Living up to Life



STELLARIS 5 WLL LAS X Basic 20211124_EN

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I. STELLARIS description and technical specifications

STELLARIS is a point scanning confocal microscope with a super resolution LIGHTNING.

Scan mode

FOV (Field Of View) Scanner

A FOV scanner is an assembly of galvanometric scanning mirrors used in single-point confocal microscope that offer the correct optical recording of a large field sizes. The galvanometric scanners are controllable in speed and position, allowing zoom and pan functions as well as tuning the scan frequency. Maximum format is 8192 x 8192 pixels.

Resonant Scanner * Optional

A resonant scanner is a type of galvanometric mirror scanner that allows fast image acquisition with single point scanning microscopes. High acquisition speed is required to track fast processes, especially in living samples. It has the additional benefit of providing a better fluorescence signal and reduced photobleaching. It works at a constant scan speed that you cannot change (8 kHz or 12 kHz).

You can switch the FOV and Resonant scanner during a software session.

Prism - based tunable multiband spectral detection

The spectral detector splits light into up to 5 spectral bands. The bands are independent and continuously tunable within the full visible spectrum. The light in each band I s detected by a HyD (Ref.: p23, 24). The arrangement of prism and mirror-slit cascaded detectors is virtually polarization-independent and highly transparent. Therefore. is features a very high photon efficiency which is needed to collect as much as possible of the fluorescence photons by applying illumination with as low as intensity as possible.



II. Start up



* Equipment appearance changes depend on the specification

1. Turn ON the PC

*Automatically log in to "LAS X User"

- 2. Turn ON the main power
- 3. Turn ON the laser power
- 4. Turn clockwise the laser key switch

5. Turn ON the Hg Lamp for eyepiece (If required)

LED: Default setting is ON, skip this step

Hg Lamp: Turn On the power switch, if required.

*When turn ON Hg lamp again, you need to wait for cooling it It takes 15- 20 min.

*Shutter switch default setting is "Remote".



6. Launch the desktop app "LAS X" 🚺



7. Bellow window will be displayed.

Select "machine.xlhw" for the Configuration and "DMi8" for the Microscope. (The "simulator.xlhw" is used to view and analyze image and not acquisition.) Click OK to start the initialization of the LAS X

	<u>Leica</u>
Leica Application Suite Advanced Fluorescence	MICROSYSTEMS
Configuration :	machine.xlhw 🗢
Microscope :	DMi8 🗢
Copyright 1997 - 2012 Leica Microsystems CMS Gr	nbH OK Cancel

9. Turning ON the Lasers

Configuration Click on the "Configuration" tab and the "Laser Config" to open the main laser control window



Turn ON the laser(s) with the appropriate laser line(s) that will excite the fluorophores you are using



*Do Never change WLL %

*OPSL is single wavelength CW laser

*WLL is wavelength tunable pulse laser

*Equipment appearance changes depend on the specification

Laser Wavelength (nm)	Fluorescent dye/protein		
405	Blue Ex. (DAPI, Hoechst etc.)		
WLL: 485-685	Green -Infrared Ex.		
	Green Ex. FITC, Alexa488, Cy2, GFP etc.		
	Yellow Ex. YFP etc.		
	Red Ex. TRITC, Alexa555, Cy3, RFP, DsRed, mCherry etc.		
	Infrared Ex. Alexa633, Cy5 etc.		
OPSL 448	Cyan Ex. CFP, Lucifer Yellow etc.		
488	Green Ex. FITC, Alexa488, Cy2, GFP etc.		
514	Yellow Ex. YFP etc.		
561	Red Ex. TRITC, Alexa555, Cy3, RFP, DsRed, mCherry etc.		
638	Infrared Ex. Alexa633, Cy5 etc.		

III. Acquire Image

Configuration	Acquire	Process	Quantify	Analysis A
Configure various hardware and software component	Configure the settings for image acquisition	Provide various tools for image editting	Provides various tools for quantitative image evaluation	2D image analyses and image processing



All images and image series are collected and shown in a directory structure.

 $\mathbf{5}$

sop acquisition.

project directory.

Start O

Navigator: The acquisition region is marked by a white box.

series and tile scan if there are defined. The images are stored in project directory. During acquisition, the marking of the button change to "Stop". You can click the button to

: Starts the experiment including z-stacks, time

III-1. The Beam Path Setting

There are all buttons and options that you will require for configuring and carrying out your experiments. You can select and set the fluorescent dyes, the lasers, the system component along the beam path and the detectors for image acquisition.



*Notch Filter Lines (Optional) attenuate the laser reflection.

*Creating a Transmitted Light Image (Ref. page 29)

*Control Panel

You can configure detector gain, laser power, Zoom, Rotation, Pinhole size and Z position (Ref. page 18 -).

III-2. Objective Selection

Single click the objective name field for display the objective selection. A list of all available objective opens. Select the objective from the list. The list close again, and the objective automatically changed.



*Equipment appearance changes depend on the specification

III-3. Configure Beam Path Settings

III-3-1. Setup a New Experiment

To set up a new experiment with the aid of the **Dye Assistant**, proceed as follows:

1. Click the Open "Dye Assistant" button (a) in the middle area of the beam path settings.





2. Click the "..." button (b. A list box of the emission spectra stored in the system opens.

3. Select a fluorescent dye. The selected fluorescent dye is listed on the left side. Another line in which you can select an additional fluorescent dye appears below the selected fluorescent dye.

As soon as you have selected two fluorescent dyes, various configuration options for the acquisition of the specimen used are suggested in the lower area of the dialog, and the detection range overlap is displayed graphically.



The displayed color bars for "Yield" illustrate the yield of the individual fluorescent dyes.

"Crosstalk" illustrates the intensity of the crosstalk into other channels.

4. Follow the same procedure for all other fluorescent dyes contained in your specimen.

5. Depending on the system configuration, a specific detector type or acquisition mode can be assigned to each fluorescent dye. To do so, click the selection field and select the desired detector type or "Selecting acquisition modes for experiment configuration" (Ref. page 23, 24) from the dropdown menu (see Right).

ALEXA 488 ALEXA 594	HyD ‡ HyD X HyD X HyD S Tau Modes FLIM STED HONDO	
	STED HyD NDD	

It is possible that individual configurations are not available. Example: If two fluorescent dyes in use are assigned to a hybrid x-detector but the system is only equipped with one hybrid x-detector, this necessarily results in a configuration in stack-by-stack or frame-by-frame sequential mode (Ref. page 13).

6. The system assigns a color look-up table to each dye automatically during the selection of multiple fluorescent dyes. The suggested colors do not follow the spectral properties of the dye but are intended to achieve a high contrast so that the fluorescent dyes are highly distinct in overlay images.



