

Nikon

Super-resolution Microscope

N-STORM

Simple Operation Manual

Introduction

Thank you for purchasing a Nikon product.

This instruction manual is written for users of the Nikon Super-resolution Microscope N-STORM. To ensure correct usage, read this manual carefully before operating this product.

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Symbols used in this operation manual

This operation manual uses the following symbols.

-  Indicates information that should be kept in mind when using this product, or which provides useful hints.

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Terminology Used in This Document

N-STORM

System that allows STochastic Optical Reconstruction Microscopy (STORM) with Nikon's inverted research microscope Ti-E.

During STORM, some fluorescent probe molecules are randomly stimulated (activated) with relatively weak light to become activated, after which their images are acquired through an EM-CCD camera (imaging). The series of images that is acquired through frequent repetition of this process is analyzed and synthesized by software, and formed into a super-resolution image.

Conventional image

Image of 256 x 256 pixels acquired using an EM-CCD camera. In STORM analysis, a set of sequentially acquired conventional images is used as material.

STORM image (2D-STORM/3D-STORM image)

Super-resolution image generated as a result of analyzing a dataset of conventional images. It includes information such as the position, size, and intensity of each individual fluorescent probe molecule.

A STORM image that only has information on the positions in the X- and Y-axis directions is referred to as "2D-STORM image," while one that also has information on the positions in the Z-axis direction is referred to as "3D-STORM image."

Dye pair

Composite dye used for STORM observation, such as the following:

Examples: Alexa405-Alexa647

Cy2-Alexa647

Cy3-Alexa647

Probe

Fluorescent molecule (dye pair or monomolecular dye) used for STORM observation.

Dataset

Set of consecutive frames (conventional images) acquired through an EM-CCD camera. A dataset is saved in ND2 file format for NIS-Elements.

Frame

Individual conventional image that is acquired through an EM-CCD camera as material to be used to generate a STORM image.

Activation frame

Frame (image) that is acquired when relatively weak laser light for activation is emitted. This activation causes some fluorescent probe molecules to become activated. Activation frames are not used for STORM analysis. Note that if images are to be acquired in continuous mode, there is no difference between the activation and imaging frames because activation and imaging are performed simultaneously.

Imaging frame

Frame (image) that is acquired when laser light for imaging is emitted. In this frame, fluorescent probe molecules fluoresce only when activation has caused them to become activated. Note that if images are to be acquired in continuous mode, there is no difference between the activation and imaging frames because activation and imaging are performed simultaneously.

Cycle

Set of frames that consist of an activation frame (normally one frame) and the subsequent imaging frames (normally three frames) on one channel during image acquisition in normal mode.

Channel

For a multistaining procedure, the cycle in which each dye is observed is called a channel.

Period

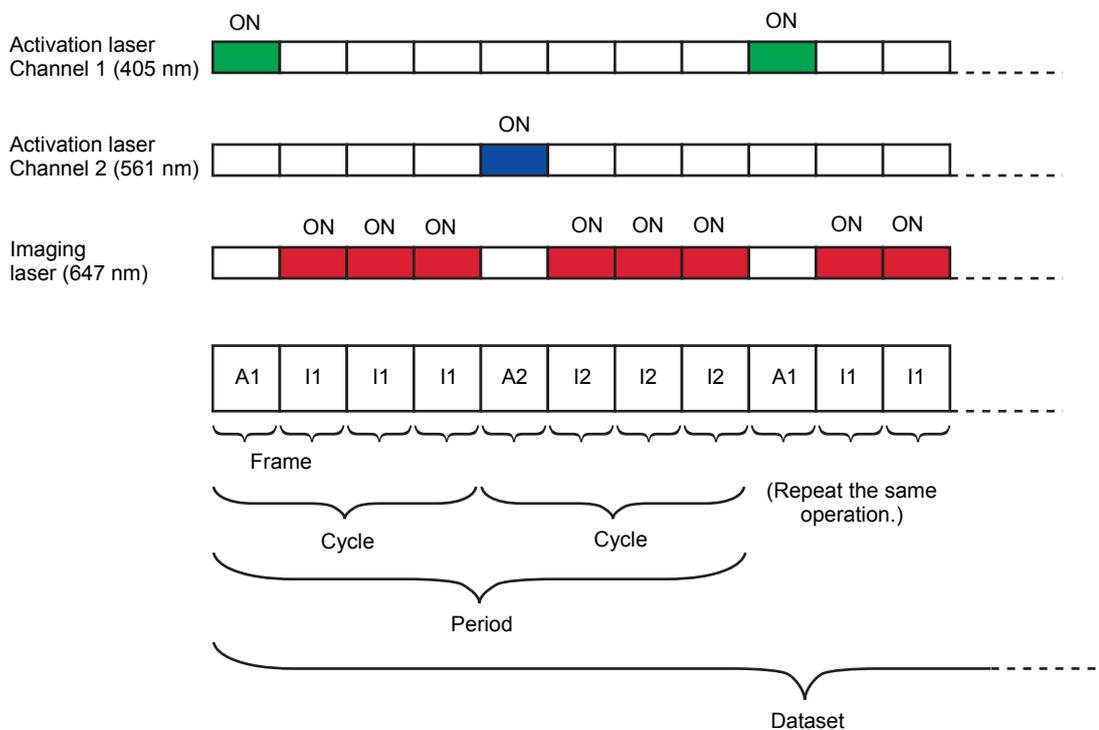
Set of cycles that are made up of one cycle of each of the multiple channels on which images are acquired in normal mode. For example, if images are acquired on two channels, one period consists of two cycles. If images are acquired on only one channel, a period is the same as a cycle.

✔ Dataset structure in normal mode (example)

Number of image acquisition channels: 2

Number of activation frames per period (per cycle) on one channel: 1

Number of imaging frames per period (per cycle) on one channel: 3



A1: Activation frame on channel 1

I1: Imaging frame on channel 1

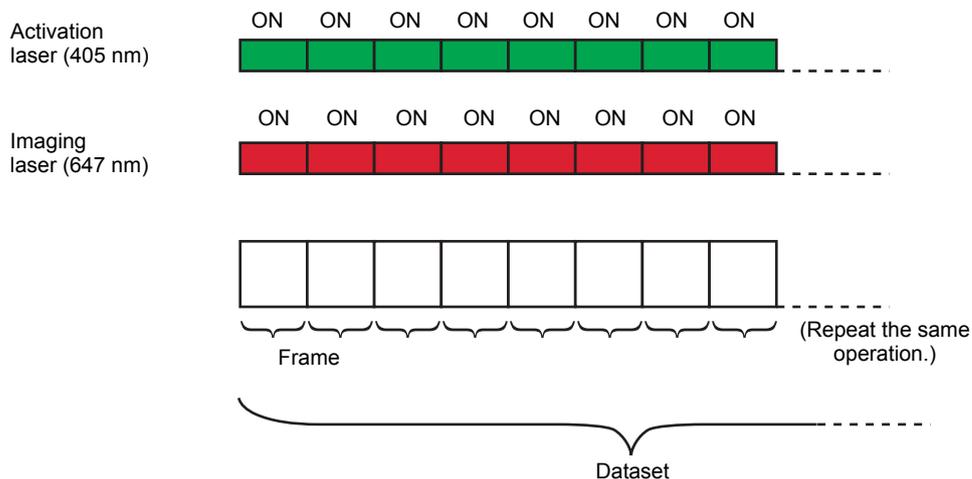
A2: Activation frame on channel 2

I2: Imaging frame on channel 2

Continuous mode

Method of acquiring STORM images using a monomolecular dye that can itself become bright or dark, instead of requiring a dye pair for activation. Since the activation and imaging laser light are emitted simultaneously to acquire images, acquisition takes less time than in normal mode. In this case, the definitions of cycle and period are not applied.

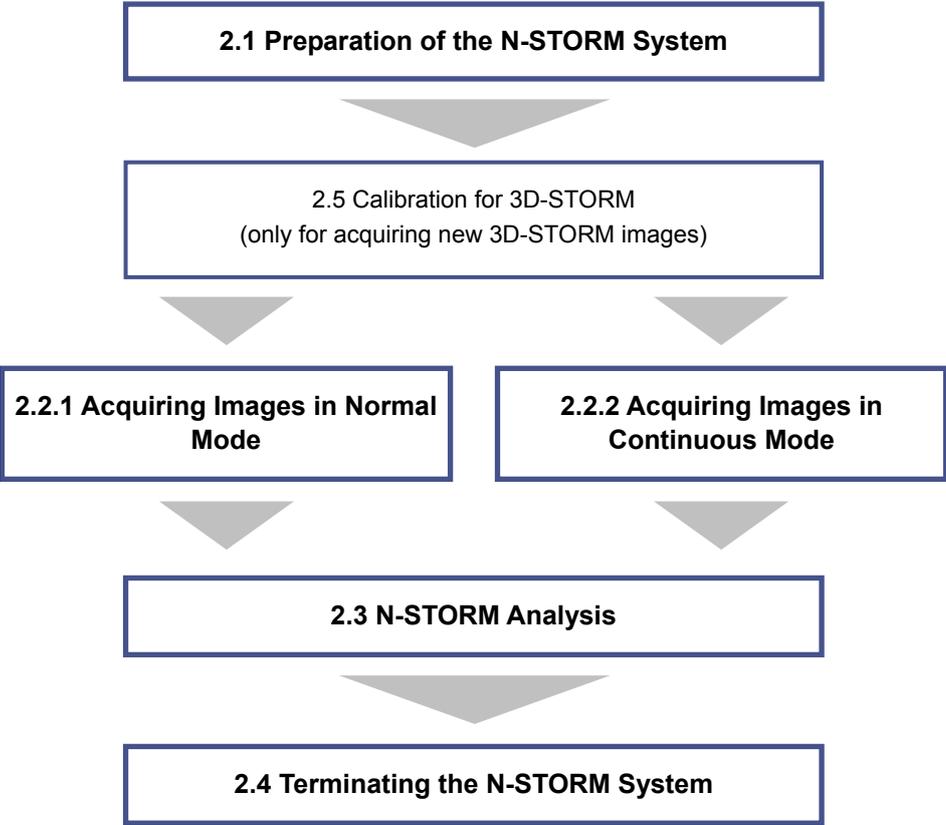
Dataset structure in continuous mode (example)



Non-specific activation (NSA)

During image acquisition in normal mode, only those molecules that are detected in the first imaging frame after activation are classified as being of the relevant channel. Also, those molecules that are not detected in the first imaging frame but in the second or subsequent frames are classified as being those of a non-specific activation (NSA) channel. Information on non-specific activation channels is used for crosstalk subtraction. (For details, see step 6 in “2.3 N-STORM Analysis,” in Chapter 2.)

The overall operational steps and their corresponding descriptions in this chapter are as follows:



The screenshots in this document are presented as an example.

2.1 Preparation of the N-STORM System

Prepare the microscope, the laser, and other peripheral devices, and then start NIS-Elements AR.

✔ Connecting the piezo Z stage

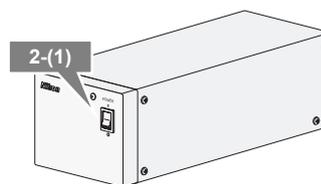
The controller for the piezo Z stage must be connected to the PC with a USB cable. Remove the analog cable that directly connects the controller to the microscope. Also, select [Manage devices...] from the [Devices] menu, and turn off [Ti PiezoZ] under [Nikon Ti].

1 Perform safety checks.

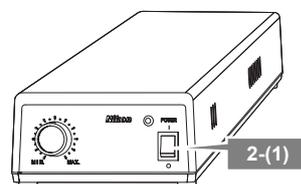
(-> Chapter 3, "Detailed Microscopy Procedure" of the user manual of the TIRF illuminator)

2 Turn the power on.

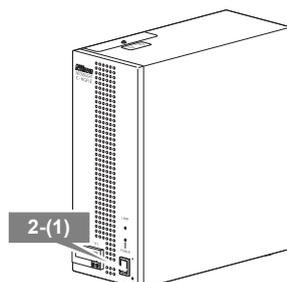
- (1) Turn on the motorized stage and the illumination light source.
- (2) Turn on the piezo Z stage.
- (3) Turn on the microscope.
- (4) Turn on each laser head. (See the user manuals for the TIRF illuminator and each laser head.)
- (5) Turn on the LU4A laser unit.
- (6) Turn on the PC.



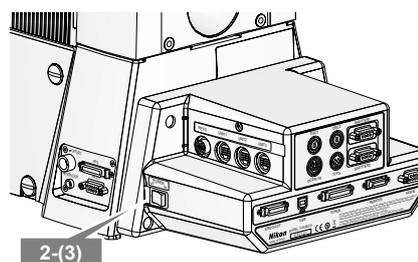
Motorized stage power supply



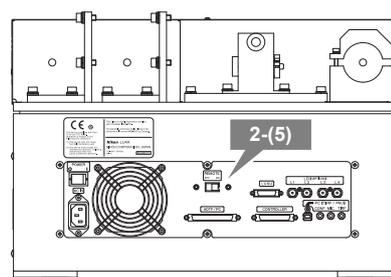
Diascopic illumination power supply



HG precentered fiber illuminator



Microscope



LU4A

✓ **When MPB Communications Inc.'s 647-nm laser is used**

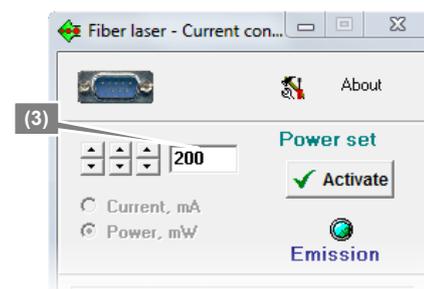
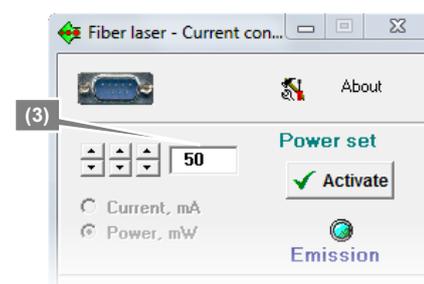
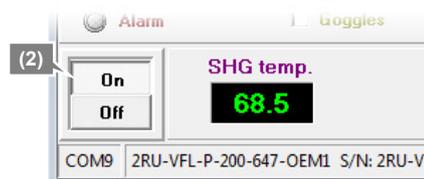
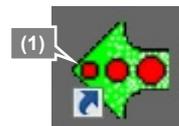
- (1) Start the laser control software GUI-VFL.
- (2) Click the [On] button in the window to turn on the 647-nm laser.
The value of [SHG temp.] is displayed.
- (3) Follow the procedure below to gradually increase the output of the 647-nm laser through GUI-VFL (procedure recommended by the laser maker).

