan speed.

### Scan magnification:

		Sets a scan magnification.
(2)	Scan Zoom Reset button	Sets the scan magnification to 1.000.
(3)	Switch button for the previous setting	Returns to the previous setting.
(4)	Scan Area window button	Displays the Scan Area window. For details of Scan Area window, see Chapter 9.

# 8.2 Relationships among Scan Area Shape, Resolution, and Scan Speed

This section describes the relationship of the resolution and scan speed in the Scan setting window, and the relationship of the scan area shape set in the navigation mode versus the resolution and scan speed.

Relationship of resolution and scan speed

Once a Scan Size (resolution) is set, the software automatically generates a list (see Table 8.2-1) of the scan speeds available with that resolution, making them selectable from the Scan Speed pull-down menu.

For example, suppose you set the resolution for the Square scan area to X = 512 and Y = 512 pixels, and select the Unidirectional scan. Then, values listed in the Scan Speed pull-down menu are: 0.06, 0.125, 0.25, 0.5, 1, or 2 (enable selecting when Fast mode is turned on), or 4 (enable selecting at 8X or higher scan magnification and when Fast mode is turned on.)

- * The performance at the scan speed is not guaranteed. It varies depending on the environment.
- * When the Band scan area is selected in the Galvano scan mode, scan speed values listed in the Scan Speed pull-down menu are guideline, which may not be the actual scan speed.

**Retention of Scan Settings for Different Scan Areas** 

For each scan area shape, the latest Scan settings are retained.

Once a scan area is selected in the navigation mode, the navigation mode displays the scan area that has been set at last, and the Scan setting window displays the set values of Scan settings.

Automatic Change of Scan Settings with Change in the Band Scan Area Shape

If the ratio of X and Y lengths of the Band scan area is changed:

Resolution: Does not change Scan speed: Changes based on the new ratio of X and Y lengths, in a manner that gives the same pixel dwell.

<i>Table 8.2-1</i>	Combinations of resolution and scan speed (Square scan area)
	Resolution

			Resolution	011		
	64 (not available in VF mode)	128 (not available in VF mode)	256	512	1024	2048 (not available in SD and VF mode)
0.03					Uni-scan	Uni-scan
0.04					Uni-scan	Uni-scan
0.06				Uni-scan	Uni-scan	Uni-scan
0.125				Uni-scan	Uni-scan	Uni-scan
0.25			Uni-scan	Uni-scan	Uni-scan	Uni-scan
0.5			Uni-scan	Uni-scan	Uni-scan	
1		Uni-scan	Uni-scan	Uni-scan		
2		Uni-scan	Uni-scan	Uni-scan ^{*1} Bi-scan		
3				Bi-scan ^{*1}		
3.5			Bi-scan			
4	Uni-scan	Uni-scan	Uni-scan ^{*1}	Uni-scan ^{*2}		
6			Bi-scan ^{*1}			
7	Uni-scan	Uni-scan ^{*1}				
8			Uni-scan *2	Bi-scan *2		
12	Uni-scan ^{*1}					
15			Bi-scan ^{*2}			

Scan speed

#### Marks in the table

'1 mark indicates that selectable scan speed when Fast mode is turned on. (For C2 and C2plus)

⁵2 mark indicates that selectable scan speed at 8X or higher scan magnification and Fast mode is turned on. (For only C2plus)

: Indicates that the combination is unavailable in the SD and VF mode.

#### For Band scan area

• The scan speed list is automatically changed depending on the Y resolution.

Example 1.

For resolution is 512 pixel and 1/2 band scan, "0.125, 0.25, 0.5, 1, 2, 4^(*1), or 8^(*2)" are listed as the scan speed.

Example 2.

For resolution is 512 pixel and 1/4 band scan, "0.25, 0.5, 1, 2, 4, 7  $^{(*1)}$ , or 15  $^{(*2)}$ " are listed as the scan speed.

Example 3.

For resolution is 512 pixel and 1/6 band scan, "1, 2, 4, 8, 12, 18 ^(*1), or 50 ^(*2)" are listed as the scan speed.

(*1) Selectable scan speed when Fast mode is turned on. (For C2 and C2plus)

(*2) Selectable scan speed at 8X or higher scan magnification and Fast mode is turned on. (For only C2plus)

• Scan in the Virtual Filter mode (VF) When the Virtual Filter mode is selected, the scan time varies with the set number of channels. ("Time calculated by the displayed scan speed" x "number of channels")

## 8.3 Scan Settings

This section describes the Scan settings.

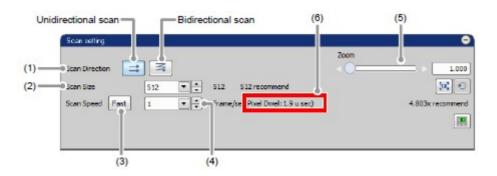


Figure 8.3-1 Scan settings

#### Table 8.3-1 Functions of Scan settings

	Name	Function
(1)	Scan Direction	Toggles between Unidirectional and Bidirectional scan. Bidirectional scan is only selectable if the Square scan area, Band scan area, or Line scan area is set. By default, Unidirectional scan is selected. Bidirectional scan is not available when the SD or VF mode is selected.
(2)	Scan Size	Sets the scan resolution in the X-direction. (Setting unit: Pixel) The resolution in the Y-direction is automatically calculated from the X to Y ratio of the scan area.
		Pull-down menu:
		Selects the desired resolution from this list.
		<b>UP and Down buttons:</b> Click these to select resolutions one after another.
(3)	Fast button	Turning on this button makes the scan speed high.
		When a Fast Galvano compatible model (C2plus) that supports high scan speed is in use, the scan speed is further increased by turning on the [Fast] button and set the scan magnification to 8X or higher.
		* For speeding-up of the scan speed, see Section 8.3.1, "Fast Mode."
(4)	Selecting Scan Speed	Sets scan speed. (Setting unit: Frame/sec or lines/sec in line scan mode)
		<b>Pull-down menu:</b> Selects the desired scan speed from this list.
		<b>UP and Down buttons:</b> Click these to select scan speeds one after another.
(5)	Scan magnification	Sets scan magnification.

#### Slider bar:

Slides to the right or left to set the scan magnification.

#### **Arrow buttons:**

Click either arrow button to increase or decrease the scan magnification stepwise.

Direct entry in scan magnification display field:

Type the desired setting value.

 (6) Pixel Dwell Indicates the laser irradiation time per pixel. This value is automatically confirmed from scan resolution and speed.

• If the Spectral Detector mode or the Virtual Filter mode is selected as the detection mode, the bidirectional scan cannot be executed.

Correcting the Image Shifting when Setting Bidirectional Scan

Image shifting correction when Bidirectional scan is selected is shown below.

When Bidirectional scan is selected from Scan Direction, the [Direction mismatch adjustment] button appears for shift correction.

Click this button to display the Align bidirectional scanner window.

can setting			Zoom	
Scan Direction	: 🗐 🦻	Direction mismatch adjustment(us)	< O	1.000
Scan Size	512 •	512 512 recommend		$\mathbf{N}$
Scan Speed Fast	2	Frame/sec(Pixel Dwell: 1.2 u sec)	4	563x recommend

Figure 8.3-2 Correcting the image shifting for Bidirectional scan

[3] Correct the image mismatch caused by Bidirectional scan.

Figure 8.3-3 Align bidirectional scanner window

There are a correcting the intage shifting for Brain cettorian sean	<i>Table 8.3-2</i>	Correcting th	he image	shifting for	Bidirectional scan
---------------------------------------------------------------------	--------------------	---------------	----------	--------------	--------------------

Item	Description
Image shift correction range	-50 to 50
Image shift correction action	Sets the correction value.

#### Slider bar:

Slides to the right or left to set the correction value.

#### **Arrow buttons:**

Click either arrow button to increase or decrease the correction value in steps of 0.1.

#### Direct entry in correction value display field:

Type the correction value.

#### 8.3.1 Fast Mode

Turning on the Fast button makes the scan speed ultra-high.

When a Fast Galvano compatible model (C2plus) that supports high scan speed is in use, the scan speed is further increased by turning on the [Fast] button and set the scan magnification to 8X or higher.

	Scan setting				-
Fast button	Scan Direction	] 4		200m	8.000
	Scan Size Scan Speed	512 • •	512 512 recommend Frame/sec(Pixel Dwelt0.2 u sec)		4.563x recommend

Figure 8.3-4 Fast mode

Notes on the use of Fast mode (when scan magnification of 8X or higher is selected)

Note the following restrictions when a scan magnification of 8X or higher is selected in the Fast mode.

- Only when 256 or 512 is selected for Scan Size, the scan speed is further increased.
- When Scan Area rotation has been set, turning on the [Fast] button resets the rotation angle.
- The Fast mode cannot be used together with the Spectral Detector mode (SD) or Virtual Filter mode (VF). (If the Optical path changeover lever for scan head is changed to Spectrum while the Fast mode is turned on, the Fast mode is automatically turned off.)

• If correction of image deviation is greatly changed in the bidirectional scan, it takes several seconds until the correction is applied, but this is no problem. Wait until the correction is applied.

To perform fast image acquisition in the Fast mode, the following conditions must be met. Otherwise, the image acquisition speed may be lower than the specified speed.

- (1) The Power Options setting of the Windows 7 OS must be set to [High Performance].
  - * To make the Power Options setting, click the [Start] button > [Control Panel] > [Power Options] to display the [Select a power plan] window. On the [Select a power plan] window, click [Show additional plans] and select [High Performance].
- (2) The network must be the onboard network of recommended PC and no other expansion network card must be installed.

 Table 8.3-3
 Details of Fast mode when scan magnification of 8X or higher is selected

 Available Scan Size
 512

Available Scan Size		256	512	
Soon Snood	Unidirectional	7 fps	4 fps	
Scan Speed	Bidirectional	15 fps	8 fps	
Available Detector		DU3 Only		
Scan Zoom		x8 to x1000	x8 to x1000	
Scan area rotation function		Unavailable		
CROP scan area		Unavailable		
ROI scan area		Unavailable		
Free line scan		Unavailable		
Photo activation experiment		Unavailable		

# 8.4 Unidirectional and Bidirectional Scan

## 8.4.1 Unidirectional and Bidirectional Scan Motion

Unidirectional scan consists of "forward paths" only, while Bidirectional scan uses both "forward and reverse paths."

Thus, Bidirectional scan takes less time to acquire a given image, but it causes shifting between the image scanned along the forward path and that scanned along the reverse path.

It is therefore necessary to correct the image shifting when Bidirectional scan is selected.

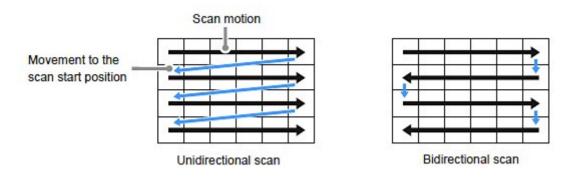


Figure 8.4-1 Unidirectional and Bidirectional scan motion

# 8.4.2 Scan Settings upon Toggling between Unidirectional and Bidirectional Scan

When you change from Bidirectional scan to Unidirectional scan, or vice versa, the new scan may not be executable with the current scan settings.

In that case, the Scan setting can be automatically changed.

# **9** Navigation Mode

The navigation mode enables to set the scan area in acquired images.

There are two types of navigation modes.

If settings on either window are changed, display of the scan area, etc., on the other window changes in an interlocked manner.

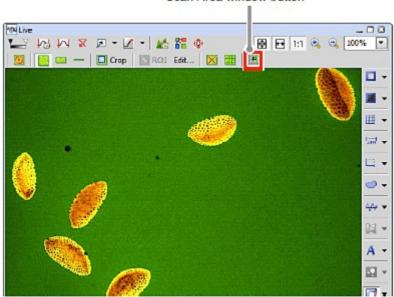
# 9.1 How to Display Navigation Mode

The procedure of how to display each window is shown as follows.

Scan Area Window

How to display the Scan Area window is shown below.

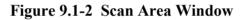
Click the button shown below to open the Scan Area window.



Scan Area window button

Figure 9.1-1 To display the Scan Area window

C2plus Scan Area x	
📴 💳 🗕 Crop	ROI Edit 🔀 🧱 🧮
Zoom: 7	1
Pixel size: 0.62	Nyquist XY -
Pixel size: 0.62 Scan size: 512 💌 Width: 512	Nyquist XY  Rotation: 0 Height: 512
Scan size: 512 💌	Rotation: 0
Scan size: 512  Width: 512	Rotation: 0



#### * Other display methods

As shown below, right-click on the gray area (without any setting window displayed) to display a menu.

*Then select* [*Acquisition Controls*] -> [*C2plus Scan Area*] *in the menu to open the Scan Area window.* 

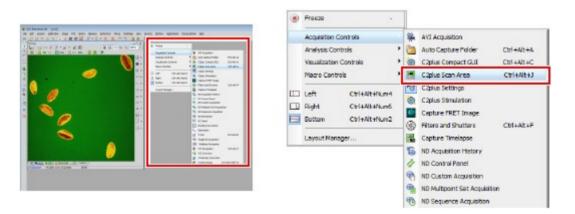


Figure 9.1-3 To display the Scan Area window

#### **Navigation Mode**

How to display the navigation mode is shown below.

The navigation mode is displayed by clicking [Show Scan Area] button in the Live window (which opens when the live image is acquired) or the Captured window (in which the live image was captured).

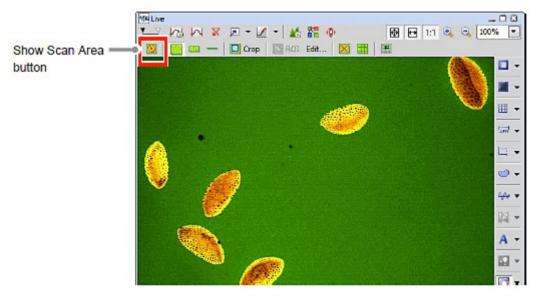
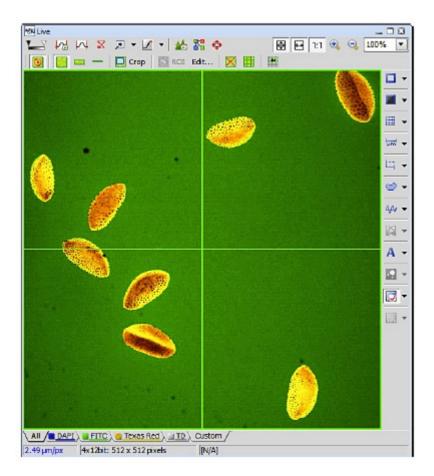
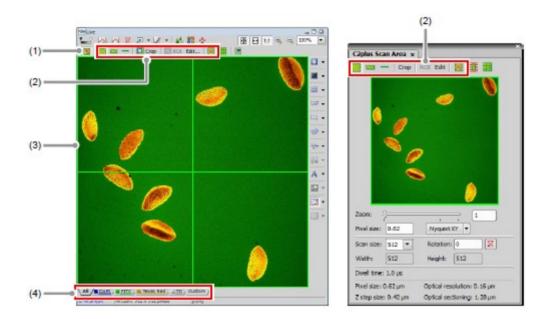


Figure 9.1-4 Live window





# 9.2 Structure of Navigation Mode

Figure 9.2-1 Navigation mode

Table 9.2-1	Summary	of navigation	mode functions
-------------	---------	---------------	----------------

	Name	Function
(1)	Show Scan Area button	Switch the Live window to the navigation mode.
(2)	Scan area setting tools	Provides tools for setting a scan area. A scan area of a selected shape can be set. Available tools vary depending on the scan mode and the type of scan area. For scan areas that can be set, see Section 9.3, "About Scan Areas."
(3)	Scan area view	A set scan area is displayed as green lines. If two or more ROI scan areas are set, colors of displayed lines are different. The lines are displayed in light blue for the Crop scan area.
		All tab - Displays the overlaid images of all channels.
(4)	Channel selection tabs	Fluorescence dye name tabs - Displays the fluorescence dye names of each channel. Clicking each tab displays only the image of the corresponding channel.

# 9.3 About Scan Areas

There are three types of scan areas according to their shape. They are the Square scan area, the Band scan area and the Line scan.

Additionally, two other types are available. They are the ROI scan area and the Crop scan area, designed to serve particular purposes.

"NIS-Elements C" allows the user to store and retrieve the scan area settings (except for ROI scan area). For storing and retrieving the [Optical Configuration] settings, see the sections concerning the optical configuration in the "NIS-Elements AR (Advanced Research) User's Guide."

### 9.3.1 Conditions for Setting Scan Areas

The following table shows conditions for setting scan areas.

Scan area	Function	Parameter limits
Square	Rotation	-90 to 90°
	Magnification	As desired (1X to 1000X)
	Resolution	Both X and Y: 64 ^(*1) , 128 ^(*1) , 256, 512, 1024, or 2048 ^(*2) pixels
	X to Y ratio	$\mathbf{X} = \mathbf{Y}$
Band	Rotation	-90 to 90°
	Magnification	1X to 1000X
	Resolution	X: 64 ^(*1) , 128 ^(*1) , 256, 512, 1024, or 2048 ^(*2) pixels
		Y: 32, 64, 128, 256, 512, or 1024 ^(*2) pixels
	X to Y ratio	X > Y
Line	Line type	Straight line only
	Magnification	1X to 1000X
	Resolution	X: 64 ^(*1) , 128 ^(*1) , 256, 512, 1024, or 2048 ^(*2) pixels

 Table 9.3-1
 Conditions for setting scan areas

^(*1) Unsettable when the Virtual Filter [VF] is selected as the detection mode.

^(*2) Usable only when the Standard Detector [DU3] is selected as the detection mode.

- The rotation angle and the Crop scan area cannot be set when the Bidirectional scan is selected.
- The "ROI scan area" and the "Crop scan area" are effective for the Square scan

area.

• Parameters of the "ROI scan area" and the "Crop scan area" depend on the scan area.

### 9.3.2 Scan Area Setting Tools

The available scan area setting tools include the Square setting tool, Band setting tool, Line setting tool, ROI setting tool and Crop setting tool.

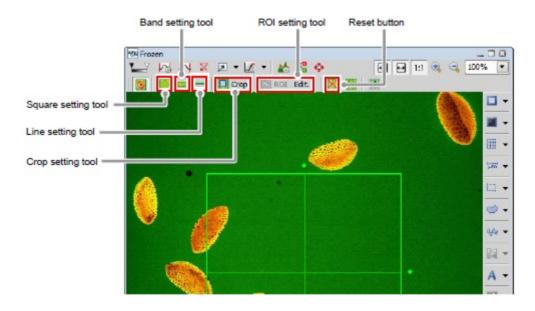


Figure 9.3-1 Scan area setting tools

Name	Function
Square setting tool	The Square scan area of a desired size can be set. The X and Y-directions are always of the same resolution.
Band setting tool	The Band scan area of a desired size can be set. The Y-direction resolution is always lower than the X-direction resolution.
	The Line scan (straight line scan) of a desired length and angle can be set. The line width is 1pixel.
Line setting tool	Images can be acquired in the X and T-directions. X and T-directions -
	Timelapse observation is carried out only to the area specified by the Line.
Crop setting tool	Enables to set a smaller rectangular scan area within the Square scan area.
	The file size of image data can be decreased without changing the

	scan speed by cutting off unnecessary parts.
	The resolution in Y direction is the same or lower than the resolution in X direction.
	* The Crop setting tool is not displayed when the Bidirectional scan is selected.
ROI setting tool	Enables to set the laser irradiation area with any shape. The scan area includes all ROIs.
Reset button	Resets the current scan area settings.

• Z-direction setting when scanning a cross section

The Z-direction will be set by NIS-Elements. For setting instructions, refer to "NIS-Elements AR (Advanced Research) User's Guide."

#### **Square Scan Area**

The Square scan area appears when the square setting tool is selected.

- By default, the Square scan area occupies the whole of the image window.
- Only one Square scan area can be set in a single image.
- The Square scan area cannot be removed.
- The Square scan area that appears upon selecting the square setting tool is the one that was set previously.
- In the image window, the Square scan area can be set to any position and any size.

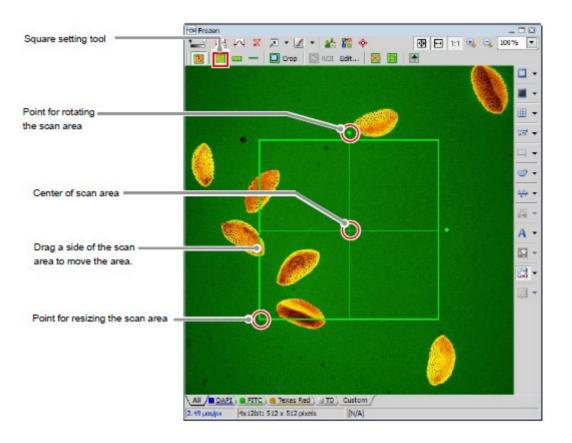


Figure 9.3-2 Square scan area

<i>Table</i> 9.3-3	Functions	of the Square	scan area	and their on	eration
14010 7.5 5	1 1110110115	of the square	scan arca	and men op	ci anon

Function	Operation
Resize scan area	Drags the point placed at each corner, or at the center of each side, of the scan area. The scan area can be enlarged or reduced to a desired size, while retaining the square form.
Rotate scan area	Places the mouse pointer over the rotation point located outside the scan area. As the pointer changes to the rotation pointer, drag it to rotate the scan area. The rotation range is -90 to 90 degrees. The scan area can be rotate only within the display area of the image window. It cannot be rotate outside the display area.
Move scan area	Places the mouse pointer on a side of the scan area. As the pointer changes to the move pointer, drag it to move the scan area. The scan area can be moved only within the display area of the image window. It cannot be moved outside the display area.

**Band Scan Area** 

The Band scan area appears when the band setting tool is selected.

• By default, the Band scan area has the X-direction length equal to the width of the image window,

and the Y-direction length equal to 1/2 of the X-direction length, with its center at the center of the image window.

- Only one Band scan area can be set in a single image.
- The Band scan area cannot be removed.
- The Band scan area that appears upon selecting the Band setting tool is the one that was set previously.
- The Band scan area can be set to any position in the image window, and to any size that meets the condition "X-direction length > Y-direction length."

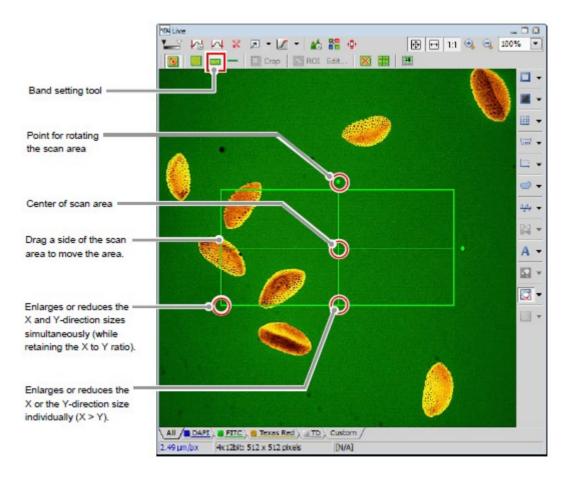


Figure 9.3-3 Band scan area

Table 9.3-4 Functions	s of the Band scan area and their operation
Function	Operation
Resize scan area	Drags the point placed at each corner, or at the center of each side, of the scan area to enlarge or reduce the size as desired.
	<b>Point at each corner -</b> The size can be enlarged or reduced as desired while retaining the ratio of X to Y-direction lengths.

	<b>Point at the center of each side -</b> The X and Y sizes can be changed individually provided that "X- direction length > Y-direction length."
Rotate scan area	Places the mouse pointer over the rotation point located outside the scan area. As the pointer changes to the rotation pointer, drag it to rotate the scan area. The rotation range is -90 to 90 degrees. The scan area can be rotate only within the display area of the image window. It cannot be rotate outside the display area.
Move scan area	Places the mouse pointer on a side of the scan area. As the pointer changes to the move pointer, drag it to move the scan area. The scan area can be moved only within the display area of the image window. It cannot be moved outside the display area.

**Resolution of Band Scan Area** 

This section describes the X and Y-direction resolution of the Band scan area.

Table 9.3-5 Resolution of Band scan area

X-direction resolution	A desired X-direction resolution is selected from $64^{(*1)}$ , $128^{(*1)}$ , $256$ , $512$ , 1024, and 2048^{(*2)} pixels
Y-direction resolution	The Y-direction resolution is automatically set as it is calculated from the ratio of X to Y-direction lengths.

#### **Example:**

If the X-direction resolution = 512, and the ratio of X-direction length to Y-direction length = 1:1/2, then the Y-direction resolution is set to "256."

^(*1) Unsettable when the Virtual Filter [VF] is selected as the detection mode.

^(*2) Usable only when the Standard Detector [DU3] is selected as the detection mode.

#### • If the Band scan area is resized:

For the X-direction, the resolution does not vary even if the X-direction length is changed. For the Y-direction, if the X-direction and/or the Y-direction length is changed, the ratio of X to Ydirection lengths varies.

Based on the new ratio, the Y-direction resolution is automatically recalculated and set.

#### **Example:**

Assume that the X-direction resolution = 512 pixels, the Y-direction resolution = 256 pixels, and the ratio of X to Y-direction length = 1:1/2.

If the Band scan area is changed and the resultant ratio of X to Y-direction lengths = 1:1/4, the Y-direction resolution is set to 128 pixels.

#### • If the X-direction resolution is changed:

The Band scan size does not vary either in the X or Y-direction.

The Y-direction resolution is automatically set as it is recalculated from the ratio of X and Y-direction lengths.

#### **Example:**

Assume that the ratio of X-direction length to Y-direction length = 1:1/2, where the X-direction resolution = 512 pixels and the Y-direction resolution = 256 pixels. If the X-direction resolution is changed to 256 pixels, the Y-direction resolution is set to 128 pixels.

#### Line Scan

The Line scan area appears when the Line setting tool is selected.