Click the box next to the detector selection in

order to select a different color look-up table from a list as necessary.

7. If one of the suggested configurations appeals to you, click the corresponding "Apply" button. The Dye Assistant is closed, and the configuration is incorporated into the beam path settings.



8. Select one of the suggested instrument configurations for your application and click the corresponding "Edit" button if subsequent optimization is still required. An additional editing window opens.



9. Adjust the settings for laser line $\,\,\mathbb{C}\,$ or

detection range $\, \mathbb{G} \,$ as necessary.

Depending on the instrument configuration, the conventional laser lines available are displayed as short gray lines that the excitation laser line locks onto.

A gray bar is displayed for instrument configurations with a white light laser; the laser line can be moved to any point on this bar.

You can change the size and position of the detection ranges @.

10. Apply the adjustments:

Apply: Adjusts the hardware for image acquisition and closes the Dye Assistant.

OK: Applies the settings to the main window of the Dye Assistant.

III-3-2. Selecting Fluorescent Dye with Drag and Drop

The fluorescent dyes are dragged and dropped into the settings. You can use the fluorescent dyes from your favorites bar or from the overall list.



Click the desired fluorescent dye and drag it (a) to the desired setting while holding down the left mouse button. While dragging the fluorescent dye, the available detectors are displayed and highlighted when you hover the mouse over them. You can drop the fluorescent dye over a detector of your choice. If you do not select a certain detector and drop the fluorescent dye into the "empty" space above the detectors, the software ensures the allocation to the most suitable detector.

As soon as you let go of the mouse button, the spectral properties of the selected fluorescent dye are displayed as a curve (b). Although the emission spectrum is displayed by default, you can also have the excitation spectrum or both displayed, see Beam Path Settings: Displaying the Excitation/Emission Spectrum.

The software analyzes the spectral properties of the fluorescent dye and automatically suggests a suitable excitation laser line and a detector with a corresponding spectral range (c).

In the Yield/Crosstalk field, the colored bars give you immediate information about the strength of the optical crosstalk to other channels and the intensity yield of the individual fluorescent dyes.

III-3-3. Assigning Fluorescent Dyes to a Detector

If you want to assign a fluorescent dye to a detector manually:

1. Click the desired detector to call up its configuration area.

2. Select the desired fluorescent dye from the drop down menu.

The corresponding emission curve is loaded. Adjust the excitation line and the detection range as necessary.

III-3-4. Assigning Laser to a Setting

1. You can insert the laser lines using the Add Laser button. Click the button, hold the left mouse button down and drag into the desired setting onto the excitation range.

2. Set the detector and detection range (Ref. next page, Setting Detector and Detection Range,

page)	•		\$ ◆ 🛩 ⊿	S	*	Add Laser
				Setting 1		
		HyD S 1	HyD X 2	HyD S 3	HyD X 4	HyD R 5
		450	500 550	600 650	700 750	800 850
		HyD S 1				HyD R 5
				Trans PMT - Off		

III-3-5. Deleting Fluorescent Dyes

Click the curve of the fluorescent dye you want to delete and then click the recycle bin icon or use the Delete key on your keyboard.

Alternatively, you can click the fluorescent dye, hold the left mouse button and drag the fluorescent dye out of the setting.

The fluorescent dye is deleted from the setting, the associated detector is switched off, and the Yield/Crosstalk display is updated accordingly.

III-3-6. Adjusting the Excitation Wavelength

1. Click the excitation laser line (@) that the software has suggested on the basis of the spectral properties of the dyes. The excitation line is displayed as active and in white. Below the setting, the configuration area expands. The shutter has been opened already **ON**.



2. Set the desired laser intensity (AOTF setting) by pulling on the Intensity slider (\oplus). Alternatively, you can also enter the intensity directly. Click the displayed value and change it in the input window.

You can adjust the power also using the mouse wheel or the control panel.

You can accordingly configure the control panel in the USB Control Panel dialog. If the parameter Smart Channel Intensity has been assigned to the control panel, the corresponding excitation line is detected automatically as soon as you select an image in the viewer.

III-3-7. Setting Detector and Detection Range

 Click the detector (①) that the software has suggested based on the spectral properties of the dyes.
 The detector is displayed as active and in white. Below the setting, the configuration area expands. The



detector has been activated already (ON).

2. Adjust the detection range as necessary. The detection range can be shifted using the mouse. You can also change the size of the detection range by dragging one of its ends or entering exact values in the entry window (double-click on the detection range).

Alternatively, you can double-click the detection range to open an entry window in which you can enter values directly.

3. Set the detector sensitivity by pulling at the **Gain** slider (\oplus). The default setting is 100%. Alternatively, you can also enter the sensitivity directly. Click the displayed value and change it in the input window. The value can also be set from the control panel.

III-3-8. Save and load settings



Save setting

You can save the settings for image acquisition for later experiments with the same or a similar specimen. The complete sequential configuration you have created is saved. This can consist of individual setting or a sequence of multiple settings.

1. Click the **L** button. The file browser is opened.

2. Enter a file name and confirm with "Save" to store the setting.

Load setting

If you have saved setting or sequence at an earlier time, you can load these to work under reproducible acquisition conditions.

- 1. Click the button. The file browser is opened.
- 2. Select the desired setting and click "OK".

III-3-9. Configure Pseudo color look-up table

In the configuration for the respective detector, single click the color field for the display of the lookup table. A list of all available look-up tables opens. Select a look-up table from the displayed list by double-clicking. The list close again, and the look-up table is assigned to the detector.

R	Setting 1	
Pseudo co	blor of look-up table	5
HyD S 1 Gain 2.50 🗢		Territoria Territoria Mada
HyD S 1 Gain 2.50 ¢	Excitation Line Dye Yield / Crosstalk Auto (491) ALEXA 438	TauContrast TauModes Operating Mode OFF Intensity * Analog *

III-3-10. Selecting the acquisition mode: Line Sequence, Frame Sequence, Stack Sequence