Set the power to 50 mW, click [Activate], and then wait until the value of [Power, mW] becomes about 50 mW.

Then, set the power to 200 mW, click [Activate], and wait until the value of [Power, mW] reaches about 200 mW. The value of [Power, mW] may change by a few percent, but this does not affect the acquisition of STORM images.

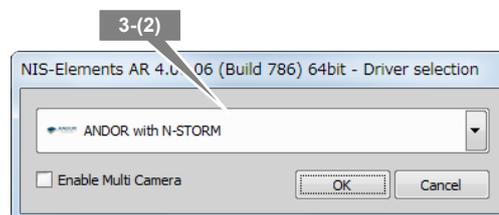
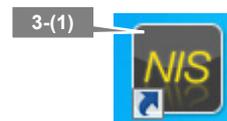
After the completion of this procedure, use NIS-Elements to adjust the laser power. It is not necessary to use GUI-VFL for adjustment.

(For details on the procedure for turning off this laser, see step 1 in "2.4 Terminating the N-STORM System.")



3 Start NIS-Elements AR.

- (1) Start NIS-Elements AR by double-clicking the corresponding icon.
- (2) When the camera driver selection dialog is displayed, select [ANDOR with N-STORM]. (To change the camera driver after NIS-Elements is started, select [Select Driver...] from the [Acquire] menu.)
- (3) Display the [N-STORM] control window and the [DU-897 Settings] (camera) control window. (Select [Acquisition Controls] from the [View] menu and then select the control windows.)



4 Place the imaging target in the visual field.

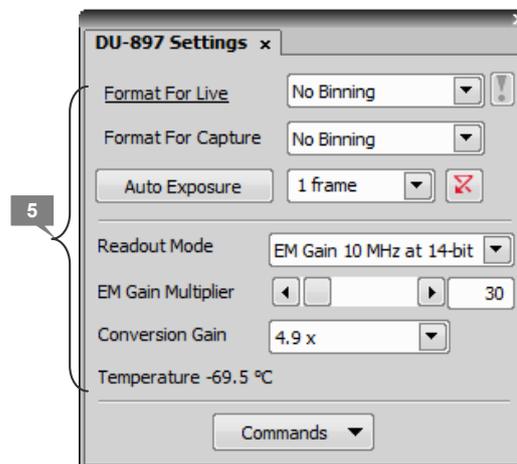
Set a specimen, direct the light path to the binocular part to perform epi-fluorescence microscopy, and then put the STORM imaging target into the visual field.

(For details on the procedure for epi-fluorescence microscopy, see the user manual for the microscope.)

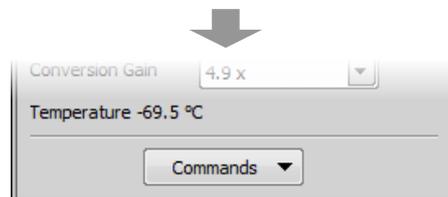
5 Configure camera settings.

In the [DU-897 Settings] control window (EM-CCD camera settings), configure the settings as follows.

[Format For Live]: No Binning
 [Format For Capture]: No Binning
 Exposure time: Any setting (50 msec or 1 frame recommended)
 [Readout Mode]: EM Gain 10 MHz 14-bit
 [EM Gain Multiplier]: 30
 [Conversion Gain]: Maximum available value
 [Desired Temperature]
 (Commands -> Advanced Camera Settings): -70°C

**6 Wait until the temperature of the camera stabilizes at about -70°C and [Desired temp. differs!] disappears.**

It takes a few minutes for the temperature of the camera to stabilize.

**7 Put the objectives for STORM into the light path.**

Objectives: CFI Apo TIRF 100x oil (NA1.49) or
 CFI Plan Apo VC 100x oil (NA1.40)

Use Nikon immersion oil Type B or Type NF for oil immersion of the objectives.

When using CFI Apo TIRF 100x oil, adjust the correction ring to suit the cover glass. If a cover glass No. 1-S (No.1.5) is used, it is recommended that the position of the correction ring be 0.165 mm.

8 Perform an observation with TIRF illumination.

- (1) Direct the light path to the EM-CCD camera.

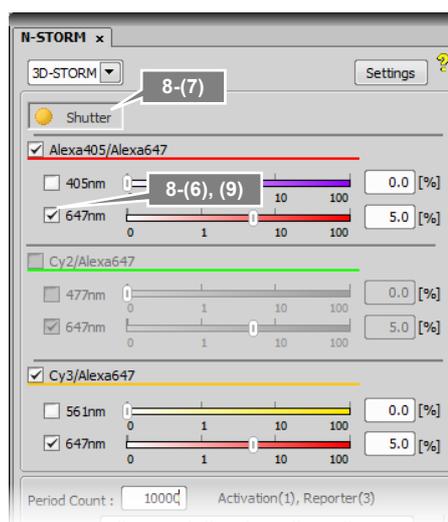
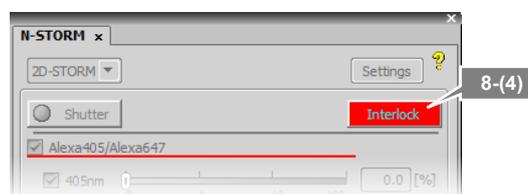
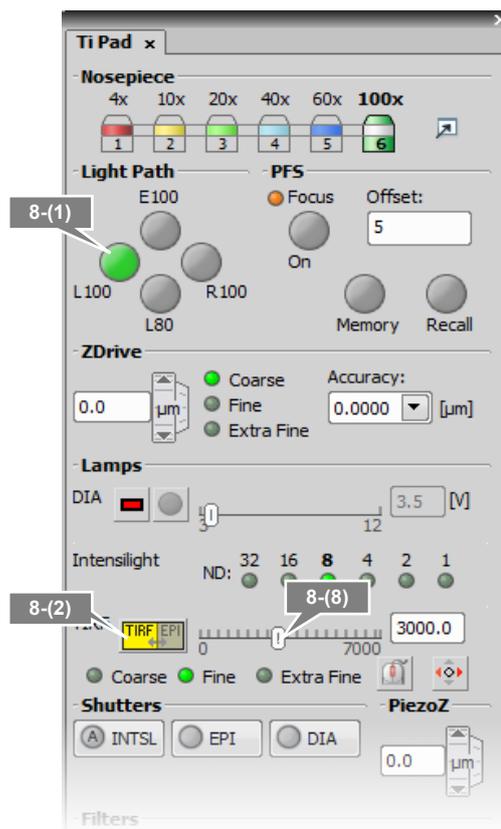
Note: Set the cylindrical lens for 3D-STORM to OUT (where it is not in the light path).

- (2) Set the episcopic illumination to [TIRF].
- (3) Put the N-STORM filter cube into the light path.
- (4) Click [Interlock] on the N-STORM control window to disable the laser interlock.

✔ If the interlock cannot be disabled

If clicking [Interlock] does not disable the interlock, the light path may not be set to the side port, or the laser safety cover of the stage may not be secured properly. Make sure that the light path is set to the side port (the EM-CCD camera for STORM), and that the laser safety cover is secured. Then, click the [Interlock] button again.

- (5) Click  [Live] of NIS-Elements to display the live image on the screen.
- (6) Select the checkbox for the 647-nm laser and set the power to about 5% to 10%. (If the power is too strong, photo-bleaching further occurs.) Deselect the checkboxes for the other lasers.
- (7) Set the shutter of the laser to OPEN in the N-STORM control window.
- (8) Set the laser position to the TIRF position (about 4200). (For details on how to adjust the TIRF, see the user manual for the TIRF illuminator.)
- (9) After TIRF observation is completed, temporarily deselect the checkbox for the 647-nm laser on the N-STORM control window.



9 Configure camera ROI settings.

Select [Camera ROI] -> [Define ROI] from the [Acquire] menu of NIS-Elements, and then configure the settings as follows.

- [Left] 128 pixels
- [Top] 128 pixels
- [Width] 256 pixels
- [Height] 256 pixels

Available range

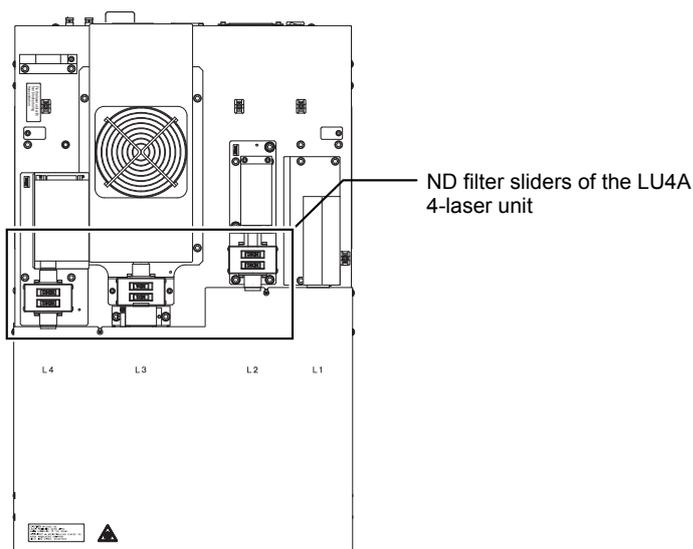
The range available for STORM is 256 x 256 pixels only.

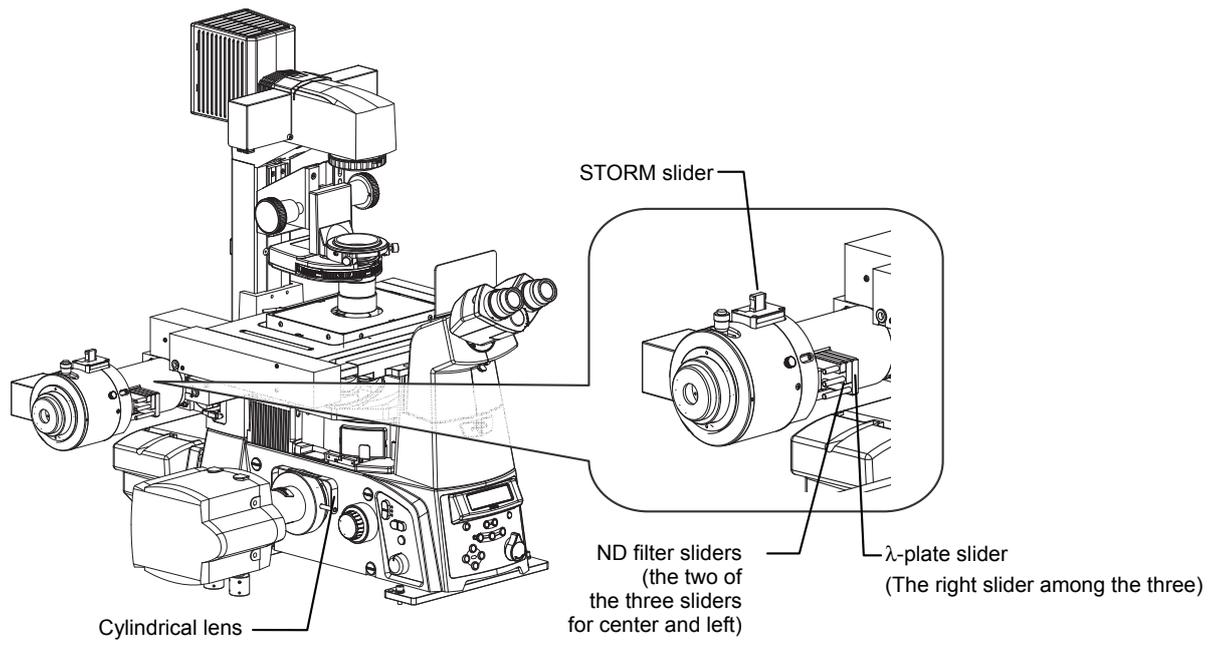
10 Configure the settings for the other optical systems for N-STORM.

	LU4A 4-laser unit	Electric TIRF illuminator TI-TIRF-E			3D-STORM port (Ti-E side port)	
	ND filter slider	STORM slider	λ -plate slider	ND filter slider	Cylindrical lens	
					N-SIM/N-STORM switching port is used	
2D-STORM	IN ^{*1}	IN	IN	OUT	OUT	1x
3D-STORM	IN ^{*1}	IN	IN	OUT	IN ^{*2}	STORM ^{*2}

IN: Included in the light path, OUT: Not included in the light path

- *1 ND filter slider of the LU4A 4-laser unit
 L2 position: Two ND32s are put into the light path.
 L3 position: Both ND2 and ND4 are put into the light path.
 L4 position: Two ND32s are put into the light path.
- *2 Cylindrical lens
 When the cylindrical lens is put into the light path, the image will be slightly blurred.





2.2 Acquiring 3D-STORM (2D-STORM) Images

To perform STORM analysis, automatically repeat light stimulation and image acquisition with the appropriate laser power to create a dataset. There are two types of mode for acquiring images: normal mode and continuous mode. In normal mode, activation and imaging are performed in separate frames. In continuous mode, they are performed simultaneously.

The following basically describes the procedure for acquiring 3D-STORM images. Any differences from the procedure for acquiring 2D-STORM images are noted together with the symbol “→”.

2.2.1 Acquiring Images in Normal Mode

In the example below, the following two types of probe are used.

- Channel 1: Alexa405-Alexa647 dye (Alexa647 activated by Alexa405)
- Channel 2: Cy3-Alexa647 dye (Alexa647 activated by Cy3)

✓ Calibration for 3D-STORM

Before the first 3D-STORM image is acquired, it is necessary to perform calibration. See “2.5 Calibration for 3D-STORM.”

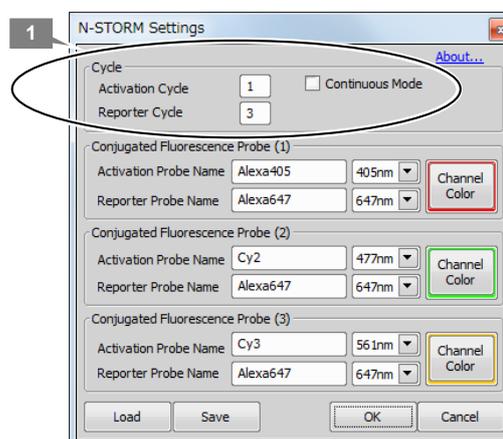
- 1 Click [Settings] in the [N-STORM] control window and then specify the number of frames for each cycle.