- The Line scan has no default value.
- Line scan drawing can be set on the Live window, but is hidden after the live image has been acquired and is displayed only on the Scan Area window.
- Only one Line scan can be set in the single image.
- The Line scan cannot be removed.
- The Line scan that appears upon selecting the line setting tool is the one that was set previously.
- In the image window, the Line scan can be set to any position and any length and angle.
- The Line scan can be used in the live acquisition and in the timelapse acquisition with no delay.



Figure 9.3-4 Line scan

Table 9.3-6 Functions of the Line scan and their operation					
Functions	operation				
Change scan line	Drags the both ends of the line to change the line length or angle. * Settable scan line length varies depending on the [Fast] button ON/OFF state in the Scan setting window. For the [Fast] button, see Section 8.3.1, "Fast Mode."				
Rotate scan line	The Line scan allows a line to be drawn with a desired length and at a desired angle on the Scan Area window, thus it does not provide a function to rotate it.				
Move scan line	Places the mouse pointer on a side of the scan line. As the pointer changes to the move pointer, drag it to move the scan line. The scan line can be moved only within the display area of the Scan Area window. It cannot be moved outside the display area.				

Table 9.3-6 Functions of the Line scan and their operation

#### **Crop Scan Area**

Enables to set a smaller rectangular scan area within the Square scan area. The file size of image data can be decreased without changing the scan speed by cutting off unnecessary parts.

- For the Crop scan area, a rectangle only can be selected.
- Only one Crop scan area can be set on an image.
- Any position and any size can only be set within the Square scan area.
- If the Crop setting tool is selected, the previously set the Crop scan area is displayed.
- The resolution in Y direction is the same or lower than the resolution in X direction.
- Unusable in the Bidirectional scan.
- When the Crop scan area is set, the acquired image becomes bright because Pixel Dwell cannot be retained.

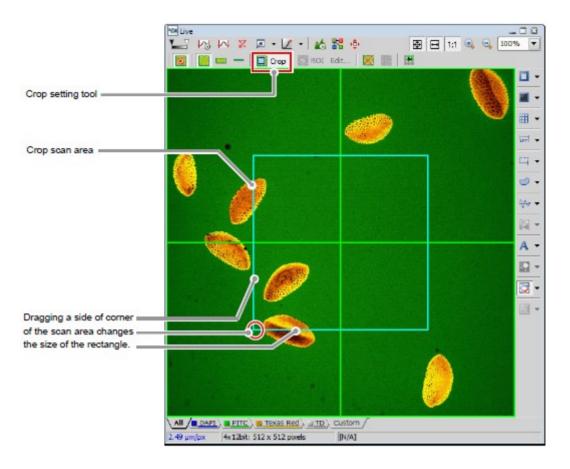


Figure 9.3-5 Crop scan area

Table 0 3 7	Functions of	the C	ron scan	aroa and	their operation
1 <i>uble</i> 9.5-7	T unclions of	ine Ci	op scan	area ana	ineir operation

FunctionsoperationResize scan areaWhen the mouse pointer is placed on a corner or side of the Crop<br/>scan area, an arrow pointer is displayed.<br/>Clicking the mouse while an arrow is displayed and dragging in the<br/>arrow direction enables to enlarge or reduce to any size.

#### **ROI Scan Area**

Clicking the [Edit ROIs inside Square Area] button of the ROI setting tool displays the ROI Editor. Use the ROI Editor to set a scan area with any shape.

- Unusable when the image resolution is 2048.
- Two or more ROI scan areas can be set on an image.

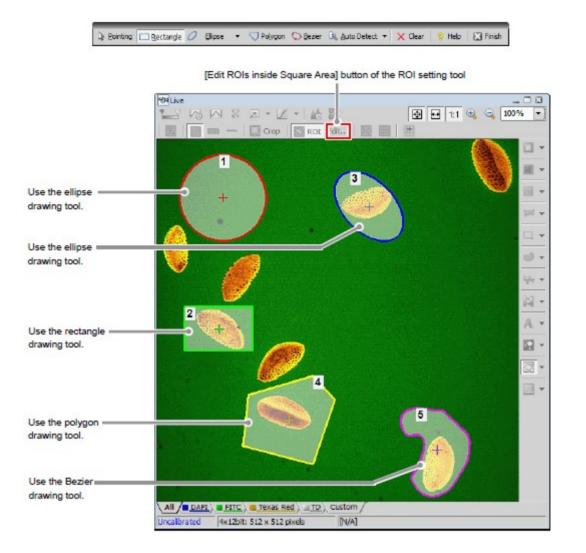
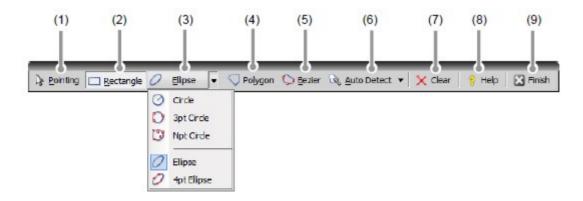


Figure 9.3-6 ROI scan area

* Drawn ROIs are hidden when the ROI Editor is closed, but you may check the scan areas on the Scan Area window.



#### Figure 9.3-7 ROI Editor

Table 9.3-8	<b>Functions</b>	of the	ROI scan	area	drawing t	ool

1000	N	0				
	Name	Functions and their operations				
(1)	Pointing		nove a drawn ROI on the window.			
(2)	Rectangle		lesignate the scan area enclosed by a rectangle.			
(3)	Ellipse	Used to a	lesignate the scan area enclosed by a circle.			
		Circle	Clicks the center of the desired circle to draw a precise circle with a radius of the dragged length.			
		3pt Circle	Designates any three points by clicking on the image to draw a precise circle that passes the three points.			
		Npt Circle	Designates multiple points by clicking on the image to automatically select several points nearest to the circle to draw a precise circle.			
		Ellipse	Clicks the center of the desired circle to draw a precise circle with a radius of the dragged length. When the base point on the top, bottom, right, or left of the circle is picked and dragged, the circle deforms to an ellipse. When the drawn circle is picked and dragged with the mouse, the circle moves to another position. Right-clicking on the drawn circle designates the circle as the ROI scan area.			
		4pt Circle	Designates any four points by clicking on the image to draw a precise circle that passes the four points.			
(4)	Polygon	Designat pointer to	lesignate the scan area enclosed by straight lines. es the start point by clicking on an image and moving the o the straight line ending position (end point) and clicking straight line. Draw straight lines subsequently to draw a			
		To close place the mouse. Double-c	the selected area by connecting straight lines to each other, mouse pointer on the start point and double-click the clicking the pointer at a position different from the start			
		1	also close the selected area.			
(5)	Bezier drawing tool	curve by For freeh mouse.	freehand drawing or for drawing a straight line or smooth placing anchor points. and drawing, click the mouse on the image then drag the a curve using anchor points, drag the mouse in the curving			
			the selected area, right-click the mouse. (Double-clicking e left button also closes the selected area.)			
(6)	Auto Detect (Adjacent similar colors)	adjacent By clicki adjacent	automatically detect and specify the similar color portion to the clicked position. ng the mouse on the image, the similar color portion to the clicked position is selected. e selected area, right-click the mouse.			
(7)	Clear	Clears th	e ROI scan area.			

(8)	Help	Displays the help for ROI Editor.
(9)	Finish	Finishes drawing and editing of the ROI scan area and closes the
		ROI Editor.

### 9.3.3 Switching Scan Area Setting Tools

The scan area setting tool can be switched by clicking the respective buttons. Before using the Crop setting tool and the ROI setting tool, turn the [Show Scan Area] button to "OFF."

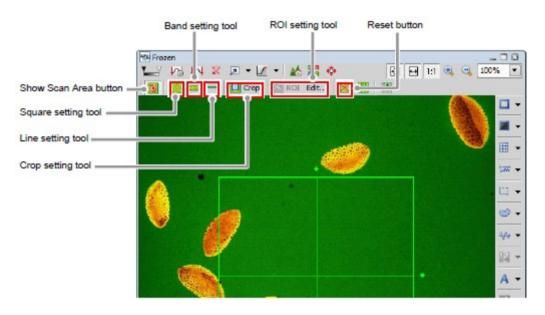


Figure 9.3-8 Scan area setting tools

When one scan area setting tool is switched to another setting tool, the information of the previous scan area is stored even after the display of the previous scan area disappears from the window.

For example, if the Square scan area is set using the square setting tool and then switches over to the band setting tool and set the Band scan area, when the square setting tool is switched back, the Square scan area appears. (As shown below)

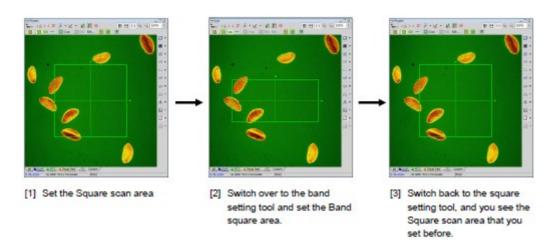


Figure 9.3-9 Storing scan area settings

### 9.3.4 Scan Area Zoom Function

The scan area zoom function is effective for the Square scan area and the Band scan area.

Set the Square or the Band scan area around the portion of the live image to be enlarged. Then, acquire the live image, and the portion set with the scan area appears enlarged.

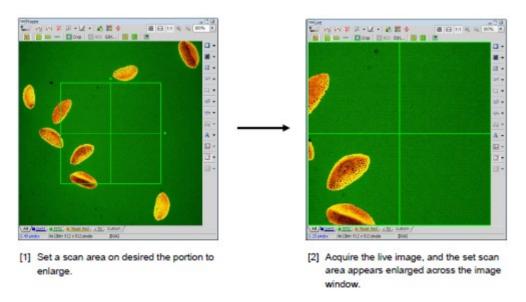


Figure 9.3-10 Scan area zoom function

#### 9.3.5 Scan Area Rotation Function

The scan area rotation function is effective for the Square scan areas and the Band scan areas.

Set a rotated square or the Band scan area around the portion of the live image to be rotated. Acquire the live image once again, and the rotated scan area appears in upright position.

This function rotates a set scan area, and at the same time, applies the scan area zoom function.

The scan area cannot be rotated when the Bidirectional scan is set.

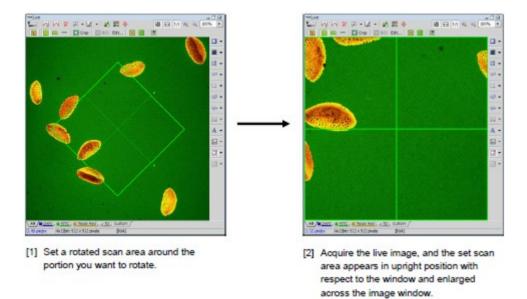


Figure 9.3-11 Scan area zoom and rotation function

# **10** Photo Activation Setting

This chapter describes the basic operation procedures to execute the photo activation experiment sequence that acquires images of target changes at a high speed while irradiating the stimulation laser beam and acquire the observed images.

- * When a three-laser unit without AOM is connected, the photo activation experiment cannot be executed.
- * When the Virtual Filter mode is selected, the photo activation experiment cannot be executed.

# **10.1** Photo Activation Setting Procedure

1. Acquire the image of sample for photo activation.

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Arraina	File and	Dje					
2 ⁸ Live	6000		Detector	829	0	lose mechanical shutte	r during experime
6	10.	Eye Port	Ch series	None	Laser	Emission	
Find Node		1	Ch1	DAPE	408.0	417-477	
2° XY			Ch2	FETC	488.0	499-529	
No XY			ch3	Texas Red	543.5	553-618	

Figure 10.1-1 Acquire a live image



Figure 10.1-2 Acquire a frozen image

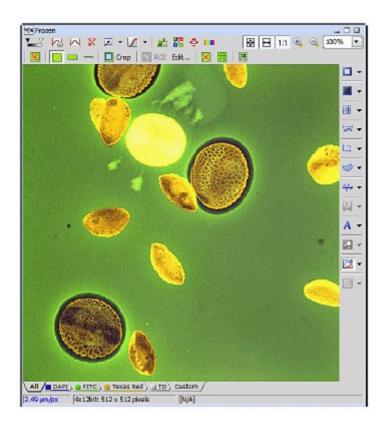


Figure 10.1-3 Frozen image

2. In the Acquisition window, set the image to be acquired through photo activation experiment observation.

Laser Power Monitor	AG Select	All Channels	HV Linear Correction
Ch 1 DAPI	Laser 405.0	Ch 2 FETC	Laser 488.0
HV 4 🚺	- 40	HV 4	
Offset		Offset 4	
Laser   4	ND 16.0 0.0		► ND 4.0 0.0
Ch 3 Texas Red	Laser 561.0		
HV 4 🔼	50		
Offset 4	- <b>•</b> •		
Laser 4 0	1.0 0.0		
Pinhole 4		ज 🗹	
561.0 V AHome	■ ■ 1.2 A.U. 30.0 µm <-	ни « О	
361.0		Offset 4	

Select the Acquisition window

#### Figure 10.1-4 Acquisition setting window

3. Specify an area, point, or line to which the photo activation is to be applied with the Simple ROI Editor.

For details of the Simple ROI Editor, see Section 10.2.3, "Simple ROI Editor."



Figure 10.1-5 Setting of a ROI area

👌 Bointing 🗆 Bectangle 🖉 Blipse 🔹 🖓 Polygon 🚫 Bezier 🔍 Auto Detect + 🛞 Auto Detect Al + 🔶 Stin. Point + 🖌 Dreve Holes 🛛 🗙 Clear 🎝 Unclo 🕼 Racto 🔗 Help 🔯 Finish

Figure 10.1-6 Simple ROI Editor

4. Specify a photo activation area. Draw a ROI area on the acquired image. Right-click on a designated ROI area to display a menu. Select [Use as Stimulation ROI: S1] from the displayed menu and designate a ROI area as the photo activation area.

If you wish to assign multiple photo activation areas to separate photo activation frames, select [Use as Stimulation ROI: S2] or [Use as Stimulation ROI: S3]. (Only when a photo activation ROI area is selected.)

* Multiple ROIs can be set for each photo activation frame. ROI can be divided into frames to set different stimulation conditions.



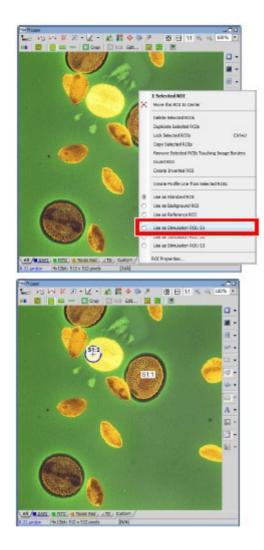


Figure 10.1-7 Setting of photo activation area

- * To specify a photo activation point, select [Stim. Point] of the Simple ROI Editor and specify a point on the image.
- * To specify a photo activation line, select [Stim. Line] of the Simple ROI Editor and specify a line on the image.
- * You can specify only one photo activation point/line and cannot specify multiple photo activation target areas.
- * You can crop the specified ROI area and set a non-photo activation area within the photo activation ROI area. Click the [Draw Holes] button of the Simple ROI Editor and then select a drawing tool. Then draw an area to be cropped on the pre-selected ROI area. (Point drawing tools are unavailable for specifying a non-photo activation area.)

5. Switch to the Photo Activation window.

Select the stimulation laser beam and set the laser power and photo activation speed. (For stimulation laser setting, see Section 10.2.2, "Laser Setting for Photo Activation.")

Acquisition     // Proto.bcbudor,Neuching.source)     Low Po	nela] HVUnear Conection	<ul> <li>Select the Photo Activation setting window</li> </ul>
Image: Contract of the second secon		
Ch3         Texas Red         Lear Xd.0           HV         4		
Introduit         Image: Control of the section o	< <u>→</u> ► <u>→</u>	

Figure 10.1-8 Switching to Photo Activation window

- 6. Select HV mode in Photo Activation.
  - * The HV mode can be set only when [DU3] is selected for the detection mode. For details of settings, see Section 10.1.2, "Setting HV Mode in Photo Activation."

	/ < Photo Activation/Bloaching(Laser::408.0/488.0/543.5/)
2	HV node during stmillator 2001-W = 1 (Select Straubion Jrea) = All consistion area out to came Manual Stri 400 recovery
₹] 408.0 ₹] 543.5	
Itimulation 5	censeting Scan Speed 125 💌 Sec (Phane (Phaildveil: 45.6 Linec)

Figure 10.1-9 HV mode

7. For each photo activation frame, set the stimulation laser beam, laser power, and photo activation speed. If you set multiple photo activation areas on the same frame, all of the specified ROIs are photo-activated at the same time.

(If two ROIs are the photo activation targets, a photo activation frame photo-activates the two ROIs. In this case, photo activation may take time even though each photo activation area (ROI) is small.)

							HV Linear Co	riection
					HV node o	turing stimulation	Zens HV	
2	3 Select	Strulation Are	a) 🗌 Alat	mulation are	e set to same	Manual Shift	t .	- 3
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¥ 943.8	-		0.0					
stimulation i	can setting				200303	32		
	Scan Speed	1 *	Sec /P	one (P	beldvel: 15	(u sec)		

#### Figure 10.1-10 Laser Setting for Photo Activation (Specify the photo activation ROI area)

* When a photo activation point/line is specified, photo activation target area is limited to only one. Therefore photo activation frame cannot be set. When a photo activation point is specified, the photo activation speed cannot be set.

	HV Unear Correction
	HV node during stimulation Zwo HV
	Manual Shift
₽ 438.0 4 <b>=)</b> > 20.0	Ø 488.6 ≤ 🐋 🕨 😰 20.0
943.9 4 🌒 🕨 👔 0.00	
Stimulation Scan setting Stimulation Spaced 30 💌 Inter / sec.	(Pouldvell: 45.6 user)

Figure 10.1-11 Laser Setting for Photo Activation (Specify the photo activation line)

8. Click the [Photo Activation] button to open the ND Stimulation window.

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N/2 Time	= 2 = () ≥ 400.0 4 m	nelect it tradition do		nalation area set			28.0

Figure 10.1-12 Photo activation button

* Other display methods

As shown below, select [Applications] -> [Define/Run Sequential Stimulation...] from the menu bar to open the ND Stimulation window.



#### Figure 10.1-13 To display the ND Stimulation window

9. In the ND Stimulation window, set the photo activation experiment sequence. (For the photo activation setting window, see Section 10.2.1, "Photo Activation Experiment Sequence Setting.")

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🗆 San	e to l'ile							Fie	cord	Deta
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		Acquisi	-	- 1		1 sec		1sec	-	2
24										

Figure 10.1-14 Experiment Sequence Setting

10. When the settings have been completed, click the [Apply Stimulation Settings] button to send the photo activation area information to the experiment sequence.



Figure 10.1-15 Experiment Sequence Setting

11. Click the [Run now] button to execute the photo activation experiment sequence.

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#### Figure 10.1-16 Experiment Sequence Setting

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#### Figure 10.1-17 Experiment sequence running

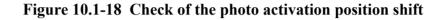
- * If the photo activation position is not correct, manually correct the photo activation position. For manual correction of the photo activation position, see Section 10.1.1"Correcting the Photo Activation Position Shift "
- * Only one of Galvano Scanner and DMD module can be used as stimulation device for Confocal. Stimulation by Galvano Scanner cannot be used if you add DMD module by Manage Device. Please change the system configuration by removing the DMD module using Manage Device when stimulation by Galvano scanner is required.

#### **10.1.1** Correcting the Photo Activation Position Shift

If the photo activation position is not correct, the photo activation position can be corrected manually.

1. Click the [Manual Shift] button on Photo Activation window to open the Manual Shift Alignment window.

			HV mod	te during stimulation	Zero HV 💌
2	3 (Select Stir	nulation Area ) 📃 All sti	mulation area set to sam	e Manual Shift	
408.0	-	▶ 20.0	☑ 488.0 ◀	• •	20.0
543.5	•				
Stimulation 5	ican setting				



2. Correct the shift by specifying the shift amount (pixel) for each photo activation group while checking the image acquired by the first photo activation sequence.

S 1	к: 0	рх у: 0	рх
52	к: 0	рх <b>у:</b> О	px
S 3	к; 0	рх у: 0	px
F		Cancel	1

Figure 10.1-19 Manual Shift Alignment window

3. After correcting the photo activation position shift, reexecute the photo activation experiment sequence.

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

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t XY		(02)	FUTC	488.0	499-529	
AY		03	Texas Rad	CEALERS .	923-632	
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XIL						W Linear Correction
					ring stimulation []	
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	1 2 3 (!	Select Stimule	tor Area) 🔲 Al straulator		Manual shift	
XY Tine	1 2 3 (;	-			Manual shift	20.3

Figure 10.1-20 Photo Activation button

4. If the previous photo activation experiment sequence setting is to be maintained, click the [Apply Stimulation Settings] button to send the photo activation area information to the experiment sequence.

	e to File							Ro	corel	Deterry
Time sch	edule (C2	plus Galvano	10	plus Gal	vano)		•	[]   +	+	×ð
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#2		Acquini	٠		33	1 вис	*	1 вес	٣	2
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#### Figure 10.1-21 Experiment Sequence Setting

5. Click the [Run now] button to execute the photo activation experiment sequence.

perne	nt: [M	194	nulation	8.	_								
	1 2	3	4 5		5	7	а ,	9		1 12	13 14	15	15 17
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			tter whe					and the		y 1 1 2 1 1			
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#### **10.1.2** Setting HV Mode in Photo Activation

The HV value mode in photo activation can be set. (This mode is settable only when [DU3] is selected for the detection mode.)

							HV Unear C	orrection
				1	HV mode dur	ing stimulation	Zero HV	
2 3	(Select Stimul	ation Area )	🔄 All stimu	lation area set	to same	[Manual Shit	t Zero HV Auto recove	ry 🔍
408.0			20.0	483.0	-		▶ 20.0	
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mulation Scar			Sec / Gran	a (Dival	السا	1000)		
	ican Speed 16	•	Sec / Fram	e (Ptxel o	well : 45.6	usec)		

Figure 10.1-23 HV mode settings

Table 10.1-1 Functions of HV mode

	Name	Function
(1)	Zero HV	The HV value is always 0 in the stimulation phase, but it becomes the user-specified HV value only in the image acquisition phase.
(2)	Auto recovery	The HV value becomes 0 when PMT overload due to photo activation occurs, but the user-specified HV value is restored at the end of the stimulation phase.

# **10.2** Setting of Each Window

This section describes setting items in each window on photo activation are explained.

### 10.2.1 Photo Activation Experiment Sequence Setting

Depending on the reaction speed, set the experiment sequence including photo activation and observation before and after photo activation is given.

Photo activation and image acquisition are executed in time series for each phase set.

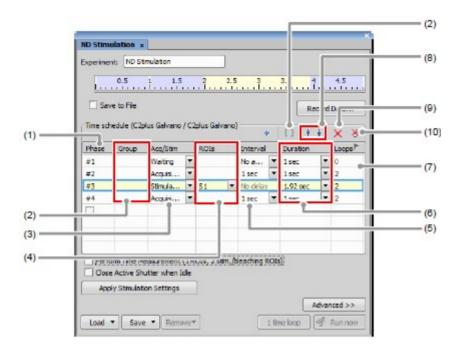




Table 10.2-1 Functions of ND Stimulation window	<i>Table 10.2-1</i>	Functions	of ND	Stimulation	window
-------------------------------------------------	---------------------	-----------	-------	-------------	--------

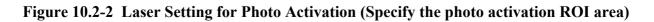
	Name	Function
(1)	Phase	Clicks to set phases of the experiment sequence.
(2)	Group	[]
		Group the phases. Selects the phases to be grouped while pressing the [Shift] key and click the Group button [] for grouping. Set the number of repeated count in [] displayed in the Group field.
(3)	Acq/Stim	Selects the items to be set from "Acquisition," "Stimulation," "Bleaching," and "Waiting."
		The FRAP experiment can also be executed by making the phase settings as follows. #1 = Acquisition / #2 = Stimulation / #3 = Acquisition
(4)	ROIs	Specifies the photo activation frame to run the set phase. Enables to select from seven choices; "S1," "S2," "S3," "S1, S2," "S1, S3," "S2, S3," "S1, S2, S3."
		e.g.)
		<ul><li>Frames 1 and 3: Select "S1" and "S3."</li><li>Frame 1 only: Select "S1."</li></ul>
		(It is set as "S1" when a photo activation point/line is specified for a photo activation area.)
(5)	Interval	Specifies the phase interval.

		<ul> <li>- "No delay": No interval</li> <li>- "No acquisition": No interval and image acquisition</li> <li>If "Stimulation" or "Bleaching" is set in the [Acq/Stim] column,</li> <li>[Interval] is fixed to "No delay."</li> </ul>
(6)	Duration	Specifies the time (length) of the selected phase. If the time (length) is designated, the number of execution times is automatically selected. (If [Interval] is set to "No delay" and [Loops] is changed, [Duration] is also changed in an interlocked manner.)
(7)	Loops	Specifies the number of execution times for the selected phase. (If [Interval] is set to "No delay" and [Duration] is changed, [Loops] is also changed in an interlocked manner.)
		* When the photo activation point or the photo activation line is designated, [Loops] cannot be set.
(8)	Move the phase one line	Ť
	lille	Brings the selected phase to one line above.
		+
		Brings the selected phase to one line below.
(9)	Remove the phase	$\times$
		Removes the selected phase.
(10)	Remove all	×
		Removes all phases.

## **10.2.2** Laser Setting for Photo Activation

The stimulation laser beam to be irradiated in the experiment sequence is set.