Sequence		
	CFP	P Ng Add Laser III 11
	Setting 1	→ → → → → → → → → → → → → → → → → → →
(a). Type WIL Supe	Frequency Shutter Intensity 200	٢
Stack Sequence	* * *	Line Sequence
Line Sequence For each row, the system switches from the parameters of one setting to the parameters of next. * The results in virtually simultaneous creation of the frames.	Frame Sequence	The method is particularly advantageous for specimens that change their position (e.g. living cells). *In this mode, the following parameters in the setting can differ: Laser setting, Detector status (ON/OFF) *When configuring the sequence, detectors are not shut off electronically when switching between the individual setting. Therefore, do not select detection range for the detectors that is under the excitation line of another setting.
Frame Sequence The system switches to the next setting after scanning complete image. * Additional application options include scanning a specimen stained multi times with a single detector.	8	First, one image is acquired with the parameters from the setting: then, the parameters from the next setting are configured and the next image is acquired. *In this mode, the following parameters in the setting can differ: Laser intensity, Frame Accumulation, Line/Frame Average, Pinhole and motCorr setting, Detector property (Detection range, Gain, Tau Mode, Operation Mode), Shutter status (ON/OFF)
Stack Sequence The system switches to the next setting after scanning entire image series (z-stack, time series, spectral series). * Additional application options include scanning a specimen stained multi times with a single detector.	٤	 First, the complete image series is acquired with the parameters from the setting: then, the parameters from the next setting are configured and the next image series is acquired. *In this mode, the following parameters in the setting can differ: Laser intensity, Frame Accumulation, Line/Frame Average, Pinhole and motCorr setting, Detector property (Detection range, Gain, Tau Mode, Operation Mode), Shutter status (ON/OFF)

III-3-11. Restore the image acquire settings

You can restore some instrument parameters regarding the selected file.



Open the dialog

You can check all information and instrument parameters regarding the selected file from the context menu (Right mouse button).

tau STED tau STED	Close Project Ctrl+W	De	escription:							
	Save Project Ctrl+S	9	Apply Text							
	Save All Ctrl+Shift+S	Ditte Gar Mail								
	Save as									
				07/04 17:40:01.10					IGROSYSTEM	
	Create Collection		Total Exposures	s: 24 (1 channels	, 24 frames)					
	Delete Del		Dimensions							
	Rename F2		Dimension	Log 512	ical Size	Physics 250.00	il Length	Physical Orig	gin .	
			Y	512		250.00		0.00 µm	0.00 µm 0.00 µm	
	Cut Ctrl+X		z	24		-11.23	m	-0.02 µm		
	Copy Ctrl+C		Channels LUT Resolution Min Bite 8 0.000000			lin Max				
	Export >					0000-000				
	a the same to be a set of the set		Time Stamps:							
	Recalibrate Image		Frame (Show A	e (Show All) Relative Time (s)			Absolute Time (h.m.s.ms) 17:40:01:125		Date 2012/07	
	Properties		24		0.058	17:40:01:103			2012/07	
	Open in new viewer		Confocal Settin	ngs						
	Open file location		Name Boan Mode Scan Direction X			Value xys xys Understimat				
	Apply image settings		Scan Speed Version Number			400 Hz				
	Best Focus		Version Nursee InSTEDActive ZPestion IsBaye/Z			5 0 -11.3 ym 0				
	Close All									
	Close All		Magnification			10				
			ObjectiveName			C PLAN 10.D	0.22 POL			
			Apply Settings						Print	

III-4. Define the parameters and Acquire image III-4-1. Live Scan and Fast Live Scan (Preview scan)



You can configure the setting for a Fast Live mode. The Fast Live mode enables you to toggle quickly back and force between two settings.

Fast Live mode: scan with bellow setting, e.g. fast navigation

Live mode: scan with defined parameters for image acquisition, e.g. define the parameters for acquisition



Move the mouse over the right area of the button. Fast Live to The configuration symbol is displayed.



The Fast Scan Setting dialog opens. Depending on which scanner your system has available (FOV and/or resonant scanner), following parameters can be set.

-O	Settings X
Fast Live (FOV Scanner)	Fast Live Resonant (RS)
Maximal Format :O 256 🗢	Maximal Format :O 256 🗢
Minimal Scan Frequency : 600 3	Minimal Scan Frequency :O 8000 🗢
Overview Zoom : O	Overview Zoom : O 1.25
Bi-Directional	Bi-Directional
No Average/Accumulation	No Average/Accumulation
No Dynamic Signal Enhancement	No Dynamic Signal Enhancement
Active Sequence Only (Between Lines Mode)	Active Sequence Only (Between Lines Mode)

* Active Sequence Only(Between Lines Mode): When the function is activated, Fast Scan Live mode is carried out only for the sequence currently in progress (default setting).

III-4-2. Control Panel

By clocking the "Control Panel configuration" button, will display control panel dialog. Default setting is bellow:



①Smart Gain:Configure selected channel Gain

②Smart Intensity: Configure selected channel laser intensity

③Smart Wavelength: Configure selected channel laser wavelength (Only WLL)

(4) Pinhole : Configure selected sequence Pinhole size

(5) **Zoom**: Configure Zoom rate

6Z position : Configure the Z position

z-Galvo: Configure super Z stage (Galvano stage)

z-Wide: Configure motorized stage (inverted microscope: objective revolver, Upright microscope: specimen stage)

Difference of z-Galvo and z-Wide (Ref. page 27)

III-4-3. Optimizing image contrast

By clicking the "Quick Look-up table" **W** button, make it possible to change the look-up table quickly.

 $\mathsf{Pseudo}\ \mathsf{Color}\ {\rightarrow}\mathsf{Grow}\ \mathsf{Over}/\mathsf{Under}\ {\rightarrow}\mathsf{Gray}\ {\rightarrow}\mathsf{Pseudo}\ \mathsf{Color}$

Grow Over/Under: Highlight grayscale values the top edge of the look-up table in "Blue" and grayscale values the low edge of the look-up table in "Green".

*In default setting "Blue" indicate saturation and "Green" indicate No signal.



III-4-4. Acquire image or image series

Buttons for Image Acquisition

Live O Fast Live O

Preview scan (Ref. page 15)

*The image is not stored

Capture Image O:: Acquires a frame of the selected channel of the current stage position. The images are stored in project directory.

Navigator: The acquisition region is marked by a white box.

Start O

Example: Starts the experiment including z-stacks, time series and tile scan if there are defined. The images are stored in project directory. During acquisition, the marking of the button change to "Stop". You can click the button to sop acquisition.

III-5. Configuration of Image Settings

III-5-1. Configure image format, scan speed, zoom



④Format : Number of pixels in the image Default setting is 512x512 Pixels. By selecting the preset format from the pulldown menu, the format was applied. By clicking the ⊕, you can set the desired format.

②Speed: Scan Speed(Hz)

Default setting is 400 Hz. By selecting the preset scan speed from the pulldown menu, the scan speed was applied. By clicking the ⊕, you can set the desired scan speed.

* The zoom factor change if the scan speed is high than 601 Hz (The zoom factor is depend on the scan speed)

* The slower scan speed, the higher S/N but easier bleach.