[Continuous Mode]: Off

[Activation Cycle] (number of activation frames): 1

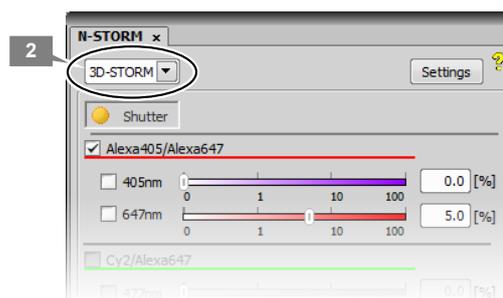
[Reporter Cycle] (number of reporter frames): 3

After making this setting, click [OK].



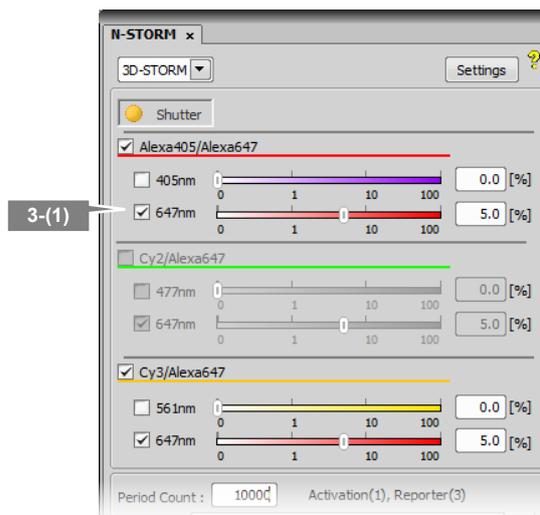
- 2 Select [3D-STORM] from the list at the top left of the [N-STORM] control window.

→ To acquire a 2D-STORM image, select [2D-STORM].

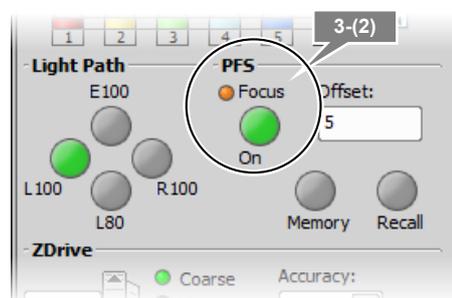


3 Finely and properly adjust the laser focal point of the TIRF illuminator.

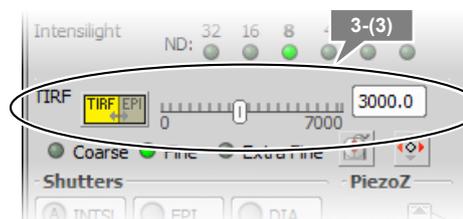
- (1) Select the checkbox for the 647-nm laser.



- (2) Turn on the Perfect Focus System (PFS) in [Ti Pad] and set the focus.

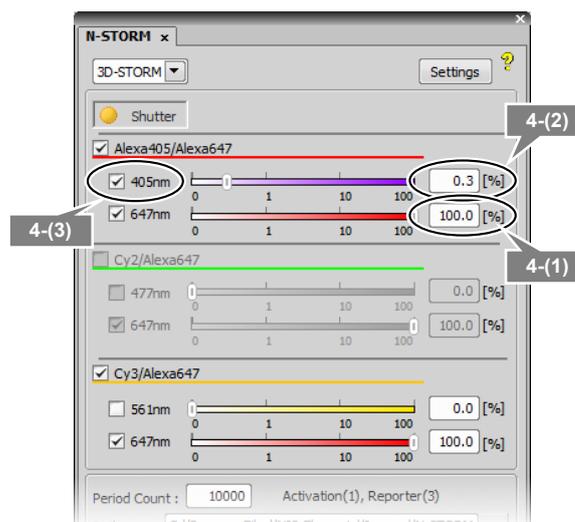


- (3) Finely adjust the laser focal point of the TIRF illuminator so that the observation target is visible.



4 Check activation.

- (1) Make sure that the checkbox for the reporter laser (647 nm) is selected, and then set the power to 100% by adjusting the power slider.
- (2) Make sure that the checkbox for the activation laser (405 nm, 457 nm, or 561 nm) is deselected, and then set the value to about 0.3% by adjusting the power slider.
- (3) Select the checkbox for the activation laser so that it emits laser light for about a second, and then immediately deselect it. Check that the live image becomes bright once and then returns to the previous state as a result of activation causing the fluorescent probe to fluoresce. When using multiple activation lasers, check this for each activation laser.



✔ If activation cannot be checked

If emission of the activation laser does not make the live image bright, this can be improved by replacing the buffer solution of the specimen with a fresh solution.

5 Configure the N-STORM acquisition settings.

The settings are as follows.

Fluorescent probe checkbox:

Turn on the probe to be used.

Output of the activation laser (405 nm, 457 nm, 561 nm):

0.2% to 0.3% (values with which proper activation is performed as shown in step 4 above)

Output of the imaging laser (647 nm):

100%

[Period Count] (number of periods for image acquisition):

Any setting (normally 5000 to 20000 ^{*1})

[Path], [File Name] (folder to which the file is to be saved and the file name):

Any setting

[STORM Image] (whether to display the STORM image during image acquisition):

If this checkbox is selected, the STORM image is previewed during acquisition. Normally, this should be selected.

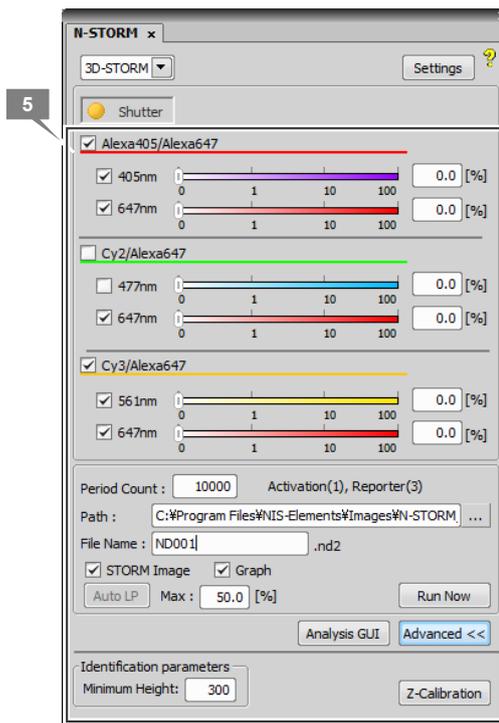
[Graph] (whether to display a graph during image acquisition):

If this checkbox is selected, a graph showing the number of bright points in the image is displayed. Normally, this should be selected.

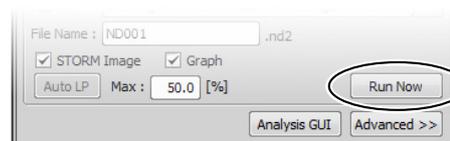
[Minimum Height] (minimum intensity):

300 ^{*2}

- *1 The above number of periods for image acquisition is a reference value. Adjust it according to the structure or coloring status of the specimen. Even when it is set to 20000 periods in advance, if it is confirmed as being large enough on the real time analysis (preview) screen, it is possible to halt the acquisition (for example, about 10000 periods) by clicking [Finish].
- *2 The above-mentioned minimum height is a reference value. Adjust it according to the amount of background light for the specimen, etc.

**6 Click [Run Now].****Disabling large image mode**

If large image mode is enabled with the ND Acquisition function of NIS-Elements, images cannot be acquired. Disable the acquisition of large images before clicking [Run Now].



- 7 Measure the gap between the boundary surface of the cover glass and the position of the observation target (Z-axis direction) using the method below.**

→ This step is not required for 2D-STORM.

- (1) Step 1:
When the right dialog is displayed, select the imaging laser (647 nm). Set the focus to the boundary between the cover glass and the specimen by using the PFS offset controller. (Adjust the power to about 50% to 100% using the slider.)

- ✔ **Set the focus to the boundary surface of the cover glass.**

When setting the focus, move the XY stage and view, as boundary marks, the dye molecules that are non-specifically adsorbed onto the boundary surface of the cover glass to identify the boundary surface.

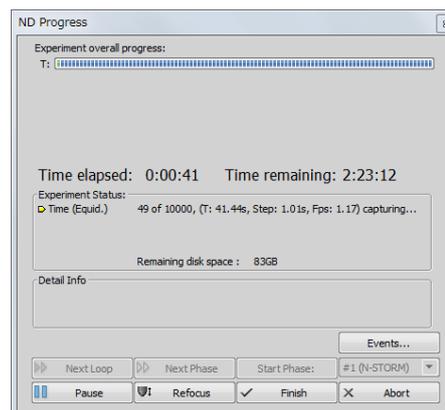
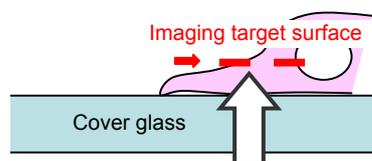
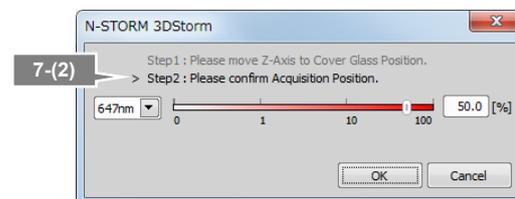
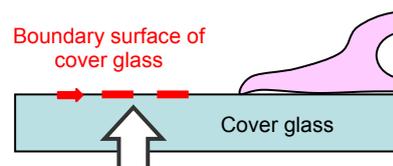
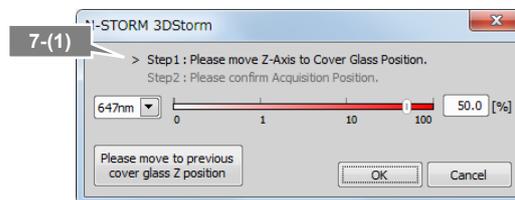
After setting the focus, click [OK]. The XY stage automatically returns to its original position.

- (2) Step 2:
When the dialog shown on the right is displayed, set the focus to the target imaging position by using the PFS offset controller.

After setting the focus, click [OK]. The measured gap (Z-axis direction) is automatically saved and used in analysis.

When acquisition is started, the dialog box shown on the right is displayed. Do not perform any operation using this dialog box until image acquisition has been completed. Clicking [Events...], [Pause], or [Refocus] prevents the acquisition of appropriate images for STORM analysis.

To halt the acquisition, click [Finish] or [Abort]. (If [Finish] is clicked, the acquired images are saved. Be careful because, if [Abort] is clicked, the acquired images will be discarded.)

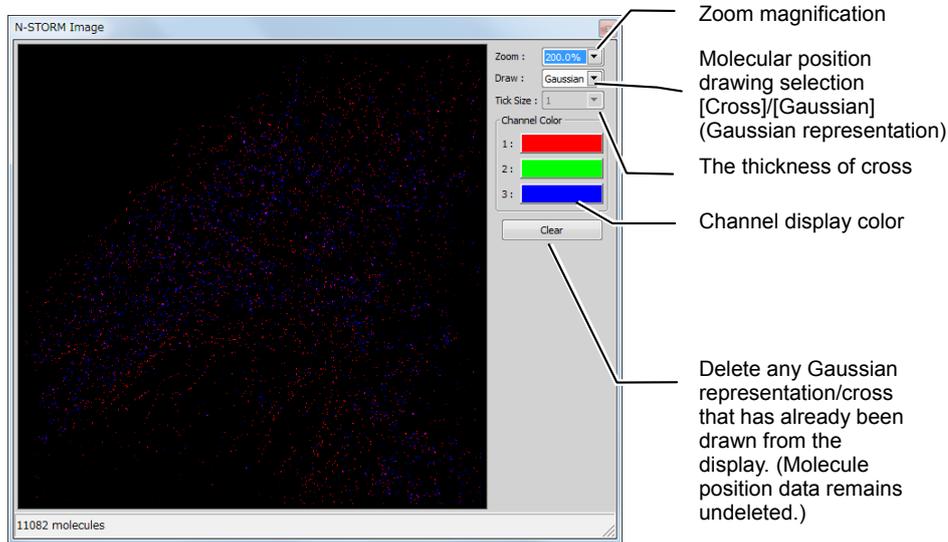
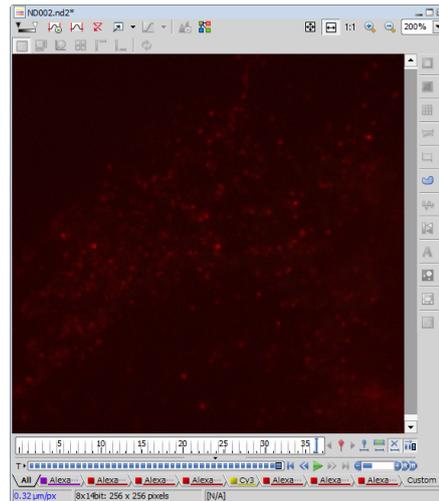


8 Adjust the power of the activation laser, if necessary, during real time analysis (preview) of the acquired images.

Image acquisition is started. The result of real time analysis (preview) and a graph are displayed.