	HV Linear Correction	
	HV mode during stimulation Zero HV	
(1)	1 2 3 Select Stimulation Area ) All stimulation area set to same Manual Shift	
(2)		(3
	☑ 543.5 ◀ 🏟 🕨 🛛 0.0	
(4) —	Stimulation Scan setting Scan Speed 1 Sec / Frame (Rixel dwell: 1.9 u sec)	



	HV Linear Correction	
	HV mode during stimulation Zero HV  (Manual Shift)	
(2) —	→	— (3)

Figure 10.2-3 Laser Setting for Photo Activation (Specify the photo activation Point)

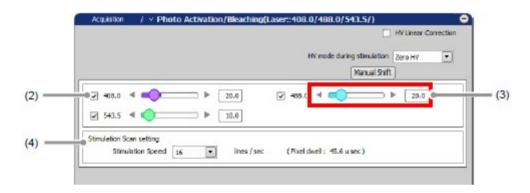


Figure 10.2-4 Laser Setting for Photo Activation (Specify the photo activation line)

<i>Table 10.2-2</i>	<b>Functions</b>	of Photo	Activation	window
---------------------	------------------	----------	------------	--------

	Name	Function
(1)	Photo activation frame tabs	Selects the photo activation frame to be set. (Only when a photo activation ROI area is selected)
(2)	Stimulation laser selection check box	Selects the stimulation laser beam to be irradiated on the sample.
(3)	Stimulation laser power output adjustment	Adjusts the output power of the stimulation laser beam to be irradiated.
(4)	Photo activation scan speed setting	Sets the photo activation scan speed. The speed is expressed in Sec/Frame when a photo activation ROI area is selected, or in lines/sec when a photo activation line is selected.

### 10.2.3 Simple ROI Editor

This section describes the functions of the Simple ROI Editor drawing tool.

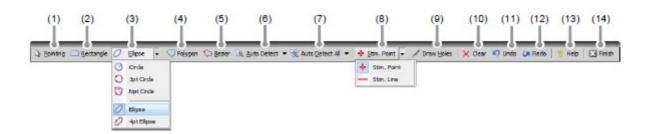


Figure 10.2-5 Simple ROI Editor

<i>Table 10.2-3</i>	Functions of the Simple ROI Editor drawing tool
---------------------	-------------------------------------------------

	Name	Functions and their operations		
(1)	Pointing	Used to a	move a drawn ROI on the window.	
(2)	Rectangle	Used to a	designate the ROI area enclosed by a rectangle.	
(3)	Ellipse	Used to o	designate the scan area enclosed by a circle.	
		Circle	Clicks the center of the desired circle to draw a precise circle with a radius of the dragged length.	
		3pt Circle	Designates any three points by clicking on the image to draw a precise circle that passes the three points.	
		Npt Circle	Designates multiple points by clicking on the image to automatically select several points nearest to the circle to draw a precise circle.	
		Ellipse	Clicks the center of the desired circle to draw a precise circle with a radius of the dragged length. When the base point on the top, bottom, right, or left of the circle is picked and dragged, the circle deforms to an ellipse. When the drawn circle is picked and dragged with the mouse, the circle moves to another position. Right-clicking on the drawn circle designates the circle as the ROI scan area.	
		4pt Circle	Designates any four points by clicking on the image to draw a precise circle that passes the four points.	
(4)	Polygon	Designat pointer to draws a s polygon. To close	the selected area by connecting straight lines to each other,	
(5)	Bezier drawing tool	mouse. Double-o point car Used for curve by For freeh mouse.	e mouse pointer on the start point and double-click the clicking the pointer at a position different from the start n also close the selected area. freehand drawing or for drawing a straight line or smooth placing anchor points. hand drawing, click the mouse on the image then drag the	
		To draw	a curve using anchor points, drag the mouse in the curving	

		direction. To close the selected area, right-click the mouse. (Double-clicking the mouse left button also closes the selected area.)
(6)	Auto Detect (Adjacent similar colors)	Used to automatically detect and specify the similar color portion adjacent to the clicked position. By clicking the mouse on the image, the similar color portion adjacent to the clicked position is selected.
		To fix the selected area, right-click the mouse.
(7)	Auto Detect All (All similar	Used to automatically detect and specify all the similar color portion to the clicked position.
	colors)	By clicking the mouse on the image, all the similar color portion to the clicked position is selected.
		To fix the selected area, right-click the mouse.
		Used to designate the photo activation point.
(9)	Stim. Point	You can specify only one photo activation point and cannot specify multiple photo activation target areas.
(8)	Stim. Line	Used to designate the photo activation straight line.
		You can specify only one photo activation line and cannot specify multiple photo activation target areas.
(9)	Draw Holes	Used to draw a non-photo activation area in a ROI area drawn by using the various tools of Simple ROI Editor.
(10)	Clear	Clears the ROI area.
(11)	Undo	Returns to the previous operation status.
(12)	Redo	Re-executes the operation returned by Undo.
(13)	Help	Displays the help for Simple ROI Editor.
(14)	Finish	Finishes drawing and editing of the ROI area and closes the Simple ROI Editor.

# **11 Using Manual Microscope**

This chapter describes how to make settings for operations from NIS-Elements with the Confocal Microscope C2 connected to Nikon manual microscope .

When using FN1 microscope, connect the C1-Y-TT Trinocular Tube (hereinafter referred to as trinocular tube). Combination of the trinocular tube and the "vertical movement device (Nikon "RFA" or Prior external Z Drive "Prior Z RFA")" enables NIS-Elements to control the manually-operated ECLIPSE FN1.

# 11.1 Setting Manual Microscope Connection

Make settings using the following procedure to synchronize NIS-Elements with the manual microscope.

# 1 Call the Manage devices window

Select [Devices] on the menu bar and then select [Manage devices...]. The Manage devices window appears.

12 12 19	格王(3) @ [	Benege devices	- Cantonie +
		Patching Russessent Probes and Filters	

Figure 11.1-1 Devices menu

lanage devices		L
nstalled devices:		
		Add
		Renove
		Cose
		L-3035-
thysical Devices		
hysical Devices	Configure De	EVICE
Connect Disconnect		evice
Connect Disconnect		

#### Figure 11.1-2 Manage devices window

# 2 Add "Manual Microscope"

1. Click the [Add] button in the Manage devices window to display the menu for devices to be added.

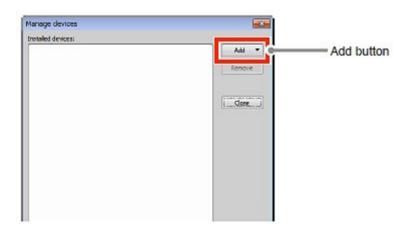


Figure 11.1-3 Manage devices window

2. Select the "Manual Microscope" form pull-down menu. "Manual Microscope" is added in the [Installed devices:] area.

nstalled devices:	Add V
	Nim OF
	Nanual Microscope
	recon unterslight
	Nikon LLHA
	Nikon LUSU
	Nikon LUGU and AGM
	Nilon Ni-E
	Nikon REA
	Nikon Ti

Figure 11.1-4 Manage devices window

lanage devices		
Instaled devices: - 49 Manual Microscope - 21 Nicoscope - 21 Nicoscope - 21 Nicoscope - 21 PitterBock(type: Turret, - 21 PitterBock(type: BPL, name: P		Add v
Physical Devices	Configure Device	
Connection Parameters	Reset	
Logical Devices		

Figure 11.1-5 Manage devices window

### 3 Add the vertical movement device (Nikon "RFA" or Prior external Z Drive "Prior Z RFA")

 Click the [Add] button to display the menu for devices to be added. Select the "Nikon RFA" or Prior external Z Drive "Prior Z RFA" form pull-down menu. (*) The Connection Parameters window appears.

Nanage devices	
Installed devices:	
	Kien C+C     Manual Microscope     Niken Enternalight     Niken LU4A     Niken LU5U     Niken LU5U and ACM     Niken LU5U and ACM
	Nikon RFA
	INKON II
Physical Devices Connect Configure Device Connection Parameters Reset	

### Figure 11.1-6 Manage devices window

- (*) Compatible Microscopes: Nikon RFA : ECLIPSE AZ100, ECLIPSE 80i, ECLIPSE FN1 Prior Z RFA : ECLIPSE Ti-U, ECLIPSE FN1
- Set the Serial port in the Connection Parameters window. Select the serial port to which "Nikon RFA" or Prior external Z Drive "Prior Z RFA" is connected from the pull-down menu of [Serial port:]. Click the [OK] button to finish the Serial port setting.

	Selects a serial por	t.
Connection	Parametei s	×
Serial port:	COM1 -	
Speed:	9600 💌	Cancel

Figure 11.1-7 Connection Parameters window

3. If you use "Nikon RFA" as the vertical movement device, set the model number.

Select "RFA" in the [Installed devices:] area and click the [Configure Devicec] button. The Model setting window appears.

	Manage devices	1
Selects a RFA. ——		
	H      Physical Devices      Connect      Disconnect      Configure Device      Configure Device      Logical Devices      Device Parameters	Configure Device button



Set the model number in the Model setting window.
 Select "99888" (*) from the pull-down menu of [Model:].
 Click the [OK] button to finish the Model number setting.

	Model setting	
Selects a Model number.	Model 99388 💌	ОК
		Cencel
	Wodelnumber           99640         100 nm step resolution, 100 µm(n           99641         100 nm step resolution, 100 µm(n           99642         100 nm step resolution, 100 µm(n           99643         874 for E50014, 50 nm step resolution, 100 µm(n           98644         874 for E50014, 50 nm step resolution           98645         874 for E50014, 50 nm step resolution           99645         874 for E-60014, 50 nm step resolution           99646         874 for E-60014, 50 nm step resolution, 201	otation otation Aution, 300 µm/hotation asolution, 100 µm/hotation skution, 100 µm/hotation skution, 100 µm/hotation

Figure 11.1-9 Model setting window

(*) The model number differs depending on the microscope. ECLIPSE AZ100, ECLIPSE 80i, ECLIPSE FN1 : 99888 5. Click the [Close] button to close the Manage devices window.



Figure 11.1-10 Manage devices window

# 4 Setting the [Manual Microscope Pad]

 Display the [Manual Microscope Pad]. As shown below, right-click on the gray area (without any setting window displayed) to display a menu. Then select [Acquisition Controls] -> [Manual Microscope Pad] in the menu.

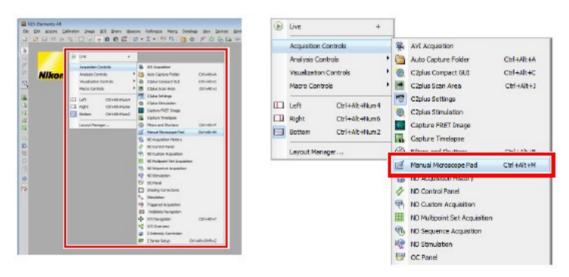


Figure 11.1-11 To display the Manual Microscope Pad

# * Other display methods

And also, select [View] on the menu bar and then select [Acquisition Controls] -> [Manual Microscope Pad] to open the control pad.

Del graune Calibration (mape 500 Genery Messure Reference Mana) Data (2) [] (2) (2) (2) (2) (2) (2) (2) (2) (2) (2)			-100	damice +	× 🖌
	19	Layout Custowize Toolbar	:		
Nikon		Dedarg Rates	•		
		Acquisition Cantrolo	1.0	A/C Acquisition	
Planual Microscope Pad x  Nosepicte		Siqualization Cantrols		Auto Capture Polder Ciplus Compact GLI Ciplus Scon Arca	Chiwaliwa Chiwaliwa Chiwaliwa
Filer Turret		Inege		Clplus Settings Clplus Stitulation	
Etter		Lävers Layers Properties 2007	•	Capiture PRET Swape Capiture Timelapee	CHANN
	1	Magnifer sizes options col estufisen	1	Manual Percoscope Pad	CS1+AR+M
		jpectral image	1	ND Cantrol Panel	
		Sies, Process Camponent		ND Cuetow Acquisition	
	977		18	ND Multipeint Sct Acquisit	kon .
				ND Sequence Acquisition	
				ND Steulation OC Panel	
				Shading Corrections	
				Stimulation	
			10	maggered acquisition	
			12	Welplate Nanipatien	
				KYZ Nevigetion	Ctil+Alt+?
				EV2 Oversiew	
			_	2 Intensity Correction 2 Series Setup	CHIHARHSHRHZ

Figure 11.1-12 To display the Manual Microscope Pad

2. Set the Nosepiece information in the [Manual Microscope Pad].

Click the 🔎 button in the [Nosepiece] area. The Nosepiece & Objectives window appears.

Manual Microscope Pad ×	×
Nosepiece	
1 2 3 4 5 6	폐
Filter Turret	

Figure 11.1-13 Manual Microscope Pad

Nosepiece position	Objective name		Z-Step (Auto focus)	Z-Step (Slices)	Working
1 .		•			[
20		-			
30		1			
40		ĨŦĨ			
50		-			
60		ĨŦ			

Figure 11.1-14 Nosepiece & Objectives window

- * When using manual microscope, information on the installed objectives cannot be read automatically by NIS-Elements. When using a manual microscope, be sure to select the objective to be used in the Nosepiece & Objectives window.
- 3. Specify the Objective in the Nosepiece & Objectives window. Select the Objective name to be used from the pull-down menu of [Objective name].

Figure 11.1-15 Nosepiece & Objectives window

loseplece position				tep focus)	Z-Step (Slices)	Working distance
1 💿 🗌		•				
20			None			
30			0.5×	+ TI		
40		=	1x	٠fi		
50		=	2x	٠Fi		
60		-	2.5x	• 5		
			4X	+ <u>P</u>		L
			5x	- N	LU PLan E	PI 5x
			10x	F.	LU PLan B	D Sx
			16x	•	LU PLan E	PI P 5x
			20x	×.	LU PLan F	luor EP1 5x
			25x		LU PLan F	luor BD 5x
			40x		LUPLANE	NOL ENT & 2X
			50x	•	AZ Plan Fl	uor 5x
			60x	+	Coolscope	5x
			100x	1		
			150x	+		

Figure 11.1-16 Selecting Objectives

4. Click the [OK] button to close the Nosepiece & Objectives window.

posi	piece ition	Objective name		Z-Step (Auto focus)	Z-Step (Slices)	Working distance	
1	۲	LU PLan Fluor EPI P 5x	-	22.20	44.40	23500.00	
2	0	Plan Apo 20x DIC M	-	0.90	1.80	1000.00	
3	0		-				
4	0		•		[		
5	0		•				
6	0		-				

Figure 11.1-17 Nosepiece & Objectives window

The connection setting procedure has been completed.

# 11.2 Manual Microscope Pad

The Microscope Control Pad for manual microscope consists of the following portions:

Manual Microscope Pad ×	×
Nosepiece	
5x 20x	
1 2 3 4 5 6	
- Filter Turret	
	Z

# Figure 11.2-1 Manual Microscope Pad Settings

#### <u>Nosepiece</u>

Select the objective located in the light path.

# **11.3** Operating the Z Drive

This section describes how to operate the Z drive of the manual microscope by NIS-Elements.

1. Display the XYZ Navigation window.

As shown below, right-click on the gray area (without any setting window displayed) to display a menu. Then select [Acquisition Controls] -> [XYZ Navigation] in the menu.

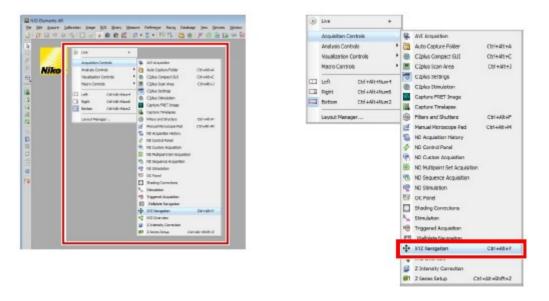
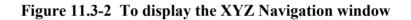


Figure 11.3-1 To display the XYZ Navigation window

And also, select [View] on the menu bar and then select [Acquisition Controls] -> [XYZ Navigation] to open the window.

Impact of the second	And the second se		
Image:	And the second se		
Andrea Carefol The Control Andrea Carefol The Control Andrea Carefol The Control Andrea Carefol The Control Andrea The Co		room diaman	and the second se
C per j 0.0 Maren + 400.0 M.M.B + m + C.nkj 0.1 Maren + 400.0 M.M.B + m + C.nkj 0.1 Maren + 400.0 M.M.B + m 2 Control Hand + 4000 M.M.B + m + m + h ann + 4000 M.M.B + m + m + h ann + 4000 M.M.B + m + m + h ann + 4000 M.M.B + m + m + h ann + 1000 M.M.B + m + m + h ann + 1000 M.M.B + m + m + h ann + 1000 M.M.B + m + m + h ann + m + m + m + m + m + m + m + m + m + m + m + m + m + m + m + m + m + m + m + m + m + m + m + m + m + m + m + m + m + m + m	* Anto Casters Felder Chin * Caters Company GUE Chin	Analysis Controls * Visualization Danimits *	
P-dan stallator     Porter 4 2000 (No. 1-40.4pm     Porter     Porter 4 2000 (No. 1-40.4pm     Porter 4 2000 (No.4000 (No	•	inga •	Here.
Im-bitater, Accorption, 2Capit     Image: Image     Image: Imag	* 🔂 Capter Tendapor Chi-	LasesProvence +	100 C
✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓         ✓			
Si KU Rufsport Set Appantine     @ 10 Unigenet Association     @ 20 University     Consultant     @ Co		2	
Label Constant ¹ / ₂ Strukture	<ul> <li>KD Kultpoint Set Apputition</li> <li>KD Exponent Explanation</li> <li>KD Explanation</li> <li>KD Explanation</li> </ul>		
	stadeg Corrections ¹⁶ ₁₆ Stevalation		
A contained of the second	A contained on the		



2. Control the Z drive position.

Click the upper and lower blue arrow buttons to move the Z drive. Each clicking of the button can move the Z drive up and down by one step (predetermined length).

XYZ Navigation : XY [um]	K		
>	Coarse		
XY step	Fine	0.1 1 10 5.0	2 drive shift button
1000.0	FOV	<b>V V V V</b>	, <u>-</u>
	100% -		
KY - XYdrive Simula			
X [um]: 0.0	Range: <-55.00	), 55.00> mm Mov	-
Y [µm]: 0.0	Range: <-35.00	), 35.00>mm	
Z - Zdrive Simulato Z (µm): 0.00	Range: <-1000	.00, 1000.00> Move	
XY=[0.0000, 0.000	0]mm, Z=0.0µm		

Figure 11.3-3 XYZ Navigation window

* The travel distance can be set with three precision levels (0.1  $\mu$ m, 1  $\mu$ m, 10  $\mu$ m) and the user-

defined step value.

* The Z drive can also be moved directly to any position by entering its coordinate in the [Z-Nikon RFA ZDrive] edit box.

# **12 Using C2+TIRF System**

By combining the observations of single molecules with laser TIRF and the sectioning capabilities of the C2, C2+TIRF System allows for multi-perspective cellular analysis.

Use the "digital imaging head for C2 for Nikon microscopes" attached to the left-side port of Nikon microscope [ECLIPSE Ti] and CCD camera attached to the back port, by switching between them.

With the C2, Z stack images (more than one) are acquired, and with the CCD camera for TIRF position (one image) is acquired.

# 12.1 Starting the C2+TIRF System

To use C2+TIRF System, you may select it at activation of NIS-Elements C or after the activation.

Steps to enable C2+TIRF System at activation of NIS-Elements C

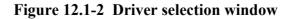
1. On the Driver selection window displayed at activation of NIS-Elements C, turn "ON" the [Enable Multi Camera] check box.

On the Driver selection window, you may select the second camera.

	NIS-Elements AR x.xx.xx (Build	d xxxxx) 64bit - Dr	iver selection
	Nikon Confocal		·
Enable Multi Camera —	Enable Multi Camera	ОК	Cancel

# Figure 12.1-1 Driver selection window

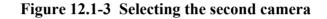
First driver:		
Nicon Confocal		-
Nixon Confocal		-
Enable Multi Camera	СК	Cancel



2. From the pull-down menu of the Second driver:, select the secondary camera.

For the CCD camera, only [ANDOR] is selectable.

Arst driver:	a ser all'ha ne e an an Arth (1997) a 1999. A
Nixon Confocal	-
econd driver:	
Nikon Confocal	-
+ ANDOR	



3. Click the [OK] button to starts the NIS-Elements C. After NIS-Elements C activates, the menu bar shows two camera operation icons.

NIS-Elements AR x.xx.xx (Bu	uild xxxx) 64bit - Driver selection
First driver:	
Nixon Confocal	•
Second driver:	
• ANDOR	•
🕅 Enable Multi Camera	OK Cancel

Figure 12.1-4 Starting the NIS-Elements C

-		ements A Acquire		n hans	Off Proces	Maaning	Deference	Vierre	Databas	an Vara	Denice	window	v Apple	abora	Decegyplation	Help
	D	-	6 %	C2plus		OF	Color Sim		à 🕘						INTR. 🥥 6PE	
40																

Figure 12.1-5 Menu bar for C2+TIRF System

Enabling C2+TIRF System after Activation of NIS-Elements C

1. Select [Acquire] on the menu bar and then select [Select Driver...]. The Driver selection window appears.

selecto	ivel		E • 00	ir 9n 🕑 👔	 Ø. E.	12 B	🙂 INTSI. 🔘
	• • • • • •		6 7 E	Curtoriza +			
Select 0	anera	•					
Camera	Settings (Cliplus)	P11					
Uve -P	ut.						
Live - Q	uality.	CB1++					
<ul> <li>Electe</li> </ul>							
ChORUS		C31+-					
0 6.0 C	oture C	tri+Epace					
Averag	(DFF)	•					
Integra	= (OPT)	· ·					
Cemera	ROE						
O RANG	otune						
AVE Acc	ubiton						
Fast Tar	elapse						
P Capture	Multicharmel Drange	•					
Capture	2-Geries	•					
Capture	Tranlapor						
Capture	Multipoint						
Custore	Acquisitor						
0720 12	ge snage tries shape.	100 C					
	ge iwage						

# Figure 12.1-6 To display the Driver selection window

ild xxxx) 64bit - Di	river selection
	-
ОК	Cencel
	ild xoox) 64bit - Di

# Figure 12.1-7 Driver selection window

2. Turn "ON" the [Enable Multi Camera] check box. On the Driver selection window, you may select the second camera.

	NIS-Elements AR x.xx.xx (Build	d xxxx) 64bit - Dri	iver selection
	Nikon Confocal		•
Enable Multi Camera	Enable Multi Camera	ОК	Cencel

Figure 12.1-8 Driver selection window

First driver:		
Nikon Confocal		-
Serond driver:		•
Enable Multi Camera	ОК	Cancel

# Figure 12.1-9 Driver selection window

3. From the menu of the Second driver:, select the secondary camera. For the CCD camera, only [ANDOR] is selectable.

Nikon Confo	cal	-
cond driver:	al	
INKON CONTO		

#### Figure 12.1-10 Selecting the second camera

4. Click the [OK] button to confirm it.

The menu bar shows two camera operation icons.

iild socce)	) 64bit - Dr	river selection
		Ţ
_		-
	OK	Cancel
	ild soost	ild xxxx) 64bit - Di





Figure 12.1-12 Menu bar for C2+TIRF System

# 12.2 Setting ECLIPSE Ti and Laser Connection

Make settings using the following procedure to synchronize NIS-Elements with the ECLIPSE Ti and LU4A (Laser).

# 1 Call the Manage devices window

Select [Devices] on the menu bar and then select [Manage devices...]. The Manage devices window appears.



Figure 12.2-1 Devices menu

Nanage devices		
Installed devices:		
		Add -
		Renove
Physical Devices		
Connect Disconnect	Configure Device	
	· · · · · · · · · · · · · · · · · · ·	
Connection Parameters	Report	
and the second	Reset	
Connection Parameters	Reset	

Figure 12.2-2 Manage devices window

#### 2 Add "Nikon Ti"

Click the [Add] button to display the menu for devices to be added. Select the "Nikon Ti" form pull-down menu. "Nikon Ti" is added in the [Installed devices:] area.

Page 7	of 24
--------	-------

anage devices		
netalled devices:		
	Add	
	Nikon Ci-E	
	Nikan FN1/D1H	
	Nikan Intensiight	
	Nikan LLMA	
	Nikan LUSU	
	Nkon LUSU and AOM	
	Nikon N-C	
	AR DEA	
	Nikan Ti	
Physical Devices	Configure Device	
Connection Parameters	Reset	
Logical Devices Device Parameters		

Figure 12.2-3 Manage devices window

lanage devices		<u> </u>
netalled devices;		
G- and Vilson Ti     Morescope     More	:EP() :D(A) inator-DD(A) inator-EP() e: Turret(1) e: Turret(2) son, neme: Emission Whee()	Close
Physical Devices	Configure Device	
Connection Parameters	Reset	
Logical Devices		

# 3 Add "Nikon LU4A"

Click the [Add] button to display the menu for devices to be added. Select the "Nikon LU4A" form pull-down menu. "Nikon LU4A" is added in the [Installed devices:] area.

- and Niloon Ti	Abd V
- Vincescope - Vincescope - Vin Tiz - Ti Piezoz	Nikon Cr-E Nikon Ret (/OIH
- XYDrive - XIDrive (type: EP), name: DVTS.)	Nikon LUHA
Shutter3(type: EPI, name: EPI)     Shutter3(type: DIA, name: DIA)     Shutter2(type: DIA, name: DIA)     Univitator EDIA(name: Illuminator EDIA)     Univitator EDIA(name: Illuminator EDIA)     Univitator EDIA(name: Illuminator EDIA)     Univitator EDIA (type: Illuminator EDIA)     Dineston Whee((type: EPI, name: Evidation W     Analyser     O Condenser     V PF5     Q TIREPArrow     TIREPosition     V Zoom	
vysical Devices	wide
Gonnect Deconnect Configure De	
Connect Deconnect Configure De	eset

Figure 12.2-5 Manage devices window

anage devices		
stalled devices:		
Shutter 1(type: CPI, nam     Shutter 1(type: CPI, nam     Shutter 2(type: DIA, nam     Uninstor-OlA(name: Ik     Uninstor-PI(name: Ik     Uninstor-PI(name: Ik     Uninstor-PI(name: Ik     Uninstor-PI(name: Ik     Uninstor-PI(name: Ik     District (type: Turret, na     District (type: Turret, nam)     PS     TIRPNamo     Shutter (type: PI, name)     Shutter	ie: DTÅ) uminator-D(A) uminator-D(A) me: Turret1) me: Turret2) isoson, name e: Emission White P[, name: Excitation White P[, name	
4 II.		
frysical Devices Connect Disconnect Connection Parameters	Configure Device	
ogical Devices		
Device Parameters		

Figure 12.2-6 Manage devices window

#### 4 Setting the Lasers (Nikon LU4A)

1. Call the LU4A Pad window.

As shown below, right-click on the gray area (without any setting window displayed) to display a menu. Then select [Acquisition Controls] -> [LU4A Pad] in the menu to open the LU4A Pad window.

