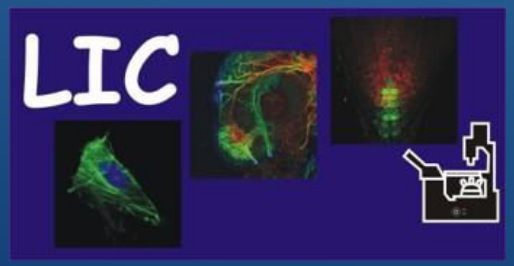
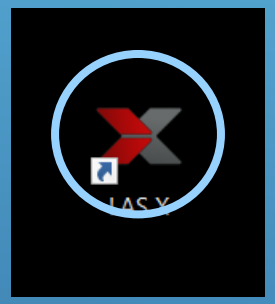
Video Tutorial - Basic

Leica Sp8-I-STED- used for STED

room 00.024

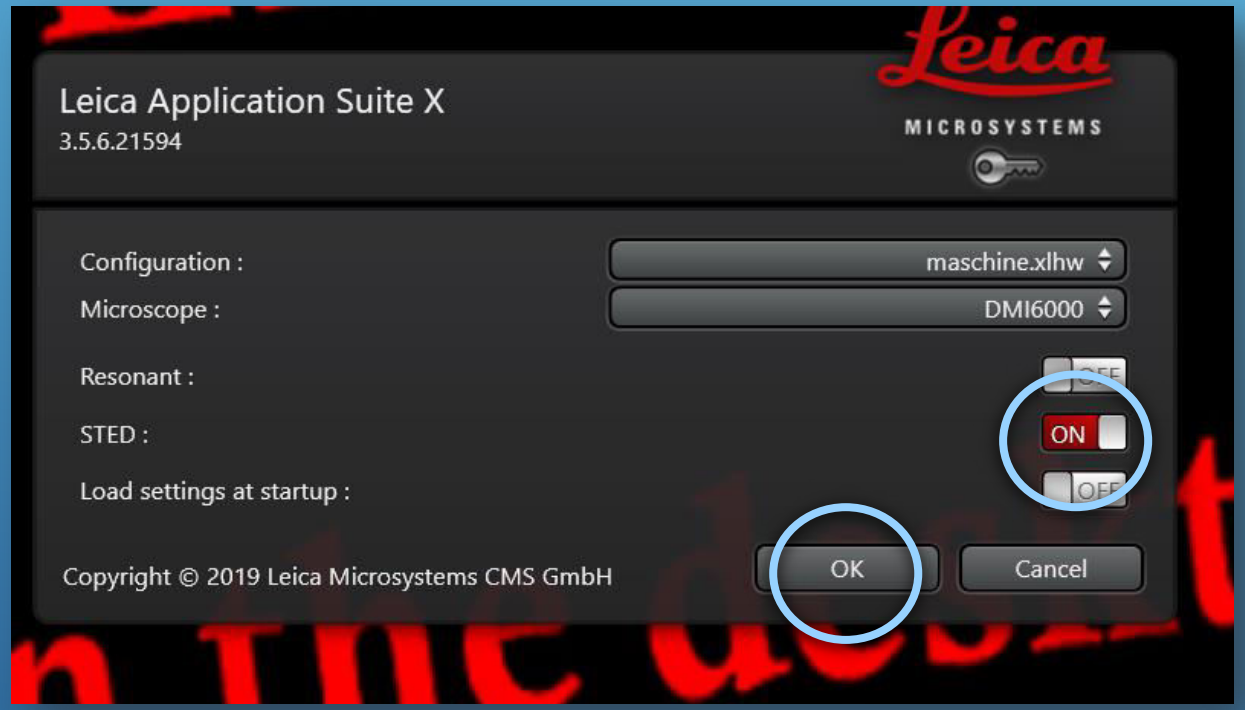