Recommended number of bright points

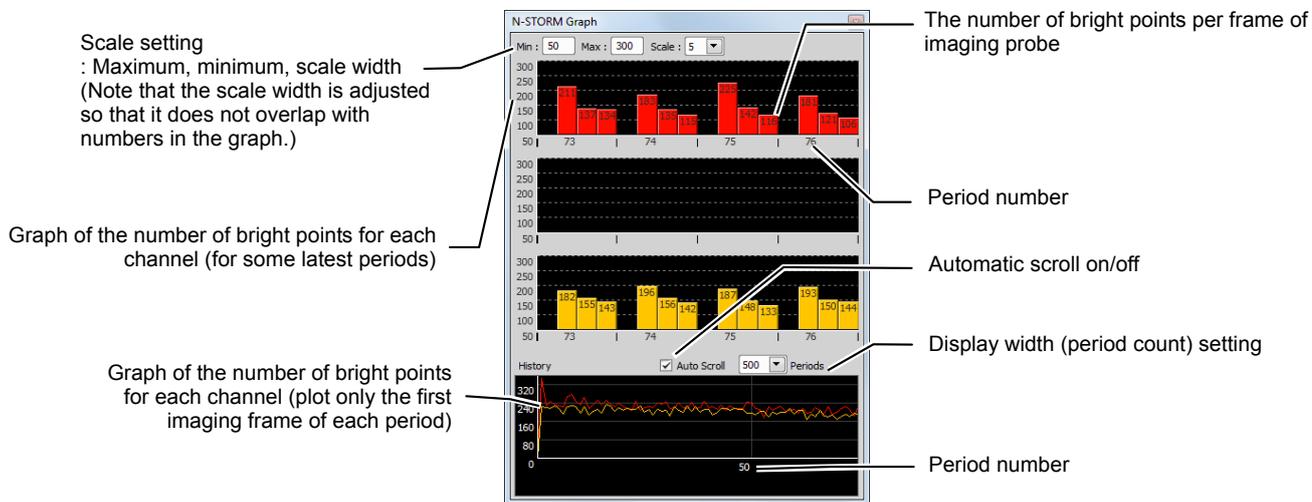
The recommended data for STORM analysis is as follows: the density of bright points is not too low but not so high that they do not overlap each other. For example, a structure that covers the entire screen (such as a microtubule in a cell) should be provided with about 100 to 200 bright points per frame in a visual field with 256 x 256 pixels. The number of bright points to be activated can be adjusted by changing the power of the activation laser.



Real time analysis (preview) screen during image acquisition

Details of real time analysis

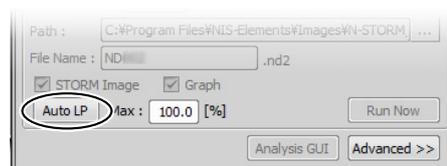
During real time analysis, the value of [Minimum Height] on the N-STORM control window is used as a threshold to analyze bright points. Real time analysis does not include drift correction.



Graphic screen during image acquisition

After the completion of acquisition, click [x] in the windows for real time analysis (preview) and graph to close the windows.

After the power of the activation laser is adjusted, clicking [Auto LP] in the [N-STORM] control window automatically adjusts the laser power so that the number of bright points is maintained. Also, the upper limit on the power for automatic adjustment(%) can be specified on the [Max] box.



2.2.2 Acquiring Images in Continuous Mode

In the example below, the following probe is used.

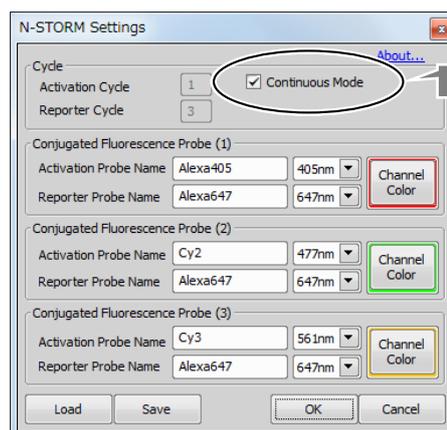
- Alexa647 dye (Alexa647 activated by Alexa405)

☑ Calibration for 3D-STORM

Before the first 3D-STORM image is acquired, it is necessary to perform calibration. See “2.5 Calibration for 3D-STORM.”

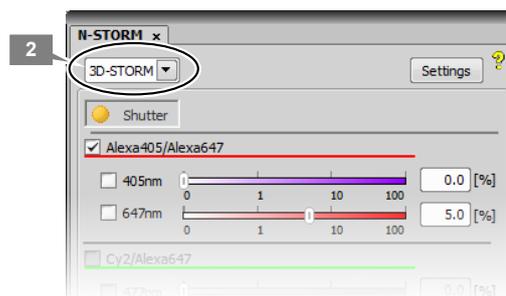
- 1 Click [Settings] in the [N-STORM] control window and then select [Continuous Mode].

After making this setting, click [OK].



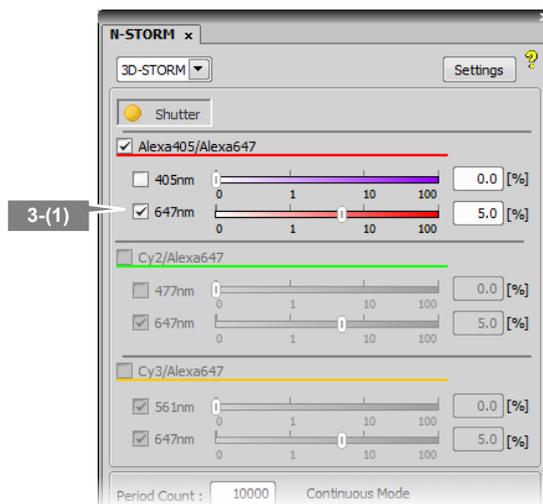
- 2 Select [3D-STORM] from the list at the top left of the [N-STORM] control window.

➔ To acquire a 2D-STORM image, select [2D-STORM].

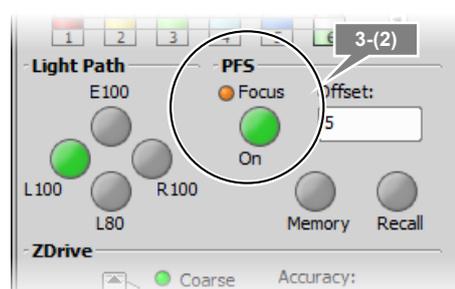


3 Finely and properly adjust the laser focal point of the TIRF illuminator.

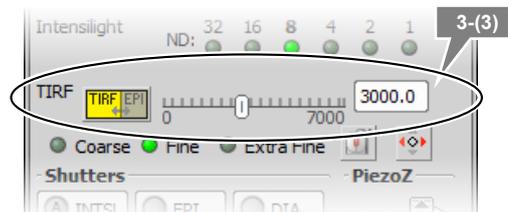
- (1) Select the checkbox for the 647-nm laser.



- (2) Turn on the Perfect Focus System (PFS) in [Ti Pad] and set the focus.

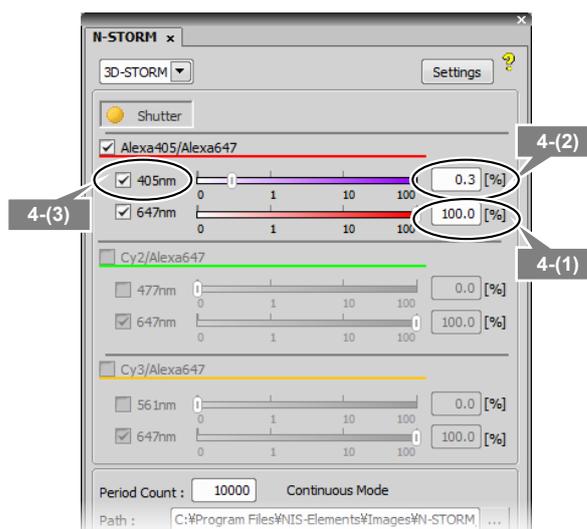


- (3) Finely adjust the laser focal point of the TIRF illuminator so that the observation target is visible.



4 Check activation.

- (1) Make sure that the checkbox for the reporter laser (the figure on the right shows an example when a 647-nm laser is used) is selected, and then set the power to 100% by adjusting the power slider.
- (2) Make sure that the checkbox for the activation laser (the figure on the right shows an example when a 405-nm laser is used) is deselected, and then set the value to about 0.3% by adjusting the power slider.
- (3) Select the checkbox for the activation laser so that it emits laser light for about a second, and then immediately deselect it. Check that the live image becomes bright once and then returns to the previous state as a result of activation causing the fluorescent probe to fluoresce.



✔ If activation cannot be checked

If emission of the activation laser does not make the live image bright, this can be improved by replacing the buffer solution of the specimen with a fresh solution.

5 Configure the N-STORM acquisition settings.

The settings are as follows.

Fluorescent probe checkbox:

Turn on the probe to be used.

Output of the activation laser (the figure on the right shows an example when a 405-nm laser is used):

0.2% to 0.3% (values with which proper activation is performed as shown in step 4 above)

Output of the imaging laser (647 nm):

100%

[Period Count] (number of periods for image acquisition):

Any setting (normally 5000 to 20000 ^{*1})

[Path], [File Name] (folder to which the file is to be saved and the file name):

Any setting

[STORM Image] (whether to display the STORM image during image acquisition):

If this checkbox is selected, the STORM image is previewed during acquisition. Normally, this should be selected.

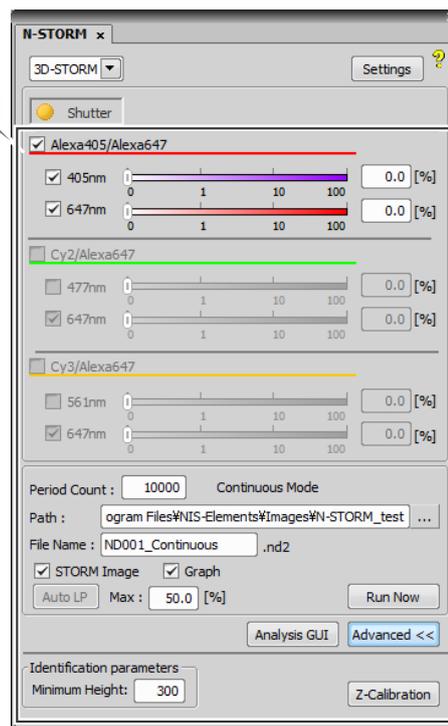
[Graph] (whether to display a graph during image acquisition):

If this checkbox is selected, a graph showing the number of bright points in the image is displayed. Normally, this should be selected.

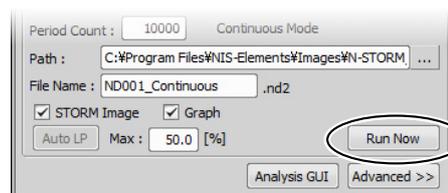
[Minimum Height] (minimum intensity):

300 ^{*2}

- *1 The above number of periods for image acquisition is a reference value. Adjust it according to the structure or coloring status of the specimen. Even when it is set to 20000 periods in advance, if it is confirmed as being large enough on the real time analysis (preview) screen, it is possible to halt the acquisition (for example, about 10000 periods) by clicking [Finish].
- *2 The above-mentioned minimum height is a reference value. Adjust it according to the amount of background light for the specimen, etc.

**6 Click [Run Now].****Disabling large image mode**

If large image mode is enabled with the ND Acquisition function of NIS-Elements, images cannot be acquired. Disable the acquisition of large images before clicking [Run Now].



- 7 Measure the gap between the boundary surface of the cover glass and the position of the observation target (Z-axis direction) using the method below.**

➔ This step is not required for 2D-STORM.

- (1) Step 1:
When the dialog shown on the right is displayed, select the imaging laser (647 nm). Set the focus to the boundary between the cover glass and the specimen by using the PFS offset controller. (Adjust the power to about 50% to 100% by using the slider.)

✔ Set the focus to the boundary surface of the cover glass.

When setting the focus, move the XY stage and view, as boundary marks, the dye molecules that are non-specifically adsorbed onto the boundary surface of the cover glass to identify the boundary surface.

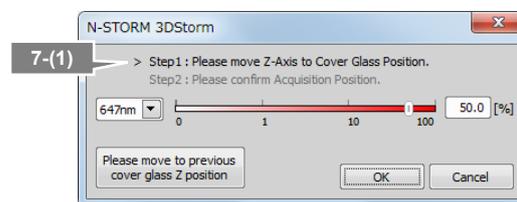
After setting the focus, click [OK]. The XY stage automatically returns to its original position.

- (2) Step 2:
When the dialog shown on the right is displayed, set the focus to the target imaging position by using the PFS offset controller.

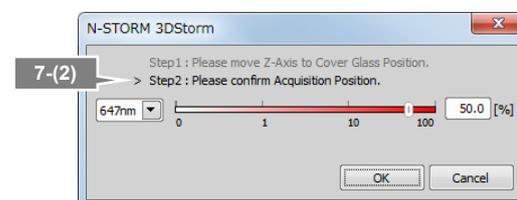
After setting the focus, click [OK]. The measured gap (Z-axis direction) is automatically saved and used in analysis.

When acquisition is started, the dialog box shown on the right is displayed. Do not perform any operation using this dialog box until image acquisition has been completed. Clicking [Events...], [Pause], or [Refocus] prevents the acquisition of appropriate images for STORM analysis.

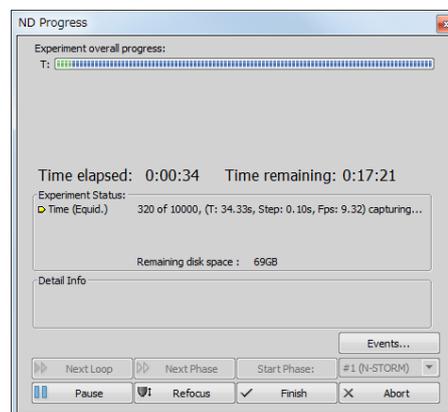
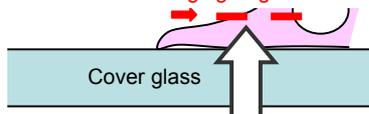
To halt the acquisition, click [Finish] or [Abort]. (If [Finish] is clicked, the acquired images are saved. Be careful because, if [Abort] is clicked, the acquired images will be discarded.)



Boundary surface of cover glass



Imaging target surface

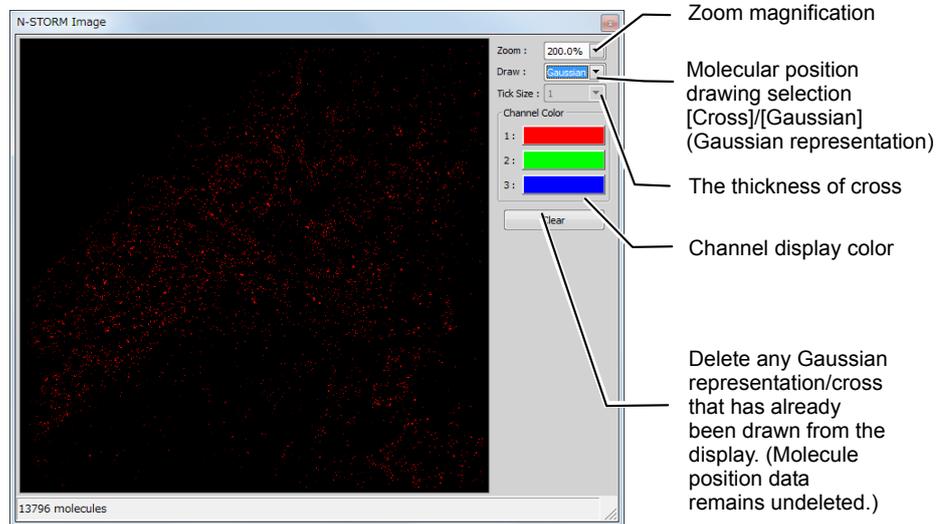


8 Adjust the power of the activation laser, if necessary, during real time analysis (preview) of the acquired images.

Image acquisition is started. The result of a real time analysis (preview) and a graph are displayed.

