ABBERIOR STED

USER MANUAL

• Switch on the 640 nm laser line (1), switch is on the back panel





• Switch on LEDs. from "coolLED" controller (2)



- All other laser lines will be switched on automatically.
- Computer should be switched on. Log on under < Abberior User >.



• Touch the screen of the Olympus microscope controller, then press "Start Operation".





• Open software (double click <Inspector> icon on the desktop). Choose <YES> in the "Do you want to switch microscope" dialog box.





It is normal to see <Hardware Errors> window. Press <Initialize for Pinhole and Camera>.

The h	ardware layer reported one or several errors:	
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 You have to initialize the laser 580 nm by turning the key 90° left and back as shown:





• Click <OK> to close <Hardware Errors> window.

CHOOSE THE OBJECTIVES

Available:

- 20x Air

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- 60x Water
- 100x Oil (by default, recommended)

Choose the objective using TOUCH SCREEN of the CONTROLLER.





ATTENTION! Always press <ESCAPE> button to bring the stage to zero position when you change the sample

SAMPLE INSTALLATION

Install your sample . Adjust position of the stage by hands.





CHOOSE THE MODE:

Available:

- Fluorescent (FL)
- BrightField (BF)
 - Camera (CAM)

Choose the mode using TOUCH SCREEN of the CONTROLLER.



• <FL> mode:

Turn on the appropriate LED source by pressing the bottom and the knob on the LED controller to switch the light source on. Rotate the knob to adjust intensity.





<u>Available:</u> -λ1 400 nm -λ2 490 nm -λ3 585 nm -λ4 635 nm • <BF> mode:

On the condenser, change position of the shutter wheel from <1> to <2>.



Adjust the lamp intensity for sufficient illumination of the field of view.



<CAM> mode:

From the software choose <New Measurement Document>.

Turn on the appropriate LED source. Adjust brightness as described above by turning the knob on the Led Controller or by changing <Exposure time> in Camera window.





Click **<Start>** to start visualization Change the **Exposure time** for a deem/bright sample.



Press **<Stop>** when you found your region of interest and placed the ROI in the centre of the camera window.

CHOOSE THE TEMPLATE

• From the software choose **<New Document from template>**. Choose template with the channel that meets your staining. You can add more channels later.



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Common Templates recomended: -1C STED 580 (excitation 488 nm) -1C STED 775 (excitation 640 nm) -Goldbeads (alignment)

GRAPHICAL USER INTERFACE (GUI)

GUI consists of <Live dialogs>, <Tool bars>, <Image windows/Measurements>.

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Different Live dialogs can be found from the main panel Window \rightarrow Live Dialogs

Tool bars



SCANNING

Example of the Confocal overview image acquisition.

- 1. Set the sample, find the region to be imaged (camera, bright field, etc modes).
- 2. Open the template for Confocal/STED imaging.
- 3. Choose the separate window for the **fast Confocal overview**. All default templates have the window for the **fast Confocal overview**. This window measurements has the setting allowing to set the area of interest and focus on the sample while performing the fast scan with the predefined pixel size (can be changed), lasers, detectors .
- 4. Set the Size of the area to be scanned and Pixel size in <Experiment Control> window in <Live Dialogs> . Defaults are: 30 um, 150 nm respectively.



5. Press <REC> to begin the scanning process. Set the Autorepetition mode to continue scanning during the focusing.

 Start scanning
 Copy measurement window

 Measurement Toolbar
 Stop
 Repetitive measurements AUTOREPETITION

CONFOCAL AND STED IMAGE ACQUISITION

6. - For precise focusing Z-position can be adjusted by changing Z in <Global Offset> in <Experiment Control> window in <Live Dialogs>. The z-value can be changed by scrolling the wheel of a mouse. <CTRL> + scrolling the mouse wheel allows even more precise (fine) focusing.

-Autofocus

To switch on the autofocus <Use Autofocus> can be chosen in <Experiment Control> window (tab)



When autofocus is used **z- position** can be controlled in the OlympusIX window (scrolling the mouse wheel).



