ABBERIOR STED

USER MANUAL

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Updated 14.04.2020

STARTING THE INSTRUMENT AND THE SYSTEM

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

On the back panel of this box

- 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>.**

STARTING THE INSTRUMENT AND THE SYSTEM

- 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>**.
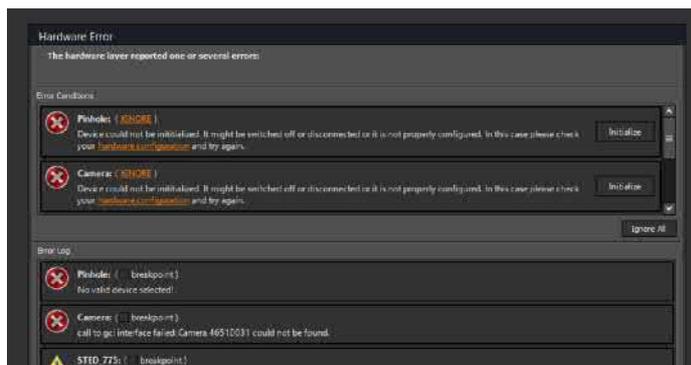
- You have to initialize the laser **580 nm** by turning the key 90° left and back as shown:

- Click **<OK>** to close **<Hardware Errors>** window.

USING THE MICROSCOPE

CHOOSE THE OBJECTIVES

(i) Available:
- 20x Air
- 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.
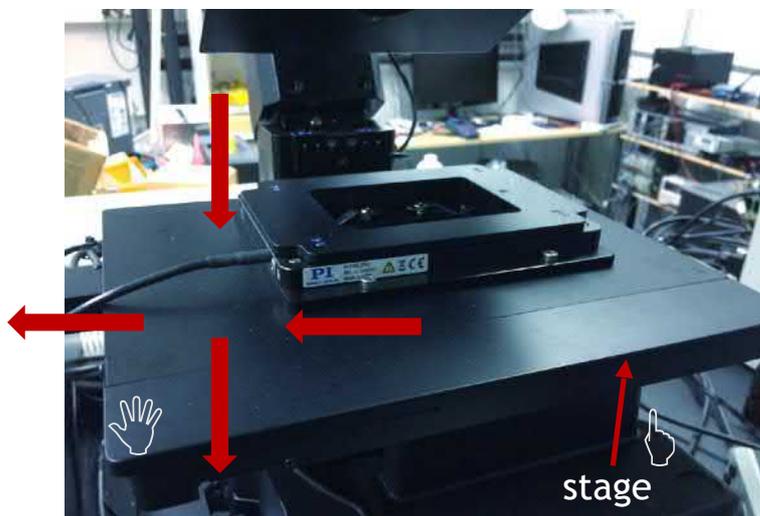
microstage

stage

ATTENTION!
The maximum load to the microstage is 0,5 kg.
Do not press the microstage by hands!

VISUALIZING THE SAMPLE

CHOOSE THE MODE:

        <u>Available:</u>

ⓘ   - **Fl**uorescent (FL)
      - **B**right**F**ield (BF)
      - **Cam**era (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.
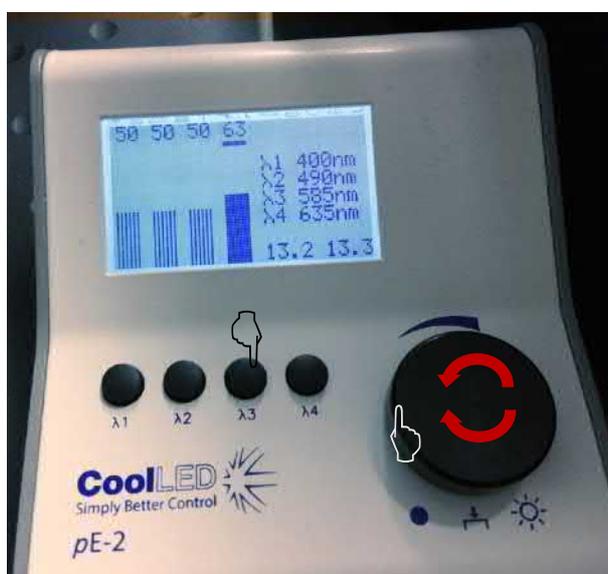
<u>Available:</u>
-λ1 400 nm
-λ2 490 nm
-λ3 585 nm
-λ4 635 nm