* Resonant scanner works at a constant scan speed that you cannot change (8 kHz or 12 kHz).

③Bidirectional X : <2x faster acquisition (need to adjust phase)</p>

The default scan mode is unidirectional where the beam scan in the X-direction, is blanked on return and again scans in the X-direction for the set number of lines. bidirectional scanning patters do not blank the beam while returning. This may reduce scan time, but may introduce some scan artifacts that must be corrected by "Phase".





④Zoom: Adjust optical Zoom factor

Adjust zoom factor various ways by following:

-Adjusting slider, -Entering specific zoom factor, -Activating "Zoom In" and -drawing ROI in image, -Zoom knob on Control panel (Ref. p16)

* The zoom factor change if the scan speed is high than 601 Hz (The zoom factor is depend on the scan speed)

(b)Line/Frame Average/Accumulation
Averaging and Accumulation remove noise
In general, less averaging required with a lower gain setting for the detectors.
Frame Average/Accumulation: The specific setting is valid for currently selected setting. When checkbox is enable, the setting is taken over for all settings in a sequence.

©Rotation : Rotate image if necessary (Optional)

You can use the image field rotation to rotate the display $+/-100^{\circ}$ in the viewer during the live image acquisition.

Rotation knob on control panel (Ref. p16)

Functions of adjusting the image display are arranged on the left edge of the viewer Generally, the selection of a suitable look-up table for a specific use depends on the user's own judgement and needs.

Auto Scale щ 29 % The look up table automatically adapted to the gray scale values of the acquisition. This makes it possible to extend the The configured value is dynamic range and, to work with entire shown in an entry window bandwidth of the color depth. Automatic below and above the scaling is applied to all channels, for zlook-up table and can be stacks, to the entire series. also entered here directly. Above and bellow the color control, the maximum measurable photon count/pixels are displayed can be detected the configured bit depth

▼ XY: 512x512	400 Hz 1.00 1.00 AU 🛛 🕕 🖈
Format :	512 x 512 + O
Speed :	400 ¢ 🕥
Bidirectional X :	OFF
Zoom Factor :	01.00
Zoom In :	OFF
Image Size :	184.52 µm * 184.52 µm
Pixel Size :	3 361.1 nm * 361.1 nm
Optical Section :	1.068 µm 😌
Pixel Dwell Time :	1,44 µs Frame Rate: 0,593/s
Line Average :	
Line Accu :	
Frame Average :	
Frame Accu :	
Rotation :	
Pinhole (4	
Dynamic Signal En	hancement (5) 🔺
Enable :	ON
Frames : 💻 🗨	11 🗢
Weight : 🗕 🗨	0.40 🗢

① XY format optimization

Select optimize xy format to set the optimal number of pixels correctly over sample (Ref. page 22).

* Resolution depends on NA of objective and pinhole size.

②Switch the function FOV Scanner and Resonant Scanner

Gray: FOV Scanner

Red: Resonant Scanner

* Resonant scanner works at a constant scan speed that you cannot change (8 kHz or 12 kHz).
* For resonant scanner, you can not use HyD S analog mode.

(5) Dynamic Signal Enhancement(DSE) : DSE is an image optimization method. It is available for time lapse series with the acquisition mode xyt, xyzt, etc. Enable: Switch the function ON and OFF here Frames: Set the number of individual images to be average here (max. 65). The number must be odd

so that, during image acquisition, the averaging calculation always includes the same number of frames before and after the current image.

Weight: You can adjust how strongly the neighboring frames are weighted in the average (value range from 0 to 2). The higher the value the stronger the stronger the weighting of the more remote images. * If you have set the Counting mode for activate detector, no fluorescence intensities are averaged; instead of counting photons are accumulated though the number of frames that are set. DSE is "Exponential weighted moving average" and improve S/N without reducing FPS

In a left Time lapse series, 14 frames are being captured (from left to right). For frames, the number 7 is set. The graphic indicates which frames are being used to calculate the averaged images.

The first raw indicates the weighted averaging for the 7th frame (Blue highlighting). Three frames before and after the 7th frame are indicated in the calculation for the averaging (red box). The frames outside the red box are not considered in the averaging. In the second row, the averaging for 8th frame is performed is performed and in the third row, for the 9th frame.



In acquisition mode during image acquisition (Start): For the fist and last images, only one half of the specific frames are averaged. The total number of images remains unchanged. Both raw data and the averaged image are saved. The calculation starts during the acquisition, as soon as sufficient data

is available.

In live mode: Here the averaging aggregates up to the specific number Frames. In the example, the 7 most recently acquired frames are

averaged if "7 frames" is set. No data is saved in live mode.

III-5-2. The Nyquist Oversampling Theorem and Pixel Size

The Nyquist-Shannon sampling thorem describes the fact that an analog signal of any form can always be restored if the sampling rate in digitizing is more than twice as high as the maximum frequency included in the signal.

This means that the pixel size must be set in such a way that sufficient pixels are available for representting the resolved structure.

- The sampling spacing is good if the pixel size equals one half of the opical resolution
- If the pixel size is no longer than one half of the optical resolution of the microscope.



III-5-3. Smooth Rendering

Switches the smoothing the images shown in the viewer on or off. This is exclusively an image processing function and has no effect on the raw data. This function is ENABLED by DEFAULT.

Disable smooth rendering
Y-Selection
Autofocus
Snapshot
Snapshot all

III-5-4. The difference of HyD S, HyD X and HyD R (Ref: Volker Schweikhard et al.,

Nature Method 2020)

Select the detectors while referencing below figure that show the Photon Detection Efficiency (PDE) of HyD S, HyD X and HyD R.



- HyD S

HyD S is silicon-based Multi-Pixel Photon Counter (MPPC) detector. This detector is the result of years of engineering at Leica. The result is enhanced image quality that sets new standards in confocal imaging.

*HyD S is available for TauSense and τ -STED, but not for FLIM and FCS.

HyD S has 3 Operation mode: Analog, Counting and Reflection (Ref. Next page)

III-5-5. About HyD (Hybrid Detector)

Operation mode

Depending on the detector, following mode are available.

-Counting: The counting mode makes it possible to carry out a quantitative image acquisition by displaying the image based on the number of photons detected per pixel. The number of photons is displayed in a look-up table, whose gray-scale value in this case corresponds exactly to the number of photons (e.g. Gray-scale value: 15 corresponds to 15 photons).

*It is used, for example, for ratio imaging, FRET or image correction.

*The Gain function is deactivated in this mode.

*If the number of detected photons per pixel is very large, the depiction capacity of the color depth can be exceeded e.g. 255 at 8 bit. In this case, switch to higher color depth, such as 12 bit or 16 bit (Ref. next page).