Recommended number of bright points

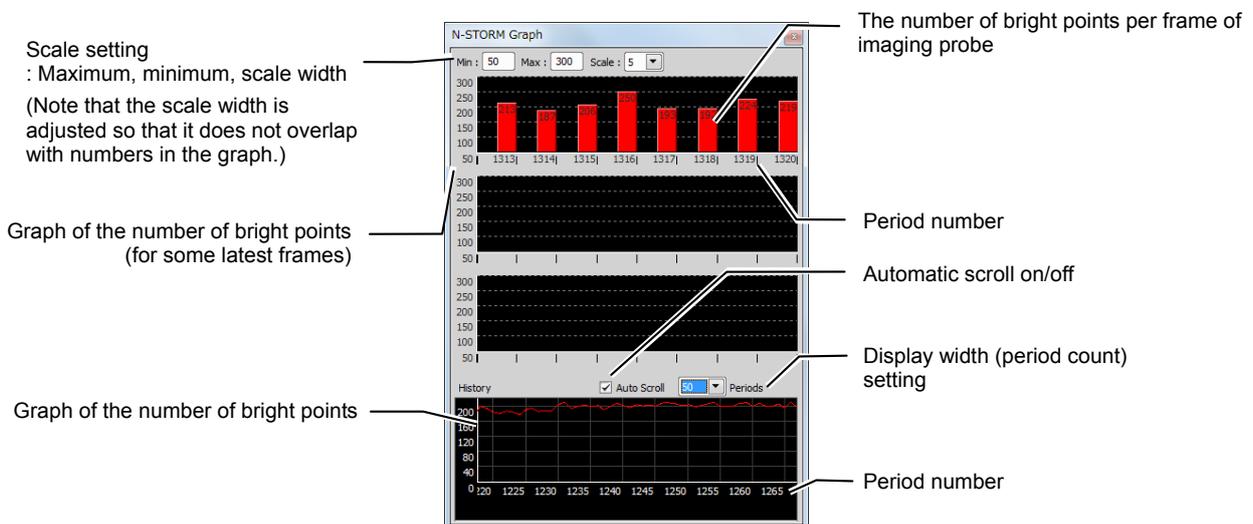
The recommended data for STORM analysis is as follows: the density of bright points is not too low but not so high that they do not overlap each other. For example, a structure that covers the entire screen (such as a microtubule in a cell) should be provided with about 100 to 200 bright points per frame in a visual field with 256 x 256 pixels. The number of bright points to be activated can be adjusted by changing the power of the activation laser.



Real time analysis (preview) screen during image acquisition

Details of real time analysis

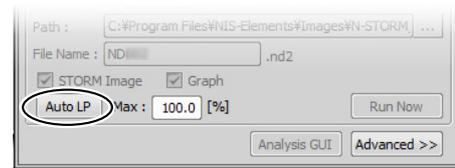
During real time analysis, the value of [Minimum Height] on the N-STORM control window is used as a threshold to analyze bright points. Real time analysis does not include drift correction.



Graphic screen during image acquisition

After the completion of acquisition, click [x] in the windows for real time analysis (preview) and graph to close the windows.

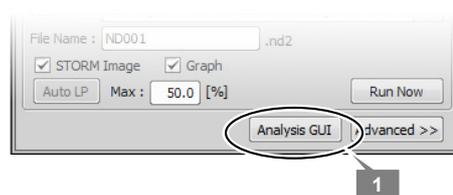
After the power of the activation laser is adjusted, clicking [Auto LP] in the [N-STORM] control window automatically adjusts the laser power so that the number of bright points is maintained. Also, the upper limit on the power for automatic adjustment(%) can be specified in the [Max] box.



2.3 N-STORM Analysis

Analyze an acquired dataset, perform drift correction, and identify the positions of the fluorescent probe molecules.

- 1 Click [Analysis GUI] to display the analysis tool.



- 2 Click  [File Open].

A dialog box for selecting a STORM image is displayed.

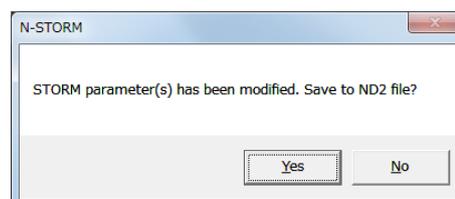
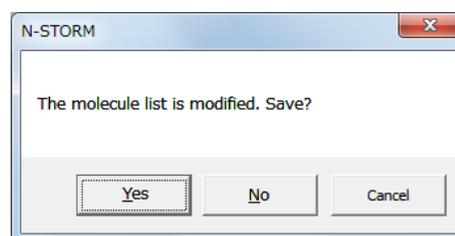


- 3 Select the image file (ND2 file) acquired for STORM analysis and then click [Open].

✔ Saving open data

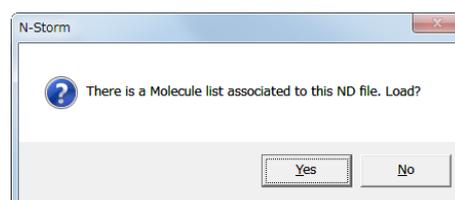
In the N-STORM analysis window, it is not possible to open two or more ND2 files at once. For this reason, if a new file is opened, the currently open file is closed. If, at this time, the file contains any information that is not saved, a dialog box is displayed.

- If the currently open molecule list (STORM image) has not yet been saved, the dialog box shown on the right is displayed. To save the molecule list, select [Yes]. If [No] is selected, the latest molecule list will be discarded.
- If the STORM parameter(s) in the currently open ND2 file have not yet been saved, the dialog box shown on the right is displayed. To save the parameter(s) to the ND2 file, select [Yes]. If [No] is selected, any changes made to the parameter(s) will be discarded.



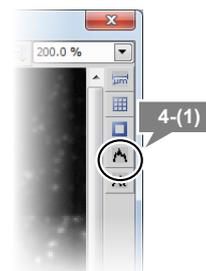
✔ Results of past analysis

If STORM analysis has already been performed on the selected ND2 file such that a corresponding molecule list file exists, a dialog like that shown on the right is displayed. To perform a new STORM analysis, select [No]. (If [Yes] is selected, the saved result of the previous analysis is read.)

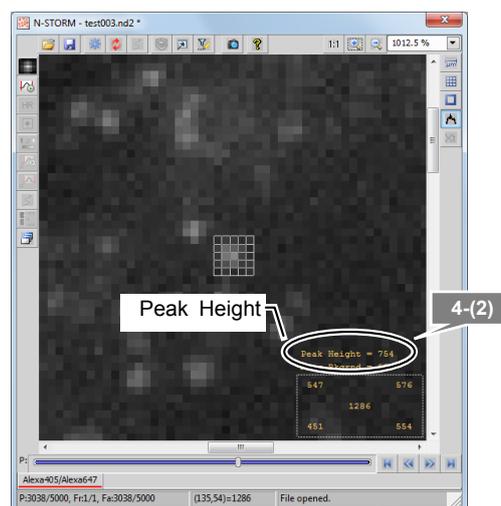


4 Check the minimum intensity of the bright points to be identified as molecules.

- (1) To check the intensity, click  [Use Peak Statistics].



- (2) Select the darkest bright point of all those to be identified as molecules, and then position the mouse pointer to its center. Read and write down the value of [Peak Height] (this value is used as the minimum intensity for identifying bright points in the next step).



5 Configure the settings for identifying bright points.

- (1) Click  [Identification Settings].



- (2) In the dialog box, configure the settings as follows.

[Minimum Height]:

Specify the minimum intensity of the bright points, which was checked in the previous step.

[Maximum Height]: 20000

[CCD Baseline]: 100

[3D]: On

➔ **For 2D**
[3D]: Off

Click [>>] to make the following detailed settings.

[Minimum Width (nm)]: 200

[Maximum Width (nm)]: 700

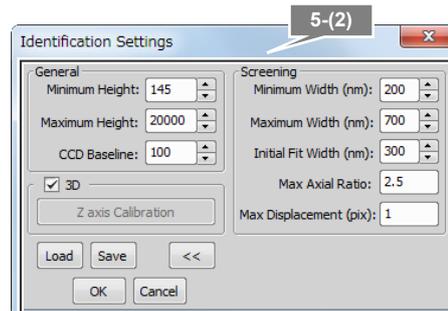
➔ **For 2D**
[Maximum Width (nm)]: 400

[Initial Fit Width (nm)]: 300

[Max Axial Ratio]: 2.5

➔ **For 2D**
[Max Axial Ratio]: 1.3

[Max Displacement (pix)]: 1



✔ Setting Screening

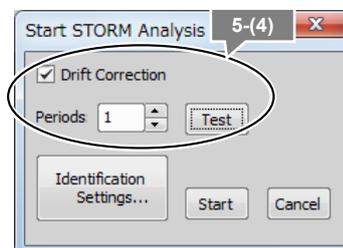
The bright point images that are excluded by Screening of Identification Settings are not displayed on any channel.

Upon the completion of setting, click [OK].

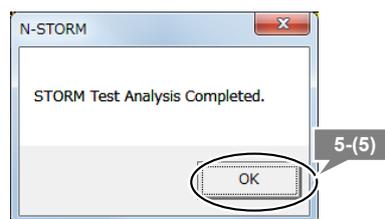
- (3) Click  [Start STORM Analysis].

- (4) Select [Drift Correction], set [Periods] for [1], and then click [Test].

Test analysis is performed for the currently displayed period.



- (5) Upon the completion of the test analysis, the confirmation dialog is displayed. Click [OK].
 Make sure that the bright points are identified correctly.



✔ If the intended result is not achieved

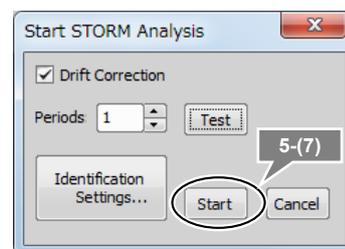
If bright points of noise are detected or if bright points of signals are not detected, reconfigure the parameters for identifying bright points in [Identification Settings] and then perform the test analysis again.

✔ To analyze only some periods

Using the period slider, the analysis target periods can be specified by right-clicking each of the start and end points of the periods to be analyzed and by then selecting [Set as Start Period] and [Set as End Period].

- (6) Click [Start STORM Analysis] again.

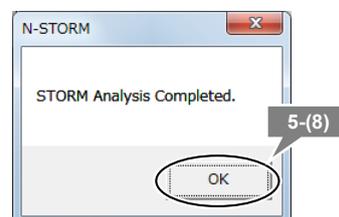
- (7) Click [Start] to start analysis of the entire dataset.
 Analysis of bright points is performed for the entire dataset. Upon the completion of the analysis, the confirmation dialog is displayed.



- (8) Click [OK].
 As soon as the analysis is complete, information on the positions of the molecules is saved in the following two formats.

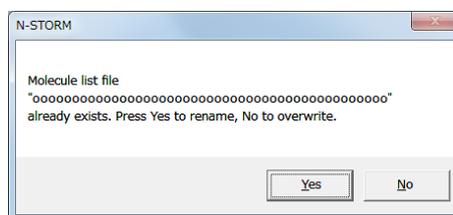
- **Molecule list in binary format**
 File name: (ND2-file-name)_list.bin
- **Drift correction information**
 File name: (ND2-file-name) + _drift.txt

Both the binary file and the text file are saved to the same location as the ND2 file of the dataset.



✔ Overwriting confirmation dialog

If the result of a past analysis of the analyzed ND file exists in the same folder, a dialog like that shown below is displayed.



To save the result of a previous analysis, select [Yes]. The file name for the results of the previous analysis is changed to [(ND2-file-name)_list-(the-current-date-and-time-yyyy-mm-dd-hh-mm-ss).bin]. The results of the latest analysis are saved as [(ND2-file-name)_list.bin].

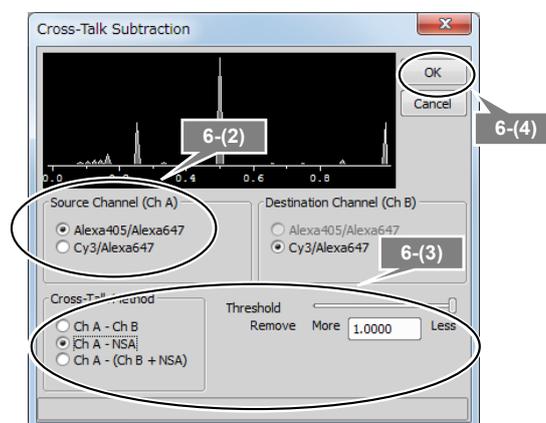
If the result of the previous analysis is unnecessary, select [No]. The result of the latest analysis is saved as [(ND2-file-name)_list.bin], overwriting the result of the previous analysis.

✔ To move or copy files

When moving or copying files for STORM, handle the ND2 file and the binary (bin) and text (txt) files of the analysis result as a group.

6 Subtract crosstalk. (Only in normal mode. The crosstalk subtraction function is not used for images acquired in continuous mode.)

- (1) Click **Xt** [Cross-Talk Subtraction].
- (2) For [Source Channel (Ch A)], select the channel for which crosstalk is to be subtracted.
- (3) Select the method and the threshold for crosstalk subtraction. Use the [Threshold] slider or input a value to specify the threshold.



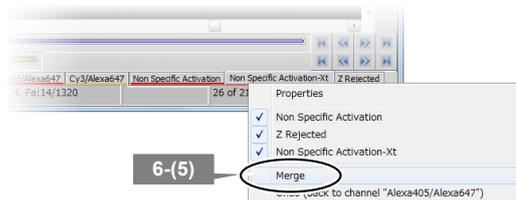
When specifying the method and the threshold for crosstalk subtraction, refer to the table below.