VISUALIZING THE SAMPLE

- **<BF> mode:**
  On the condenser, change position of the shutter wheel from **<1>** to **<2>.**
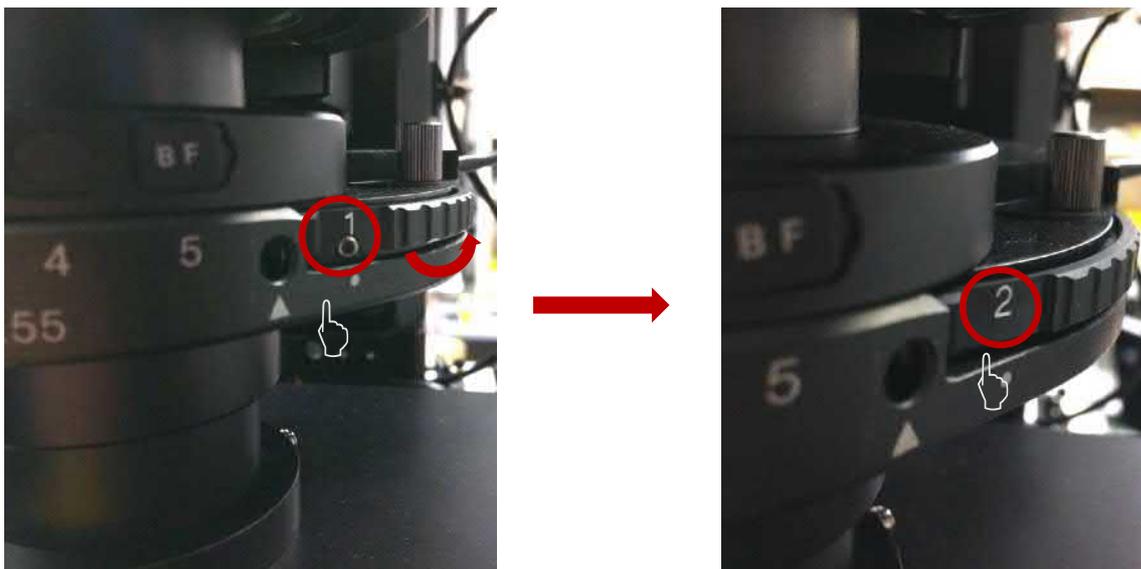
Adjust the lamp intensity for sufficient illumination of the field of view.
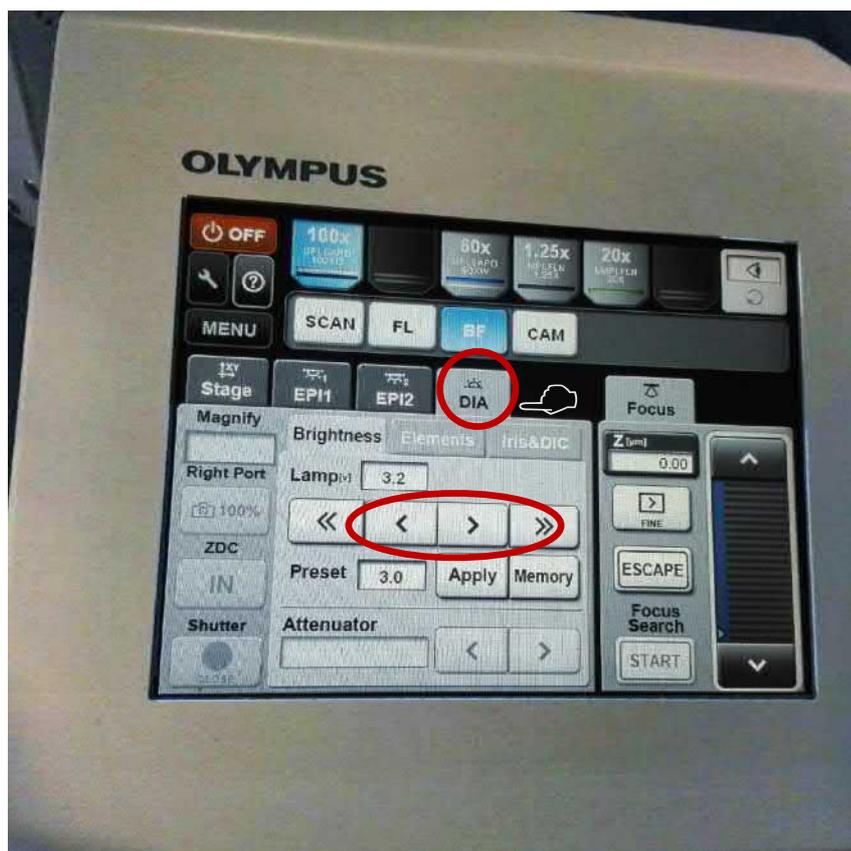
VISUALIZING THE SAMPLE

