From Eye to Insight

LIGHTNING
Manual

20190919_EN

Depending on the licese, available method and wizard are different.

	Image optimization during image acquisition	Configure settings for image optimization (After image acquisition)
Lightning Process	×	○
Lightning Expert	○	○
Lightning	○	×

＊LAS X Small is not available for Lightning

＊Call up Seq. and Apply are not available for Lightning wizard.

So, Call up Seq. or Apply in "TCS SP8", then go to Lightning wizard.

Lightning wizard

The wizard uses the Lightning or Lightning Expert license

1. Click **TCS SP8** in the menu bar at the top left.

2. Select **Lightning** entry.

   The Lightning wizard starts with **Acquire** step.

The Lightning wizard contains 5 steps.
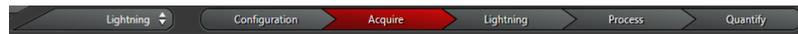
1. You can choose between 4 scan modes in the **Acquisition Mode** dialog of the Lightning wizard.

   xyz, **xyzt**, **Mark and Find**, **Tilescan**

2. In the Lightning wizard, sequential acquisition is enable by default. The **Between Frames** mode is preset as the scan mode in the Sequential Scan dialog. You can also acquire image using the **Between Stacks** or **Between Lines** mode. In the **Pinhole** are of the XY dialog, the average defined wavelength for the respective sequence is automatically applied as the **Emission** λ **[nm]** emission wavelength.
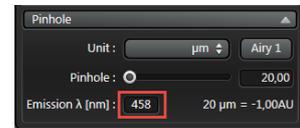
3. You have 2 options for configuring image acquisition parameter setting:

A. Coupling **Speed** and **Resolution** in the **Lightning Grade** dialog

   1. Make sure that coupling is enabled in the **Lightning Grade** dialog. The chain has to highlighted in red. This is enable by default.

   2. Drag the slider to the correct position to define whether the image is to be acquired at maximum speed (**Speed** direction) or with maximum resolution (**Resolution** direction)

Note: You can define the individual value ranges or the parameters in the Configuration steps, See Page 10, "Lightning Configuration"

B. Without coupling **Speed** and **Resolution** in the XY dialog:

   1. Click the chain to disable coupling in the **Lightning Grade** dialog.

   2. Configure your instrument parameter setting in the XY dialog.

4. Enter the required data for **Mounting Medium** and **Refractive Index**, and define the image optimization **strategy**

Adaptive	On the basis of the SNR, the setting for optimum image are determined automatically
Global	This strategy is recommended as default for all image
LowSignalToNoise	This strategy is optimized for high noisy image.

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5. There are 2 buttons for live mode at the lower edge of the screen:

Live	Enable normal live mode
Fast Live	Enable fast live mode, 512x512 pixel、600Hz、Bidirectional

6. If you execute the image acquisition by clicking **Start**, a data record in the original (ex. Series002) and data record for the optimized images with the _Lng ending (ex. Series002_Lng) are created.
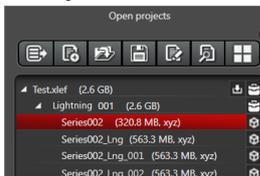
Lightning Process

The **Lightning Process** and **Lightning Expert** license gives you the option to optimize previously acquired images in the LASX outside of the lightning wizard.

This is done in the Process step:

Lightning Process	Process menu
Lightning Expert	
Lightning	You can change Strategy or Refractive index of mounting medium
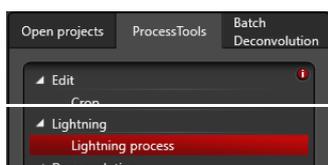
In the tab **Process / Lightning**, select the tool **Lightning process**. (Lightning Process license)

Select **Lightning** menu (Lightning Process license)