Method of crosstalk subtraction	Recommended uses	Destination of the molecules exceeding the threshold (subtracted molecules)	Recommended threshold (ratio of surrounding molecules)
Ch A – Ch B	If the result of imaging is predicted (Ch A and Ch B are found to be in clearly separate areas). (Can be used for 2-channel operation only.)	Ch B	0.51
Ch A – NSA	If the result of imaging is not predicted.	NSA	0.49
Ch A – (Ch B + NSA)	If the result of imaging is not predicted. (Can be used for 2-channel operation only.)	NSA	0.49

If Ch A – NSA is selected, information on the molecules of Ch B is not used. Molecules that are regarded as NSA by referring to the NSA frames (the second and third imaging frames of Ch A) are removed.

- (4) Click [OK].
The molecules that exceed the threshold are removed from their channel, and a new channel in which they are to be stored is created. The channel is named [destination-channel-name-Xt]. ("Xt" stands for crosstalk.)

- (5) Check the details of the [destination-channel-name-Xt] channel tab. If there is no problem in merging with the destination channel, right-click the tab and then select [Merge].



To cancel the destination, right-click the [destination-channel-name-Xt] channel tab and then select [Undo]. The state existing before crosstalk subtraction is restored.

- (6) If STORM images are acquired on two channels, subtract crosstalk for the other channel in the same way, by following steps (1) to (5).

In this case, perform the steps up to (5) for the first channel, and then return to (1) and perform the same processing for the second channel.

7 Click [File Save] to save the STORM parameters.

The parameters for STORM analysis are saved to the ND2 file.

Type of file used by N-STORM

The file formats used by the N-STORM software are as follows.

File type	File name	Description
ND2 file	(ND2-file-name).nd2	Set of conventional images acquired through NIS-Elements. Parameters for STORM analysis (e.g., Identification Settings, Display Options, Filter Settings) are saved into the ND2 file by selecting the ND2 format in the [File Save] dialog box of the N-STORM analysis window.
Result of STORM analysis in binary format	(ND2-file-name)_list.bin	STORM image (molecule list) created through analysis. This is automatically created by performing N-STORM analysis. It can also be saved by using the [File Save] dialog box in the N-STORM analysis window.
Result of STORM analysis in text format	(Any-file-name).txt	A molecule list can be exported in text format by selecting text format in the [File Save] dialog box of the N-STORM analysis window. This is used to share the results of analysis with other software.
Drift correction data	(ND2-file-name)_drift.txt	This file is automatically created by performing drift correction. It is internally used by the N-STORM software.

Open the STORM image on the main screen of NIS-Elements

The currently displayed image can be captured and opened on the main screen of NIS-Elements by clicking  [Create New Elements Document from Current View].

Set ROI and zoom in on it

ROI can be set by clicking  [Use ROI]. Right-clicking within the specified ROI range displays the ROI range according to the size of the window.

2.4 Terminating the N-STORM System

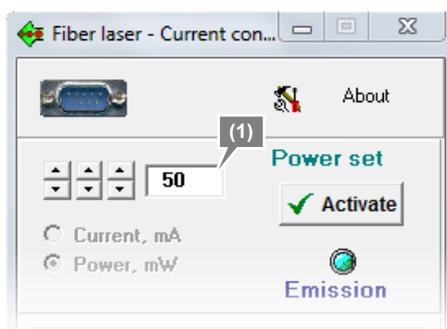
Upon completion of the necessary operation, terminate NIS-Elements AR and turn off the microscope, the laser, and other peripheral devices.

1 Terminate NIS-Elements AR.

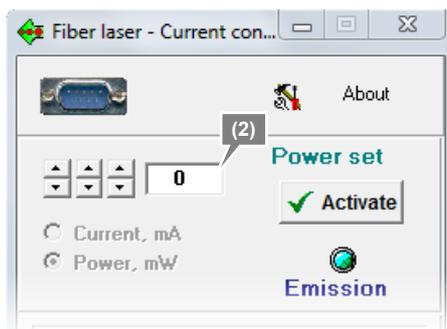
✓ When MPB Communications Inc.'s 647-nm laser is being used

Follow the procedure below to gradually decrease the output of the 647-nm laser through the GUI-VFL software, and then turn the power off (procedure recommended by the laser maker).

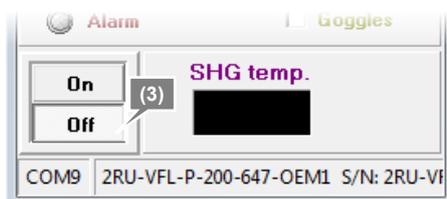
- (1) Set the power to 50 mW, click [Activate], and then wait until the value of [Power, mW] becomes about 50 mW.



- (2) Then, set the power to 0 mW, click [Activate], and then wait until the value of [Power, mW] becomes 0 mW.



- (3) Click [Off] in the Fiber laser window to turn off the 647-nm laser.



- 2 Shut down the PC.
- 3 Turn off each laser head.
- 4 Turn off the microscope.
- 5 Turn off the piezo Z drive.
- 6 Turn off the motorized stage and the illumination light source.

2.5 Calibration for 3D-STORM

To perform 3D-STORM analysis, it is necessary to carry out calibration in advance so that the positions in the Z-axis direction are analyzed correctly. For calibration, a dataset (ND2 file) acquired through a predetermined method is used. This calibration associates the positions in the Z-axis direction with the ellipticity ratio of an unfocused bright point image, allowing the analysis of positions in the Z-axis direction.

1 Make preparations for acquiring 3D-STORM images according to “2.1 Preparation of the N-STORM System.”

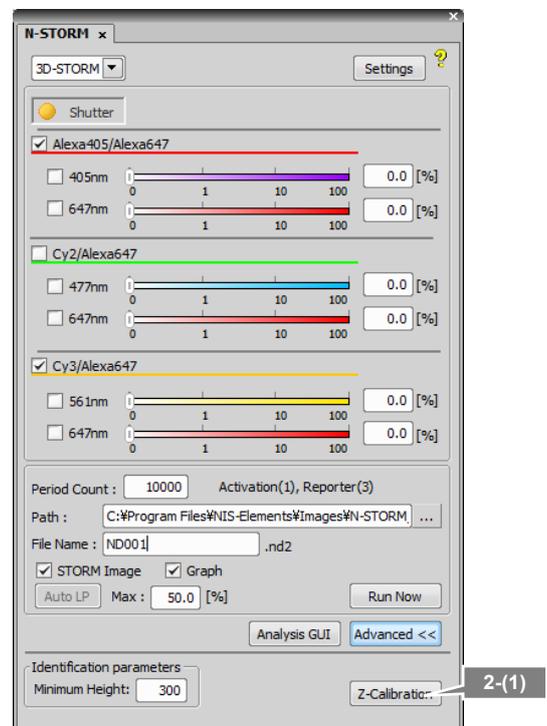
A fluorescent bead specimen is used for calibration.

Recommended fluorescent beads:

TetraSpeck Microspheres, 0.1 μm Fluorescent Blue/Green/Orange/Dark Red T-7279 (Molecular Probes)

2 Acquire images for calibration.

- (1) Click [Z-Calibration] in the [N-STORM] control window. (If [Z-Calibration] is not displayed, it is displayed by clicking [Advanced].)



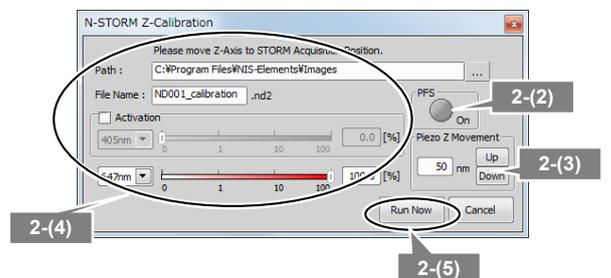
- (2) Turn off the PFS.

Click [PFS] in the [N-STORM Z-Calibration] dialog box to gray out the button.

✓ PFS settings

To perform calibration, it is necessary to turn off the PFS.

- (3) Click [Up] and [Down] in [Piezo Z Movement] to set the focus.



- (4) Configure the N-STORM acquisition settings.

Specify the folder in which the ND2 file is saved, as well as the file name.

Deselect the [Activation] checkbox. (During calibration with fluorescent beads, the activation laser is not used.)

Set the output of the imaging laser (647 nm) to about 10%.

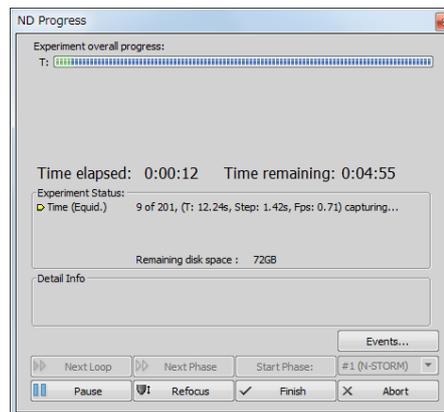
- (5) Click [Run Now] to acquire images.

The dialog box on the right is displayed. Do not perform any operation with this dialog box until image acquisition is completed.

201-frame image acquisition is automatically performed according to the following steps.

- 20 frames at the focal position
- 161 frames from -400 nm to +400 nm with the focal position regarded as being the origin (Z stack)
- 20 frames at the focal position

- (6) When image acquisition is completed, click [Cancel] in the [N-STORM Z-Calibration] window to close the window.



- 3** Click [Analysis GUI] to display the N-STORM analysis window.

- 4** Click  [File Open]

A dialog box for selecting a STORM image is displayed.

- 5** Select the image file (ND2 file) acquired for calibration and then click [Open].

- 6** Make sure that the data is appropriate for calibration.

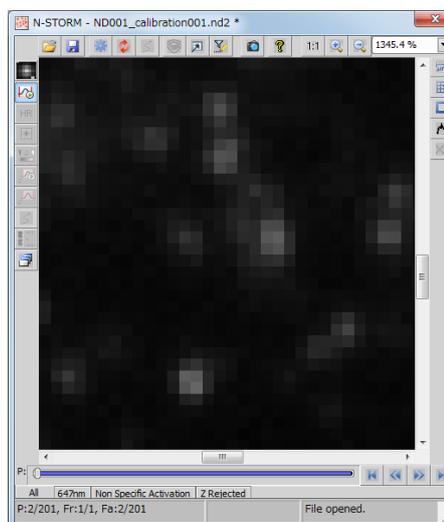
Check that the bright point image is not elliptical but circular in the first and last 20 frames.

✓ Automatically adjusting the contrast

The contrast is automatically adjusted by right-clicking the image and then selecting [Auto Scale for Full Image].

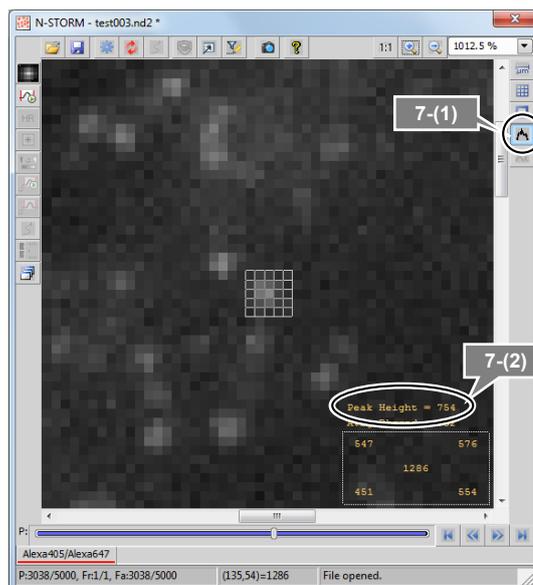
✓ If the bright point image is elliptical in the last 20 frames

If the bright point images are circular in the first 20 frames, but they are elliptical in the last 20 frames, this may be because the specimen position has been shifted. Make sure that the specimen is fixed properly and then acquire images again.



7 Check the minimum intensity of bright points

- (1) Click  [Use Peak Statistics].
- (2) Select the darkest bright point of all those to be identified as molecules, and move the mouse pointer onto its center. Read and write down the value of [Peak Height]. (This value is used as the minimum intensity for the identification of bright points in the next step.)

**8 Configure the settings for the identification of bright points.**

Click  [Identification Settings].

In the dialog box, configure the settings as follows.

[Minimum Height]:

Specify the minimum intensity, checked in (2) of the previous step.

[Maximum Height]: 20000

[CCD Baseline]: 100

Select the [3D] checkbox.

Click [>>] to configure the following detailed settings.

[Minimum Width (nm)]: 200

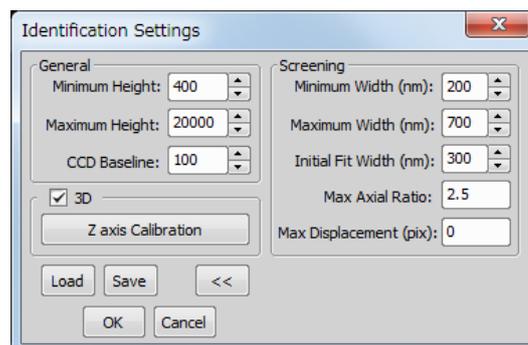
[Maximum Width (nm)]: 700

[Initial Fit Width (nm)]: 300

[Max Axial Ratio]: 2.5

[Max Displacement (pix)]: 0 (This allows bright points recorded in multiple frames to be analyzed as separate molecules, increasing the accuracy of calibration.)

Click [OK].

**Restore the Max Displacement setting**

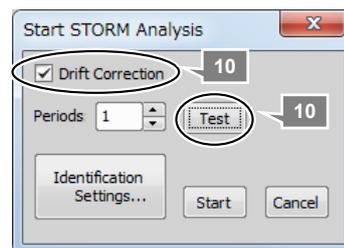
When performing analysis except for calibration, reset [Max Displacement (pix)] to the default value of 1.

9 Click  [Start STORM Analysis].

10 Select [Drift Correction], set [Periods] to [1], and then click [Test].

Test analysis is performed for the currently displayed period.

Upon completion of the analysis, the confirmation dialog is displayed.

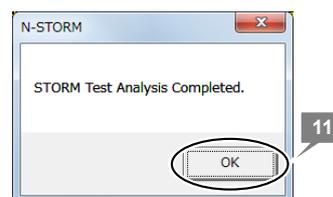


11 Click [OK].

Make sure that the bright points are identified correctly.