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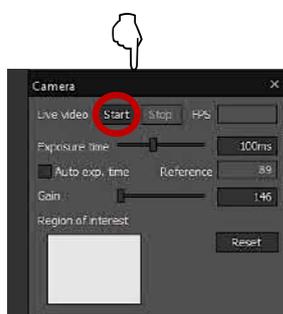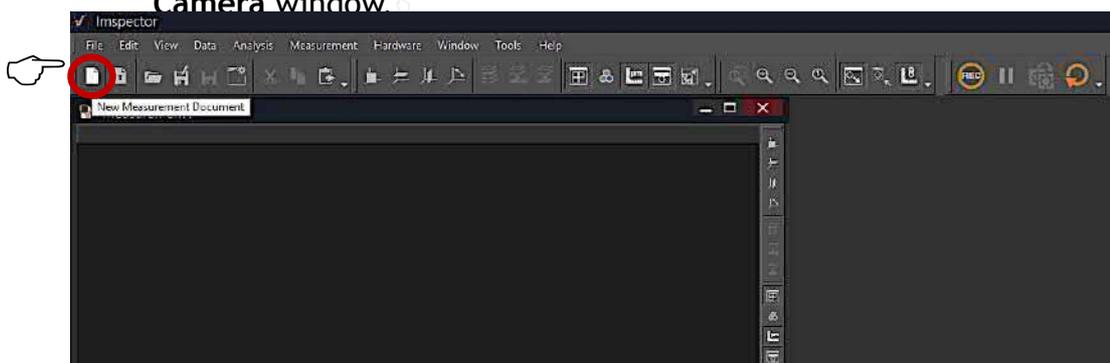
Click **<Start>** to start visualization
Change the **Exposure time** for a deem/bright sample.

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

CONFOCAL AND STED IMAGE ACQUISITION

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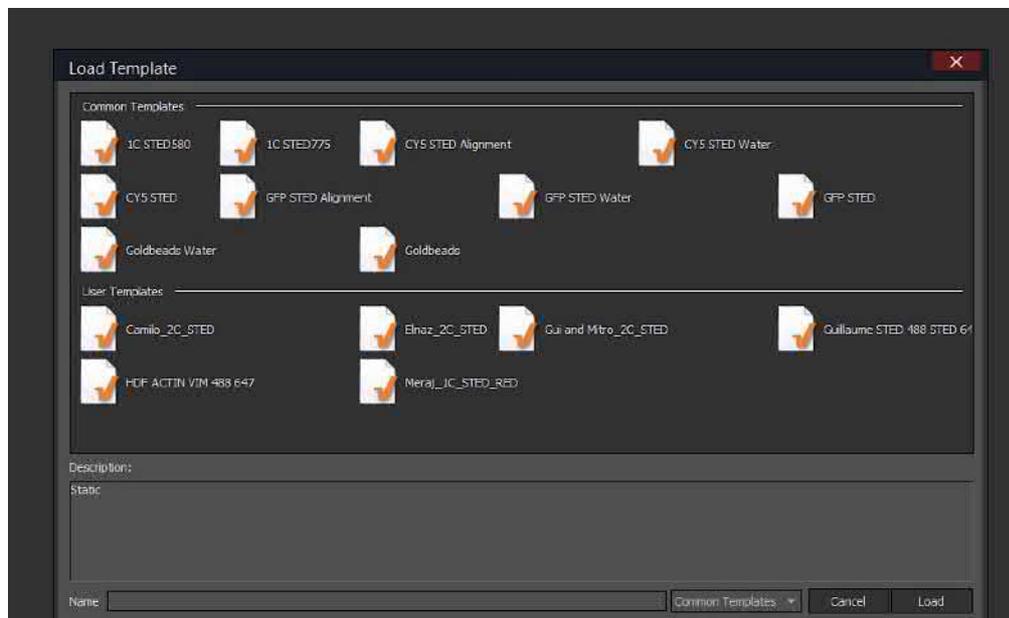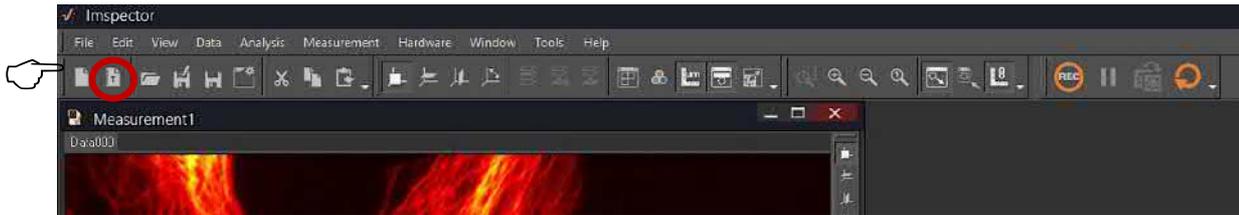
Common Templates recomended:
-1C STED 580 (excitation 488 nm)
-1C STED 775 (excitation 640 nm)
-Goldbeads (alignment)