At the bottom of the Process display window, a dialog appears the Lightning setting, and you can adjust setting here.
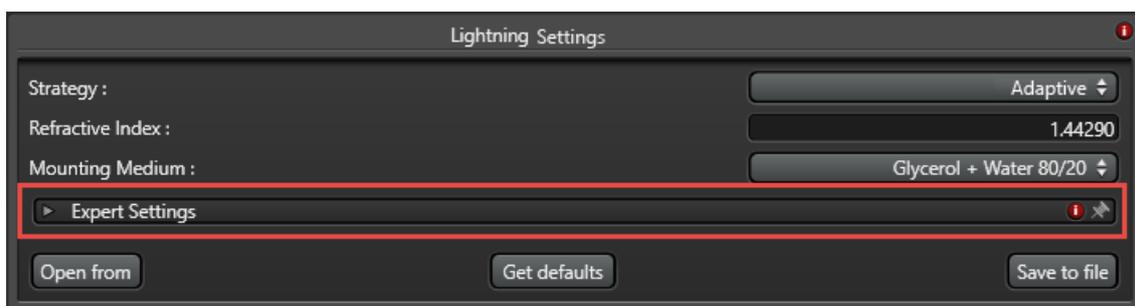
Strategy: Select a strategy for which application the automatic image optimization is to be executed.

Adaptive	On the basis of the SNR, the setting for optimum image are determined automatically (default)
Global	This strategy is recommended as default for all image
LowSignalToNoise	This strategy is optimized for high noisy image.

If necessary, adapt the Lightning setting, for this purpose, see next page "Lightning Expert Setting".

Expert Setting: Opens the dialog the deconvolution parameter, see next page "Lightning Expert Setting"

Open from	Opens a file browser for selecting the file with stored setting
Get defaults	Reset the setting to the default values.
Save to file	Opens a file browser for selecting the storage location and designation of the file.

Click **Apply** to start the image optimization.

Expert Settings

Apply to all channels	Apply parameters to all channel
Type	Automatically selected from Confocal, STED and Multi-photon
Number of iterations	**Lightning** is based on the iterative algorithm. In general, the following applies: The higher number of iterations, the better the results. Large numbers of iteration, however, tend to lead to more artifacts, which means that there is always an optimal number of iteration. In the **Lightning** automatic mode, the optimal number of iteration is determined so that good deconvolution quality is ensured while also keeping artifact to a minimum. You can define the number of iterations yourself.

Contrast Enhancement	The contrast of the measurement data is increased before the deconvolution.　You can adjust the contrast with the slider (0 - 1)
Cut off (%)	Before deconvolution, voxel values below a certain threshold value set to 0.　You can adjust the threshold value with the slider (0 - 1).
Regularization Method	Regularization reduces the effect of noise.　These are 2 methods, both of which presume good smoothness in the original image in the definition smoothness: **Total Variation** and **Good' Roughness**.
Regularization Parameter	This enables you to set the degree of regularization.　The lower the value, the more severely the noise is reduced.
Optimization	These are used to optimize the processing noisy images. These are 5 optimization level available (**None**, **Low**, **Medium**, **High**, **VeryHigh**) for smoothing the initial image. If an image is very noisy, it is advisable to set the optimization to High (default setting).
Post-Filter	Select a smoothing filter for the image after image optimization. None, Bilateral (Edge-preserving filter), Gaussian (Gaussian filter)

Excitation Wavelength (nm)		Wavelength of the excitation laser.	
Emission Wavelength (nm)		Detected wavelength which lie at a distance of 15% from the left edge of the detection range	
pinhole (AU)		The width of the pinhole.　This is usually configured to 1 AU.	
Normalization		Select how the signal value are scaled from 2 methods	
	Range	Scaled in a 16-bit range. The signals remain available, since they are standardization by sum total of intensity.	
	Photon Count	Scaled in terms of the detected number of photons that survive.　Hence, the result is quantifiable in regards to the detected photons.	
Objective		Adapt the following preset values individually	

		for your experiments.
	Numerical Aperture	Numerical Aperture NA
	Immersion Reflective Index	Refractive index of the immersion medium for the objective
	Magnification	Objective magnification
	Z-Offset (μm)	z-position during the start of the acquisition relative to the coverglass.
	Objective Design Parameters	The value of the objective characteristic and the coverglass are preset here and cannot be changed
		Immersion Reflective Index Design	Refractive index of the immersion medium for the objective
		Cover Slip Thickness Design	Thickness of the coverglass
		Cover Slip Reflective Index Design	Refractive index of the coverglass for the objective
Cover Slip		
	Refractive Index	Refractive index of the utilized immersion medium for the objective
	Thickness (μm)	Thickness of the coverglass
Mounting Medium		
	Mounting Medium	Select the utilized mounting medium from the dropdown menu here
	Refractive index	Refractive index of the mounting medium
Saving and Loading Setting		You can save the setting made and reload them for additional experiments. To do so, the following button are available
	Open from file	Opens a file browser for selecting the file the stored settings.
	Save to file	Opens a file browser for selecting the location and designation of the file

＊Batch Processing

Select all raw data by pressing the Control key while making your selection, then Apply.