✔ If the intended result is not achieved

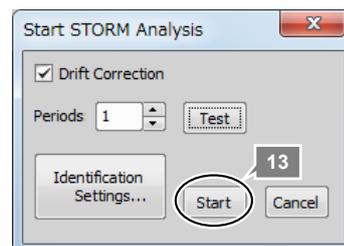
If bright points of noise are detected or if bright points of signals are not detected, reconfigure the parameters for identifying bright points in [Identification Settings] and then perform the test analysis again.



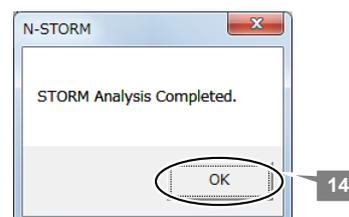
12 Click  [Start STORM Analysis] again.

13 Click [Start] to start analysis.

Analysis of bright points is performed for the entire dataset. Upon completion of the analysis, the confirmation dialog is displayed.



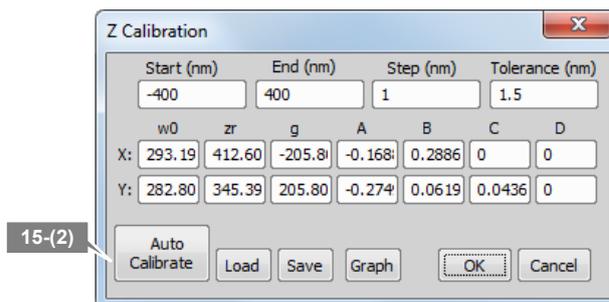
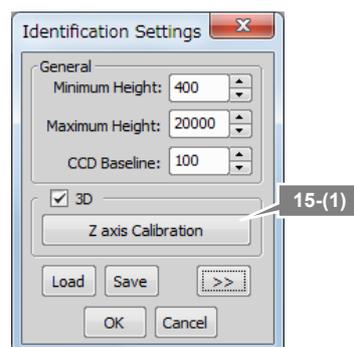
14 Click [OK].



15 Calculate the parameters for calibration and then save them as settings.

- (1) Click  [Identification Settings] to display the dialog box and then click [Z axis Calibration].
- (2) Click [Auto Calibrate].

The calibration results (coefficients of the calibration curve function) obtained through the procedure above are automatically saved as internal settings in the N-STORM software, and subsequently are automatically applied to 3D-STORM analysis.



✓ Check the results of calibration on the graph

Clicking [Graph] in the [Z Calibration] dialog box displays a graph showing the results of calibration. Display the graph of [Wx/Wy vs. Z] if necessary, and check that Wx and Wy intersect nearly at 0 on the horizontal axis and that there is no large gap between the plot data (distributed points) and the fitting data (lines).

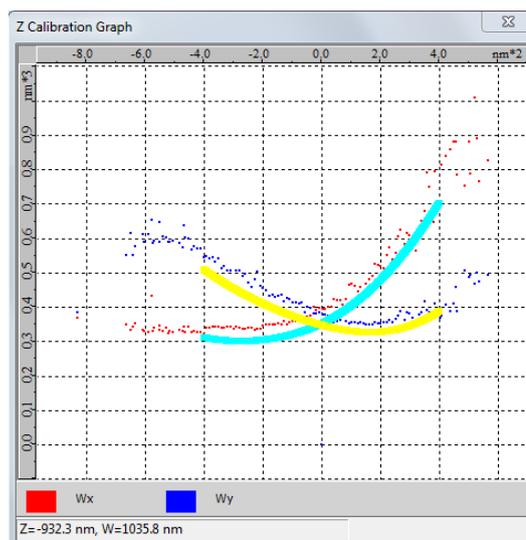
✓ Apply the results of calibration to an existing dataset

To apply the new calibration result to an existing dataset, apply the following procedure.

- (1) Click [Save] in the [Z Calibration] dialog box to save the calibration result as a file.
- (2) Open the existing dataset.
- (3) Display the [Z Calibration] dialog again and then click [Load] to read the calibration result file to be applied. Click [OK] twice to close the dialog box.
- (4) Click  [Start STORM Analysis] to perform analysis again.

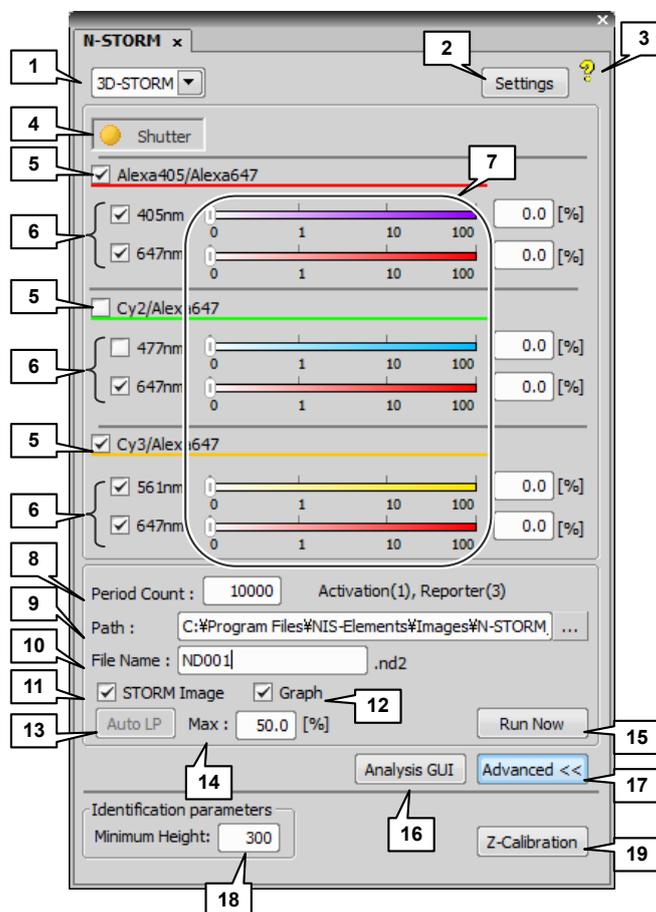
✓ Timing of calibration

When the objective has been switched or the composition of the STORM buffer has been changed, perform calibration again.



3.1 N-STORM Control Window

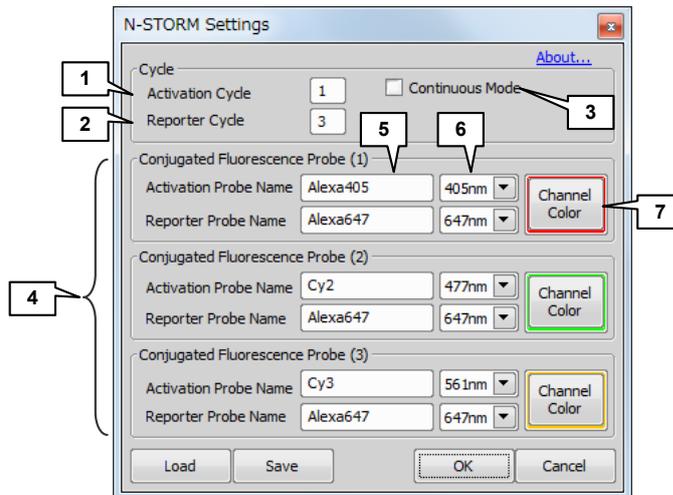
Window that is used for the acquisition of STORM images.



No.	Name	Description
1	2D-STORM/3D-STORM switching button	Switches between the 2D-STORM/3D-STORM modes.
2	[Settings] button	Displays the [N-STORM Settings] dialog box, in which the number of frames for each cycle and the type of fluorescent probe are specified.
3	Help button	Displays help.
4	[Shutter] button	Collectively opens or closes laser shutters.
5	Fluorescent probe checkbox	Selects the fluorescent probe to be used.
6	Laser checkbox	Turns the laser on and off through the AOTF in the laser unit.
7	Laser power slider	Adjusts the laser power through the AOTF in the laser unit.
8	[Period Count]	Specifies the number of periods for the dataset to be acquired.
9	[Path]	Specifies the storage location of the dataset to be acquired.
10	[File Name]	Specifies the file name of the dataset.
11	[STORM Image] checkbox	Selects whether to display the real time analysis (preview) screen during acquisition of the dataset. To display the real time analysis, select this checkbox before clicking [Run Now].

No.	Name	Description
12	[Graph] checkbox	Selects whether to display a screen of graph of the number of bright points during acquisition of the dataset. To display the graph, select this checkbox before clicking [Run Now]. Also, when using Auto LP, select this checkbox.
13	[Auto LP] button	Automatically adjusts the power of the laser so that the number of bright points is constant in the imaging frames. The number of bright points obtained when this button is clicked is regarded as the reference value. (Can be used during image acquisition only.)
14	[Max]	Specifies the maximum of the adjustment range when the laser power is automatically adjusted using the [Auto LP] button.
15	[Run Now] button	Starts image acquisition according to the settings configured in the N-STORM control window.
16	[Analysis GUI] button	Displays the N-STORM analysis window.
17	[Advanced] button	Displays detailed settings.
18	[Minimum Height]	Specifies the threshold for the difference in intensities (between the peak of each bright point and the background) that is used when molecules are identified during real time analysis (preview). If the intensity exceeds the threshold, the relevant bright point is identified as being a molecule.
19	[Z-Calibration] button	Performs calibration for 3D-STORM.

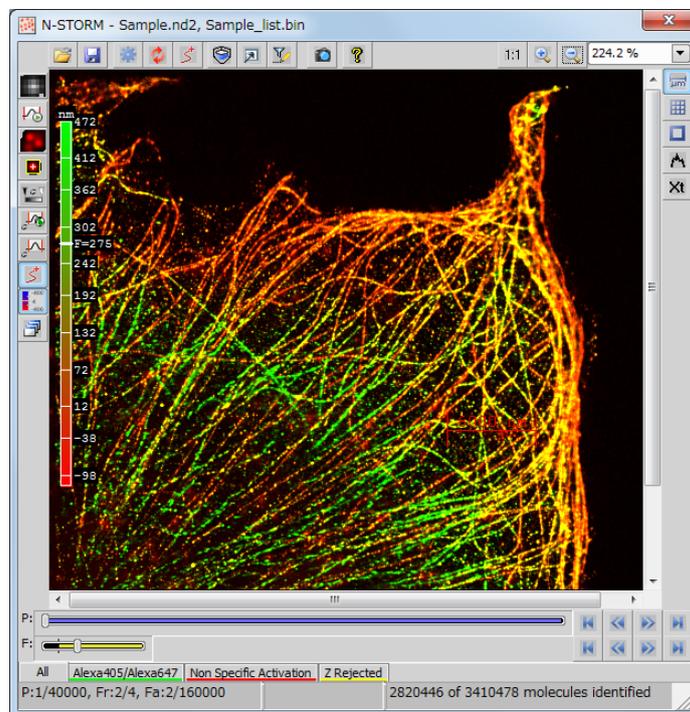
3.2 N-STORM Settings Window



No.	Name	Description
1	[Activation Cycle]	Specifies the number of activation frames for each cycle. (This cannot be specified if continuous mode is used to acquire images.)
2	[Reporter Cycle]	Specifies the number of imaging frames (frames that capture the light emission of the reporter) for each cycle. (This cannot be specified if continuous mode is used to acquire images.)
3	[Continuous Mode]	Selected when continuous mode is used.
4	[Conjugated Fluorescence Probe] area	Specifies the following: the fluorescent probe to be used, the wavelength of the laser to be used, and the display color.
5	[Activation Probe Name]/ [Reporter Probe Name]	Specifies the names of the activation and reporter fluorescent probes for each channel.
6	Laser wavelength	Selects the excitation wavelength to be used.
7	[Channel Color] (channel display color)	Specifies the color to be used for display.

3.3 N-STORM Analysis Window

Window that is used to perform STORM analysis on an acquired dataset.



3.3.1 Tool Bar

Top tool bar

Icon	Name	Description
	[File Open]	Opens a dataset (file extension: nd2) or a binary-format molecule list (file extension: bin).
	[File Save]	Saves an ND2 file and molecule list for STORM with specified names. Data is saved as follows, depending on the type of file selected in the saving dialog. [ND2 files]: ND2 files that include analysis parameters (e.g., Identification Settings, Display Options, Filter Settings) (file extension: nd2) [Molecule List (binary) files]: Binary-format molecule list (STORM image) (file extension: bin) [Molecule List (text) files]: Text-format molecule list (file extension: txt) Note: An ND2 file only contains parameters for STORM analysis and the results of analysis (STORM image) are not stored in the file. The results of analysis are saved into a binary file as a molecule list.
	[Identification Settings]	Specifies the intensity and the size of a bright point image for STORM analysis. Also click this button when switching between 2D and 3D for analysis or when performing calibration for 3D-STORM.

Icon	Name	Description
	[Start STORM Analysis]	Starts STORM analysis and drift correction.
	[Apply Drift Correction]	Only performs drift correction.
	[Display 3D STORM Image]	Displays a 3D image (3D-STORM only).
	[Display Options]	Determines how to display an image.
	[Filter Settings]	Sets the conditions for displaying only some molecules.
	[Create New Elements Document from Current View]	Outputs a new NIS-Elements document from the current view. (The state of zoom and the display modes of conventional images and STORM images affect the output image.)
	[Help]	Displays help.
	[1 to 1 Scaling]	Adjusts the zoom level so that one pixel of the image matches one pixel of monitor.
	[Zoom In]	Zooms in on the display of the image.
	[Zoom Out]	Zooms out on the display of the image.

Left tool bar

Icon	Name	Description
 	[Show conventional low resolution image]	Selects whether to display a conventional image.
	[Auto Scale conventional low resolution image]	Selects whether to enable or disable auto scale (auto adjustment) of the intensity for displaying a conventional image. (If auto scale is enabled while ROI is being used, it is adjusted according to the ROI range.)
   	[Switch STORM high resolution image display mode]	Determines how to display a STORM image ([None] (Not displayed)/[Cross]/[Gaussian] (Gaussian representation)/[Both])
	[Mark molecules identified in current frame]	Marks, with a yellow square, a molecule identified in the current frame of the conventional image.
	[Adjust Gaussian rendering parameters]	Displays a dialog in which settings are configured for the Gaussian representation. Adjusts the Gaussian representation according to the intensity and size of the bright point images.
	[Continuous automatic Gaussian rendering parameters adjusting mode]	Set this button to on to automatically adjust the Gaussian representation according to the current display range.
	[Single shot automatic Gaussian rendering parameters adjustment]	Clicking this button automatically adjusts the Gaussian representation according to the current display range (the ROI range if ROI is used).
	[Drift corrected coordinates display]	Applies drift correction to the displayed image (only available for STORM images on which drift correction is performed).