GRAPHICAL USER INTERFACE (GUI)

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

ⓘ   Different **Live dialogs** can be found from the main panel **Window →Live Dialogs**

CONFOCAL AND STED IMAGE ACQUISITION

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.

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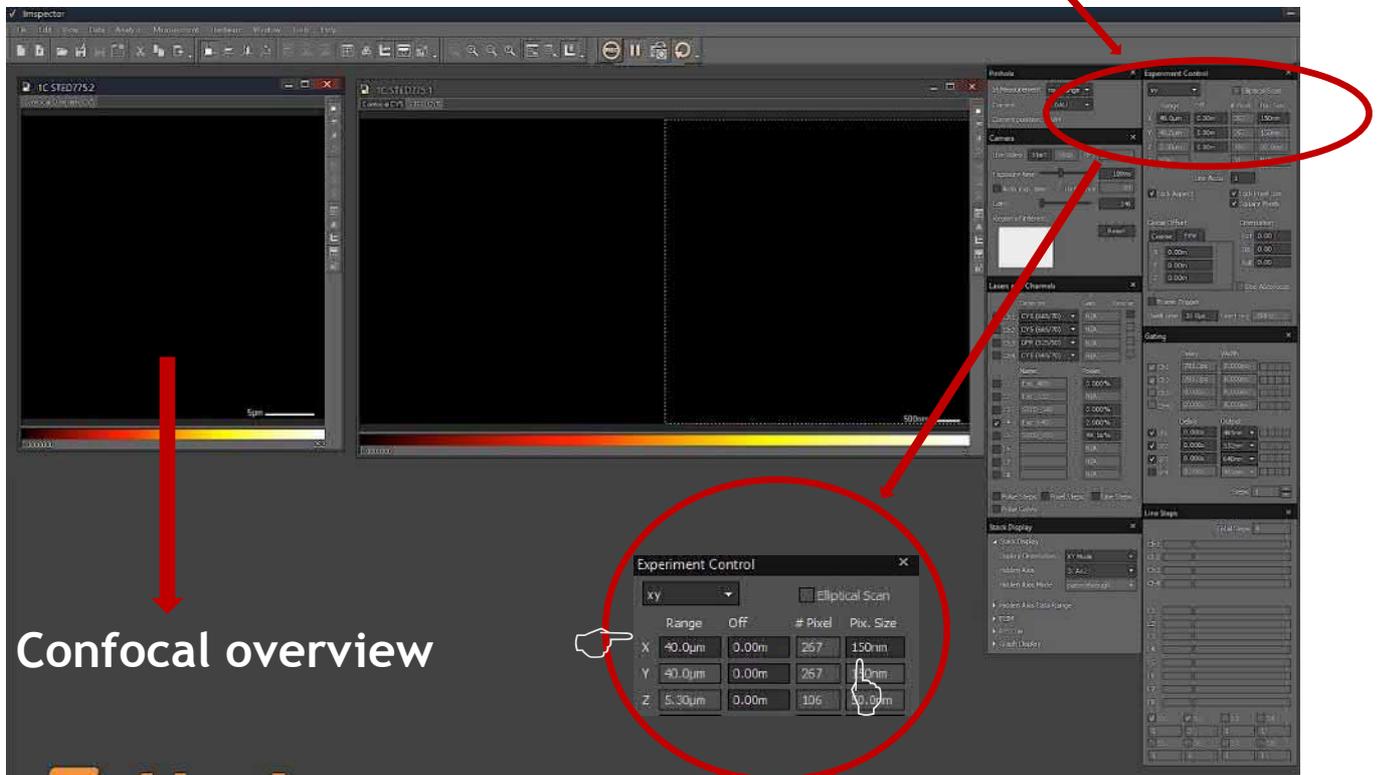
5. Press **<REC>** to begin the scanning process. Set the Autorepetition mode to continue scanning during the focusing.