Life Imaging Center 2020





Start Software

Click on LASX Icon to start **LASX** Software



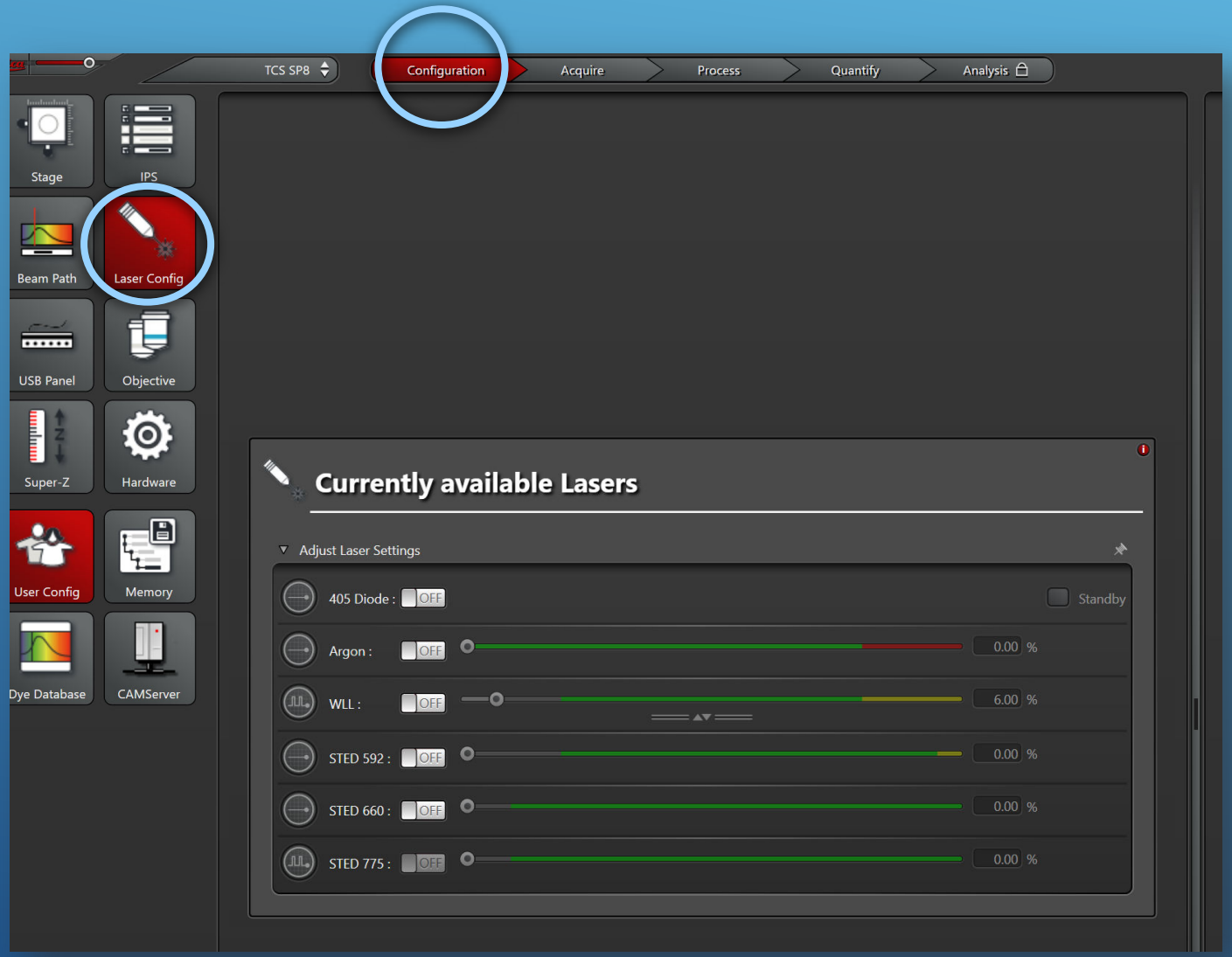
Start Software

Configuration: maschine