*For evaluation in other program, you can export the images as ASCII files (Ref. page 44).

-Analog (HyD S): This operating mode can be used for the regular image acquisition. To adapt the image acquisition to the light intensity of the specimen, you can set the gain of the light signal.
 -Reflection(HyD S): This mode is primarily intended for reflection image or extremely high signal intensity. This mode should be used in special case only.



Acquire image using Counting mode

▼ XY: 512x512 400 Hz	1.00 1.00 AU 🔹 🔶
Format:	512 x 512 🌲 💽
Speed :	400 Hz 🌲 🖸
Bidirectional X:	
Zoom factor : 💿	1.00
Zoom In:	OFF
Image Size :	184.52 µm * 184.52 µm
Pixel Size :	361.10 nm * 361.10 nm
Optical Section:	0.895 µm 🕤
	Optimize xy Format
Pixel Dwell Time: 1.44 µs	Frame Rate: 0.258/s
Line Average : 1 Line Accu: 1 Frame Average : 1 Accu: 1	

-Adjust laser power with Analog/Digital mode (Gain 50 - 100).

-Change to Counting mode

-Adjust image intensity with Scan peed and/or Accumulation (Not adjust laser power)

Lower scan speed, longer pixel dwell time, higher photon number.

*Resonant scanner cannot change scan speed.

In counting mode, look-up table corresponds exactly the photon number. Then Line/Frame Accumulation make it possible to show high photon number image.

*To decide the accumulation number, check the gray scale value with "Auto Scale" (Ref. 19). Then, set the accumulation number not to exceed the color depth coding. Or switch color depth 8 Bit to 12 or 16 Bit (Ref. Next page).

Switch the color depth

Switch to the "Configuration"

Select "Hardware"



In the dialog field "Hardware Settings" in Resolution, select the suitable color depth in the dropdown menu "Bit Depth".

step

🔯 Hardware Settings	
Panning Step Size 3	Line Average during Live Acquisition Line Average during Live Acquisition
Data Transfer Mode	Resolution
Direct Enhanced	Bit Depth 8 \$
Direct Overflow Maximum Integration Time	
 Online Maximum Projection during Acquisition 	▼ Z-Movement
Online Maximum Projection during Acquisition	Activate Z-Movement Limitation Bidirectional XYZT Acquisition
Image Orientation	Manual Microscope Control
Flip X	Enable during Acquisition of Series
LDM/Wizard Stop Behaviour	Detector Auto Selection
Wait until frame is ready	Simultaneous use of internal and external Detectors

III-5-6. Z stack (XYZ)



1. Select the appropriate "Acquisition Mode", XYZ or XYZT

2. Use the control panel or Smart move to move the focus position to begging position of the stack

3. Click ①"Begin" to set the start position
4. Use the control panel or Smart move to move the focus position to end position of the stack
5. Click ①"End" to set the start position
①Use arrows to move to the set Begin or End position

②Delete the Begin and End positions③Z Size show the distance from the Begin to End.

④ Stack Direction (Z): Change direction of acquisition of the stack

(5) Select "z-Galvo" to indicate the focus drive of the microscope stage will be used to control z position during the stack (Use only Control panel to define the begin and end, Ref. next page).

©Define the number of the z-stack Nr. of Steps: Manually define the number of

images of z-stack

Z-Step Size: Manually define the distance between images System Optimized: Optimized automatically

6. Acquire the image

Start O Click the "Start" to acquire the z-stack image

III-5-7. Selecting the z-actuator (z-Galvo, z-Wide)

Enable the suitable z-actuator for moving specimen in a vertical detection.

■z-Galvo(Optional)

Galvanometer-driven fine focusing stage. It is used for a low stack thickness with high resolution in the z-direction. Maximum travel distance: 500 um (Restrict Range) or 1500 um

(Whole Range)

Configure with Control panel (if z-Galvo was selected)



-Control panel and LAS X window show the current Z position, but there do not recognize the motor-driven focus position. Then, do not change the Z position using Focus knob or Smart Move, after definition of the z-stack positions. If you change it, acquiring are was moved from desired position

Z-Wide

motor-driven focus of the microscope stand. It is suitable for very thick stacks that exceed the positioning range of the focus. However, the resolution in the z-direction is lower. Maximum positioning range: Corresponding to the free working distance of the objective used Configure with Focus knob, Smart Move and/or control panel (if z-wide was selected)

-Control panel (Ref. 16)

You can configure both z-Galvo and z-Wide, but NOT same time



III-5-8. Time Series (XYT, XYZt etc.)

1. Select the appropriate "Acquisition Mode" that include time (t), XYT or XYZT



2. ①Set the "Time Interval"

For no delay between images, select "Minimize"

3. (2) (3) Define the parameter for acquisition to stop

4. Acquire the image

Start



III-5- 9. BF (Bright Field), DIC (Differential Interference Contrast), PH (Phase Difference) image

First, configure the settings for image acquisition. Click the detector bar for "Trans PMT". The configuration area is expanded.

E	Setting 4	
450 500 HyD S 1 HyD S 3	550 600 650 700 750 800	850
0	Trans PMT - On	
()	Trans PMT Gain B0.00 🗢 Offset 0.00 🗢	\bigcirc
Transmitted light Detector ON/	OFF Transmitted light Detector Gain Transmitted light Detector Offs	et

Select the contrast type

Scan-BF: To make images in bright field mode

Scan DIC: To make DIC image, the polarizer and the analyzer are required for DIC image acquisition.



Differential Interference Contrast (DIC) image

1. Select "Scan-DIC" or "Scan-DIC-Pol"

2. You can set the prism combination (Prism) and DIC prism position (Bias) here.



By moving the Bias slider to the left or the right, you can find the optimum prism position for your experiment. Depending on the prism position, the image appears with greater or less contrast. 3. If necessary, adjust laser power and/or gain value.

*Proper Prism for the objective is required, if not "Scan-DIC" and "Scan-DIC-Pol" were not displayed.

PH (Phase difference) image

- 1. select "Scan-PH"
- 2. If necessary, adjust laser power and/or gain

Tips for Transmitted Light Imaging

Specimens that alter polarized light, such as birefringent specimens and plastic culture dishes, are not suitable for DIC. Phase contrast is still the best method for examining cells in plastic culture dishes. The excitation laser power and wavelength affect on the brightness of these image. Köhler illumination alignment is required for all transmitted light imaging.