Start scanning

Copy measurement window

Stop

Repetitive measurements
AUTOREPETITION

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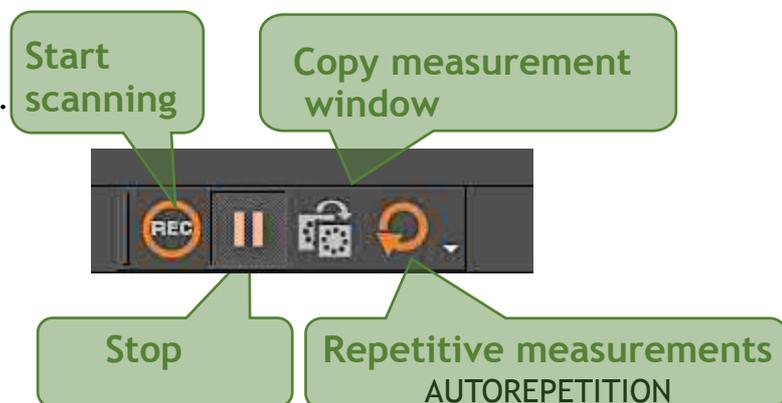
6. - For precise focusing **Z-position** can be adjusted by changing Z in **<Global Offset>** in **<Experiment Control>** window in **<Live Dialogs>**. The z-value can be changed by scrolling the wheel of a mouse. **<CTRL> + scrolling the mouse wheel** allows even more precise (fine) focusing.

-**Autofocus**
To **switch on** the autofocus **<Use Autofocus>** can be chosen in **<Experiment Control>** window (tab)
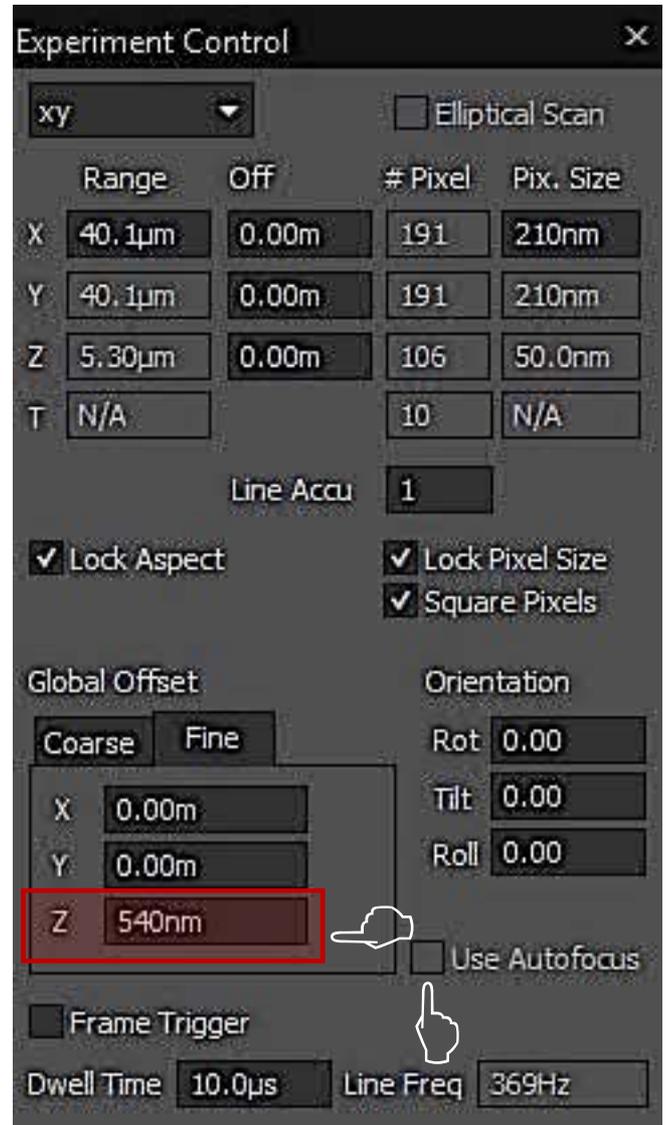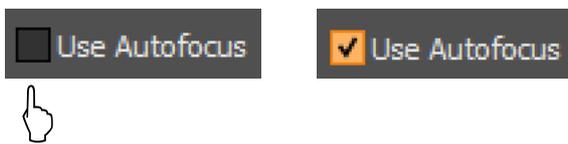
When autofocus is used **z- position** can be controlled in the OlympusIX window (scrolling the mouse wheel).
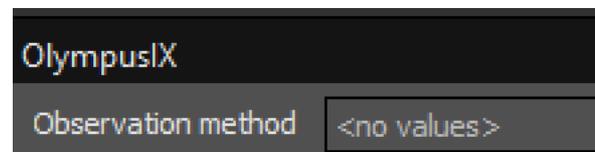
CONFOCAL AND STED IMAGE ACQUISITION