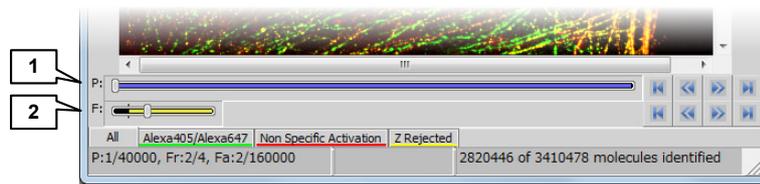
Icon	Name	Description
	[Height map]	Performs color-coding on a STORM image according to the position in the Z direction (3D-STORM only).
	[Batch STORM Analysis]	Continuously performs multiple rounds of STORM analysis. Used to automatically perform multiple rounds of analysis at night.

Right tool bar

Icon	Name	Description
	[Scale Bar]	Displays the scale. Right-clicking displays the property setting dialog.
	[Grid (Graticules)]	Shows a grid for approximate measurement. Right-clicking displays the property setting dialog.
	[Use ROI]	Displays ROI. The ROI affects the auto-scale display of the intensity and histogram, among others.
	[Use Peak Statistics]	Displays the peak detection tool.
	[Cross-Talk Subtraction]	Displays the dialog box to be used for crosstalk subtraction.

3.3.2 Slider

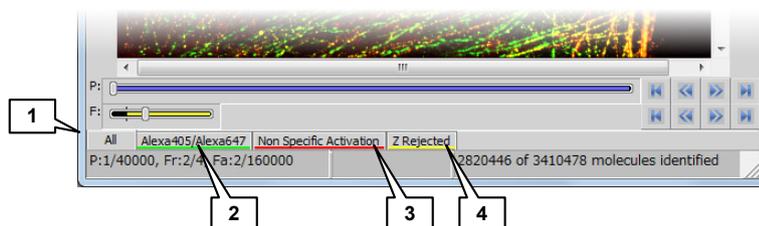
A slider is used to move between the frames of conventional images.



No.	Name	Description	
1	[P] (period)	Moves to a frame that is placed in the same ordinal position as the current position in any of the adjoining periods. (Moves to the third frame of the previous or next period if the third frame is currently displayed.)	
	Right-click Period Slider	[Set As Start Period]	Specifies the start point of the range for STORM analysis.
		[Set As End Period]	Specifies the end point of the range for STORM analysis.
		[Select Entire Range]	Considers the entire dataset to be in the range for STORM analysis.
2	[F] (frame)	Moves between frames. If the [All] channel tab is selected, clicking in the first frame (or in the last frame) of a cycle allows you to move to the last (or first) frame of the adjoining period. Cannot move between channels. If a channel tab other than [All] is selected, clicking in the first frame (or in the last frame) of a period allows you to move to the adjoining frame regardless of the channel.	

3.3.3 Channel Tab

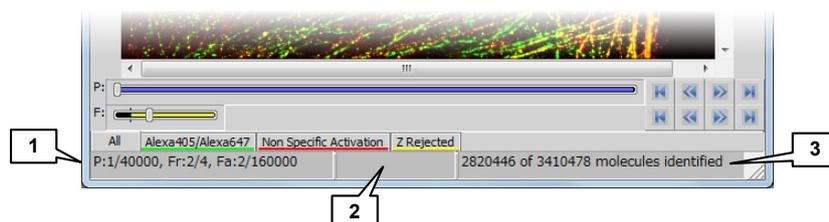
A channel tab is used to change the display of multiple channels included in a dataset. Right-clicking a channel tab allows you to set the property of the relevant channel. It is also possible to select whether to display the [Non Specific Activation] channel and the [Z Rejected] channel.



No.	Name	Description
1	[All]	Displays all the molecules for every channel. If the tabs of the [Non Specific Activation] and [Z Rejected] channels are displayed, the molecules in these channels are also included in the [All] tab.
2	Fluorescent-probe-specific channel tab	If an image is acquired on multiple channels, the channel-specific tab is displayed.
3	[Non Specific Activation]	In a cycle, molecules that are detected not in the first imaging frame but in the second or subsequent imaging frame are classified in a non-specific activation (NSA) channel. Information on non-specific activation channels is used for crosstalk subtraction.
4	[Z Rejected]	As a result of 3D-STORM analysis, molecules that are plotted outside the calibration range in the Z-axis direction, and molecules whose ratio of X- and Y-axis direction image widths exceeds the Max Axial Ratio of the Identification Settings are classified in the Z Rejected channel. (For 3D-STORM only)

3.3.4 Status Bar

Left side



No.	Description
1	<p>(Normal)</p> <p>[P: current-period/number-of-all-periods-for-the-dataset, Fr: current-frame/number-of-all-frames-for-the-current-cycle, Fa: current-frame/number-of-all-frames-for-the-dataset]</p> <p>If the current frame is an activation frame, [(A)] is displayed.</p> <p><input checked="" type="checkbox"/> Cycle</p> <p>A cycle is a set of frames for one channel that are included in one period.</p> <p>(If a filter by Z Stepping is used)</p> <p>[Z slice: (displayed-Z-range) nm]</p>
2	<p>[(X-coordinates, Y-coordinates) = intensity]</p> <p>The position of the mouse pointer on a conventional image (the top-left and bottom-right of the screen are "1, 1" and "256, 256", respectively) and the intensity.</p>
3	<p>(During analysis)</p> <p>The numbers of analyzed frames and identified molecules are displayed.</p> <p>(After completion of analysis)</p> <p>[x=xxx nm, y=xxx nm]</p> <p>Once STORM analysis is completed, the position of the mouse pointer is displayed in nanometers. Also, during the measurement of distance, the interval between two points is displayed as follows: [dx= xxx nm, dy= xxx nm, d= xxx nm] dx and dy indicate the interval between two points on their respective axes, while d indicates the slant distance.</p> <p>(If the mouse pointer is outside the image)</p> <p>[(the-number-of-currently-displayed-molecules) of (the-number-of-all-analyzed-molecules) molecules identified]</p> <p>The number of currently displayed molecules is displayed.</p>

Image context menu (right-click on the image)

Menu Name	Description
[Auto Scale for Full Image]	Automatically adjusts the display intensity of conventional images based on the entire image.
[Auto Scale for ROI]	Automatically adjusts the display intensity of conventional images based on the part selected as ROI.
[Zoom to ROI]	Zooms in so that the entire ROI is displayed.
[Properties]	Displays the image properties (Experiment Data, Recorded Data, Molecule Statistics).

En **Symbol for separate collection applicable in European countries**
 This symbol indicates that this product is to be collected separately. The following apply only to users in European countries.
 • This product is designated for separate collection at an appropriate collection point. Do not dispose of as household waste.
 • For more information, contact the retailer or the local authorities in charge of waste management.



No **Symbol for kildesortering i europeiske land**
 Dette symbolet indikerer at produktet skal kildesorteres.
 De nedenstående punktene gælder for alle europæiske brugere.
 • Dette produktet skal kildesorteres og inleveres til dedikerede innsamlingspunkter. Må ikke kastes med normalt husholdningsaffald.
 • For mer informasjon, ta kontakt med din forhandler eller lokale myndigheter.



De **Symbol für getrennte Wertstoff-/Schadstoffsammlung in europäischen Ländern**
 Dieses Symbol zeigt an, dass dieses Produkt separat entsorgt werden muss.
 Folgendes gilt für Verbraucher in europäischen Ländern:
 • Dieses Produkt darf nur separat an einer geeigneten Sammelstelle entsorgt werden. Eine Entsorgung im Hausmüll ist unzulässig.
 • Wenn Sie sich für nähere Informationen bitte an Ihren Händler oder die örtlich für Abfallentsorgung zuständigen Behörden.



Se **Symbol för separat upphämtning i europeiska länder**
 Den här symbolen anger att produkten måste hämtas separat.
 Följande gäller bara användare i europeiska länder.
 • Den här produkten är avsedd för separat upphämtning vid ett lämpligt uppsamlingsställe. Produkten får inte kastas i hushållsaffall.
 • För mer information, kontakta återförsäljaren eller de lokala myndigheter som ansvarar för avfallsantering.



Fr **Symbole pour la collecte sélective applicable aux pays européens**
 Ce symbole indique que ce produit doit être collecté séparément. Les mesures suivantes concernent uniquement les utilisateurs européens.
 • Ce produit doit être jeté séparément dans un point de collecte approprié. Ne jetez pas ce produit dans une poubelle réservée aux ordures ménagères.
 • Pour plus d'information, contactez le détaillant ou les autorités locales responsables de la gestion des ordures.



Fi **Erillisen keräyksen merkki Euroopan maissa**
 Tämä merkki osoittaa, että tuote kerätään erikseen.
 Seuraavat maininnat koskevat vain eurooppalaisia käyttäjiä.
 • Tämä tuote kerätään erikseen asianmukaista keräyspisteistä. Älä hävittä tuotetta talousjätteen mukana.
 • Lisätietoja saat jällemyyntijäliltä tai paikallisista jätehuoltoviranomaisilta.



Es **Símbolo para recogida separada aplicable en países Europeos**
 Este símbolo indica que este producto se recogerá por separado. Lo siguiente sólo se aplica en países Europeos.
 • Este producto ha sido designado para ser recogido en un punto de almacenamiento apropiado. No lo tire como un deshecho doméstico.
 • Para más información, contacte con el vendedor o autoridades locales al cargo de la gestión de residuos.



Ru **Символ сортировки мусора, использующийся в европейских странах**
 Данный символ означает, что этот продукт должен утилизироваться отдельно от других.
 Приведённые ниже информация касается только пользователей из стран Европы.
 • Данный продукт должен утилизироваться отдельно от других в соответствующих приемных пунктах. Не выбрасывайте данный продукт вместе с бытовым мусором.
 • Дополнительную информацию Вы можете получить у продавца или у местных властей, отвечающих за утилизацию мусора.



Dk **Symbol for special bortskaffelse af denne type produkter i de europæiske lande**
 Dette symbol angiver, at dette produkt skal bortskaffes specielt.
 Det efterfølgende er kun til forbrugere i de europæiske lande.
 • Dette produkt skal bortskaffes på fx en genbrugsplads af lign. Det må ikke smides væk som normalt husholdningsaffald.
 • For yderligere information kontakt din forhandler eller de lokale myndigheder, som fx teknisk forvaltning.



Gr **Σύμβολο για την ξεχωριστή αποκομιδή απορριμμάτων στις Ευρωπαϊκές χώρες**
 Αυτό το σύμβολο υποδηλώνει ότι η αποκομιδή αυτού του προϊόντος πρέπει να γίνει ξεχωριστά.
 Τα κάτωθι απευθύνονται μόνο σε Ευρωπαϊκούς χρήστες.
 • Αυτό το προϊόν είναι σχεδιασμένο έτσι ώστε να γίνεται η αποκομιδή του σε ειδικά σημεία. Μην το πετάτε μαζί με τα υπόλοιπα απορρίμματα.
 • Για περισσότερες πληροφορίες, επικοινωνήστε με τον διανομέα του προϊόντος ή με τις υπεύθυνες τοπικές αρχές για θέματα διαχείρισης απορριμμάτων.



Nl **Symbol voor gescheiden inzameling zoals dat wordt gebruikt in Europese landen**
 Dit symbool betekent dat dit product apart moet worden ingezameld. Het volgende is alleen van toepassing op gebruikers in Europa.
 • Dit product dient gescheiden ingezameld te worden op een daartoe bestemd inzamelpunt. Niet wegwerpen bij het normale huishuis.
 • Neem voor meer informatie contact op met het verkooppunt, of met de lokale instantie die verantwoordelijk is voor het verwerken van afval.



Pl **Symbol oznaczający segregowanie odpadów, stosowany w krajach Europy**
 Ten symbol oznacza, że produkt musi być wyrzucany oddzielnie. Poniższe uwagi mają zastosowanie tylko dla użytkowników z Europy.
 • Ten produkt jest przeznaczony do oddzielnej utylizacji i powinien być dostarczony do odpowiedniego punktu zbierającego odpady. Nie należy go wyrzucać z odpadami gospodarstwa domowego.
 • Aby uzyskać więcej informacji, należy skontaktować się z przedstawicielem przedsiębiorstwa lub lokalnymi władzami odpowiedzialnymi za zarządzanie odpadami.



Pt **Símbolo para recolha de resíduos em separado utilizado nos países Europeus**
 Este símbolo indica que este produto é para ser recolhido separadamente.
 Esta norma aplica-se só para os utilizadores nos países Europeus.
 • Este produto está designado para recolha de resíduos em separado num recipiente apropriado. Não deitar no caixote do lixo doméstico.
 • Para mais informações, contactar o revendedor ou as autoridades locais responsáveis pela gestão dos resíduos.



Hu **Európai országokban érvényes "Elkülönített hulladékgyűjtés" jelzése**
 Ez a jelzés azt jelenti, hogy ez a termék elkülönítve kell gyűjtene.
 Az alábbiak csak az európai országokban élő felhasználókra érvényes.
 • Ezt a terméket a megfelelő hulladékgyűjtőhelyen, elkülönítve kell gyűjtene. Ne dobja ki háztartási hulladékként.
 • További információért forduljon a forgalmazóhoz, vagy a helyi hatóság hulladékgyűjtését felelőséhez.



It **Simbolo per la raccolta differenziata applicabile nei paesi europei**
 Questo simbolo indica che il prodotto va smaltito separatamente.
 La normativa che segue si applica soltanto agli utenti dei paesi europei.
 • Il prodotto è designato per lo smaltimento separato negli appositi punti di raccolta. Non gettare insieme ai rifiuti domestici.
 • Per maggiori informazioni, consultare il rivenditore o gli enti locali incaricati della gestione dei rifiuti.



Cz **Symbol pro oddělený sběr odpadu platný v evropských zemích**
 Tento symbol znamená, že tento produkt se má oddělně oddělně. Následující pokyny platí pro uživatele z evropských zemí.
 • Tento produkt se má oddělně na místě sběru k tomuto účelu určeném. Neadhazujte spolu s domácím odpadem.
 • Více informací o způsobu zacházení s nebezpečným odpadem vám poskytne příslušná místní instituce.




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 このマークは、中国のお客様に向けたもので、電子情報製品分野における環境保護を目的としています。

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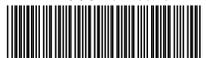
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