Microscope: DMI6000

For STED use

Resonant	**OFF**
STED	**ON**
Load settings	**OFF**

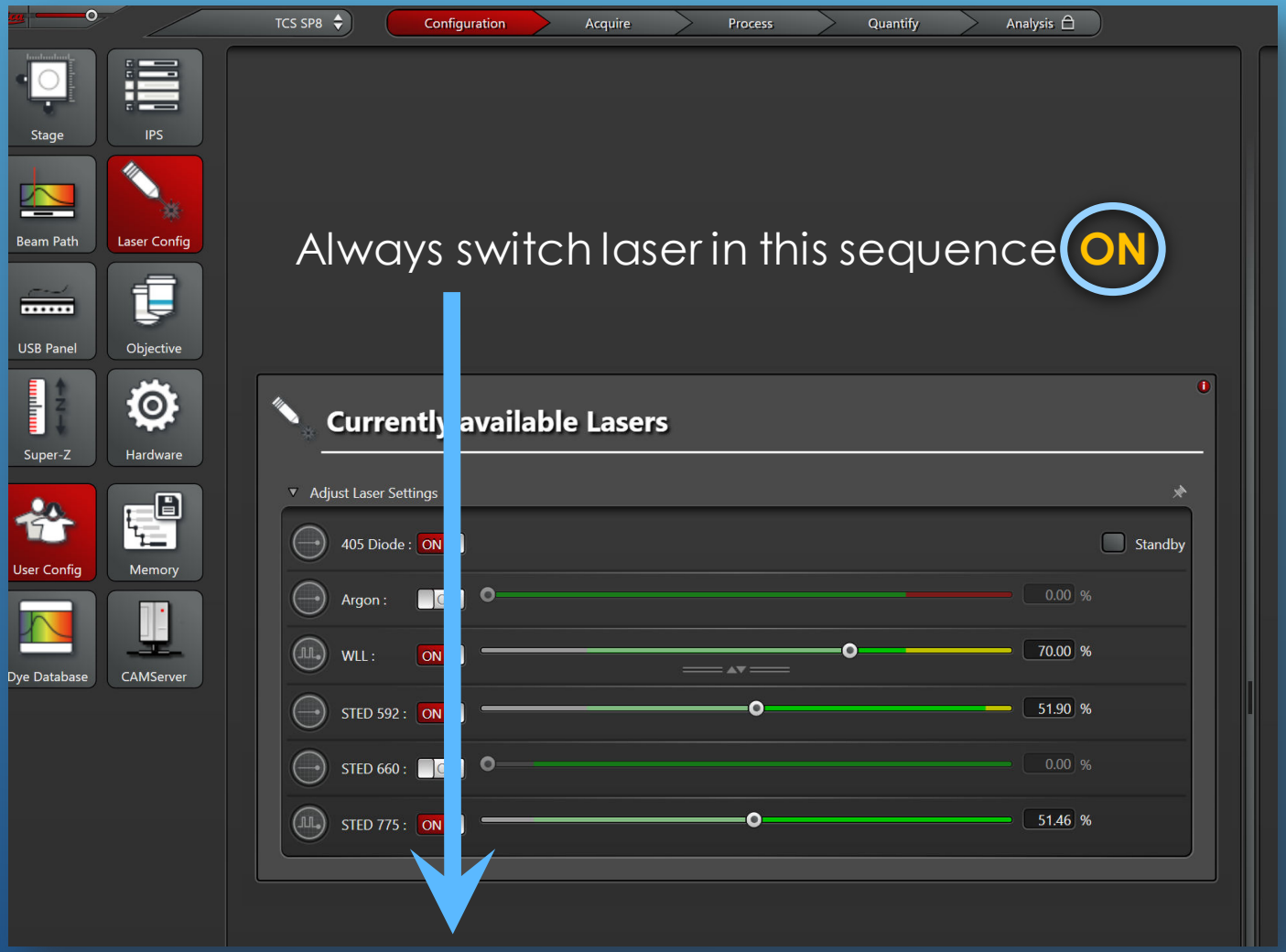
Press **Ok**



Turning on Lasers

Click on the **Configuration** tab