By this moment you could meet 2 ERRORS:

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 → too high signal. Change the laser power in a **Lasers and Channels Live Dialog** window.
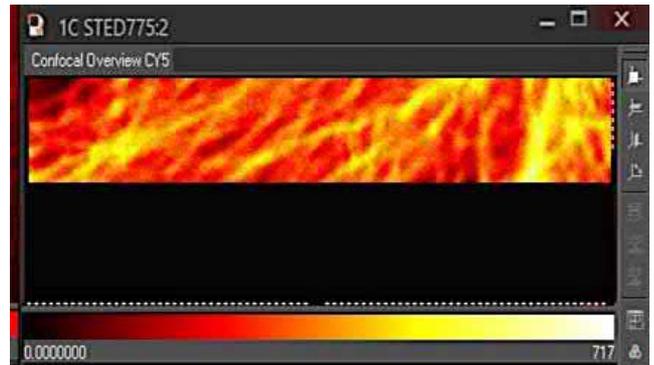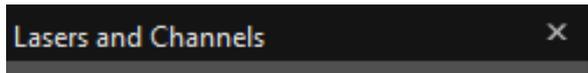
CONFOCAL AND STED IMAGE ACQUISITION

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 →  Use as ROI ( or Ctr+Shift+U)` and focus if needed. You can continue scanning in this mode to correct the focus.
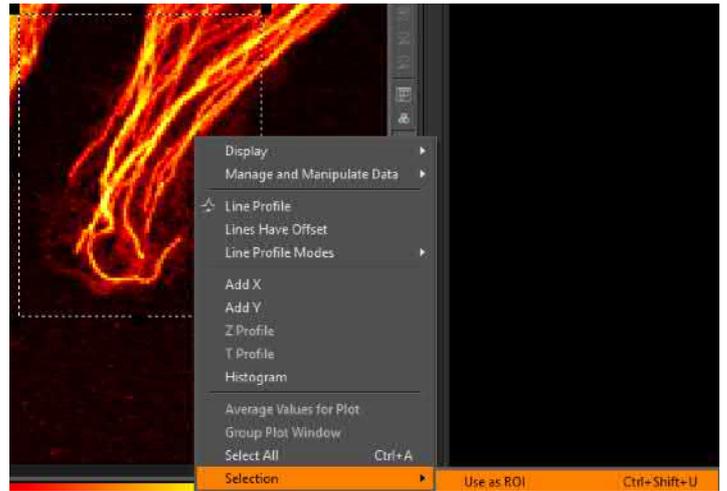
2. By selecting **Selection → Copy as ROI (or Ctr+Shift+R)** the area can be transferred into the measurement window for Confocal / STED (eyedropper cursor left-click the second window).
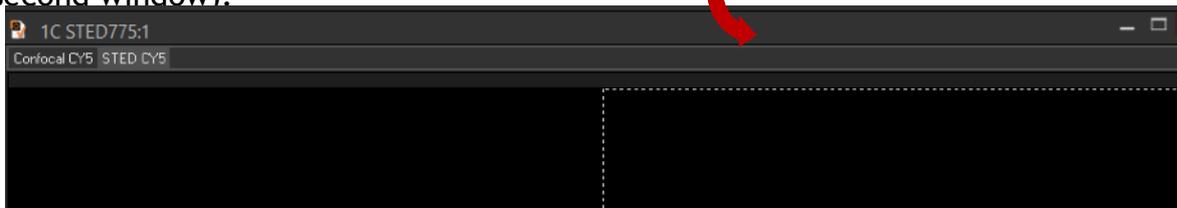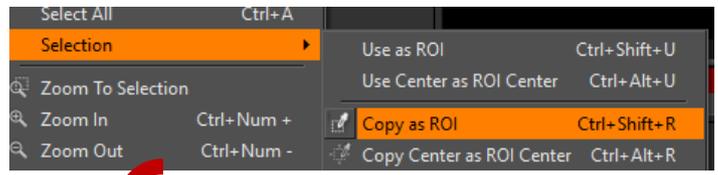
When the window for Confocal / STED images (measurements) activated (left click of the mouse), **<<Line Step>>** and **<<Laser and Channels>>** windows represent the settings for the measurements.