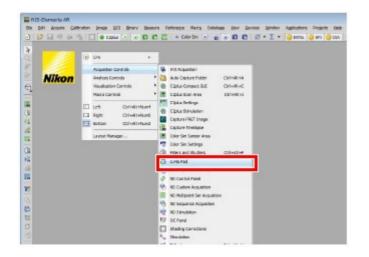


Figure 12.2-7 To display the LU4A Pad window

 Open the LU4A Configuration window. Click the [Configure...] button in the LU4A Pad window. The LU4A Configuration window appears.

12         6.33 mm         0.0         [Ni]           21         405 nm         0.0         [Ni]           22         405 nm         0.0         [Ni]           24         437 nm         0.0         [Ni]           44         477 nm         0.0         [Ni]           54         488 nm         0.0         [Ni]           52         534 nm         0.0         [Ni]           54         543 nm         0.0         [Ni]           543 nm         0.0         [Ni]         1           543 nm	2 405 nm 2 457 nm 4 407 nm 4 477 nm 4 477 nm 4 477 nm 4 477 nm 4 477 nm 5 458 nm 5 554 nm 5 554 nm 5 555 nm 5 556 nm, 100 Manual Test Shot				
ti 495 mm 0.0 [1%]	It = 405 nm It = 457 nm It = 457 nm It = 477 nm It = 477 nm It = 488 nm It = 488 nm It = 488 nm It = 514 nm It =			0.0	[%]
1         457 mm         0.0         [Mail           1         457 mm         0.0         [Mail           4         477 nm         0.0         [Mail           4         477 nm         0.0         [Mail           5         458 mm         0.0         [Mail           5         543 mm         0.0         [Mail           5         543 nm         0.0         [Mail	12 437 mm 14 477 mm 15 488 mm 15 488 mm 15 514 mm 16 513 mm 17 513 mm 16 518 mm	1 1	1	1.1	1.00
31:         457 mm         0.0         [Me]           41:         477 mm         0.0         [Me]           5:         483 mm         0.0         [Me]           5:         483 mm         0.0         [Me]           5:         514 mm         0.0         [Me]           5:         513 mm         0.0         [Me]           5:         513 mm         0.0         [Me]           Manual Test Shot         [S38 mm, 100%]	12 437 mm 4 477 mm 12 438 mm 12 438 mm 12 514 mm 13 514 mm 14 513 mm 15 514 mm 15 518 mm 1			0.0	[96]
41         477 mm         0.0         [1%]           51         488 mm         0.0         [1%]           52         514 mm         0.0         [1%]           54         543 mm         0.0         [1%]           54         543 mm         0.0         [1%]           5         543 mm         0.0         [1%]           Manual Test Shot         [538 mm, 100%],         Manual Test Shot	400 mm           12         400 mm           12         514 mm           13         514 mm           14         543 mm           15         543 mm           16         543 mm           17         543 mm           18         514 mm           19         543 mm           10         Manual Test Shot           Pulse Test Shot         Pulse times	1.1	1	1.1	1.1
41         477 mm         0.0         [1%]           51         488 mm         0.0         [1%]           52         514 mm         0.0         [1%]           54         543 mm         0.0         [1%]           54         543 mm         0.0         [1%]           54         543 mm         0.0         [1%]           5         543 mm         0.0         [1%]           Manual Test Shot         [538 mm, 100%],         Manual Test Shot	41         477 nm           12         488 nm           14         544 nm           15         544 nm           16         543 nm           17         543 nm           18         544 nm           19         543 nm           10         543 nm           10         543 nm           10         Manual Test Shot           Pulse Test Shot         Pulse times			0.0	[%]
1         488 mm         0.0         [%]           5:         488 mm         0.0         [%]           5:         534 mm         0.0         [%]           7:         543 mm         0.0         [%]           5timulation         538 mm, 100%],         Manual Test Shot	te 488 mm te 514 mm te 514 mm Stimulation Settings [558 mm, 100 Manual Test Shot Pulse Trait Shot	4	11	1.1.1	1.1.1
5: 483 mm 0.0 [1%]	t: 488 nm t: 514 nm t: 514 nm t: 514 nm t: 513 nm t: 513 nm t: 513 nm t: 513 nm t: 513 nm t: 514 nm			0.0	[96]
543 mm         0.0         [54]           543 mm         0.0         [56]           543 mm         0.0         [56]           Stimulation         5         5           Settings         [538 mm, 100%],         Manual Test Shot	Stimulation Settings Manual Test Shot Pulse Test Shot Pulse Test Shot	1 1	1	1.1	1
51         514 mm         0.0         [5%]           7         543 mm         0.0         [5%]           Stimulation         5         5         5           Settings         [538 nm, 100%],         100%],         100%],	te 514 nm 543 nm Stimulation Settings [538 nm, 100 Manual Test Shot Pulse Treat Shot Pulse Treat Shot			0.0	[%]
543 nm         0.0         [56]           Stimulation	Stimulation Settings Manual Test Shot Pulse Test Shot Pulse Test Shot			1.1	1.1
7.         543 nm         0.0         [56]           Stimulation	h 543 nm Stimulation Settings (538 nm, 100 Manual Test Shot Pulse Test Shot			0.0	[96]
Settings (538 nm, 100%), Manual Test Shot	Stimulation Settings (538 nm, 100 Manual Test Shot Pulse Test Shot Pulse times	1 1	1	1 1	1.20
Stimulation Settings (538 nm, 100%), Manual Test Shot	Stimulation			0.0	[%]
	Pulse Teat Shot Pulse tine:	538 mm,	nn, 1	.00%],	
Pulse Text Shot Pulse times 200 [msec]	L				
	Seam Sauther	ulse tim	e trie	100	[msec]
		10	1		
	Contocal	1	-	140	_
Confocal	hutters				
	ACTE SH5				
Conficul TRP	0 638 mm 0 +05 mm 0 Aroon				

# Figure 12.2-8 LU4A Pad window

3. Select the laser to be used.

Select the laser to be used from the pull-down menu of each laser. After selecting the laser, click the [OK] button to confirm the settings.

L4		L3 L2 L1	Select the lase
543 nm	• 457, 477,	488, 514 nm 💌 (405 nm 💌 638 nm 💌	
aser Lines			to be used.
	Name	Wavelength [nm]	
1.	638 mm	638	
2	405 nm	405	
3.	457 nm	457	
4	477 nm	477	
5.	488 mm	488	
6.	514nm	514	
	[ mun		
7.		543	
Cogarithm		543	
Cogarithm			
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Detions bow Shutte 638 nm	ric scale		
Degenithm options show Shutte 638 nm	ric scale	solber	
Logarithm lptions how Shutte 638 m Show AC	ric scale	solber	
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Department Deptions Show Shutte G38 mm Show AO asser Ports	rs on Devices to	solbar Argon ☐ 543 mm I SH5	
Logarithm Options Show Shutte 638 nm Show AC Left port	rs on Devices to	solbar Argon ☐ 543 mm I SH5	
Logerithn Dotions Show Shutte G38 mm Show AC Left ports Left port	IL scale	olbar Argon 543 nm 🕑 SH5	
Logerithn Dotions show Shutte 638 nm Show AO Left ports Left port Info Si Firmwa	nic scale rs on Devices to 405 nm TF Shutter Confocal	olbar Argon 543 nm 🕑 SH5	



# 12.3 Optical Configuration Setting

Register the settings for the confocal image acquisition with C2 and the TIRF image acquisition with CCD camera to the Optical Configuration files.

This section describes the setting of laser optical path switching (set on the LU4A Pad window) and setting of device selection (set on [Ti Pad]) for image acquisition that are to be configured only for C2+TIRF system.

# 12.3.1 Optical Configuration Setting for C2

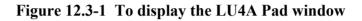
#### 1 Switch the laser optical path on the LU4A Pad window

1. Call the LU4A Pad window.

As shown below, right-click on the gray area (without any setting window displayed) to display a menu.

Then select [Acquisition Controls] -> [LU4A Pad] in the menu to open the LU4A Pad window.

Nikon	Lins + Acquisiter Controls Andress Controls Visuelization Controls Macra Cantrols	Capius Compact Rull Conference
	Andrato Controls VaueRation Cantrols	Aufe Capiture Frolder     Centralit-IA     Capiture Company: BUE     Centralit-IA
	VasuRation Cantrols	Capius Compact Rull Conference
	Magra Carsoit P	Capital Scan Wild
	Let Ostatitunt	10 Elsis Brilings
	I Rote: Col+At+Numi	Cpte Strukten
		Caphure FRGT Image
		Capiture Threadport
	Lavout Nenager	Color Sin Sensor Area
		🐨 Color Sin Sattings
		A descent and and a descent and a d
		🗇 siya kut
		AD Carbid Panel
		45 ND Custom Acquisition
		30 Multipoint Set exculation
		MD Bequares Acquisitor
		10 Involution 10 OC Panel
		C Pand blacking Carrectores



2. With Beam Switcher in the LU4A Pad window, select the laser optical path. For the optical configuration of the C2, select [Confocal].

	LU4A Simulator Pad x		
	1: 🛄 638 mm	0.0	[%]
		1.18.11	1
	21 🛄 405 mi	0.0	[%]
	0	1.112.11	1.
	3: 457 mm	0.0	[%]
	() _ · · · · · · · · ·		1
	-41 🛄 -477 nm	0.0	[96
	Q. T.	( User	- 553
	5: 433 mm	0.0	[%
	P	1	
	6: []] 514 mm	0.0	[%
	Q		_32
	71 🛄 543 mm	0.0	[%]
	Q-, , , , , , , , , , , , , , , , , , ,		
	Stimulation		
	Settings  638 ref	. 10096].	
	Manual Test Shot		
	Pulse Text Shot Pulse to	202	Inter
	[] Puige #	act Level	Jused
	Ream Switcher		
gure the C2 setting,	Confocal	TIRP	_
onfocal]	Shutters		
1.60	ACTE SH5		
		. Le	
	638 nm 405 nm	argon 10	543 ni
	🔮 Canfigure		

Figure 12.3-2 LU4A Pad window

# 2 Select the device to be used for image acquisition with [Ti Pad]

# 1. Call the [Ti Pad].

As shown below, right-click on the gray area (without any setting window displayed) to display a menu.

Then select [Acquisition Controls] -> [Ti Pad] in the menu to open the [Ti Pad].

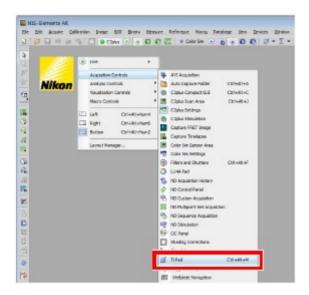


Figure 12.3-3 To display the Ti Pad

 Select the device for [Ti Pad]. For the optical configuration of the C2, select [EPI].

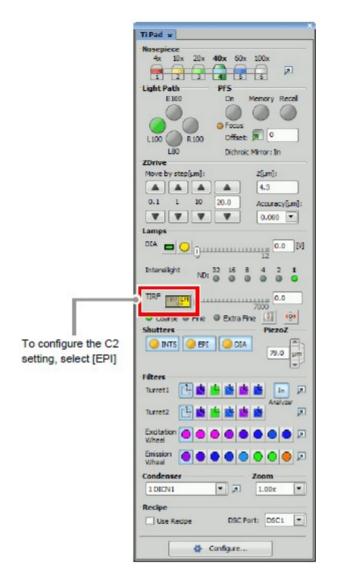


Figure 12.3-4 Ti Pad

3. Configure laser power and other settings on [C2plus Settings], and register the configuration to the Optical Configuration file. For storing and retrieving the [Optical Configuration] settings, see the sections concerning the optical configuration in the "NIS-Elements AR (Advanced Research) User's Guide."

# 12.3.2 Optical Configuration Setting for CCD Camera

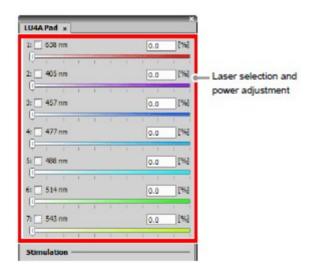
# 1 Switch the laser optical path on the LU4A Pad window

- 1. Call the LU4A Pad window.
- 2. With Beam Switcher on the LU4A Pad window, select the laser optical path. For the optical configuration of the CCD, select [TIRF].

LU4A Pad x			
1: 638 mm	0.0	[25]	
0			
21 🛄 405 nm	0.0	[96]	
	1 1 1	1	
3: 🔄 457 mm	0.0	[95]	
0	1. 1. 1	1	
41 🗌 477 nm	0.0	[96]	
	1. 1. 1.	1	
5: 🗌 455 m	0.0	[76]	
	1 1 1	1.17	
6: 514 mm	0.0	[94]	
U	1 1 1	1 1	
71 🔄 543 nm	0.0	[96]	
Stimulation [538]	nm, 10096],	-	
Manual Test Shot			
Pulse Test Shot Pulse	time: 300	[msec]	
Beam Switcher			
Confocal	TRP	- C	To configure the CCD camera
			setting, select [TIRF]
Shutters			
ACITE SH5			
@ 638 nm @ 405 nm @	Argon	543 nm	
🖉 Config	rt		



3. Select the lasers for CCD camera image acquisition and make power adjustments.





# 2 Select the device for image acquisition on [Ti Pad]

- 1. Call the [Ti Pad].
- 2. Select the device for [Ti Pad].

For the optical configuration of the CCD, select [TIRF].

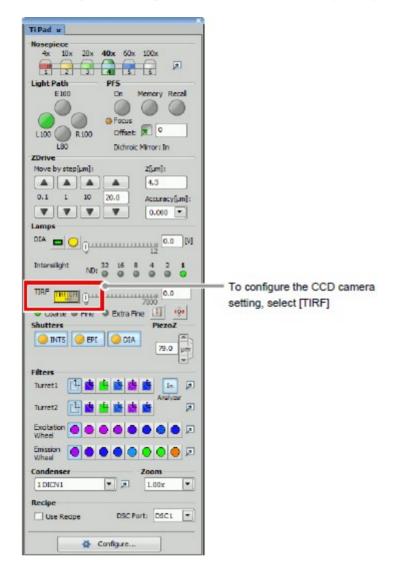


Figure 12.3-7 Ti Pad

3. Register the Optical Configuration file for the CCD camera. For storing and retrieving the [Optical Configuration] settings, see the sections concerning the optical configuration in the "NIS-Elements AR (Advanced Research) User's Guide."

# **12.4 Procedure of Image Acquisition**

Acquire the images by using the registered Optical Configuration files.

# 1 Call the ND Acquisition window

Select [Applications] -> [Define/Run ND Acquisition...] from the menu bar to open the ND Acquisition window.

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B	otton	N/A		Mo	re to TIRP	
	µn (⇔ 0.	E.S.C.	Steps	Range:	N/A	μm
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Step: 1.000	-	and the second se				
Bottore N/A	hu	Tapi N/A	thus	Topi	IN/A	Jun
Step: 1.000 Bottorrc N/A Z Device: Ti 2Dr	- 5	Tapi N/A	hu	Topi Battomi	-	µn un
Bottore N/A	- 5		hw		-	=
Bottorrc N/A Z Device: Ti ZDr	tve	•	Ioni 🛞 E	Battom ottom to 1	IN/A Top	=
Bottore N/A	tve	•	Ioni 🛞 E	Battom	IN/A Top	=

#### Figure 12.4-1 ND Acquisition window

# 2 Set the Z stack for image acquisition with C2

1. Turn "ON" the [Z Series] tab check box.

Σ:[				
Seve to file		Record De	sta	
Order of Experiment + Timing				
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IXX		RF		
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Step: 1.000 µm 🗢 0.175µm 31 Ste	Range	e: INGA	μm	
Battorn: N/A µm Tapi N/A µm	Relati	ve Positions:		
Z Device: Ti ZDrive	Тарі	NúA	μm	
	Battor	mi (NJA	μn	
Close active Shutter during Z Movement Direction:	Bottom t	р Тар		
	Top to B	ottom		

Figure 12.4-2 ND Acquisition window

2. Set the Z stack for image acquisition with C2. For Z stacks settings, refer to "NIS-Elements AR (Advanced Research) User's Guide."

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	pa ange: 30.00 µm	
Close active Shutter during 2 Movement Direction:	Bottom to Top     Top to Bottom     Advanced >>     1 time loop     If Run now	

Figure 12.4-3 Z stacks settings

# 3 Set the TIRF position for image acquisition with CCD camera

1. Turn "ON" the [TIRF] check box.

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Order of Experim	ent • Timing	i i					
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and the second second	hu			_	Relative	Positions:	
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Bottorre 0.00 2 Device: Ti 2	prive	Тари	30.00	]µm	Relative Topi Bottomi	Positions: +14,00 -16.00	]µn ]µn

Figure 12.4-4 Select the TIRF

2. Set the TIRF position for image acquisition with CCD camera. Adjust the Z stage to the TIRF position. You may adjust the Z stage to the TIRF position with either of the following methods: (1) Manually handle the Z stage.

(2) Into [ZDrive] for Ti Pad, input the TIRF position to operate the Z stage.

After adjusting the Z stage to the TIRF position, click [Set TIRF Posc]. The Z stage is set to the TIRF position.

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Close active Shutter during 2 Move	ment	Directo	n: 🛞 B	ottom to T	op	
			От	ap ito Bott	a m	
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#### 4 Register the devices for image acquisition

1. Turn "ON" the [Lambda] tab check box.

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Optical Conf.	Name	Comp. Calor	2 Pos.	* X 8	
1 days artists the	itter during Filter C	hanna			

## Figure 12.4-6 Select the Lambda tab

2. Register the C2 and CCD camera.

Click below the [Camera] column, and then register the first device. Click on the second line, and then register the second device.

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	and the second se			a . LT	Large Image		
	Setup				* 00	++	XX
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	C2plus	The config.	<b>T</b>	DAPI		AI	
egistration of device				FITC			
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						Adve	nced >>

Figure 12.4-7 Registration of device

# 5 Select the Optical Configuration file

Select the Optical Configuration files for the C2 and CCD camera from the pull-down menu for Optical Conf.

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Figure 12.4-8 Select the Optical Configuration file for C2

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		Texas Red					
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	e snutter during Hiter Define Ratio		efræ PRET	Adv	anced >>		camera



# 6 Setting the Z phase for TIRF

Select the [TIRF] from the pull-down menu of [Z Pos.] for CCD camera. The C2 acquires images of all positions configured with Z stacks, thus the setting remains as [All] without change.

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Color 💌				All Home	0	Select the [TIRF
Color 💌						
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C Close active	Shutter during Filte		efre PRET			
C Close active			efre PRET	TIRF	ced >>	

Figure 12.4-10 Z phase settings

#### 7 Start the image acquisition

Click the [Run now] button to start the image acquisition.

After image acquisition, two image windows for C2 confocal image and CCD camera TIRF image are displayed.

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Figure 12.4-11 Run now

# **12.5** Merge the Confocal Image and TIRF Image

When the C2+TIRF System is in use, a confocal image acquired with C2 and a TIRF image acquired with CCD camera can be merged and acquired as one image.

#### Merge Camera

Turn "ON" the [Merge Camera] check box of the Advanced menu on the Lambda tab and click the [Run now] button to execute the image acquisition to get merged images.

Note that images are simply merged without any regard for the difference in the sizes of the C2 confocal image and the CCD camera TIRF image even if they are not in the same size, thus it is recommended to adjust the image sizes in advance.

#### Merge Camera + Stretch Camera Image to Same Size

To coordinate the confocal image and TIRF image sizes, turn "ON" both the [Merge Camera] check box and [Stretch Camera Image to Same Size] check box and then perform image acquisition.

This makes the smaller one expanded to the larger image size and a merged image is acquired with the same size.

However, even in that case, note that the image is simply expanded for size coordination regardless of what is represented in the image, thus it is recommended to adjust the image sizes in advance.

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# Figure 12.5-1 ND Acquisition window

# 13 C2plus Compact GUI

NIS-Elements allows you to use the C2plus Compact GUI window, which is a simplified configuration screen supporting functions equivalent to those of "C2plus Settings," so that you can sufficiently use the window spaces.

This chapter describes how to show the screen and the functions available on it.

# 13.1 Displaying the C2plus Compact GUI

As shown below, right-click on the gray area (without any setting window displayed) to display a menu. Then select [Acquisition Controls] -> [C2plus Compact GUI] in the menu to open the C2plus Compact GUI window.

(If you are using a Galvano scanner that does not support the Fast Galvano, select [Acquisition Controls] -> [C2 Compact GUI] from the menu.)

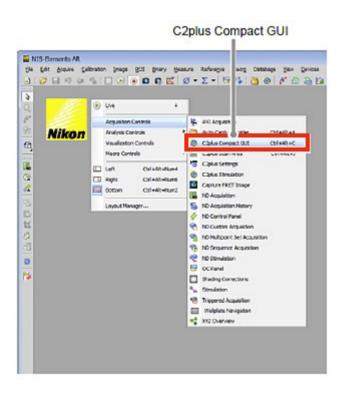


Figure 13.1-1 To display the C2plus Compact GUI

C2plus Compact GUI ×

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Fps:	0.353; Frame	Time: 2.8 sec 🔹 Settings 🔹
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🗹 Indo-	-1/Ca2+saturat	ed Laser 405.0 nm 0.0
<mark>⊮ Indo</mark>	-1/Ca2+saturat	ed Laser 405.0 nm 0.0
8	-1/Ce2+saturat	
HN	-1/Ca2+sabrat	
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HV Offset	0	0 0 9,00 Laser 458.0 nm 0.0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
HV Offset	0 e pH 7.4 0 e o pH 7.4 0 c o c o c o c o c o c o c o c o c o c o c o c o c o c o c o c c c c c c c c c c c c c	
HV Offset ● 405 ✓ OFP/ HV Offset ● 458 ✓ FluoS HV Offset ● 561 ✓ TD	0 e pH 7.4 0 e o pH 7.4 0 c o c o c o c o c o c o c o c o c o c o c o c o c o c o c o c c c c c c c c c c c c c	O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O     O

Figure 13.1-2 C2plus Compact GUI (DU3-use)

# 13.2 Functions of the C2plus Compact GUI

The C2plus Compact GUI window allows you to configure settings for use of the Confocal Microscope C2 in the same manner as you configure with [C2plus Settings].

The following shows an example of the screen where DU3 is selected for Detector mode.

For details of each item by detector, see the appropriate chapter.

	(1) (2) (3) (5)	
	C2plu Compat GUI =	
	Scan Capture Find	
(4)	City Eye Port AG	
		1000
(6)		(7)
(8)	Control by:  Pixel Dwel Frame/ser	(10)
(9)	Fast Mode 1.9 4.8 10.3 21.6 44.2	(10)
(11)	Size	
(11)	64 <u>128 256 512</u> 1024 2048	
	Normal Ø 2x v 2 2x v	(12)
(13)	3h Series	(14)
		(15)
(16)	<ul> <li>Fps: 0.353; Frame Time: 2.8 sec</li> <li>Settings -</li> </ul>	(17)
(18)	Pinhole 1.2 1.2 AU	(19)
(20)	● AU calculated for: 561.0 ▼ 30.0 µm	
(21)		(22)
/	Indo-1/Cs2+seburated Leser 405.0 nm 0.0	
(	HV 00	
	Offset0	
	CFP/pH 7.4 Laser 458.0 nm 0.0	
(23)		
7 A A A		
	● 458 · ND 9.00	
	FluoSpheres red fluor Leser 561.0 nm 0.0	
	HV ()0	
	Offset0	
(	9.00	
(24)	о ъ <u>с</u>	
	HV () (0)	
	Offset 0	

Figure 13.2-1 C2plus Compact GUI (DU3-use)

Table 13.2-1 Functions of the C2plus Compact GUI

	Name	Function
(1)	Scan button	Starts/stops live image acquisition.
(2)	Capture button	Captures the image.
(3)	Find button	Starts/stops live image acquisition in Find mode. Find mode is the mode where the live image acquisition is executed by temporarily switching to the high-frame-rate setting in order to ease the detection of the observation object such as a cell.

		For details, see Section 4.1.2, "Find Mode."
(4)	Eye Port button	Changes optical path to eye port. Each function on the C2plus Settings window becomes non- selectable when the Eye Port button is selected.
(5)	Auto Gain	<b><u>DU3-use</u></b> Automatically adjusts the HV value (HV gain) of the currently selected channel to the optimum values.
		* For details, see the following.
		DU3: Section 5.2.5, "Auto Gain." <u>SD or VF-use</u> Automatically adjusts the Si HV value (Si HV gain) to the optimum values.
		* For details, see the following.
		SD: Section 6.2.4, "Auto Gain."
		VF: Section 7.2.4, "Auto Gain."
(6)	Laser power monitor button	Displays the laser power value (integer obtained after A/D conversion divided by 10) of the current channel by clicking this button.
		* When a laser unit of the LU-N series is in use, the value displayed in the monitor does not increase over a certain value with the increase in the laser power value, but this is not a problem.
(7)	Scan Direction	Toggles between Unidirectional and Bidirectional scan. Bidirectional scan is only selectable if the Square scan area, Band scan area, or Line scan area is set. By default, Unidirectional scan is selected.
		The button is enabled only when the Bidirectional scan is selected. Clicking this button displays the Align bidirectional scanner window allowing correction of image shifting.
		* For details, see the following. Section 8.3, "Scan Settings."
(8)	Control by:	Switches the Scan Speed selection form.
(9)	Fast Mode	Turning on this button makes the scan speed high. When a Fast Galvano compatible model (C2plus) that supports high scan speed is in use, the scan speed is further increased by turning on the [Fast] button and set the scan magnification to 8X or higher.