The difference of the Scan-DIC and Scan-DIC-POL

Scan-DIC consist of 1 polarizer and 2 prisms, whereas Scan-DIC-POL consist of 2 polarizer and 2 prisms. Insertion of these components to the optical path reduce the fluorescence signal and the fluorescence image resolution.

*Confocal microscope uses coherent laser as excitation, so 1 polarizer is enough for DIC image acquisition.

IV. TauSense (Ref: M Julia Roberti et al., Nature Research 2020)

TauSense analyze the photon arrival time and provide image independent of intensity.

This allows you to remove unwanted signals, such as reflections, from images and separates different species in your specimen based on their fluorescence lifetimes.

* A pulsed laser is required (e.g. WLL)

			S	etting 5					×
	*		Clic	k the det	ector, c	onfiguration	n 📄		
		550	area	a expand	S			1 800	850
HyD S 1	I HARRISON OF THE OWNER OWNER OF THE OWNER OWN			HyD S 4					
			Trans	PMT - Off					
\bigcirc	HyD S 2 Gain	2.50 🗢 Excitation Line	e Dye	Yield / Crosstall	TauContrast	I TauModes	Operating Mode		6
	ON O	Auto (499) 🗘	ALEXA 488 🗢		ON	TauSeparation 🗘 🖸	Counting \$		U

①TauContrast: Ref. Next page

②TauMode

TauModes	
Intensity 🗘 🚺	Ð
None	
Intensity	
TauGating	
GateScan	
TauScan	
TauSeparation	

In the detector configuration area, the left image shown modes are available. Click the icon to open the TauMode dialog. **None:** Only if TauContrast function is switched ON. The viewer only displays the lifetime image and not intensity image of the specimen. **Intensity**: Without gating. The viewer displays the full intensity image of the specimen. Default setting.

TauGating: Ref. page 33 TauScan: Ref. page 34 TauSeparation: Ref. page 35

IV-1. TauContrast

The "TauContrast" function is used to measure the average photon arrival time per pixel in the detector and display is as a colored lifetime image. Two images are acquired per detector and displayed in the viewer: The first image the fluorescence intensity is shown through the brightness and the second image additionally uses colors to represent the average photon arrival time. * TauSense does not require additional laser irradiation

Start your experiment by clicking Start or Capture.



IV-2. TauGating

You can set the number of gates per channels as you please. Every channel is displayed in a separate partition of the viewer.

Using TauGating mode, you can create multiple channels for one detector to eliminate, for example, unwanted signals such as reflections or background noise from the acquisition. You can set number of gates per channel and the number of channels as you please.

Three channels a with one time gate b each are preset. Each line represents one channel, each



detection range one time gate. You can add both more channels and time gates per channel.a: Each channel is represented in one line. Every channel is displayed in a separate partition of the viewer.

(): Each time gate is displayed as a detection range. You can use the mouse to adjust the size of the time gates and to move them with \leftrightarrow . In this way, you can define the beginning and end of the image acquisition (based on the laser pulse). If a channel has multiple time gates, the viewer collects them in one partition.

(D: Using the "Plus" and "Minus" buttons on the right margin, you can add more time gates to the respective channel or delete the currently selected time gate. A maximum of three time gates can be configured per channels.

e: Time scale. Photon arrival time after the laser pulse in nanoseconds. Here you can read the defined time gates.

Add Channel: In doing so, you add another channel below.

Remove Channel: By doing this, you delete the currently selected channel.

Start your experiment by clicking Start or Capture.
IV-3. TauScan

You can obtain a diagram of the fluorescence lifetime components. This curve indicates how frequently lifetime components appear in the entire image. With the aid of the diagram, the lifetime components in the different species of the specimen can be determined. In this way, you can also determine whether a dye contains more than one lifetime component, for example.



In this dialog, you can break down the entire range of detectable lifetime components into a selectable number of sections. One channel is assigned to each section. The channel is displayed as a separate intensity image in a viewer partition. With the aid of this, you can assign the different species in your specimen to individual lifetime components. In addition, you can determine whether species contains more than one lifetime component. You can use the TauSeparation (Ref. next page) method to separate the various species in your specimen if necessary.

(a): Lifetime diversity curve Graphical display of the frequency of lifetime components (lifetime measured in ns) in the entire image. Each peak indicates a lifetime component present in the image.

O: Set the number of channels or images here. This number corresponds to the breakdown of the entire range of detectable lifetime components in O.

©: Entire range of detectable lifetime components.

(D: Time scale Lifetime in nanoseconds, broken down to the number of images set under **(D**). The double arrow marks a section of detectable lifetime components.

Start your experiment by clicking Start or Capture.

IV-4. TauSeparation

You can carry out a separation (e.g. dye separation) of species present in the specimen based on different fluorescent lifetime that you have determined, e.g. in a preceding TauScan (Ref. Previous page). For each lifetime component, an intensity image is generated in a separate channel and displayed in a viewer partition.



You can use the TauSeparation method to separate species present in the specimen based on their fluorescence lifetimes and acquire the resulting in separate channels. If a species has multiple lifetime components, you can also detect them separately. Configure the setting for species separation in this dialog.

(a): Lifetime diversity curve Graphical display of the frequency of lifetime components (lifetime measured in ns) in the entire image. Each peak indicates a lifetime component present in the image.

(**b**: Each line represents a channel. Each species is assigned a separate channel.

 \bigcirc : The line represents a lifetime component for the respective channel. Move this line below a peak to assign a lifetime component to a channel and, consequently, to a species.

(D: You can adjust the color for the channel display. Double-clicking the color field opens a dialog for selection of a LUT.

e: Using + and – buttons, you can add further channels or species and remove them.

 \oplus : By clicking "Find", the analysis is carried out for all configured channels, and optimum setting for the lifetimes are determined.

Start your experiment by clicking Start or Capture.

Tips

It will be easier to separate lifetime component in bellow conditions.

- There are big lifetime difference
- There are no intensity difference
- \cdot The lifetime components localization are big different

TauSeparation: There are cases in which divided images are saturated, even in entire image is not saturated. Then, you can change the bit color depth to 12 bit or 16 bit (Ref. page 25).

V. Viewer V-1. Change acquired image Pseudo color, adjust scaling

Change Pseudo color: Double clicking the color bar. A list of all available look-up table opens. Double click to select a different look-up table from the left area. Adjusting the Scaling Range: You can adjust the scaling. This makes it possible to extend the dynamic range and, therefore, to work with the entire bandwidth of the color depth. this is primary helpful in evaluating dark images



Show the Acquisition mode, format and the memory size
*Show units for the image size. By clicking button, you can switch between "µm" and "pixel"

If a time or z series is displayed in the viewer, a control element bar appears in the lower or right edge of the viewer with a slider used to navigate through the various acquisition times.