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.
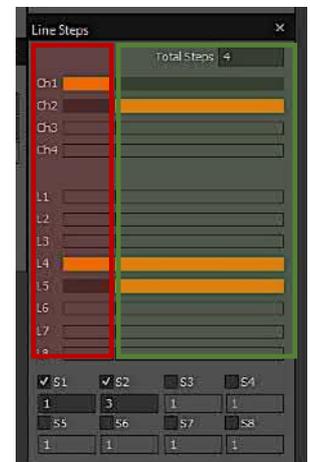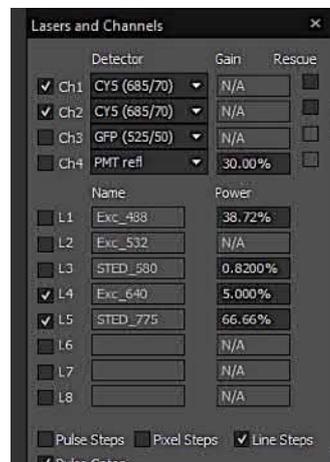
CONFOCAL AND STED IMAGE ACQUISITION

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.

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 → **Save as ,** then choose your folder and save as **.msr**
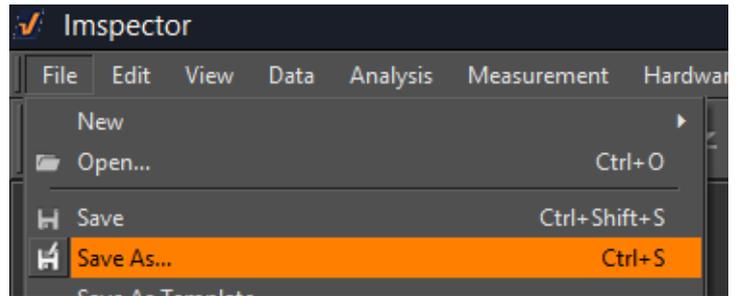
As an alternative, you can export a separate image in most of the known graphical formats:
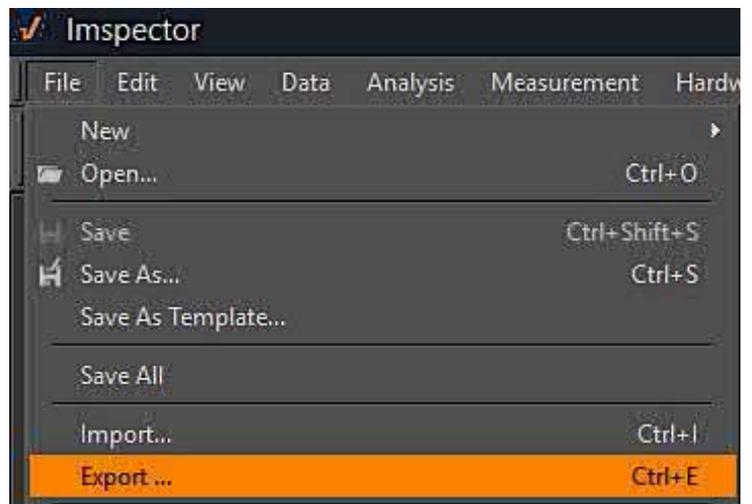
 Acquired images **are not stored** in the memory and every next acquisition will overwrite the previous one, so **you have to save every image/project** that you want to keep .