	* For speeding-up of the scan speed, see Section 8.3.1, "Fast Mode."
(10) Scan Speed	Selects scan speed. (Setting unit: Frame/sec or lines/sec in line scan mode)
	* When the Band scan area is selected, scan speed values shown in each Scan Speed button are guideline, which may not be the actual scan speed.
(11) Scan Size	Selects the scan resolution in the X-direction. (Setting unit: Pixel)
	The resolution in the Y-direction is automatically calculated from the X to Y ratio of the scan area.
(12) Scan Area window button	Displays the Scan Area window. For details of Scan Area window, see Chapter 9.
(13) Ch Series button	Settable only in DU3-use.
	When the [Ch Series] button is turned on, channel series is set allowing the user to set a desired order of channels to be scanned.
	Click $\checkmark$ to the next of the [Ch Series] button and select [Flexible setupc] to open the Line Channel Series Setup window. Set the scan order on this window.
	Channel series can also be applied to the image acquisition phase in the photo activation experiment sequence (but cannot be applied to the stimulation phase).
	* For details, see the following. Section 5.1.4, "Selecting the Channel Series."
(14) Ch.Setup check box	Displayed when the [Ch Series] button is ON.
	When checked, the setting by the channel is facilitated.
	Automatically enters the state where only one channel is selectable.
(15) Channel Series scan order	Displays the channel scan order when Channel Series is set.
(16) Fps:	Indicates the current scan settings.
(17) Settings button	Displays the menu for various settings such as HV Linear
	Correction.
	* Select [Show AUX Detector] from the menu that appears when the [Settings] button is clicked to make an external detector available. While an external detector is in use, the [AUX] icon is displayed on the Compact GUI window. For details, see Chapter 16.
(18) Pinhole	Adjusts the pinhole size.
	Sets a pinhole size in Airy units (units of airy disk size).
	Slider bar:
	Slides to the right or left to set the pinhole size. (Unit:

	A.U.)
	<b>Direct entry in pinhole size display field:</b> Type the desired setting value.
	* For details, see the following.
	<b>DU3:</b> Section 5.2.3, "Setting the Pinhole."
	<b>SD:</b> Section 6.2.3, "Setting the Pinhole."
(19) AU button	VF: Section 7.2.3, "Setting the Pinhole." Changes the pinhole to the predetermined home position. The value of the home position can be changed in the A.U. Calculation Settings window.
	* For details, see the following.
	<b>DU3:</b> Section 5.2.3.1, "Calculation Settings for Pinhole Size."
	<b>SD:</b> Section 6.2.3.1, "Calculation Settings for Pinhole Size."
	VF: Section 7.2.3.1, "Calculation Settings for Pinhole Size." The A.U. Calculation Settings window is displayed by selecting [AU settingsc] from the setting menu displayed by the [Settings] button.
(20) Reference excitation wavelength for the pinhole size calculation	Selects the excitation wavelength as the reference of the automatic calculation of the pinhole size from the laser wavelengths, or enter it manually in the A.U. Calculation Settings window.
	* For details, see the following.
	<b>DU3:</b> Section 5.2.3.1, "Calculation Settings for Pinhole Size."
	<b>SD:</b> Section 6.2.3.1, "Calculation Settings for Pinhole Size."
	VF:

Section 7.2.3.1, "Calculation Settings for Pinhole Size." Opens the Optical path window.

(21) Optical path Setting

button	To use, select the detector and the dichroic mirror, the channels as well as the fluorescence dye, laser, and for each channel.
(22) Detection mode indicator/selection button	Selects/displays the Detection mode for use. When the optical path changeover lever on the C2 scan head is set to the [Spectrum] position, [SD] or [VF] can be selected as the detector mode. When the optical path changeover lever on the C2 scan head is set to the [Standard] position, the detector mode is fixed to [DU3].
	* For details of each item for each Detection mode, see the

* For details of each item for each Detection mode, see the following.



Chapter 5, "Detection Mode DU3."

05	
50	
20	

Chapter 6, "Detection Mode SD."



Chapter 7, "Detection Mode VF."

(23) Brightness adjustment for each channel For each of the channels (Ch1 to Ch3), use the HV, Offset, Laser, and ND filter IN/OUT controls to adjust the brightness of the live image.

Note that these items vary depending on the selected detector.

* For details, see the following.

#### **DU3:**

Section 5.2.1, "Structure of Acquisition Window."

#### SD:

Section 6.2.1, "Structure of Acquisition Window."

#### VF:

Section 7.2.1, "Structure of Acquisition Window."

(24) Brightness adjustment for transmitted detector For the transmitted detector, use the HV and Offset controls to adjust the brightness of the live image.

* When acquiring the transmitted image (TD image) only, see the following.

#### **DU3:**

Section 5.2.1.2, "When Acquiring Transmitted Image Only."

VF: Section 7.2.1.2, "When Acquiring Transmitted Image Only."

# 13.3 Photo Activation Setting Using the C2plus Compact GUI

This section describes how to set the photo activation in the C2plus Compact GUI window. For procedures common to the C2plus Settings window, see Chapter 10, "Photo Activation Setting."

#### **1** Acquire the image of sample for photo activation.

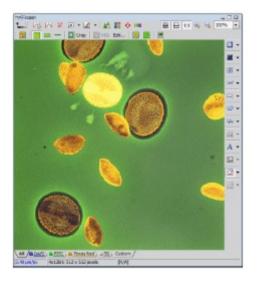


Figure 13.3-1 Frozen image

2 Adjust the brightness of the live image.

☑ DAPI         Laser 405.0 m           Offset         □           ◙ 405         □           0 405         □           ☑ FITC         Laser 433.0 m           HV         □           ○ffset         □           ○ 405         □           ○ FITC         Laser 433.0 m           HV         □           ○ ffset         □           ○ ffset         □	m 0.0
Offset	
A405	89
✓ FITC         Laser 488.0 m           HV         →           Offset         →	0
HV	25.21
	m 0.0
	89
0483	0
*	11.29
Texas Red Laser 561.0 n	m 0.0
ни	72
Offset	0
	3.39
<b>⊡</b> TD	
HV(	120
0ffset	0

Figure 13.3-2 The C2plus Compact GUI window

3 Specify a photo activation area, point, or line.



Figure 13.3-3 Setting of photo activation area

#### 4 Set the stimulation laser.

 To manually display the C2plus Stimulation window, right-click on the gray area (without any setting window displayed) to display a menu as shown below. Then select [Acquisition Controls] -> [C2plus Stimulation] in the menu.

Visualization Controls • (0) Capus Compact Gui Crittevit+C Macro Cantrols • (2) Capus Son Area Cet+Alt+3 Eignature Controls • (2) Capus Son Area Cet+Alt+3		-G i		000	2	•	Σ • 19 15 1 👸	<ul> <li>M 2</li> </ul>	80	44 48	2
Acta Explaint Controls Acta Explains Folder Cell+Alt+A Analysis Centrols  Acta Explaint Cell+Alt+A Vasialization Controls  Acta Explaint Som Area Cell+Alt+C Macro Controls  Acta Explaint Som Area Cell+Alt+1 Explaints Centrols  Acta Explaint Som Area Cell+Alt+1		۲	Live		+	1					
Acta Explain Son Tools Acta Explain Folder Carl+Alt+A Analysis Controls • Ark Acquisition Visualization Controls • Captas Campact GUE Carl+Alt+C Macro Controls • Captas Son Area Carl+Alt+1 Explaints Controls • Carl+Alt+2			Paste								
Visualization Controls • (0) Capus Compact Gui Crittevit+C Macro Cantrols • (2) Capus Son Area Cet+Alt+3 Eignature Controls • (2) Capus Son Area Cet+Alt+3	11111011		Acquisition Co	alatine		-	Auto Capture Polder	CH+Alt+A			
Visualization Controls • (0) Capus Compact Gui Crittevit+C Macro Cantrols • (2) Capus Son Area Cet+Alt+3 Eignature Controls • (2) Capus Son Area Cet+Alt+3			Analysis Cont	sola	٠	4	AVE Acquisition				
Signature Controls + HT Citate Settions	122		Visualization (	Controls	٠	0	C2plus Compact GUE	CIVI+AIT+C			
			Macro Contro	de .	٠		C2plus Scan Area	CH+4H+J			
			Rightane Co	ntrole		145	Citche Gattings	10000000			
I Let Ctd+Alt+Num4				-	-	۲	C zplus atmulation				
						nn.	Illunination Sequence				
Illunization Sequence			Botton	Ctil+Alt+Nu	n2	-					



2. Determine whether to set the stimulation laser for each stimulation area (photo activation frame) or apply the same setting to all frames.

Select the [Synchronize Lasers] check box to apply a single setting to all frames.

C2plus Stimulation ×	ļ		
Synchronize Lesers	HV Mode:	Zero HV	•
Stimulation Area 1			
0 405	0		21.90
0 458	0		14.75

Figure 13.3-5 C2plus Stimulation window

3. Select the laser for photo activation and set the laser power.

C2plus Stimulatio	ers Hy Mode: Ze	NO HV
Stimulation Area 1		
0 405	0	21.90
0 488		14.75
0 561	0	9.99
Scan Speed 1	Pps; Poxel Dwe	I: 1.9(512x512)
Stimulation Area 2		
0 405	0	8.07
0 488	0	15,63
0 561		32.72
Scan Speed 1	+ Fps; Pixel Dwe	I: 1.9(512x512)
Stimulation Area 3		
0 415	-0	20.07
0 403		
0 498	0	8.07
	0 0	8.07
0 458	0 0 • Pos; Posel Dwe	25.40

4. Set the scan speed for photo activation.

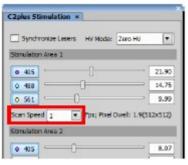


Figure 13.3-7 Scan speed

- 5. Select the HV mode in photo activation.
  - * The HV mode can be set only when [DU3] is selected for the detection mode. For details of settings, see Section 10.1.2, "Setting HV Mode in Photo Activation."

				-
Synch	ronize Lasen	s HV Mode:	Zero HV	-
tinulatio	n Area 1			
o 405	-	0		21,90
0 455		0	[	14.75
0 561	-	0		9.99

Figure 13.3-8 HV mode

#### 5 Open the ND Stimulation window.

Select [Applications] -> [Define/Run Sequential Stimulation] from the menu bar to open the ND Stimulation window.

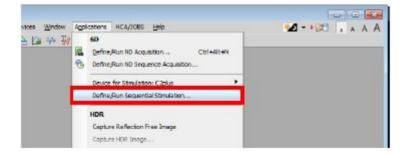


Figure 13.3-9 To display the ND Stimulation window

## 6 Set the photo activation experiment sequence.

For the photo activation setting window, see Section 10.2.1, "Experiment Sequence Setting for Photo Activation Observation."

# 14 External Trigger Output

This chapter describes the external trigger output function of NIS-Elements.

This function allows trigger signals of (e.g., Acquisition or Photo Activation experiments) to be sent to an external device connected with the C2 controller.

* For trigger input to receive trigger signals from an external device, see Chapter 15.

# 14.1 Trigger Signal Output

Trigger signals are output frame by frame when an image is acquired.

One external trigger signal channel is available. Note that the external trigger settings are unchangeable during Live or experiment.

# 14.1.1 Procedure for External Trigger Output Settings

# 1 Call the External Trigger Output Settings window

- 1. [Trigger] check box to turn it "ON." The external trigger output function is turned on, and [Trigger] button becomes effective. If trigger signals output is not to be executed, uncheck the [Trigger] check box.
- 2. Click the [Trigger] button to open the External Trigger Output Settings window.

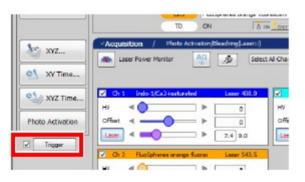


Figure 14.1-1 Trigger check box

# 2 Set the Polarity for the output trigger signal channels

1. Check the trigger signal output setting. [Acquisition] and [Photo Activation] are displayed only when the trigger signal output is ON. 2. Specify the level to output as the trigger signal.

*U* Sets the rising edge of the TTL level signal as the trigger signal.

**I** Sets the falling edge of the TTL level signal as the trigger signal.

3. Click the [OK] button to finish the trigger signals polarity settings.

	CHI L	
Acquisition	ON	
Photo Activation	ON	

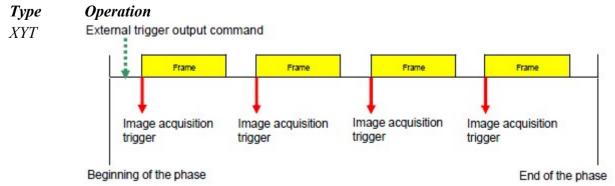


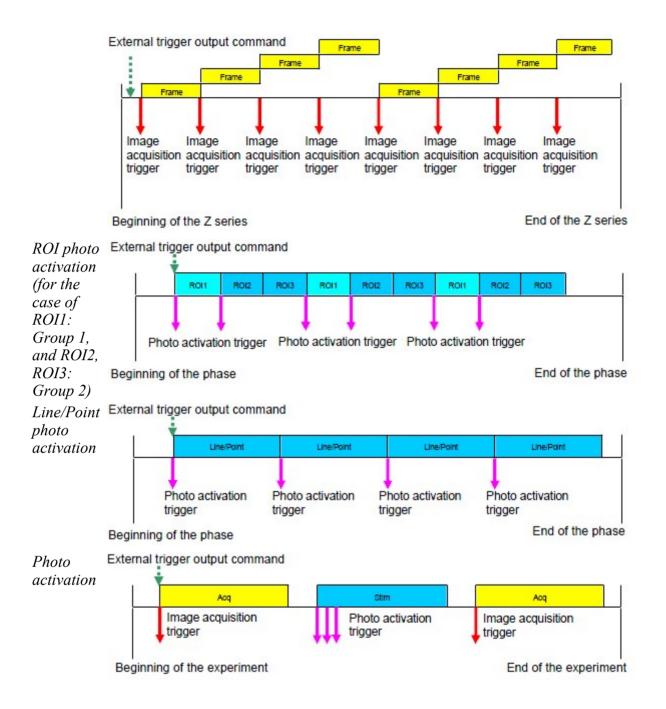
Allocation of trigger signal polarity is memorized for each user and applied when the external trigger settings window opens next.

# 14.1.2 External Trigger Output Operation List

The external trigger output operations are listed below.

Table 14.1-1 External trigger output operation list





# 15 Using Data Acquisition Device (NIDAQ)

This chapter describes operations of the NIS-Elements when the Data Acquisition Device (hereafter NIDAQ) of National Instruments is connected to the PCI Express slot in PC for Confocal Microscope C2. (The NIDAQ is connected to the PCI slot in PC for Confocal Microscope C2, but this slot is provided for use in the system and is not available for external triggers.)

Use of the NIDAQ connected to the PCI Express slot allows the user to start and stop an experiment (including image acquisition) by using trigger signals from external devices connected to the C2 controller.

# **15.1 NIDAQ Connection Settings**

Make settings using the following procedure to synchronize the NIS-Elements with NIDAQ.

#### 1 Call the Manage devices window.

Select [Devices] -> [Manage devices...] from the menu bar. The Manage devices window appears.

1 1 17 14 E (1 (a)	Earrage derives	- Cantonie -
	Patching Rusessant Probes and Filters	

Figure 15.1-1 Devices menu

Manage devices		-
Installed devices:		Add  Renave Cope
Physical Devices	Configure Device	1
Connection Parameters	Reset	
Logical Devices		

Figure 15.1-2 Manage devices window

#### 2 Add NIDAQ.

Click the [Add] button to display the menu for devices to be added. Selecting NIDAQ from the menu adds "NIDAQ" to the [Installed devices:] area and opens the NIDAQ Configuration window.

Nikon	n 90, 80, 01H n G-E n PNL/DIH n Intensight
Nikon Nikon Nikon Nikon Nikon Nikon Nikon Nikon Nikon Nikon Nikon Nikon Nikon	n 90, 80, 01H n G-E n PNL/DIH n Intensight
Nikon	n G-E n PNL/DDH n Internilight
Nikon Nikon Nikon Nikon Nikon Nikon Nikon Nikon Nikon Nikon Nikon	n PNLIDIH n Internelight
Nikon Nikon Nikon Nikon Nikon Nikon Nikon Nikon Nikon Nikon	n Internilight
Nikon I Nikon I Nikon I Nikon I Nikon I Nikon I Nikon I Nikon I Nikon I	Sector March Torrest
Nikon I Nikon I Nikon I Nikon I Nikon I Nikon I Nikon I Nikon I	
Nakon Nakon Nakon Nakon Nakon Nakon Nakon Nakon	n L200N(D),(L300N(D)
Nikon Nikon Nikon Nikon Nikon Nikon	n LU4A
Nikon I Nikon I Nikon I Nikon I Nikon I	n LUSU and AON
Nikon 1 Nikon 1 Nikon 1 Nikon 1 Nikon 1	n LV series
Nikon 1 Nikon 1 Nikon 1 Nikon 1	n MA series
Nikon 1 Nikon 1 Nikon 1	n MN+00/900
Nikon 1 Nikon 1	n N-E
Nikon	n Shutter (NE-SH-CON)
	n TE2000
Manua	n Tì
	ual Nicroscope
NIDAQ	AQ

Figure 15.1-3 Manage devices window

#### **3** Perform advanced settings for trigger signals.

1. Select the trigger signal method to be added from the [Available devices] area and click the [Add - ->] button.

Select [TTL Input] to use NIDAQ for inputting trigger signals from external devices.

Select [RealTime TTL Input] to use NIDAQ for acquiring timestamp information. (For settings for acquiring timestamp information, see Section 15.2, "Trigger Signal Input Using NIDAQ.")

The selected trigger signal method is added to the [Installed devices:] area and the Configuration window for the trigger signal appears.

vailable devices	Installed devices
Inslog input Inslog Output Zaithrated Analog Input Zaithrated Analog Output LealTime TTL Input Inutter Instance Inc. Add.	->
Iumination Device Exposure Signal TTL In	move
NIDAQ V Free resources: Analog [mout: 32	/9.0.0
Analog Output: 4 TTL I/0: 47	
Triggering	
PCI-6713 (Dev1):	Occupied
PCIe-6353 (Dev3): (Dev3/PFI0	▼ Close

## Figure 15.1-4 NIDAQ Configuration window

- * Since the NIDAQ connected to the PCI slot is used in the C2 system, "Occupied" appears on the window and the 1st NIDAQ is not be selectable for trigger signal input.
- 2. Set details of the trigger signal in the Configuration window and click the [OK] button to confirm the settings.

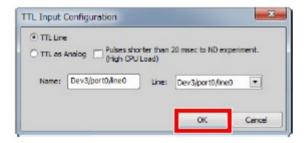


Figure 15.1-5 Trigger Signal Configuration window

- 3. Repeat steps 1 to 2 for each trigger signal method to be added.
  - * To register individual trigger signal lines to be used for each action, repeat steps 1 to 2 and select the line to be used from the [Line:] menu.



Figure 15.1-6 Trigger Signal Configuration window

4. Click the [Close] button to confirm the settings.

		Manoge devices	and the second se
NIDAQ Configuration		Tretalled devicers	AH *
Bunination Device	Installed devices TTL Input (varie: Dev3/port5/(vari5) TTL Input (varie: Dev3/port5/line2) Id ->	The Depart Dame: Devideon table	eab
NDA Free resourcesi Analog Duptur 32 Analog Duptur 4 Tit 1/0: 45 Triggering PCI-4713 (Dev1): PCI-4323 (Dev3): Dev3/Pri	2 v5 0.0 Configure Decupied TD V Close	Hinstal Devices Connect Connector Parameters Lagical Devices Device Parameters	Carifgers Device

Figure 15.1-7 Confirming advanced settings for trigger signals

* On completion of the NIDAQ connection settings with [TTL Input] registered, the [I/O] tab is added to the ND Acquisition window.

Order of Experime	int ▼ III xY □ II Z	<b>√</b> 4 1/0		
	anges Then Do Ac		00 ++	× ð
Device Type	Name	Condition	Action	
- On Experiment B	Event Set Device C	+	0 0 + +   Output	× 8
	nt   Device Type	e jivane	Touque	_ sait

Figure 15.1-8 ND Acquisition window

# 15.2 Trigger Signal Input Using NIDAQ

Use of the NIDAQ connected to the PCI Express slot allows the user to start and stop image acquisition and so on by inputting trigger signals from external devices.

When NIDAQ is installed in the PCI Express slot, trigger signal output can be set using this NIDAQ but the trigger signal output is delayed from the actual timing in some cases. For trigger signal output, follow the descriptions in Chapter 14.

## 15.2.1 Procedure for Setting External Trigger Input for Image Acquisition

This section describes how to input trigger signals during an image acquisition experiment.

The following describes the procedure for setting external trigger input using an example of acquiring an image on the ND Acquisition window.

#### 1 Call the ND Acquisition window and select the [I/O] tab.

- 1. Select [Applications] -> [Define/Run ND Acquisition...] from the menu bar to open the ND Acquisition window.
- 2. Select the [I/O] tab from switching tabs of the ND Acquisition window, and then check the check box.

	and the second se	ent 🔹	-	Record Data	I/O ta
	Device Type	Name	Candition	Action	
rigger input —— etting field		Event Set Device Outpu			
			∲   Nane	0 0 + + × ×	
	Load V Sa	ve • Remove*		tme loop	

Figure 15.2-1 ND Acquisition window

#### 2 Set the trigger signal input timing in the trigger input setting field.

1. Click the [Device Type] first line. (If several signal methods are registered, select the method of the trigger signal to be input in [Device Type].)

Order of Experime	₩xy 🔲 @ z	<b>_</b> .P	λ 🔽 🗳 1/	0		
When Device Ch	anges Then Do Act	ion	+	10	<b>1</b> + +	XX
Device Type	Name		Condition		Action	
M 111	Jev3/port0/line	0 -	Faling	-	Start Experin	ient 1
- On Experiment E	Event Set Device Ou	itput —		A	AT 1.1	52 50
Experiment Ever	nt Device Type	Na	Te .	1 69	Output	X X

Figure 15.2-2 ND Acquisition window
-------------------------------------

2. Click the [Name] first line and select the line of the trigger signal to be input.

Save to File				Record Data	i
Order of Experime	ent 🔻				
O Time	⊞xr	P ) 🗹 🔗 1/4	2		
When Deutes Ch	anges Then Do Action				
Concert Device Cr		+	Ø	🗇   † †   🗙	20
Device Type	Name	+ Condition		습기   + +   🗙 Action	20
	Name				20 -
Device Type	Name	Condition		Action	

#### Figure 15.2-3 ND Acquisition window

3. Click the [Conditions] first line and select the polarity of the trigger signal to be input.

**Rising**: Sets the rising edge of the TTL level signal as the trigger signal.**Falling**: Sets the falling edge of the TTL level signal as the trigger signal

Save to File			Record Data	
order of Experime	ent 👻			
Bure	⊞xr ∎⊖z □.	0, 7 4 1/0		
	anges Then Do Action		مد عد الد الغر	
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	anges Then Do Action	ے ا	n fj + + × > cton tart Experiment	
When Device Ch Device Type	Name	L A	,ction	Select the polarity o

Figure 15.2-4 ND Acquisition window

4. Click the [Action] first line and select the timing of the trigger signal to be input.

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and the second se	anges Then Do Action			× *	
Device Type	Name	Condition	Action		
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			Start Experim Finish Experim Start Phase N Move To Next Insert User Ev	Phase	Select the timing of the trigger signal
- On Experiment E	vent Set Device Outp	wt 🔶 🕴	00 ++	XX	
Experiment Even	nt Device Type	Nane	Output	Walt	
Load V Sav	e V Remove V		me loop	Run now	

Figure 15.2-5 ND Acquisition window

5. Click the [Action] second and subsequent lines to register two or more trigger actions, and then repeat steps 1 to 2 for each action.

	11 xr	3 z 🗖	S.	λ 🗹 🧳	1/0	1_				
When Device Ch	anges Then I	Do Action			•	1	ð	+ +	×	ð
Device Type	Name			Condition	1		Actio	n		
✓ TL.	Dev3/por	t0/line0	-	Faling		-	Start	Experin	ent	1
	Dev3/por	rt0/line2	•	Rising		•	Finish	n Experir	nent	
On Experiment 6	Event Set De	vice Outpu	rt—	4		٥	0	+ +	×	8
Experiment Eve	nt Device	Type	Nat	ne			Out	out	0	ait

Figure 15.2-6 ND Acquisition window

6. Proceed to image acquisition settings and other necessary settings, and then click the [Run now] button.

Clicking the [Run now] button opens the ND Progress window and executes experiment sequence.

: Cave to Pile	xr 🛛 🕫 z 🗖	160	) 🗹 🖉 1/4	2	Record D	Data
	nges Then Do Action		+		8 ++	x a
Device Type	Name	_	Condition		Action	
Π	Dev3/port0/line0	-	Faling	7	Start Experiment	nt 1
⊻m. □	Dev3/port0/line2		Rising	7	Finish Experime	nt
On Experiment Ex	ent Set Device Outp	ut	÷	10	0   + +	X X
Experiment Event	Device Type	Na	ne		Output	Wait

#### Figure 15.2-7 ND Acquisition window

* When the [Run now] button is clicked with [Start Experiment] set for [Action], the ND Progress window opens but the experiment sequence stops temporarily in the following state. If a trigger signal is input from an external device in this state, the experiment sequence restarts.