OlympuslX

Observation method

<no values>

CONFOCAL AND STED IMAGE ACQUISITION

By this moment you could meet 2 ERRORS:



1. Software shows the "Error: Shutter" message when you try to scan. Return "Safety shutter" to a position <1>.



2. Scanning continues but the image is only partly shown \rightarrow too high signal. Change the laser power in a Lasers and Channels Live Dialog window.



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Confocal Overview CY5	
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CONFOCAL AND STED IMAGE ACQUISITION

Choosing the ROI and acquiring the STED image

1. When the Confocal overview is acquired choose the ROI by drawing with the mouse and right click Selection \rightarrow Use as ŘOI (or Ctr+Shift+U) and focus if needed. You can continue scanning in this mode to correct the focus.



Select All 2. By selecting Selection \rightarrow Copy as ROI Selection ъ (or Ctr+Shift+R) the area can be Use Center as ROI Center Zoom To Selection transferred into the measurement Ctrl+Num + Copy as RO window for Confocal / STED Zoom Out Copy Center as ROI Center Ctrl+Alt+R (evedropper cursor left-click the second window). 1C STED775:1 _ □ Confocal CY5 STED CY5

When the window for Confocal / STED images (measurements) activated (left click of the mouse), <<Line Step>> and <<Laser and Channels>> windows represent the settings for the measurements. Confocal **STED**

In <<Line Steps>> the sequence of excitation and STED laser beams over time and the detection channels for every pixel of the image can be set.

Lasers an	d Channels		×	Line Step	s	
	Detector	Gain Res	cue			Total Steps
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✓ Ch2	CY5 (685/70) 🔻	N/A	周 吉	Ch2		
Ch3	GFP (525/50) 🔻	N/A		Ch3		
Ch4	PMT refl 🔹	30.00%		Ch4		
	Name	Power		b1	_	
11	Exc_488	38.72%		1.2	_	
LZ	Exc_532	N/A		L3		
L3	STED_580	0.8200%		14		
✓ L4	Exc_640	5.000%	1	15		
🗸 L5	STED_775	66.66%		16	_	
L6		N/A		19		
L7		N/A		122.04	1000	61
L8	ř.	N/A		1	3	1
				55	56	57
Pulse	Steps Pixel Ste	ps 🖌 Line Ste	eps	1	1	1

Ctrl+Shift+U

Ctrl+Alt+U

Ctrl+Shift+R

3. After activation the window for Confocal / STED images by pressing **<REC>** the images can be obtained with recommended parameters (by default both Confocal and STED).

To adjust the dynamic range of the image <**F9**> and <**F10**> can be used for min and max pixel value respectively.

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Remember to check that you are still in focus before acquiring your final image as some focus drift may occur (can be corrected in fast Confocal image acquiring measurement).

SAVING THE FILE

The best way to save images is to save it as a **.msr** project file that will contain acquired images and all measurement information, for that:

File \rightarrow Save as , then choose your folder and save as .msr



As an alternative, you can export a separate image in most of the known graphical formats:

File	e Edit	View	Data	Analysis	Measurement	Hardv				
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Acquired images **are not stored** in the memory and every next acquisition will overwrite the previous one, so **you have to save every image/project** that you want to keep .

ASQUARING 3D STACK

Method 1



- 1. Change the XY scan to XYZ.
- 2. Define dimensions of the stack in all 3 dimensions.
- 3. Set Pixel size for all 3 dimensions. By setting Z pixel size the thickness of the slices can be changed.
- 4. Press **<REC>** to start 3D stack acquisition.



ASQUARING 3D STACK

Method 2



- 1. Change the XY scan to XYZ.
- 2. Press **<REC>** to acquire the image in XZ plane.



- 3. Choose the ROI by drawing the rectangle by the mouse, thus the size of the future 3D stack can be chosen.
- 4. Set Pixel size for all 3 dimensions. By setting Z pixel size the thickness of the slices can be changed.
- 5. Press **<REC>** to start 3D stack acquisition.

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	z	1.65µm	350nm	15	110nm					
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			Line Accu	1						