ASQUARING 3D STACK

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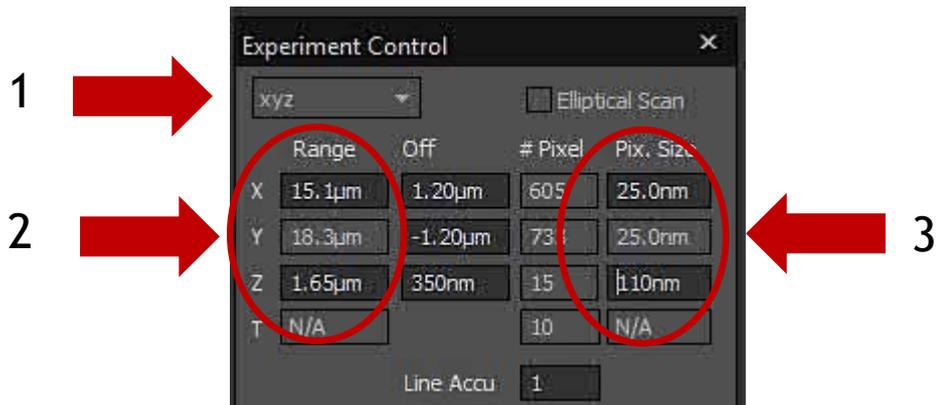
1. Change the XY scan to XYZ.
2. Define dimensions of the stack in all 3 dimensions.
3. Set Pixel size for all 3 dimensions. By setting Z pixel size the thickness of the slices can be changed.
4. Press **<REC>** to start 3D stack acquisition.

ASQUARING 3D STACK

Method 2
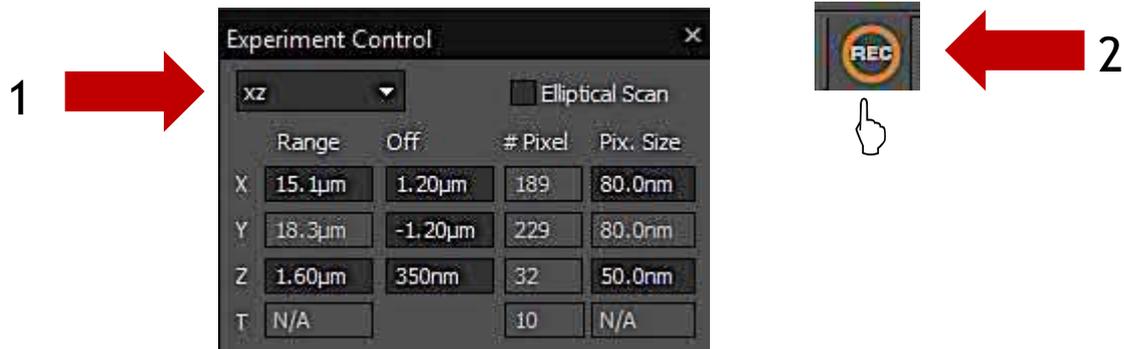
1. Change the XY scan to XYZ.
2. Press **<REC>** to acquire the image in XZ plane.
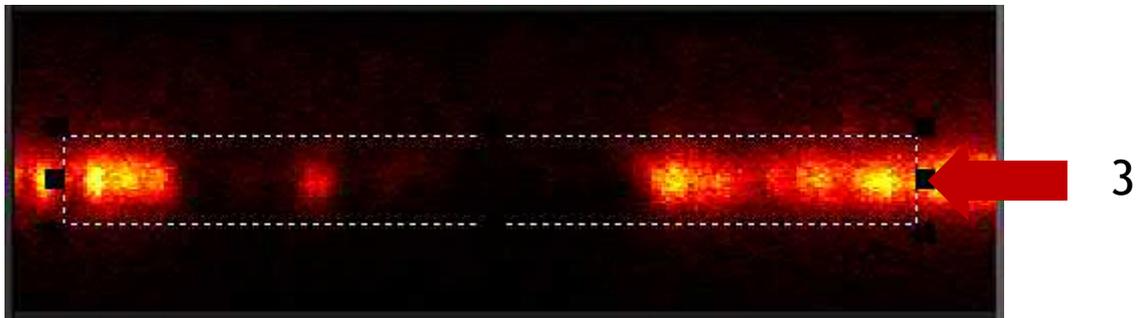
3. Choose the ROI by drawing the rectangle by the mouse, thus the size of the future 3D stack can be chosen.
4. Set Pixel size for all 3 dimensions. By setting Z pixel size the thickness of the slices can be changed.
5. Press **<REC>** to start 3D stack acquisition.