Expe	riment overall p	wogress:		
	me elapsed eriment Status	l: 0:00:00 T	lime remaining	j: N/A
	al Info			
Det				
Det				Events
Det	Next Loop	DD Next Phase	Start Phase:	Events

Figure 15.2-8 ND Progress window

# **16 Using External Detector Unit**

This chapter describes how to set an external detector unit connected with the Si port of the Confocal Microscope C2 by using NIS-Elements.

* External detector units are usable only when a C2 system without a spectral detector is in use. When C2si (system with a spectral detector) is in use, no external detector is usable.

# 16.1 Procedure for Settings on the C2plus Settings Window

- 1 Acquire the Square scan area image.
- 2 Switch the mode to the external detector usage mode.
  - 1. Switch the optical path changeover lever on the C2 scan head to the [Spectrum] position.



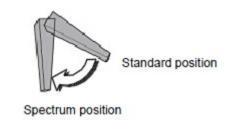


Figure 16.1-1 Optical path changeover lever for C2 scan head

2. On the Acquisition window, the [External Port] check box is turned ON and the system is switched to the external detector usage mode.

Laser Power Monit	tor AG	ß Select.	Al Channels [	HV Linear Correction
Ch 1 3ndo-1/Ca2	+saturated	Laser 405.0	Ch 2 CFP/pH 7.4	Laser 458.0
2ffset 4		0	Offset 4	► 0 ND 0.0 0.0
Ch 3 SBFL(Na+fn	ae	Laser 405.0		
Offset 4		0 0 0.0 0.0		
inhole 4	> >> >> >> >> >> >> >> >> >> >> >> >	1.4 A.U. 30.0 um <-		



* Do not perform the photo activation experiment in the external detector usage mode.

#### **3** Set the scan settings.

1. Display the C2plus Aux Control window.

As shown below, right-click on the gray area (without any setting window displayed) to display a menu. Then select [Acquisition Controls] -> [C2plus Aux Control] in the menu to open the C2plus Aux Control window.

	10		100		
	۲	Live +			
		Acquisition Controls	*	AVI Acquisition	
Nikon		Analysis Controls Visualization Controls Macro Controls Others		C2plus ALD/ Control C2plus Compact GUI C2plus Compact GUI	Ctri+Art+C Ctri+Alt+J
		Left Ctrl+Alt+Num4 Right Ctrl+Alt+Num4 Bottom Ctrl+Alt+Num2	20 00 10 10 10 10 10 10 10 10 10 10 10 10	Ciplus Settings Ciplus Stimulation Capture FRET Image Illumination Sequence	

#### Figure 16.1-3 To display the C2plus Aux Control window

2. Select the scanning method.

#### [Points]:

Multipoint scan can be set. Set the capturing time per point in the [Capture] field. (Go to step 4.)

#### [Normal]:

A scan area (such as square) can be set.

Set the capturing time per scan area or the number of frames in the [Capture] field. (Go to step 5.)

lus Aux Co	ntrol ×	
Normal	Points	
Capture:	10 sec	

#### Figure 16.1-4 C2plus Aux Control window

#### 4 Set points.

1. Open the Scan Area window or Live window and then select a point setting method from the Point (s) Scan Area tool.



Figure 16.1-5 Point setting method

Select a point setting method as shown below and points are drawn.

#### Free

Draws one point in the FOV.

Dragging the drawn point moves the point.

Double-click the mouse in the FOV to add points.

#### 2x2:

Draws 2x2 grid points in the FOV.

#### 4x4:

Draws 4x4 grid points in the FOV.

#### 8x8:

Draws 8x8 grid points in the FOV.

#### 16x16:

Draws 16x16 grid points in the FOV.

#### MxN:

Specifying the number of vertical and horizontal points on the AUX Point Grid Setup window that appears when this item is selected draws grid points in the FOV.

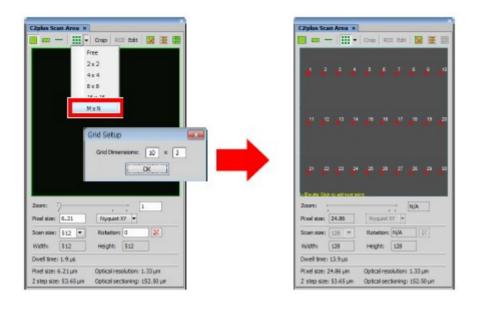


Figure 16.1-6 Point drawing

- * When the mouse pointer is set at the lower-right corner point of drawn grid points, the pointer appears to expand or reduce the entire grid area. Furthermore, dragging a point (except for the lower-right corner point) on the entire grid moves the entire grid.
- * Even if multiple points are set as grid points, a point to be scanned during the live imaging is only the active point displayed in .
- * To make a grid point to an active one, select [Set As Active] from the menu that is displayed by rightclicking on the grid point. To delete a grid point, select [Delete Point].
- * This grid point setting can be registered in the optical configuration.

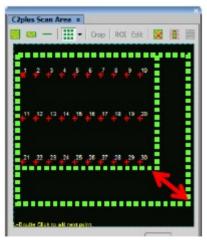
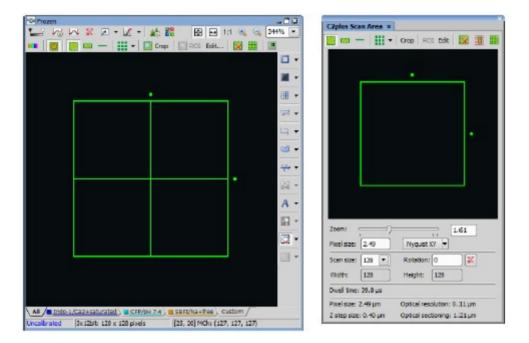


Figure 16.1-7 Scan Area window

Go to step 6.

#### 5 Set the scan area.



Open the Scan Area window and draw the scan area.

Figure 16.1-8 Scan area settings

#### 6 Adjust the laser.

Adjust the laser power on the Acquisition window.

Apquisition / Phote Advancements      Leser Power Menitor     Ag     Select.	All Channels HV Linear Correction
Ch 1         Indo-1/Ca2+solurated         Laser +00.0           HV         Image: Image	Ch Z         CPP/(cH 7.+         Laser 458.0           HV         Image: Complexity of the second sec
Chi 3         Stift/Me+Free         Laser 400.0           HV         ↓         ↓         ↓           Pffunt         ↓         ↓         ↓           Laser         ↓         ↓         ↓           Laser         ↓         ↓         ↓	
Princie  Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princie Princ	✓ Extensi Peri.

* While an external laser is connected, the laser power cannot be adjusted on the Acquisition window.

#### 7 Acquire an image on the ND Acquisition window.

Make settings on the ND Acquisition window and perform an experiment.

- * When an experiment is performed by using ND Acquisition, a black image is acquired on the NIS-Elements side because signals have been sent to the external detector.
- * When [Interval] and [Duration] are set on the ND Acquisition window for point scan, actual operation is performed with "capturing time set by AUX Control" x "specified number of points" x "number of Loop."
- When using external detector in Compact GUI Select [Show AUX Detector] from the menu that appears when the [Settings] button is clicked to make an external detector available. While an external detector is in use, the [AUX] icon is displayed on the Compact GUI window.

C2plus Compact GUI ×	*	
Scan Capture Pind		
StrePort AG		
Control by:  Pixel Dwel	Reference Contraction of the Con	
Past Mode 7.2 18	39.8	
Size		
64 128 256 512 100	2048	
Normal Ø2x + 2 2x +		
Ch Series • [1]->[-	1-31-4	
	🖄 Settings -	
Pinhole	Acquisition mode Close mechanical shutter during experiment	
**	AU settings XYZ settings	
Indo-1/Ca2+maturated     Laner 4     HN	Fast piezo 2 control node Auto Gain settings	
offeet	Find made settings	
0405	Manual shift alignment settings	
CFP/pH 7.4 Loser +	Linescan setup 1DT	
SBFLNa+fee Laser+	<ul> <li>Activate view channel on edit</li> </ul>	
	Sidebar setting	1
-	HV control	ſ
	the second se	-
r	Show ALIX Detector	

Figure 16.1-10 Compact GUI

# **17 NIS-Elements C-ER Package**

The following functions are available only for people who purchased the "NIS-Elements C-ER" package.

For other functions, see the "NIS-Elements C Instructions" and the "NIS-Elements AR (Advanced Research) User's Guide."

# **17.1** Automatic Deconvolution

When Automatic Deconvolution is executed for Capture image, Live image, or an acquired image (in ND2, Tiff, JP2, or Lim format), the software automatically determines the best processing method to acquire an image of an enhanced resolution.

# 17.1.1 Procedure of Deconvolution

#### **1** Acquire the sample image for perform automatic deconvolution.

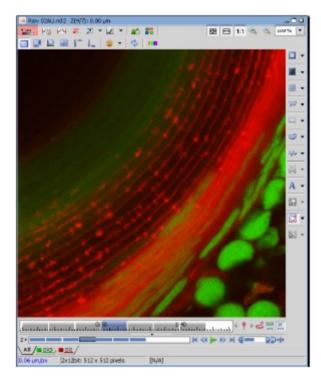


Figure 17.1-1 Image Acquisition

# 2 Perform the automatic deconvolution processing.

Set the pre-scan mode if you want to increase the point scan accuracy in the external detector unit.

1. Click the [Automatic Deconvolution] button. Starts automatic deconvolution processing.



# Figure 17.1-2 Automatic Deconvolution

* Selecting [Show deconvolution GUI] that appears by clicking - [Show deconvolution GUI] button makes it possible to display the Automatic Deconvolution window when e is clicked.

When the Automatic Deconvolution window is displayed

The Automatic Deconvolution window is used to confirm and set parameters necessary to execute deconvolution.

This window shows different items for 2D and 3D images.

## [Basic Settings] field:

(for 2D and 3D images)

This field shows parameters for acquiring the original image.

If you want to change them, select or enter values for each item.

## [Channels] field:

(for 2D and 3D images)

This field shows channels for which deconvolution is to be executed. (Channel names at the time when the original image was acquired are shown.) Deconvolution is disabled for channels whose checkbox is cleared.

# [Use Spherical Aberration Correction] checkbox:

(only for 3D images)

Selecting this checkbox enables processing considering spherical aberration at the time of deconvolution.

# [Acquisition Depth] field:

(only for 3D images) Enter the distance from the cover glass to the sample. (The default value is shown originally.)

## [Default] button:

(only for 3D images)

Used to show the value of the default position (Z stack center).

# [Sample Refractive Index] field:

(only for 3D images)

Select or enter a refractive index of the sample. (The default value is shown originally.)

## [Create new document] checkbox:

(for 2D and 3D images)

Selecting this checkbox creates a new file for deconvolution from the original image.

# [Convert document to floating point data format] checkbox:

(for 2D and 3D images, available only for 16-bit)

Selecting this checkbox enables Float conversion at the time of deconvolution. [Do not show this dialog for images with valid metadata] checkbox:

(for 2D and 3D images)

Selecting this checkbox does not show the Automatic Deconvolution window even when with the selected.

Automatic Deconvolution

**Basic Settings** 

#### [OK] button:

(for 2D and 3D images)

Used to start deconvolution processing.

#### [Close] button:

(for 2D and 3D images)

Used to close this window without performing deconvolution processing.

utomatic Deconvolution		-	Immersion Refractive Index: 1.51		-
Basic Settings			Calibration: 0.06		µm/s
Magnification: 10	l0x	-	Z-Step: 0.120)	μm	
Numerical Aperture: 1.	49		Pinhole Size: 12.77	μm	
Immersion Refractive Index: 1	51	•	Channels		
Calbration: 0.	06	μπ		ExW	EmW
Dahah Car. 12	77			488	525
Pinhole Size: 12	.77 µm		Dr.	561	525 595
	.77 µm ExW	EnW		561	
Channels		EmW 525	Dr.	561	
Channels Name	ExW		Dt Use Spherical Aberration Cor Acquisition Depth: 0.36	561	595
Channels Name I Dio Diat	EXW 468	525	Dt  Dt  Use Spherical Aberration Cor  Acquisition Depth: 0.36  Sample Refractive Index: 1.35	561	595
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Channels Name I Dio Diat	468 561 point data for	S2S S9S	Dt  Dt  Use Spherical Aberration Cor  Acquisition Depth: 0.36  Sample Refractive Index: 1.35	561 rrection	Default

(for 2D Images)

(for 3D Images)



## 17.1.2 Post-Deconvolution Processing

**Undoing/Redoing image processing** 

If an image has not been saved, it can be returned to the pre-image processing state with the (Undo) button or to the post-image processing state with the (redo) button.



Figure 17.1-4 Undo/Redo

**Displaying parameter values** 

Parameter values used for deconvolution are shown on the image window after processing. Right-clicking on these parameters or image window displays [Show Deconvolution Info]. Clearing this checkbox hides the parameters.

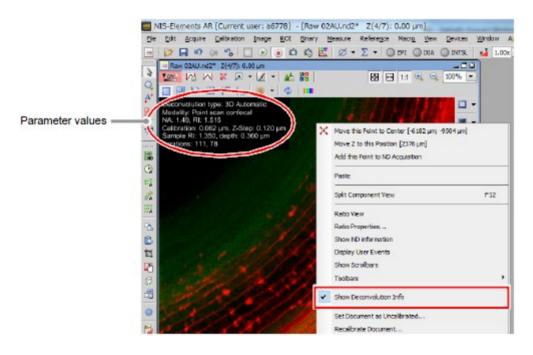


Figure 17.1-5 Show Deconvolution Info

Storing processed images

When a processed image is saved using the  $\square$  button, the image file is overwritten in the post-image processing state. When storing an image file with another name, select [File] > [Save As] from the menu bar.



Figure 17.1-6 Undo/Redo

# **18** Simple Si Detector

This chapter describes operations that are different from operations performed when other detectors are used in settings for using the Simple Si detector (C2-DUVB GaAsP Detector Unit).

When the "C2-DUVB-OP GaAsP 2nd channel Kit" is used, Channel 1 can be used an option channel.

Descriptions in this chapter are based on windows when a laser unit "LU-N4" is used.

When using the Standard Detector (DU3), see Chapter 5.

# **18.1 Optical Path Changeover Lever of C2 Scan Head for Simple Si**

When using the Simple Si detector, switch the optical path changeover lever on the C2 scan head to the [Spectrum] position.



Figure 18.1-1 Optical path changeover lever for C2 scan head

# **18.2** Combinations of Detection Modes and Functions for Simple Si

The following table lists settable functions in each detection mode.

 Table 18.2-1
 Combinations of Detection Modes and Functions

Function

Detection Mode CB VB

Unidirectional		Y	Y
Bidirectional		Y	Y
Channel Series		N	Y
Chamiler Series			
Multi position acquisition			in all detection modes. , no photo activation
(Chapter 4)			it is available.)
Fast mode (Chapter 8)		Y	Y
HV linear correction		Y	Y
Pinhole setting		Y	Y
Photo activation (Chapter 10)		Y	Y
Particular line sequential	2Ex1Em	Ν	Ν
(Chapter 5)	4Ex4Em	Ν	Ν
Using external detector (Chapter 16) *		Ν	Ν
			Y: Available N: Unavailable

* External detector units are usable only when a C2 system without a spectral detector or the Simple Si detector is in use. When C2si (system with a spectral detector) or the Simple Si detector is in use, no external detector is usable.

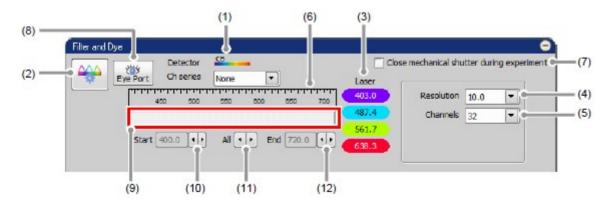
# **18.3 Detection Mode CB**

This chapter describes the settings for the Continuous Bandpass mode (CB).

In the Continuous Bandpass mode, spectral images of up to 32 channels are sequentially acquired for each specified wavelength resolution of each channel.

# 18.3.1 Filter and Dye Window

This window enables to set the Optical path.



# Figure 18.3-1 Filter and Dye window (CB-use)

Table 18.3-1 Functions of Filter and Dye window (CB-use)

	Name	Function
(1)	Detector	When Simple Si detector is in use, indicates that the Detector mode [CB] of Simple Si detector is used when the optical path changeover lever on the C2 scan head is set to the [Spectrum] position and the Continuous Bandpass mode (CB) is selected as the detection mode in the Optical path window.
(2)	Setting button	Opens the Optical path window. To use, select the detector, the dichroic mirror, the excitation laser, fluorescence dye for each excitation laser and others.
(3)	Status	Indicates the excitation laser.
(4)	Resolution	Selects a wavelength resolution.
		<b>10 nm</b> : Images of up to 32 channels are acquired in step of 10 nm within a wavelength range of 400 nm to 720 nm.
		<b>20 nm</b> : Images of up to 16 channels are acquired in step of 20 nm within a wavelength range of 400 nm to 720 nm.
(5)	Channels	Selects the number of channels (number of PMTs). When 20 nm is selected in Resolution, up to 16 channels are selectable. When 10 nm is selected in Resolution, up to 32 channels are selectable.
(6)	Wavelength range information	Displays the wavelength range information.
(7)	Close mechanical shutter during experiment	If unchecked, the shutter remains open during the ND image acquisition. As the shutter is not opened/closed every image acquisition, the time for the image acquisition can be shortened.
		* During the interval period, laser power is automatically changed to the minimum but the laser cannot be shut off completely because the shutter is left open.
(8)	Eye Port button	Changes optical path to eye port. Each function on the C2plus Settings window becomes non- selectable when the Eye Port button is selected.
(9)	Acquiring wavelength range setting bar	Sets a wavelength range to be acquired in a wavelength range from 400 nm to 720 nm. Sets a range by shifting the wavelength range setting bar to the right or left or by enlarging or reducing it. (Linked with the above setting of the number of channels to be acquired.)
		* A part of the wavelength range may be displayed in black depending on the setting conditions. In the wavelength range displayed in black, no wavelength range can be set.

(10) Start	Displays the start wavelength of the wavelength range currently selected. Enabled to enlarge or reduce the range in units of wavelength resolution with the right or left button. This start wavelength can be set within a wavelength range of 400 to 710 nm.
(11) All	In the currently selected wavelength range, enables shifting to the right or left in units of 1 nm without changing the width of the wavelength.
(12) End	Displays the end wavelength of the wavelength range currently selected. Enabled to enlarge or reduce the range in units of wavelength resolution with the right or left button. This start wavelength can be set within a wavelength range of 410 to 720 nm.

• Optical Configuration

Individual data items set in the Continuous Bandpass mode (CB) can be managed collectively with the Optical Configuration window. For storing and retrieving the [Optical Configuration] settings, see the sections concerning the optical configuration in the "NIS-Elements AR (Advanced Research) User's Guide."

# 18.3.2 Setting the Optical Path

Click the [Setting] button of the Filter and Dye window to display the Optical path window.

The Continuous Bandpass mode [CB] setting screen is displayed when the optical path changeover lever on the C2 scan head is set to the [Spectrum] position and the Continuous Bandpass mode (CB) is selected as the detection mode in the Optical path window.

Setting button -	Detector Chiseries	None	•	Clos	e mechanical ahu	tter durin	ig experiment
	450 500	560 600	950 700 007 00	and the second	Resolution Channels		•
	Start 400.0 + +	AI + + I	End 720.0	561.7			

Figure 18.3-2 Filter and Dye window (CB-use)

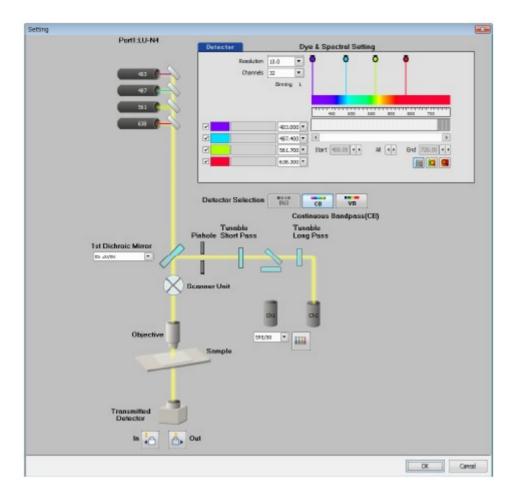


Figure 18.3-3 Optical path window (CB-use)

# 18.3.3 Optical Path Window

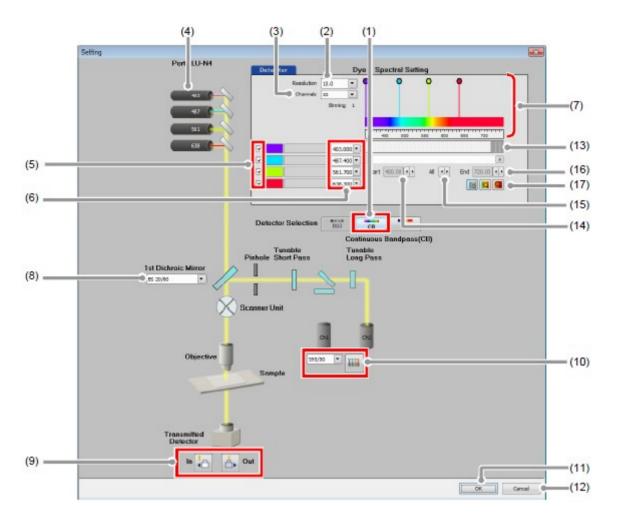


Figure 18.3-4 Optical path window (CB-use)

<i>Table 18.3-2</i>	<b>Functions</b>	of Optical	path window	(CB-use)
---------------------	------------------	------------	-------------	----------

	Name	Function	
(1)	Detection mode selection button		Continuous Bandpass mode (CB) In this mode, spectral images of up to 32 channels are sequentially acquired for each specified wavelength resolution of each channel.
(2)	Resolution	Selects a w	avelength resolution.
		0	of up to 32 channels are acquired in step of within a wavelength range of 400 nm to 720
		•	of up to 16 channels are acquired in step of within a wavelength range of 400 nm to 720

(3)	Channels	Selects the number of channels (number of PMTs). When 20 nm is selected in Resolution, up to 16 channels are selectable. When 10 nm is selected in Resolution, up to 32 channels are selectable.
(4)	Excitation laser indicator	Displays the current setting for the laser. The currently set laser icon is displayed in a large size, and the optical path is indicated.
(5)	Excitation laser selection check box	Enables to select the excitation lasers to be used.
(6)	Excitation laser wavelength select	Enables to set the laser wavelength that is set with the software configuration, regardless of the setting of the Filter block display/select.
(7)	Rainbow chart	Provides the following information:
		<ul> <li>Wavelength band for which to acquire images (shown in color and value for each excitation laser)</li> <li>Spectral profile of fluorescence dye</li> <li>Excitation laser for fluorescence dye</li> <li>A color band indicating the wavelengths in the entire band (400 to 720 nm)</li> <li>Scale of the wavelengths in the entire band (400 to 720 nm)</li> </ul>
(8)	1st Dichroic mirror select	Enables to manually select the 1st Dichroic mirror to be used. If a 1st Dichroic mirror that the NIS-Elements does not recommend is selected in the combination of the selected laser wavelength and the 1st Dichroic mirror, "!" appears following the 1st Dichroic mirror name
(9)	Transmitted detector selection button	
		Brings the transmitted detector into the Optical path, to enable the ability.
		Brings the transmitted detector out of the Optical path, to disable the ability.
(10)	Filter cube setting button	
		Used to exchange the filter cube of option dichroic mirror, create new filter cube information, or change the details of the registered filter cube. Clicking this button displays the Filter Cube Setting window. For the editing procedure, see Section 18.3.3.1, "Filter Cube Setting Window."

Through

Used to display/select the filter cube name installed in the filter cube of option dichroic mirror.

(11) OK button	Confirms the Optical path settings applied and closes the Optical path window.
(12) Cancel button	Discards the Optical path settings applied and closes the Optical path window.
<ul><li>(13) Acquiring wavelength range setting bar</li></ul>	Sets a wavelength range to be acquired in a wavelength range from 400 nm to 720 nm. Sets a range by shifting the wavelength range setting bar to the right or left or by enlarging or reducing it. (Linked with the above setting of the number of channels to be acquired.)
	* A part of the wavelength range may be displayed in black depending on the setting conditions. In the wavelength range displayed in black, no wavelength range can be set.
(14) Start	Displays the start wavelength of the wavelength range currently selected. Enabled to enlarge or reduce the range in units of wavelength resolution with the right or left button. This start wavelength can be set within a wavelength range of 400 to 710 nm.
(15) All	In the currently selected wavelength range, enables shifting to the right or left in units of 1 nm without changing the width of the wavelength.
(16) End	Displays the end wavelength of the wavelength range currently selected. Enabled to enlarge or reduce the range in units of wavelength resolution with the right or left button. This start wavelength can be set within a wavelength range of 410 to 720 nm.
(17) Enlarge button	Enlarges the rainbow chart. The display is switched in three levels.

# • About switching between CB and VB

**CB** -> **VB**:

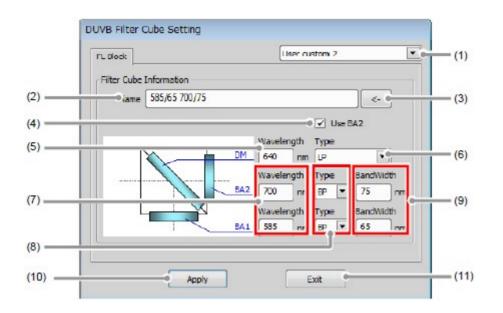
The last settings in the Variable Bandpass mode (VB) are recalled.

**VB -> CB:** 

The last settings in the Continuous Bandpass mode (CB) are recalled.