V-2. Function in the context menu



ROI	Save: Open the file browser for selection of a storage location. All ROIs are		
Reference ROI	saved to a location defined by you with (ROI) or without (Reference ROI) the		
	information on the coordinates.		
	Load: Open the file browser. in which you can select the storage location of		
	the desired ROI. ROI: The loaded ROI replace the existing ROI, Reference		
	ROI: The loaded ROI added in the center of the viewer.		
Snapshot	An image file is created from the image you have right-clicked. ROIs and		
	annotations of images are saved, where appropriated.		
Snapshot All	If multiple images are displayed in the viewer: An image file is created from		
	the image you have right-clicked. ROIs and annotations of images are saved,		
	where appropriated.		
Properties	Open the shape properties: For each plotted annotation, you can open a dialog		
	field for displaying the properties.		
	General: Adjust the designation and set the size		
	Color: Define the color Store later		
	Font: Configure the literal and select font, font		
	size and style		

V-3. Functions in the Viewer

(Ref. Advanced Manual)

Annotations	Adds the functions for image explanation to the list of buttons.	
\swarrow	Select single ROI	
ŧ	Clear selected ROIs	



	Show overlay image of selected channels: For multi-channel acquisitions, switches
Fa	the overlay view on and off. This allows you to superimpose the color channels of
	an image on one another or show them separately.
	Maximum projection Direction of view
	Calculates a maximum projection for z-series and
	presents it in a viewer.
F	*The image is just preview, you cannot save it.
MRX	*To save maximum projection image Ref. Next page
	Intensity Maximum
	Orthogonal Sectioning
	Creates a display of the z-stack in xy, xz and yz-direction. Using the crosshairs,
	precise positions can
	be reached in all three
	dimensions within the XY
	stack
×	
	* The image is just XZ
	preview, you cannot
	save it.
	* Saving an orthogonal
	section in the xz or zy-direction: Right-click on the XZ/ZY-direction of the z-stack
	to open the context menu, "Snapshot".
3D	Open in 3D viewer
	opens the 3D viewer for 3D image processing
	Show/Hide Image Channel information: Indicate the number of the associated
	setting and information on the detector and/or detection channel and lifetime (for Tau modes) and the dye with designation and color of the detection range on each
	partition insofar as this information is present on the image
	Example: Setting No. I Detector name or gate or lifetime I Fluorescent Name I Dye
	Pseudo color
	*You can rename the "Setting name". Double-click the setting name in Beam
	path and enter desired name.
	Setting 3 HyD S 2 ALEXA 488 🕒

V-4. Creating Maximum Projection in the Process step

Configuration Acquire Process Quantify

1. Proceed to step "Process" menu.

2. Under "open projects", select the image series you want to view as the maximum projection.

3. Select the "Projection" option in ProcessTools.

4. At the bottom edge of the window, click "Apply" to create a maximum projection using the current settings.

It is then displayed in the both the preview and the viewer. The created projection is also listed in the project directory.



* In Process menu, TauContrast image were consist of 2 channels: I and AAT.

"I" has intensity information and "AAT" has average photon arrival time information.

Ch1 I2 AAT2

Left image is example. Capture 2 channel images, Channel 1: Intensity image, Channel 2: TauContrast image. Then TauContrast image divided into I2 and

AAT2.

* In older versions LAS X Small, TauContrast image divided into just channels not I and AAT.

VI. Quantify



The values measured in the	ROI are displayed as
graphs on the "Graph" tab ar	nd as tables on the
"Statistics" tab.	

Statistics

Line Profile: Measure gray scale value in images and in the individual images
of image series along linear ROI.
Stack Profile: Determine the mean gray scale values in the individual images of
image series based on ROI. If you have not drawn any ROI, the mean gray
scale value of the entire individual image (Frame) are measured.
Histogram: Determine the distribution of gray scale values in images and in the
individual images of image series based on ROI.

Graphs

⊽ S	elect Channels and	ROIs		() 🖈
	ROI.01		Channel.00	01
	ROI.02			
▼ S	ort charts by Chann	els and R	Ols	* ()
5	•••••	•_		_
FO.	Sort Channels			
\mathbf{O}	Sort ROIs			

Select Channels and ROIs: You can select ROI and color channels for the quantitative evaluation of images and image series. ROI/Frame: For ROI to be available for selection here, you have to draw it in the viewer. Select the desired ROI for the quantitative evaluation.

Channel: It provides you with ROI-Channel combination. This function is available only if you combine multi-channel acquisition in

the viewer.

Sort charts by Channels and ROIs: You can define how the result of the quantitative evaluation of images and image series are displayed.

Sort Channels: The results are sorted by color channels

Sort ROIs: The results are sorted by color ROI

All in One: The results are combined into one display

Report: To save the evaluation results as a report further use. The report contains the evaluated



image, including the ROI used. The evaluation results displayed as graph and in table form in the report. Furthermore, the report contains information about the detailed information about the image or image series and the systems settings used during image acquisition.

VII. Data Handling

VII-1. Tool Bar

All of images are displayed in the "Open Project"



 Changes to the tree and directory structure of "Work in Folders". You can use button to return to the project structure.

- ② Create the new project
- ③ Open the file manager for selecting an existing project
- 4 Save ALL opened project

(5) Applies the instrument parameters of the highlighted file (Ref. page 14).

⁽⁶⁾ You can use this button to call up the project browser dialog. The file browser searches and browses for project data created using LAS X and displays a preview of the included images in a viewer.

O Show/Hide a gallery with thumbnails of all images in a folder.

VII-2. Save data

All images and image series you have acquired are collected in a project and shown in a directory structure. When saving the project data, a corresponding folder structure is created in the project directory on the hard drive.

Project: Data record containing all of the data for experiment.

Image series: Can be a 2D single image or a multi-dimensional image series

Metadata: Every data object can have metadata where hardware setting, LUT, image display settings. project settings, ROIs etc. are saved.

All of acquired images and image series are temporarily stored in project.

VI-3. Export image



Right-click on the desired image to open the context menu, "Export image".

1 Image: Exporting image acquisitions

Movie: Exporting videos 2 Destination Folder: Select the storage location for the exported images/videos ③ Store in separate folders: This option is available only if you have selected multiple elements. Checkbox enable: The project is saved with the corresponding folders and subfolders. ④ Select the export format: Image: TIFF、ImageJ TIFF、JPEG、ASCII (Export intensity value)、BMP、PNG Movie: Avi, QuickTime, MPEG-4, WMV (5) Lossless compression/Quality: TIFF: Losslessly compress the images for the export JPEG/Quicktime: Set the Desired compression rate using the Quality 6 Export LUT: The color look-up table (LUT) of the selected object is saved in the corresponding metadata subfolder. (7) Select Channels: In the case of multi-channel acquisitions, you have the option of exporting

individual channels. By clicking the desired button,

you can include the corresponding channel in the export (Red button) or exclude it (Gray button). (8) Export Channels: When the checkbox is enabled, each selected channel is saved as a separate image.