STED

If you selected the **STED** method under the type **STED Setting,** the following STED parameters are displayed.
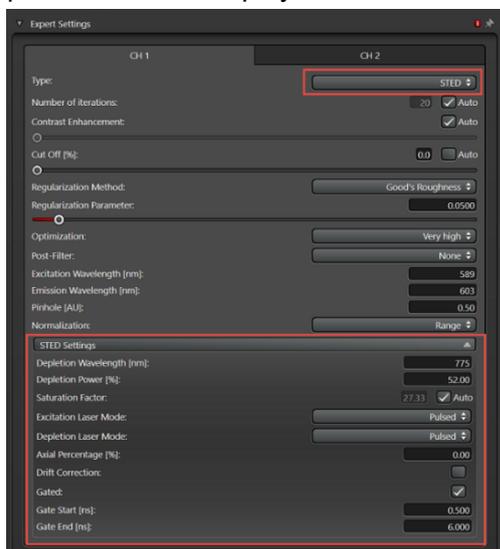
Depletion Wavelength [nm]	Depletion wavelength
Depletion Power [%]	Intensity of the depletion laser
Saturation Factor	The saturation factor indicated how intensely the fluorescence is suppressed by the depletion laser. If the **Auto** check box for the automatic configuration is disable, you can adjust the saturation factor in the input fields.
Excitation Laser Mode	Dropdown menu for selecting of the laser mode for excitation: Pulsed (**Pulsed**) or continuous (**CW**)
Depletion Laser Mode	Dropdown menu for selecting of the laser mode for excitation: Pulsed (**Pulsed**) or continuous (**CW**)
Axial Percentage [%]	Indicates the ratio of the intensities of the axial and lateral STED laser (0: only lateral; 1: only axial)
Drift Correction	In the event of cell movements, this compensates a linear drift in the xy-direction for the image capture of z-stacks.
Gated	When the function is enabled, the detection is carried out within a defined time gate. Only the photon that reach the detector during this time gate are detected.
Gate Start [ns]	Start of the time gate
Gate End [ns]	End of the time gate

Multi-Photon

If you selected the **Multi-Photon** method under the type of **Multi-Photon Settings**, the following multi-photon parameters are displayed.
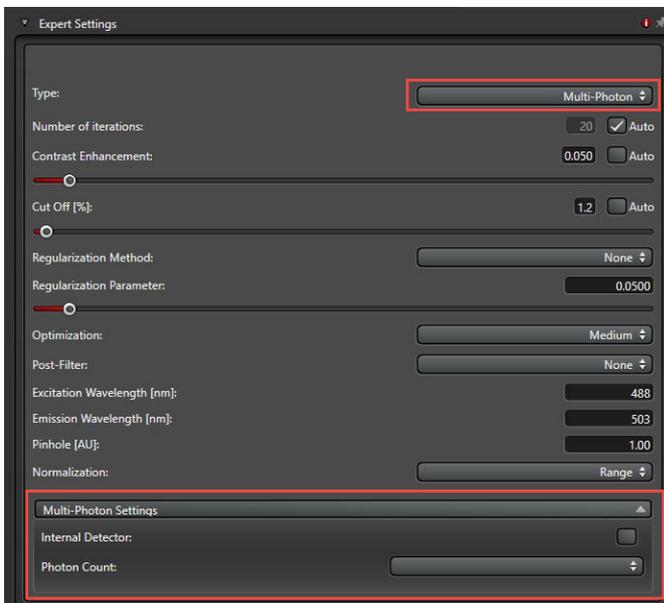
Internal Detector	You can set with which detectors the multiphoton system should work. When the function is activated, the detection performed with internal detection (descanned detection), when deacvated, with external detection (non-descanned detection).
Photon Count	You select whether the excitation should be done with 2 or 3 photons.

Lightning Configuration

In this dialog, you can configure the specific value ranges for the parameters of the **Lightning Grade** slider.

Dragging the grab points on the sliders change the limit values.   Alternatively, you can enter that fill within the specified value ranges into the input fields or change them by clicking the arrow keys.