#### 18.3.3.1 Filter Cube Setting Window

This window opens when the [Filter cube setting] button is clicked on the Optical path window. The new filter cube to be created or the content of the registered filter cube can be changed.



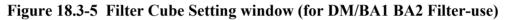


Table 18.3-3	<b>Functions</b>	of the	Filter	Cube	Setting	Window

1 401	Name	Function
(1)	Preset data	Selects a set of the Filter cube for option dichroic mirror recommended by Nikon.
(2)	Filter cube name display/Edit	Displays/edits the Filter cube name in the combination of the dichroic mirror and the barrier mirror. (Input words: 1 - 24 (in Alphabetical letters))
(3)	Filter cube name creation button	Generates the filter cube name automatically using Wavelength, Type, and BandWidth of the barrier filter.
(4)	Use BA2 check box	When checked, the second barrier filter (BA2) can be used.
(5)	Dichroic mirror wavelength input	Inputs the wavelength of the dichroic mirror. (Input range: 400 - 720 nm)
		The following wavelength is transmitted according to the selected Type.
		<b>[LP] Long pass:</b> Wavelength equal to or longer than the Wavelength value is transmitted.
		<b>[SP] Short Pass:</b> Wavelength equal to or shorter than the Wavelength value is transmitted.
(6)	Filter type select	Selects the dichroic mirror type.
(7)	Barrier filter wavelength input	Inputs the wavelength of the barrier filter (Input range: 400 - 720 nm)
		The following wavelength is transmitted according to the selected Type.

	<b>[LP] Long pass:</b> Wavelength equal to or longer than the Wavelength value is transmitted.
	<b>[SP] Short Pass:</b> Wavelength equal to or shorter than the Wavelength value is transmitted.
	[ <b>BP</b> ] <b>Band Pass</b> : Wavelength of the Wavelength value ?} BandWidth value/2 is transmitted.
(8) Barrier filter type select	Selects the barrier filter type. ([LP] Long pass/[SP] Short Pass/[BP] Band Pass)
(9) Barrier filter band input	When the barrier filter type is [BP], inputs the range from the center wavelength for the barrier filter. [Input range: 1 - 350 nm normally (but not limited to)]
(10) Apply	Confirms the Filter Cube Settings.
(11) Exit	Closes the Filter Cube Setting window. If the setting has not been confirmed with the Apply button, a confirmation dialog box appears.

# 18.3.4 Acquisition Window

The Acquisition window enables to set PMT brightness (detection sensitivity), laser power, and pinhole size.

# 18.3.4.1 Structure of Acquisition Window

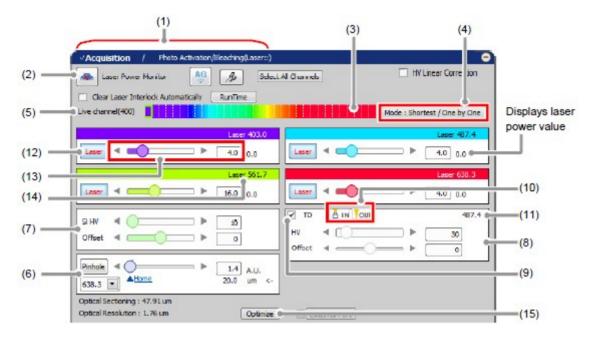


Figure 18.3-6 Acquisition window (CB-use)

Table 18.3-4	Functions	of Acauisition	window	(CR-use)
<i>Tuble</i> 10.5 4	1 unchons	of mequisition	window	(CD use)

1 401	Tuble 10.5 + T unetions of frequisition withdow (CD use)				
	Name	Function			
(1)	Acquisition/Photo Activation window switching	Switches between the Acquisition and Photo Activation windows.			
		For the Photo Activation window, see Chapter 10.			
(2)	Laser power monitor button	Displays the laser power value (integer obtained after A/D conversion divided by 10) of the current excitation laser by clicking this button. During the image acquisition, the laser power cannot be measured and this button is grayed out.			
(3)	Excitation laser color	Displays the excitation laser color of each channel specified in the Optical path window. When displaying the live image, the image of only the clicked channel is displayed on the Live window. The channel whose image is displayed can be changed during Live.			
(4)	Custom Acquisition Setting button	Click this button to open the DUVB Custom Acquisition Settings dialog box. Sequence of scan channels and excitation/dimming timing of the excitation laser can be set in the DUVB Custom Acquisition Settings dialog box. For details, see Section 18.3.4.2, "DUVB Custom Acquisition Settings Dialog Box." The current acquisition control setting is shown on this button.			
(5)	Live channel indication	Displays the wavelength of the channel that indicates the live image.			
(6)	Pinhole	Adjusts the pinhole size.			

(7)	Si HV and Offset	For pinhole size, see Section 18.3.4.4, "Setting the Pinhole." Adjusts HV and Offset of the Spectral Detector. This function can be adjusted during acquisition of the live image.
		Slider bar: Slides to the right or left to set the value.
		Arrow buttons: Click either arrow button to increase or decrease the value stepwise.
		<b>Direct entry in value display field:</b> Type the desired setting value.
(8)	Brightness adjustment for transmitted detector	For the transmitted detector, use the HV and Offset controls to adjust the brightness of the live image. Sets the offset value and the voltage (HV) to be applied to the transmitted detector.
		Slider bar: Slides to the right or left to set the value.
		Arrow buttons: Click either arrow button to increase or decrease the value stepwise.
		<b>Direct entry in value display field:</b> Type the desired setting value.
(9)	TD channel selection	Enables to acquire TD images by checking the check box. When fluorescence is excited, all excitation lights are detected by the transmitted detector (TD). If the BA filter for 405 laser is installed in the TD, excitation light is not detected by the TD.
(10)	TD IN/OUT button	Sets/removes the motorized transmitted detector in/from the optical path. (IN = Set in the optical path/ OUT = Remove from the optical path)
		As for the case where the TD IN/OUT button is not displayed, it will be displayed when the motorized transmitted detector is set in the optical path in the Optical path window.
(11)	TD scan	Displays the wavelength of the TD image scanning laser. The TD image is acquired together with the channel using the same laser wavelength.
(12)	Laser ON/OFF button	Selects whether the laser is emitted or not.
	(*1)	CN status The laser is emitted.
		Laser The AOTF shutter closes and the laser power value

OFF status

The AOTF shutter closes and the laser power value becomes 0.

When switched from OFF to ON, the laser power

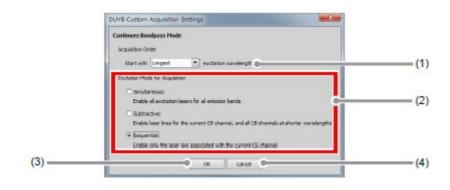
Page 13	of 40
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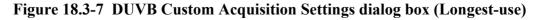
	value set in the previous ON status is applied.
(13) Laser (*1)	Sets the laser power value.
	Slider bar: Slides to the right or left to set the value.
	Arrow buttons: Click either arrow button to increase or decrease the value stepwise.
	<b>Direct entry in value display field:</b> Type the desired setting value.
	* When "C2-DUVB-OP GaAsP 2nd channel Kit" is in use, if a standard channel that uses the same laser wavelength as the option channel exists, the laser power value interlocks.
(14) Laser wavelength indication	The currently selected laser wavelength is indicated.
(15) Optimize button	Click this button to display the XYZ Size Setup window. In the XYZ Size Setup window, the calculation method of the recommended values of the resolution, zoom magnification, and Z stack step size can be set. For the XYZ Size Setup window, see Section 18.3.4.3, "Recommended Value Indication/Automatic Application."
(*1) When III NW is in	use this button is ground out and is disabled while the button on the f

(*1) When LU-NV is in use, this button is grayed out and is disabled while the button on the front panel of the laser unit is OFF or blinking.

# 18.3.4.2 DUVB Custom Acquisition Settings Dialog Box

This dialog box is used to set the sequence of scan channels and excitation/dimming timing of the excitation laser.





Cantinues Bandpass Hode Accuistion Order Start with Stortest 💌 excitation wavelength	
Excitation Made for Acquisition	(2)
Cusulative: Enable laser lines for the current CB channel, and all CB channels at shorter wavelengthe	
Gequential     Enable only the laser line associated with the current CB channel	

# Figure 18.3-8 DUVB Custom Acquisition Settings dialog box (Shortest-use)

Tabl	le 18.3-5 DUVB Custo	om Acquisition Settings dialog box
	Name	Function
(1)	Acquisition Order	Select the sequence of scan channels.
		Longest: In the order of long wavelength -> short wavelength
		<b>Shortest:</b> In the order of short wavelength -> long wavelength
(2)	Excitation Mode for Acquisition	Set the sequence of scan channels and excitation/dimming timing of the excitation laser.
		Simultaneous radio button: All lasers are excited. Lasers are not dimmed even after channels in the wavelength range have been acquired.
		Subtractive radio button: Acquisition starts with all lasers excited, and lasers are dimmed from those that have acquired channels in the wavelength range. This radio button is shown only when [Longest] is selected in Acquisition Order.
		Cumulative radio button: Lasers are not dimmed even after channels in the wavelength range have been acquired, and a laser of the next wavelength is excited. This radio button is shown only when [Shortest] is selected in Acquisition Order.
		Sequential radio button: After channels in the wavelength range have been acquired, the excited laser is dimmed and a laser of the next wavelength is excited.
(3)	OK button	Confirms the settings applied and closes this dialog box.

(4) Cancel button Discards the settings applied and closes this dialog box.

#### 18.3.4.3 Recommended Value Indication/Automatic Application

By the function of the recommended value indication/automatic application, the recommended values of the appropriate resolution, zoom magnification, and Z stack step size are calculated based on the objective type and the selected excitation wavelength.

Using the calculated recommended values enables the image acquisition clearer and with less damage to the sample.

**Recommended Value Automatic Application** 

To automatically apply the recommended values to the parameters, set the [Nyquist XY] button of the Scan Area window to ON.

C2plus Scan Area x	
👿 🚥 — Crop   ROI Edit   👿 🗃 🛃	
Zoom: ()	
Phael size:         0.62         Nyculat XY           Scan size:         512         Rotation:         0           Wdth:         512         Height:         512	Nyquist XY button
Owell time: 1.9 µs	
Pixel size: 0.62 µm Optical resolution: 0.13 µm Z step size: 0.42 µm Optical sectioning: 1.26 µm	

Figure 18.3-9 Scan Area window

	Scan setting Scan Direction	<b>: </b>		Zoom	● >	
Indicates the = recommended value of the resolution.	Scan Size Scan Speed	512 • ÷		u sec)	4.563x recomment	Indicates the recommended value of the scan
			Hone	1.4 A.U. 20.0 um <-		magnification.

Figure 18.3-10 Location of Recommended Value Indication

* When the laser or objective in use is changed, the recommended values are recalculated, and newly indicated and automatically applied.

**Recommended Value Settings** 

Detailed settings of the recommended values are made in the XYZ Size Setup window that is displayed by clicking the [Optimize] button of the Acquisition window.

If the [Nyquist XY] button of the Scan Area window is ON, the recommended values are automatically applied to the parameters.

Or if the [Nyquist XY] button is OFF, the recommended values of the scan size and zoom are indicated in the Scan setting window.

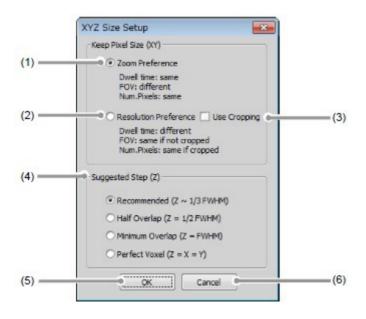


Figure 18.3-11 XYZ Size Setup window

	Name	Function		
(1)	Zoom Preference	When the [Nyquist XY] button is ON, keeps the scan size and applied the recommended value of the zoom.		
(2)	Resolution Preference	When the [Nyquist XY] button is ON, keeps the zoom and applied the recommended value of the scan size.		
(3)	Use Cropping	Fits the scan size in	detail by using Crop Scan.	
		Sets the Z step size	calculation method.	
		Recommend (Z ^{~1/3} FWHM)	Approximately one third of the thickness of optical section (FWHM value).	
(4)	Suggested Step (Z)	Half Overlap (Z=1/2 FWHM)	One half of the thickness of optical section (FWHM value).	
		Minimum Overlap (Z=FWHM)	The thickness of optical section (FWHM value).	
		Perfect Voxel (Z=X=Y)	Value same as the pixel size.	
(5)	OK button	Confirms the XYZ Size Setup window.	Size Setup applied and closes the XYZ	
(6)	Cancel button	Discards the XYZ S Size Setup window	Size Setup applied and closes the XYZ	

Table 18.3-6 Functions of XYZ Size Setup window

# **18.3.4.4** Setting the Pinhole

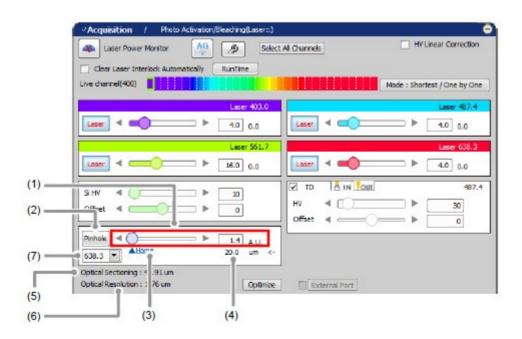


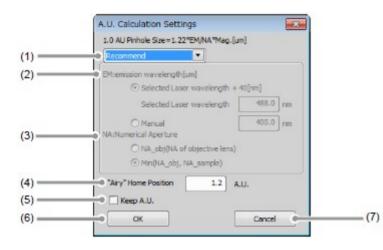
Figure 18.3-12 Setting the Pinhole (CB-use)

Tabl	Table 18.3-7       Pinhole setting functions (CB-use)			
	Name	Function		
(1)	Pinhole size setting	Sets a pinhole size for C2 system.		
		Slider bar: Slides to the right or left to set the pinhole size. (Unit: A.U.)		
		Arrow buttons: Click either arrow button to increase or decrease the pinhole size stepwise.		
		<b>Direct entry in pinhole size display field:</b> Type the desired setting value.		
(2)	Pinhole button	Displays the A.U. Calculation Settings window to calculate the pinhole size.		
		(For A.U. Calculation Settings, see Section 18.3.4.5, "Calculation Settings for Pinhole Size.")		
(3)	Home	Changes the pinhole to the predetermined home position. The value of the home position can be changed in the A.U. Calculation Settings window. (For A.U. Calculation Settings, see Section 18.3.4.5, "Calculation		
(4)	Pinhole size	Settings for Pinhole Size.") Indicates pinhole size of C2 system. (Unit: μm)		
(4)	Optical Sectioning	Indicates the FWHM (full width at half maximum) of z airy disk.		
(6)	Optical Resolution	The actual size of 1 pixel square calculated from the optical information (for objectives and scan parameters) and the size acquired from an image.		
(7)	Reference excitation wavelength for the pinhole size calculation	Selects the excitation wavelength as the reference of the automatic calculation of the pinhole size from the laser wavelengths, or enter it manually in the A.U. Calculation Settings window. (For A.U. Calculation Settings, see Section 18.3.4.5, "Calculation Settings for Pinhole Size.")		

# 18.3.4.5 Calculation Settings for Pinhole Size

This section describes the setting window for calculating the pinhole size.

Click the [Pinhole] button in Acquisition window, the A.U. Calculation Settings window appears. (Usually, the [Recommend] is selected to enable automatic calculation. [Recommend] calculates the A.U. value by using the Nikon-recommended EM and NA values.)



# Figure 18.3-13 A.U. Calculation Settings window

Table 18.3-5 A.U. Calculation Settings window

	Name	Function
(1)	Select calculation method	<b>Recommend -</b> Sets parameters automatically. (Nikon recommended)
		User Setting - Allows the user to manually set parameters.
(2)	EM:emission wavelength[µm]	Selected Laser wavelength - Calculates parameters by using the laser wavelength selected in the pinhole combo box of the Acquisition window as the emission wavelength (EM value). The wavelength displayed in the combo box is to be the laser wavelength set in the Optical Setting window.
		<b>Manual -</b> Allows the user to manually set parameters. (The parameter is calculated with the input value as the emission wavelength (EM value).) Enter the value directly from the keyboard.
(3)	NA:Numerical	Sets refractive index of the objective.
	Aperture	<b>NA_obj (NA of objective lens)</b> - Regardless of whether or not the objective NA value exceeds the refractive index of the sample, executes calculation by using the objective NA as the calculation parameter.
		<b>Min(NA_obj, NA_sample)</b> - When the objective NA value does not exceed the refractive index of the sample, executes calculation by using the objective NA as the calculation parameter. When the objective NA value exceeds the refractive index of the sample, executes calculation by using the sample refractive index.
(4)	"Airy" Home Position	Sets a home position of pinhole.

# **Direct entry in offset value display field:** Type the desired setting value.

		The pinhole size can be selected from six types in C2. Therefore, if the entered value does not match any of the types, the size that is larger than and the closest to the entered value is set as the home position.
(5)	Keep A.U. check box	When checked, the pinhole size is fixed by the A.U. when the selected wavelength or objective is changed. (However changes by the $\mu$ m.) When unchecked, the pinhole size is fixed by the $\mu$ m. (However changes by the A.U.)
		The pinhole size can be selected from six types in C2. Therefore, if the to-be-fixed A.U. value does not match any of the types, the size that is larger than and the closest to the A.U. value is selected.
(6)	OK button	Confirms the A.U. Calculation Settings applied and closes the A.U. Calculation Settings window.
(7)	Cancel button	Discards the A.U. Calculation Settings applied and closes the A.U. Calculation Settings window.

#### 18.3.4.6 HV Linear Correction

When HV changes, Gain changes as shown in the graph captioned "Without HV Linear Correction."

As HV increases, the gain variation (the variation of image brightness) is gradual initially, and it becomes steep beyond a certain point.

The gain variation can be automatically corrected to be linear with HV variation by the function called "HV Linear Correction." With this correction, gain varies at the same rate as the HV adjustment.

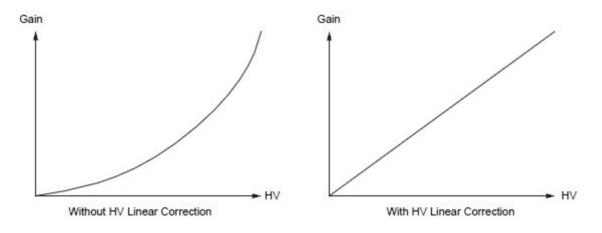


Figure 18.3-14 Gain vs. HV

To enable HV Linear Correction, check the [HV Linear Correction] check box.

Acquisition / Photo Activation/Eleaching(Laser::)     Laser Power Monitor     Age     Select.	All Channels
Clear Laser Interlock Automatically RunTime	Mode : Shortest / One by One
Leser 403.0	Laser 487.4
Laoor 561.7	Laor 638.3
S HV 4 10 Offset 4 0	ID         AIN
Pinhole ◀ () 638.3 ▼ ▲Home ► 1.4 A.U. 20.0 um <-	
Optical Sectioning : 47.91 um Optical Resolution : 1.76 um Optimize	External Port

Figure 18.3-15 HV Linear Correction

- When HV Linear Correction is enabled or disabled, HV is reset to 0 V once.
- If the Offset slider bar is moved, the accurate correction is not performed.

# **18.4 Detection Mode VB**

This chapter describes the settings for the Variable Bandpass mode (VB).

In the Variable Bandpass mode, images capturing light in any wavelength range can be acquired in up to 5 channels.

# 18.4.1 Filter and Dye Window

This window enables to select the desired channel series and set the Optical path.



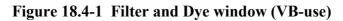


Table 18.4-1 Functions of Filter and Dye window (VB-use)

	Name	Function
(1)	Detector	When Simple Si detector is in use, indicates that the Detector mode [VB] is used when the optical path changeover lever on the C2 scan head is set to the [Spectrum] position and the Variable Bandpass mode (VB) is selected as the detection mode in the Optical path window.
(2)	Eye Port button	Changes optical path to eye port. Each function on the C2plus Settings window becomes non- selectable when the Eye Port button is selected.
(3)	Setting button	Opens the Optical path window. To use, select the detector, the dichroic mirror, the excitation laser, fluorescence dye for each excitation laser and others.
(4)	Status	Indicates for the settings for each channel (fluorescence dye name, laser wavelength, and wavelength band to be acquired). The wavelength band to be acquired can also be changed using the acquisition wavelength range for each channel slider bar. (When an option channel is in use, the wavelength band of Ch1 is not changeable.) * When "C2-DUVB-OP GaAsP 2nd channel Kit" is in use, Ch1 is an option channel and Ch2 to Ch5 are standard channels.
(5)	Ch series	Ch series is available only when "C2-DUVB-OP GaAsP 2nd channel Kit" is in use. Specifies the scan sequence of option channel and selected standard channel (one of four channels Ch2 to Ch5).
		None: Ch series is not used. When None is selected, perform scanning by simultaneously firing all lasers (option channel and selected standard channel) for the channels to be used.
		1->4: Scan starts in the order of option channel (Ch1) -> selected standard channel (one of four channels Ch2 to Ch5).
(6)	Close mechanical shutter during experiment	<ul> <li>4-&gt;1: Scan starts in the order of selected standard channel (one of four channels Ch2 to Ch5) -&gt; option channel (Ch1).</li> <li>If unchecked, the shutter remains open during the ND image acquisition.</li> <li>As the shutter is not opened/closed every image acquisition, the time for the image acquisition can be shortened.</li> </ul>
		* During the interval period, laser power is automatically changed to the minimum but the laser cannot be shut off completely because the shutter is left open.
(7)	AUX button	Displays the AUX Settings window to use the external detector

		unit. For details of the AUX Settings window, see Chapter 17.
(8)	PreScan button	Makes settings of the pre-scan mode for correction of the position shift in a zoom change or of the image acquisition area shift in the photo activation by the prior scanning.

• Optical Configuration

Individual data items set in the Variable Bandpass mode (VB) can be managed collectively with the Optical Configuration window. For storing and retrieving the [Optical Configuration] settings, see the sections concerning the optical configuration in the "NIS-Elements AR (Advanced Research) User's Guide."

# **18.4.2** Setting the Optical Path

Click the [Setting] button of the Filter and Dye window to display the Optical path window.

Select the Variable Bandpass mode (VB).

	Elecond	)f		323				-
	[AAA]	1	Detector	27		Clo	se mechanical shu	tter during experiment
Setting button —		Sym Port	Ch series	None	-	Laser	Emission	
	Second Second	1	Chi	)——		- 403.0	570-620	
			Ch2	-		- 487.4	429-500	
			tha			561.7	500-580	
			Ch4				580-650	
			0-6		4	638.3	500-720	

Figure 18.4-2 Filter and Dye window (VB-use)

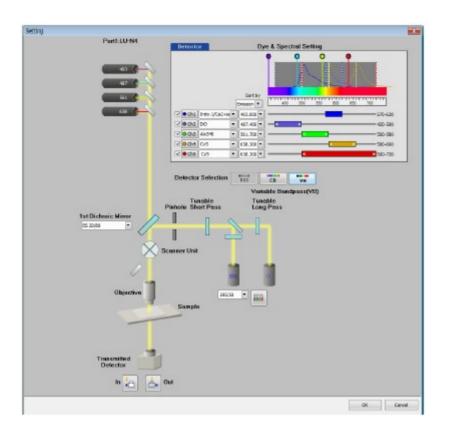


Figure 18.4-3 Optical path window (VB-use)

# 18.4.3 Optical Path Window

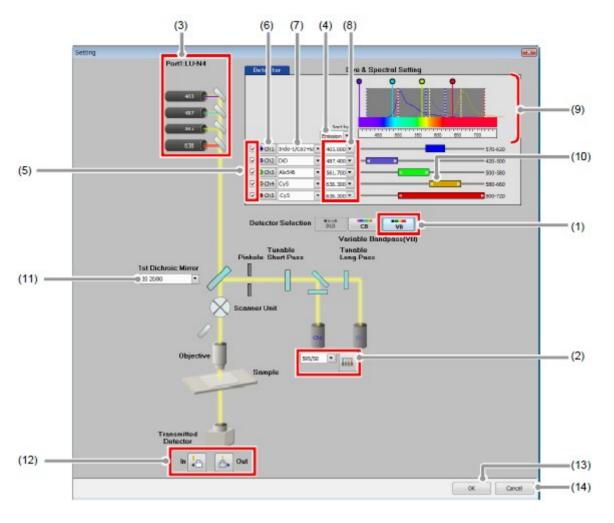


Figure 18.4-4 Optical path window (VB-use)

<i>Table 18.4-2</i>	Functions of	of Optical	path window	(VB-use)
---------------------	--------------	------------	-------------	----------

1000		
	Name	Function
(1)	Detection mode selection button	Variable Bandpass mode (VB) In this mode, images capturing light in any wavelength range can be acquired in up to 5 channels.
(2)	Scan mode select	Selects the scanner unit to be used.
		<b>Galvano</b> Galvano scan mode allows high-quality imaging of up to 4096 × 4096 pixels.
		Resonant
		Resonant scan mode allows high-speed imaging of 420 frames per second.
(3)	Excitation laser wavelength select	Enables to set the laser wavelength that is set with the software configuration, regardless of the setting of the Filter cube display/select.