Raw image: The images are saved without having been processed. Burn-in options are not available. (Options for TIFF/ImageJ TIFF)

Scaled Viewer Image: Available only for multi-channel acquisition. The images are saved as a shown in the viewer. (Options for TIFF/ImageJ TIFF)

(9) Export Overlay as scaled Viewer Image: When the checkbox is enable, all selected channels are superimposed and saved in one file.

⁽¹⁰⁾ Save Multipage: Option for TIFF images with more than two dimensions. When the checkbox is enabled, each image is saved in a searchable, multi-page file. Otherwise, each image is saved in a separate file.

(1)Reduce Tilescan Size: You can use this slider to reduce the relative size of a composed mosaic acquisition (as a percent). Otherwise, the composed mosaic acquisition is exported in its full size.
(2)Burn in to scaled Viewer Image: In this dialog, you can configure the visualization of additional image information that is displayed in the exported images or videos. When the respective checkbox is clicked, the desired image information will also be exported. The button opens another dialog with setting options for the desired image information.

⁽¹³⁾Save: When "Save" is clicked, the export is started with the configured settings and exported images/movies are saved at the specified path.

VII-4. Organization of the data in projects

All images and image series are collected in Projects and shown in a directory structure, referred to

Close Projec	t
Save Project	Ctrl+S
Save All	Ctrl+Shift+S
Save as	
Create Colle	ction
Delete	Del
Rename	F2
Cut	Ctrl+X
Сору	Ctrl+C
Export	•
Properties	
Open in new	v viewer
Open file loo	ation
Close All	

in the following as the project directory.

Close Project: Close the selected project

Save Project: Save the selected project

Save All: Saves all opened projects and files in the configured format Save as: Opens the save dialog for selecting storage location and storage format

Delete: Delete the selected file completely. Cannot be restored. *Not "Hide"

Rename: Makes it possible to rename a selected file Cut: Cuts the selected file and places it in the clipboard Copy: Copies the selected file into the clipboard

Export: Opens the Export dialog (Ref. previous page)

Properties: Opens the Properties dialog (Ref. page 14)

Open in new viewer: Opens the selected file in a new viewer

Open file location: Open the storage location of the selected file in windows explorer

Apply image settings: The instrument parameters of selected image are restored (Ref. page 14)

Close All: Close all open projects and files

VII-5. Free viewer software LAS X Small (Win10)

This module has full image viewing capabilities, including annotations, image overlay and image comparison.

Search for "Leica LAS X Core" and download the installer from "Download free LAS X Core Offline version for Life Science".



- Download Leica LAS X Brochure (English)
- Download the LAS X Mobile Connection app free of charge

*ImageJ, FIJI and Imaris are available to open the lif file.

*ImageJ requires the plugin, "Bio-Format".

VIII. Online Help

Click on the help symbol 🔍 located in the top right corner of every LAS X dialog field.

The online help opens in your default web browser. An overview of the dialog topic is displayed,

where you will find links to all relevant topics.



IX. Shut down

1. Laser OFF

Select Configuration Configuration menu and Laser Config Configuration Acquire Proces Quantify Configuration Acquire Proces Quantify Configuration Config

Turn off all lasers with single click

*Do never change WLL%

Currently available Lasers	0
Adjust Laser Settings Diode 405 : ON	Do Never change WLL%
WLL: ON	0 70.00 %

- 2. Shut down the LAS X software
- 3. Turn off the Hg lamp

- 5. Turn counter-clockwise Laser security key switch
- 6. Turn off the laser switch
- 7. Turn off the main power switch





How to Clean Immersion Objectives

It is important to remove the immersions after use Never use kimwipes on objective

Oil Objective

- 1. Just simply wipe the oil off the lens with lens cleaning paper.
- 2. Put 100% ethanol on lens paper and wipe of remaining oil.
- 3. Repeat step 2 several times with fresh part of lens cleaning paper.

Water Objective

- 1. Just simply wipe the water off the lens with lens cleaning paper.
- 2. Put 100% ethanol on lens paper and wipe of remaining water.
- 3. Repeat step 2 several times with fresh part of lens cleaning paper.

Glycerol Objective

- 1. Just simply wipe the glycerol off the lens with lens cleaning paper.
- 2. Put 100% ethanol on lens paper and wipe of remaining glycerol.
- 3. Repeat step 2 several times with fresh part of lens cleaning paper.

Confirm the chiller solution amount

When the "LOW FLUID INDICATOR" lamp is ON, add the below liquid.



X. How to use DMi8

1. Movement in XY-direction and Focusing





X-2. Change Objective using Touch Screen



(1)Press

All available objective is shown. The droplet mark indicates **63** immersion objective. Selecting objective were turn red.

②Press desired objective

If it is different type immersion objective, the icon flashes. Touch again automatically change and turn red.

X-3. Setting the Transmitted Light using Touch Screen



microscope left side (See below)

2 Press BF (Bright Field)

1) Press

③ Close/Open the shutter with "TL-Shutter" button
 *The shutter control is available from microscope left
 side (See below)

④The brightness control from the knob in





You can confirm the brightness (FIM) from touch

screen Press

*FIM can be adjusted 5 steps: 10, 17, 30, 55, 100%

X-4. Setting the Fluorescence using Touch Screen

	Transmitted	incident	Combi
	РН	FLUO	FLUO-DIC (
SO			
	POL (
Ŷ			1
	IL-Shutter		
	RHOD	DAPI FIT	C DA/FI/TX
Ē			•••••

1)Press

2 Press "FLUO"

③All available filter were shown, press desired filter.

④Close/Open the shutter with "IL-Shutter" button*The shutter control is available from microscope leftside (Same as TL, See previous page)

⑤The brightness control from the knob in microscope left side (Same as TL, See previous page)

Filter Name	Fluorescence	Ex. Range	Ex. Filter	Dichroic	Em. Filter
DAPI	DAPI	UV	BP 350/50	400	BP 460/50
FITC	FITC	Blue	BP 480/40	505	BP 527/30
FITC LP	FITC	Blue	BP 470/40	510	LP 515
RHOD	Rhodamine	Green	BP 515-560	580	LP 590

Specification

*BP: Band-Path, LP: Long-Path



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