Pinhole AU	Image resolution in the xy-direction. The value specified here correspond to a multiplication factor that is applied to the parameters configured in the **Acquire** step for image resolution. You can use this function to acquire images with the maximum conductive resolution for image optimization.
XY Oversampling	Image resolution in the xy-direction. The value specified here correspond to a multiplication factor that is applied to the parameters configured in the **Acquire** step for image resolution. You can use this function to acquire images with the maximum conductive resolution for image optimization.
Z Oversampling	Image resolution in the z-direction. The value specified here correspond to a multiplication factor that is applied to the parameters configured in the **Acquire** step for image resolution. You can use this function to acquire images with the maximum conductive resolution for image optimization.
Line Average / Accumulation	In PMT or HyD detectors with **Standard** method, the slider in the **Lighting Grade** dialog affects the **Line Average** function. In HyD detector with Counting method, the slider affects the **Line Accumulation** function, which is more suitable for counting photons.
Speed	You can select the scan speed for image acquisition

Tips

＊Optimization： There are used to optimize the processing of noisy images. Six optimization levels are available for smoothing the initial imaging. If an image is very noisy, it is advisable to set the optimization High or Very High.

For image with high S/N no regularization is necessary. For image with low S/N, no or low regularization can cause noise to interpret as signal and appear as an artifact in the image.

High S/N　None > Very Low > Low > Normal > High > Very High　Low S/N

Optimization:	Medium ⬍

(④) (②)

Result of LIGHTNING at High S/N. Intensity distribution at High S/N before (①) and after LIGHTNING(②). Result of LIGHTNING at Low S/N. Intensity distribution at Low S/N before(③) and after LIGHTNING(④). W/O regularization (Blue), w/ regularization (Orange).
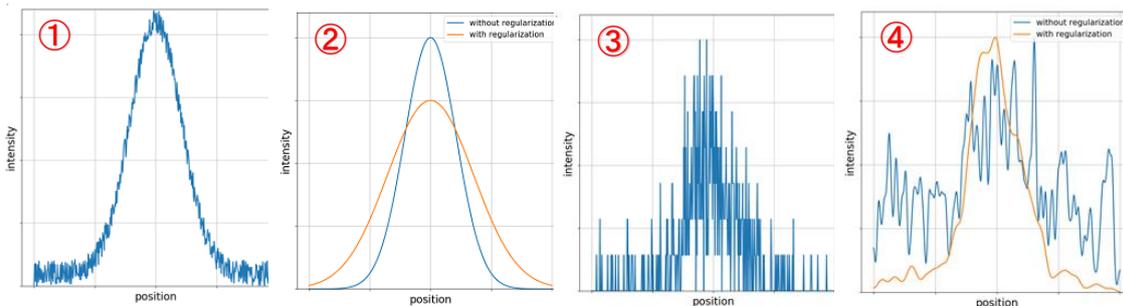
＊Iteration: The higher number of iterations, the better the results. Large number of iteration, however, tend to lead to more artifacts. You can identify the iteration number from Properties.

Number of iterations:	20 ✓ Auto
Regularization Parameter:	0.0500

＊Cut Off: Before LIGHTNING, the set threshold value is subtracted from all voxels. This improved the result of the deconvolutions.

Contrast Enhancement:	0.050 ☐ Auto
Cut Off [%]:	1.2 ☐ Auto
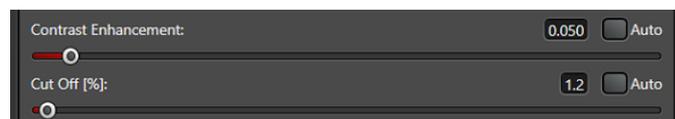
＊Batch process:
Select files while pressing CTRL key and Apply LIGHTNING.

＊ Reflactive index of mount media

Mounting Medium	Refractive Index
Water	1,333
100% PBS	1,335
Glycergel®	1,38
50% Vectashield ® + 50% PBS	1,39
50% PBS + 50 % Glycerol	1,406
80% Glycerol + 20 % Water	1,451
100% Vectashield ®	1,452
Mowiol®	1,46
ProLong® Gold / Diamond	1,47
Kaiser's Glycerol Jelly	1,47
Fructose (80,2%)	1,49
DPX	1,525
CFM-3	1,52
ProLong® Glass	1,52
TDE	1,33 – 1,52