# 18. Simple Si Detector

(4)	Sorting fluorescence dye list	Sorts the fluorescence dye list according to the selected type.
		<b>ABC</b> : Displays the list in alphabetical order.
		<b>Emission</b> : Displays the list in the order of peak wavelength of fluorescence intensity.
		<b>Excitation</b> : Displays the list in excitation wavelength order.
(5)	Channel selection check box	Enables to select the channels to be used. (Up to 5 channel.)
		* When "C2-DUVB-OP GaAsP 2nd channel Kit" is in use, Ch1 is an option channel and Ch2 to Ch5 are standard channels.
		* When using the Ch series, select the option channel (Ch1) and then select one of standard channels Ch2 to Ch5. (Ch series is available only when "C2- DUVB-OP GaAsP 2nd channel Kit" is in use.)
(6)	Channel color setting button	Displays the Color Selection window, enables to set the desired color for each channel.
(7)	Fluorescence dye selection/input:	Selects the in-use fluorescence dye name for each channel or enters an arbitrary channel name.
(8)	Excitation laser wavelength select	Enables to set the laser wavelength that is set with the software configuration, regardless of the setting of the Filter cube display/select.
(9)	Rainbow chart	Provides the following information:
		<ul> <li>Wavelength band for which to acquire images (shown in color and value for each excitation laser)</li> <li>Spectral profile of fluorescence dye</li> <li>Excitation laser for fluorescence dye</li> <li>A color band indicating the wavelengths in the entire band (400 to 720 nm)</li> <li>Scale of the wavelengths in the entire band (400 to 720 nm)</li> </ul>
(10)	Acquisition wavelength range for each channel slider bar	Specifies the fluorescence wavelength range to be acquired for each channel. Overlapping wavelength range can be specified between channels.
		* When the "C2-DUVB-OP GaAsP 2nd channel Kit" is in use, the wavelength range of the option channel (Ch1) cannot be changed using the slider bar.
(11)	1st Dichroic mirror select	Enables to manually select the 1st Dichroic mirror to be used.
		If a 1st Dichroic mirror that the NIS-Elements does not

recommend is selected in the combination of the selected laser wavelength and the 1st Dichroic mirror, "!" appears following the 1st Dichroic mirror name

(12) Transmitted detector selection button

•

Brings the transmitted detector into the Optical path, to enable the ability.



Brings the transmitted detector out of the Optical path, to disable the ability.

(13) Filter cube setting button

(14) OK button

(15) Cancel button

# 

Used to exchange the filter cube of option dichroic mirror, create new filter cube information, or change the details of the registered filter cube. Clicking this button displays the Filter Cube Setting window. For the editing procedure, see Section 18.4.3.1, "Filter Cube Setting Window."

Through 💌

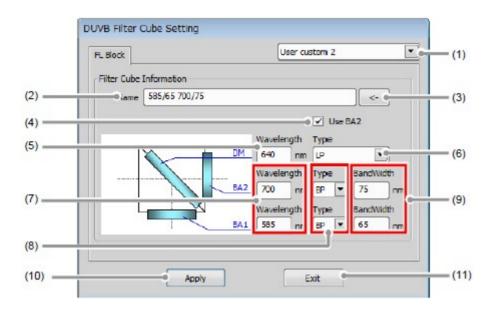
Used to display/select the filter cube name installed in the filter cube of option dichroic mirror.

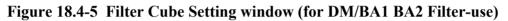
Confirms the Optical path settings applied and closes the Optical path window.

Discards the Optical path settings applied and closes the Optical path window.

# 18.4.3.1 Filter Cube Setting Window

This window opens when the [Filter cube setting] button is clicked on the Optical path window. The new filter cube to be created or the content of the registered filter cube can be changed.





1000	Name	Function
(1)	Preset data	Selects a set of the Filter cube for option dichroic mirror recommended by Nikon.
(2)	Filter cube name display/Edit	Displays/edits the Filter cube name in the combination of the dichroic mirror and the barrier mirror. (Input words: 1 - 24 (in Alphabetical letters))
(3)	Filter cube name creation button	Generates the filter cube name automatically using Wavelength, Type, and BandWidth of the barrier filter.
(4)	Use BA2 check box	When checked, the second barrier filter (BA2) can be used.
(5)	Dichroic mirror wavelength input	Inputs the wavelength of the dichroic mirror. (Input range: 400 - 720 nm)
		The following wavelength is transmitted according to the selected Type.
		<b>[LP] Long pass:</b> Wavelength equal to or longer than the Wavelength value is transmitted.
		<b>[SP] Short Pass:</b> Wavelength equal to or shorter than the Wavelength value is transmitted.
(6)	Filter type select	Selects the dichroic mirror type.
(7)	Barrier filter wavelength input	Inputs the wavelength of the barrier filter (Input range: 400 - 720 nm)
		The following wavelength is transmitted according to the selected Type.

	<b>[LP] Long pass:</b> Wavelength equal to or longer than the Wavelength value is transmitted.
	<b>[SP] Short Pass:</b> Wavelength equal to or shorter than the Wavelength value is transmitted.
	[ <b>BP</b> ] <b>Band Pass</b> : Wavelength of the Wavelength value ?} BandWidth value/2 is transmitted.
(8) Barrier filter type select	Selects the barrier filter type. ([LP] Long pass/[SP] Short Pass/[BP] Band Pass)
(9) Barrier filter band input	When the barrier filter type is [BP], inputs the range from the center wavelength for the barrier filter. [Input range: 1 - 350 nm normally (but not limited to)]
(10) Apply	Confirms the Filter Cube Settings.
(11) Exit	Closes the Filter Cube Setting window. If the setting has not been confirmed with the Apply button, a confirmation dialog box appears.

# 18.4.4 Acquisition Window

The Acquisition window enables to set PMT brightness (detection sensitivity), laser power, and pinhole size.

When "C2-DUVB-OP GaAsP 2nd channel Kit" is in use, Ch1 is an option channel and Ch2 to Ch5 are standard channels.

### 18.4.4.1 Structure of Acquisition Window

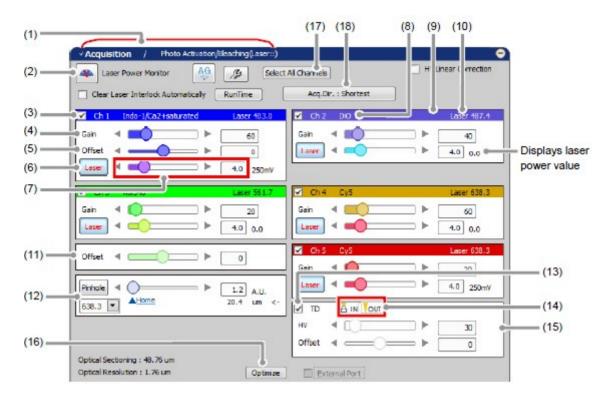


Figure 18.4-6 Acquisition window (VB-use)

1000	v	
	Name	Function
(1)	Acquisition/Photo Activation window switching	Switches between the Acquisition and Photo Activation windows.
		For the Photo Activation window, see Chapter 10.
(2)	Laser power monitor button	Displays the laser power value (integer obtained after A/D conversion divided by 10) of the current excitation laser by clicking this button. During the image acquisition, the laser power cannot be massured and this button is ground out
		measured and this button is grayed out.
(3)	Channel selection	Selects the channels (Ch1 to Ch5) to acquire the desired images.
		Do this by adding a check mark.
		* When using the Ch series, select the option channel (Ch1) and then select one of standard channels Ch2 to Ch5. (Ch series is available only when "C2-DUVB-OP GaAsP 2nd channel Kit" is in use.)
(4)	Gain	Sets the PMT Gain.
		Slider bar: Slides to the right or left to set the gain value.

#### **Arrow buttons:**

Click either arrow button to increase or decrease the gain value stepwise.

#### Direct entry in gain value display field:

Type the desired setting value.

 (5) Offset for option channel
 This function is shown only when "C2-DUVB-OP GaAsP 2nd channel Kit" is in use.
 Sets the offset value of the option channel (Ch1).

#### Slider bar:

Slides to the right or left to set the offset value.

#### **Arrow buttons:**

Click either arrow button to increase or decrease the offset value stepwise.

#### Direct entry in offset value display field:

Type the desired setting value.

(6) Laser ON/OFF button Selects whether the laser is emitted or not.

[	Laser	
0	N sta	tus

The laser is emitted.

	The AOTF s
Laser	becomes 0.
OFE status	When switch

The AOTF shutter closes and the laser power value becomes 0.

When switched from OFF to ON, the laser power value set in the previous ON status is applied.

(7) Laser (*1)

(*1)

# Sets the laser power value.

#### Slider bar:

Slides to the right or left to set the value.

#### **Arrow buttons:**

Click either arrow button to increase or decrease the value stepwise.

#### Direct entry in value display field:

Type the desired setting value.

- * When "C2-DUVB-OP GaAsP 2nd channel Kit" is in use, if a standard channel that uses the same laser wavelength as the option channel exists, the laser power value interlocks.
- (8) Fluorescence dye name indication
  (9) Channel color
  (10) Channel color<
- (10) Laser wavelength The currently selected laser wavelength is indicated.
- indication
- (11) Offset for standard channel

Sets the offset value of the standard channel. Sets the PMT gain of standard channels Ch2 to Ch5 when "C2-

	DUVB-OP GaAsP 2nd channel Kit" is in use.
	Slider bar: Slides to the right or left to set the offset value.
	Arrow buttons: Click either arrow button to increase or decrease the offset value stepwise.
	<b>Direct entry in offset value display field:</b> Type the desired setting value.
(12) Pinhole	Adjusts the pinhole size. For pinhole size, see Section 18.4.4.4, "Setting the Pinhole."
(13) TD channel selection	Enables to acquire TD images by checking the check box. When fluorescence is excited, all excitation lights are detected by the transmitted detector (TD). If the BA filter for 405 laser is installed in the TD, excitation light is not detected by the TD.
(14) TD IN/OUT button	Sets/removes the motorized transmitted detector in/from the optical path. (IN = Set in the optical path/ OUT = Remove from the optical path)
	As for the case where the TD IN/OUT button is not displayed, it will be displayed when the motorized transmitted detector is set in the optical path in the Optical path window.
(15) Brightness adjustment for transmitted detector	For the transmitted detector, use the HV and Offset controls to adjust the brightness of the live image. Sets the offset value and the voltage (HV) to be applied to the transmitted detector.
	Slider bar: Slides to the right or left to set the value.
	Arrow buttons: Click either arrow button to increase or decrease the value stepwise.
	<b>Direct entry in value display field:</b> Type the desired setting value.
(16) Optimize button	Click this button to display the XYZ Size Setup window. In the XYZ Size Setup window, the calculation method of the recommended values of the resolution, zoom magnification, and Z stack step size can be set.
	For the XYZ Size Setup window, see Section 18.4.4.3, "Recommended Value Indication/Automatic Application."
(17) Select All Channels button	Selects all channels for acquiring images.
(18) Custom Acquisition Setting button	Click this button to open the DUVB Custom Acquisition Settings dialog box.

The sequence of scan channels and the option channel acquisition timing can be set in the DUV-B Custom Acquisition Setting dialog box. For details, see Section 18.4.4.2, "DUVB Custom Acquisition Settings Dialog Box." The current acquisition control setting (sequence of scan channels) is shown on this button.

(*1) When LU-NV is in use, this button is grayed out and is disabled while the button on the front panel of the laser unit is OFF or blinking.

#### 18.4.4.2 DUVB Custom Acquisition Settings Dialog Box

Set the sequence of scan channels and the option channel acquisition timing.

variable Band	pass Mode
Acquisition Or	ler
Start with	Shortest 💌 excitation wavelength
Eived Bandhas	s (FB) Channel acquisition
If excitation	wavelength is not the same for both VB and FB channels:
acquire	FB and VB channels sequentially
🔿 acquire	FB channel simultaneously if the VB channel uses an excitation wavelength higher than 500 nm

### Figure 18.4-7 DUVB Custom Acquisition Settings dialog box

Table 18.4-5 DUVB Custom Acquisition Settings dialog box

	Name	Function
(1)	Acquisition Order	Select the sequence of scan channels.
		<b>Longest:</b> In the order of long wavelength -> short wavelength
		Shortest: In the order of short wavelength -> long wavelength
(2)	Fixed Bandpass (FB) Channel acquisition	Sets the option channel acquisition timing. (This setting is enabled only when "C2-DUVB-OP GaAsP 2nd channel Kit" is in use.)
		The option channel is acquired concurrently with other standard channels to which the same laser wavelength is assigned in principle, but is acquired at the timing specified below if the same wavelength is not present.

		acquire FB and VB channels sequentially radio button: The option channel is acquired at other independent timing.
		<ul> <li>acquire FB channel simultaneously if the VB channel uses an excitation wavelength higher than [Numerical entry] nm radio button: The option channel is acquired at the same timing as channel that uses laser with a longer wavelength than the wavelength specified in the numerical entry field by the user.</li> <li>If such channel does not exist, the option channel is acquired at other independent timing.</li> </ul>
(3) OK b	utton	Confirms the settings applied and closes this dialog box.
(4) Cance	el button	Discards the settings applied and closes this dialog box.

#### 18.4.4.3 Recommended Value Indication/Automatic Application

By the function of the recommended value indication/automatic application, the recommended values of the appropriate resolution, zoom magnification, and Z stack step size are calculated based on the objective type and the selected excitation wavelength.

Using the calculated recommended values enables the image acquisition clearer and with less damage to the sample.

**Recommended Value Automatic Application** 

To automatically apply the recommended values to the parameters, set the [Nyquist XY] button of the Scan Area window to ON.

C2plus Scan Area x	
🧱 🚥 — Crop ROI Edit 🔯 🗃 🧱	
Zoom: () 1	
Zoom: 1 Pixel size: 0.62 Nyquist XY	Nyquist XY button
Scan size: 512  Rotation: 0	
Width: 512 Height: 512	
Owell time: 1.9 µs	
Pixel size: 0.62 µm Optical resolution: 0.13 µm Z step size: 0.42 µm Optical sectioning: 1.25 µm	

Figure 18.4-8 Scan Area window

	Scan sating Scan Direction	Zoom	• • • 1.000	
Indicates the		512 recommend /sec(Pixel Dwell:4.8 u sec)	3x € 4.563x recomment	Indicates the recommended value of the scan
	Prihale 4 0 638.3 V	ome 1.2] A.U. 20.4 um	«-	magnification.
	Optical Sectioning Optical Resolution		ze	

Figure 18.4-9 Location of Recommended Value Indication

* When the laser or objective in use is changed, the recommended values are recalculated, and newly indicated and automatically applied.

## **Recommended Value Settings**

Detailed settings of the recommended values are made in the XYZ Size Setup window that is displayed by clicking the [Optimize] button of the Acquisition window.

If the [Nyquist XY] button of the Scan Area window is ON, the recommended values are automatically applied to the parameters.

Or if the [Nyquist XY] button is OFF, the recommended values of the scan size and zoom are indicated in the Scan setting window.

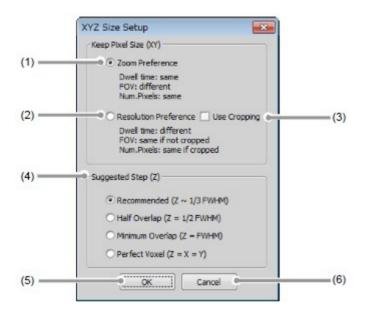




Table 18.4-6 Functions of XYZ Size Setup window

	Name	Function	
(1)	Zoom Preference	1	XY] button is ON, keeps the scan size permended value of the zoom.
(2)	Resolution Preference		XY] button is ON, keeps the zoom and uended value of the scan size.
(3)	Use Cropping	Fits the scan size in	detail by using Crop Scan.
		Sets the Z step size	calculation method.
		Recommend (Z ^{1/3} FWHM)	Approximately one third of the thickness of optical section (FWHM value).
(4)	Suggested Step (Z)	Half Overlap (Z=1/2 FWHM)	One half of the thickness of optical section (FWHM value).
		Minimum Overlap (Z=FWHM)	The thickness of optical section (FWHM value).
		Perfect Voxel (Z=X=Y)	Value same as the pixel size.
(5)	OK button	Confirms the XYZ Size Setup window	Size Setup applied and closes the XYZ
(6)	Cancel button	Discards the XYZ S Size Setup window	Size Setup applied and closes the XYZ

# **18.4.4.4** Setting the Pinhole

Laser Power Monitor	/ <b>1</b> /2 Sel	ect Al Channels	HV Linear Corre
Clear Laser Interlock Automatically	RunTime	Acq.Dir. : Shortest	]
Ch 1 Indo-1/Ca2+eab_mated	Laser 403.0	🗹 0h2 0k0	Lasor 4
Gah 🖪 📫	60	Gain 🔺 🔟	► 40
Offset 4	0	Laser 4	■ ► 4.0 0.
	4.0 250mV		
Ch 3 Abx546	Laser 561.7	🗹 Ch 4 Cy5	Laser 6
Gan 🖪 🌔 🕨 🕨	20	Gain 🖪 🛄	► 60
	4.0 0.0	Laser 🛛 🖛	■ ► 4.0 0.
		🗹 Chisi Cys	Laser 6
	0	Gain 🔺 🌔	▶ 20
Pinhole 4	1.2 A.U.	Laser 4	1.0 25
638.3 • Home	204 um <		
		HV 4 CO	▶ 30
		Offset 4	
Optical Sectioning : 4 75 um			
Optical Resolution : 1 76 um	Optimiz	e External Port	

Figure 18.4-11 Setting the Pinhole (VB-use)

Table 18.4-7	Pinhole	setting functions	(VB-use)
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	Name	Function
(1)	Pinhole size setting	Sets a pinhole size for C2 system.
	-	Slider bar:
		Slides to the right or left to set the pinhole size. (Unit: A.U.)
		Arrow buttons:
		Click either arrow button to increase or decrease the pinhole size stepwise.
		<b>Direct entry in pinhole size display field:</b> Type the desired setting value.
(2)	Pinhole button	Displays the A.U. Calculation Settings window to calculate the pinhole size.
		(For A.U. Calculation Settings, see Section 18.4.4.5, "Calculation Settings for Pinhole Size.")
(3)	Home	Changes the pinhole to the predetermined home position. The value of the home position can be changed in the A.U. Calculation Settings window.
		(For A.U. Calculation Settings, see Section 18.4.4.5, "Calculation Settings for Pinhole Size.")

(4)	Pinhole size	Indicates pinhole size of C2 system. (Unit: µm)
(5)	Optical Sectioning	Indicates the FWHM (full width at half maximum) of z airy disk.
(6)	Optical Resolution	The actual size of 1 pixel square calculated from the optical information (for objectives and scan parameters) and the size acquired from an image.
(7)	Reference excitation wavelength for the pinhole size calculation	Selects the excitation wavelength as the reference of the automatic calculation of the pinhole size from the laser wavelengths, or enter it manually in the A.U. Calculation Settings window. (For A.U. Calculation Settings, see Section 18.4.4.5, "Calculation Settings for Pinhole Size.")

# 18.4.4.5 Calculation Settings for Pinhole Size

This section describes the setting window for calculating the pinhole size.

Click the [Pinhole] button in Acquisition window, the A.U. Calculation Settings window appears. (Usually, the [Recommend] is selected to enable automatic calculation. [Recommend] calculates the A.U. value by using the Nikon-recommended EM and NA values.)

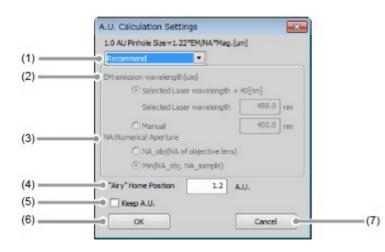


Figure 18.4-12 A.U. Calculation Settings window

Table 18.4-5 A.U. Calculation Settings window

	Name	Function
(1)	Select calculation method	<b>Recommend -</b> Sets parameters automatically. (Nikon recommended)
		User Setting - Allows the user to manually set parameters.
(2)	EM:emission wavelength[µm]	<b>Selected Laser wavelength</b> - Calculates parameters by using the laser wavelength selected in the pinhole combo box of the Acquisition window as the emission wavelength (EM value).

		The wavelength displayed in the combo box is to be the laser wavelength set in the Optical Setting window.
		<b>Manual -</b> Allows the user to manually set parameters. (The parameter is calculated with the input value as the emission wavelength (EM value).) Enter the value directly from the keyboard.
(3)	NA:Numerical	Sets refractive index of the objective.
	Aperture	NA_obj (NA of objective lens) - Regardless of whether or not the objective NA value exceeds the refractive index of the sample, executes calculation by using the objective NA as the calculation parameter.
		<b>Min(NA_obj, NA_sample)</b> - When the objective NA value does not exceed the refractive index of the sample, executes calculation by using the objective NA as the calculation parameter. When the objective NA value exceeds the refractive index of the sample, executes calculation by using the sample refractive index.
(4)	"Airy" Home Position	Sets a home position of pinhole.
		<b>Direct entry in offset value display field:</b> Type the desired setting value.
(5)	Keep A.U. check box	When checked, the pinhole size is fixed by the A.U. when the selected wavelength or objective is changed. (However changes by the $\mu$ m.) When unchecked, the pinhole size is fixed by the $\mu$ m. (However
		changes by the A.U.)
(6)	OK button	Confirms the A.U. Calculation Settings applied and closes the A.U. Calculation Settings window.
(7)	Cancel button	Discards the A.U. Calculation Settings applied and closes the A.U. Calculation Settings window.

# 18.4.4.6 HV Linear Correction

When HV changes, Gain changes as shown in the graph captioned "Without HV Linear Correction."

As HV increases, the gain variation (the variation of image brightness) is gradual initially, and it becomes steep beyond a certain point.

The gain variation can be automatically corrected to be linear with HV variation by the function called "HV Linear Correction." With this correction, gain varies at the same rate as the HV adjustment.

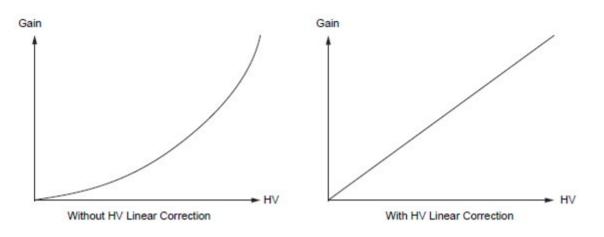


Figure 18.4-15 Gain vs. HV

To enable HV Linear Correction, check the [HV Linear Correction] check box.

Acquisition / Photo Activation(Bleaching(Laser::)					
Laser Power Monitor	All Channels				
Clear Laser Interlock Automatically RunTime	Acq.Dir. : Shortest				
Ch 1 Indo-1/Ca2+saturated Laser 403.0	Ch 2 DiO Laser 487.4				
Gan 4 60	Gain 4				
Offset 4	Laser 4 - + 4.0 0.0				
Leser 4 4.0 250mV					
Ch 3 Ab:546 Laser 561.7	Ch 4 Cy5 Laser 638.3				
Gan ◀ () > 20 Laser ◀ () + 4.0 0.0	Gain ◀				
	Ch 5 Cy5 Laser 638.3				
Prince 4					
	HV 4				
Optical Sectioning : 48.75 um Optical Resolution : 1.76 um Optimize	Offset 4 0				

Figure 18.4-16 HV Linear Correction

- When HV Linear Correction is enabled or disabled, HV is reset to 0 V once.
- If the Offset slider bar is moved, the accurate correction is not performed.

# **Appendix** Lists of Filter Cubes

#### The Filter Cube Information for the Optical Path

The Dichroic mirror or the Barrier filter to be registered on the Optical path window for the Confocal C2 is listed below.

Note:

Excitation lights are unrestricted.

**1st Dichroic mirror** 

The 1st Dichroic mirror provided for the Optical C2 is shown below.

Table 1. 1st DM List

#### Name

- 1 BS 20/80
- 2 408/488/543
- 3 405/488/561
- 4 408/488/594
- 5 440 (457)/514/594
- 6 405/488/543/640
- 7 405/488/561/640
- 8 405 (408)/457 (440)/561/640 (633)
- 9 440 (457)/514/561/640 (633)
- 10 405 (408)/457 (440)/543/640 (633)

#### **Detector filter cube**

The Dichroic mirror and the Detector filter cube provided for the Optical C2 is shown below.

#### Table 2. DM/BA filter List

	2nd DM	1st BA	3rd DM	3rd BA	2nd BA
1	455	None	None	None	None
2	482	438/24	540LP	585/65	494/41
3	511	447/60	560	561LP	510/84
4	540LP	482/35	593	594LP	514/30
5	560	494/41	648	635LP	525/50
6		510/84			537/26
7		514/30			550/49
8		525/50			585/65

9

537/26

593/40

**Registered filter cube name** 

The Detector filter cube combined with the standard filter cube is shown below. 1 – 8 (9 for 2nd Filter Cube Set) ndicates the preset setting, whereas 9 (10 for 2nd Filter Cube Set) - 11 indicates the User registration, and 12 indicates no filter required. The available Filter cube is equivalent with the Filter cube tab of the "Filter Cube Setting" window.

Table 3. Registration filter cube name List 1 1st Filter Cube Set

	Name	Registration number	2nd DM	BA1
1	438/24	1	455	438/24
2	447/60	2	482	447/60
3	482/35	3	511	482/35
4	494/41	4	540LP	494/41
5	510/84	5	560	510/84
6	514/30	6	540LP	514/30
7	525/50	7	560	525/50
8	537/26	8	560	537/26
9	User 3	9	None	None
10	User 4	10	None	None
11	User 5	11	None	None
12	Through	12	Through	Through

#### Table 4. Registration filter cube name List 2 2nd Filter Cube Set

	Name	Registration number	3rd DM	3rd BA	2nd BA
1	494/41,585/65	1	540LP	585/65	494/41
2	510/84,561LP	2	560	561LP	510/84
3	514/30,585/65	3	540LP	585/65	514/30
4	525/50,561LP	4	560	561LP	525/50
5	525/50,594LP	5	560	594LP	525/50
6	537/26,561LP	6	560	561LP	537/26
7	550/49,594LP	7	593	594LP	550/49
8	585/65,635LP	8	648	635LP	585/65
9	593/40,635LP	9	648	635LP	593/40
10	User 2	10	None	None	None
11	User 3	11	None	None	None

Page 4	4 of	£4
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12	Through	12	Through	Through	Through	
----	---------	----	---------	---------	---------	--