at the top of the application

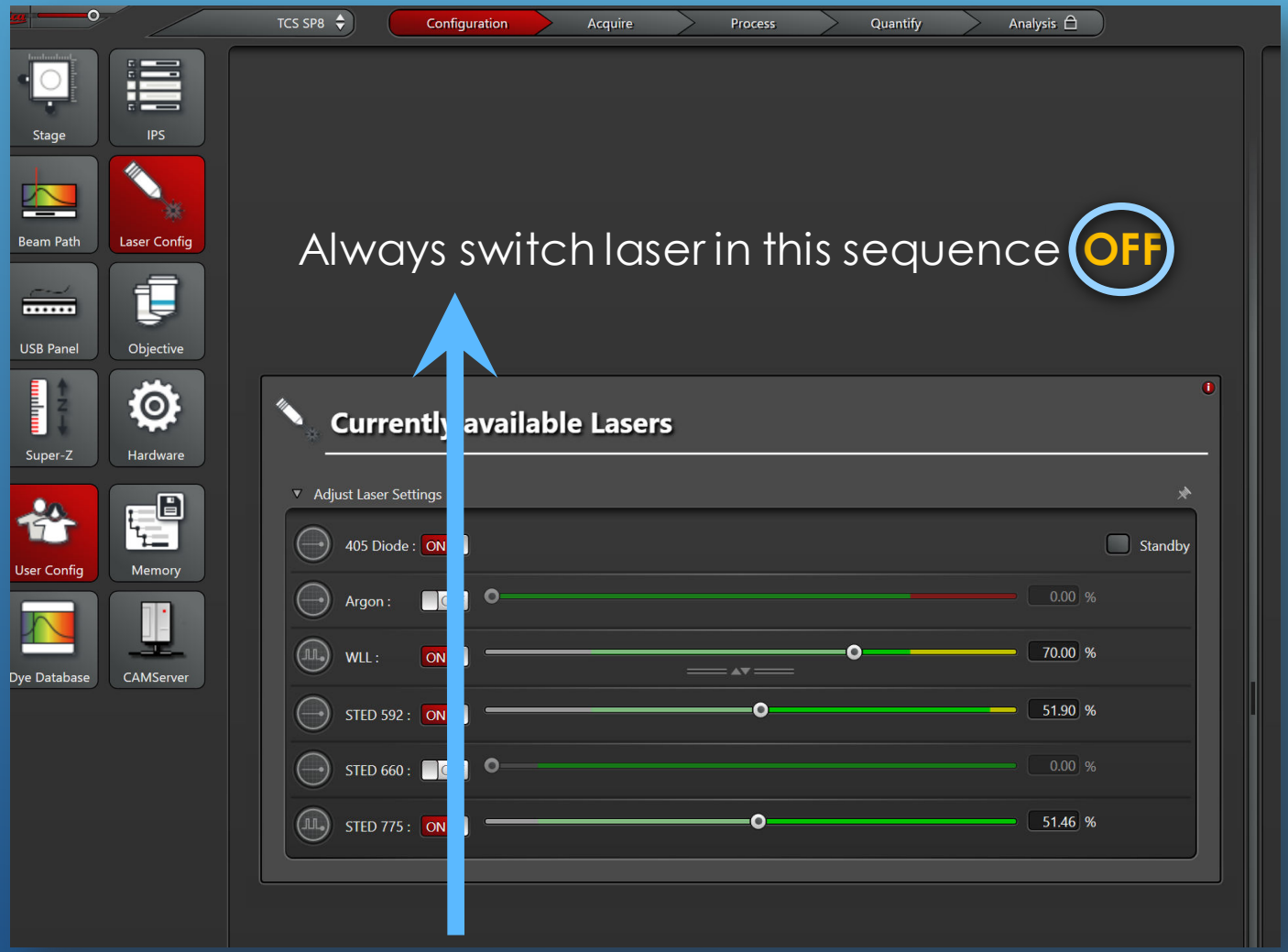
Click on the "**Lasers Config**" **button** within configuration



Turning ON Lasers

Turn **ON** only needed laser

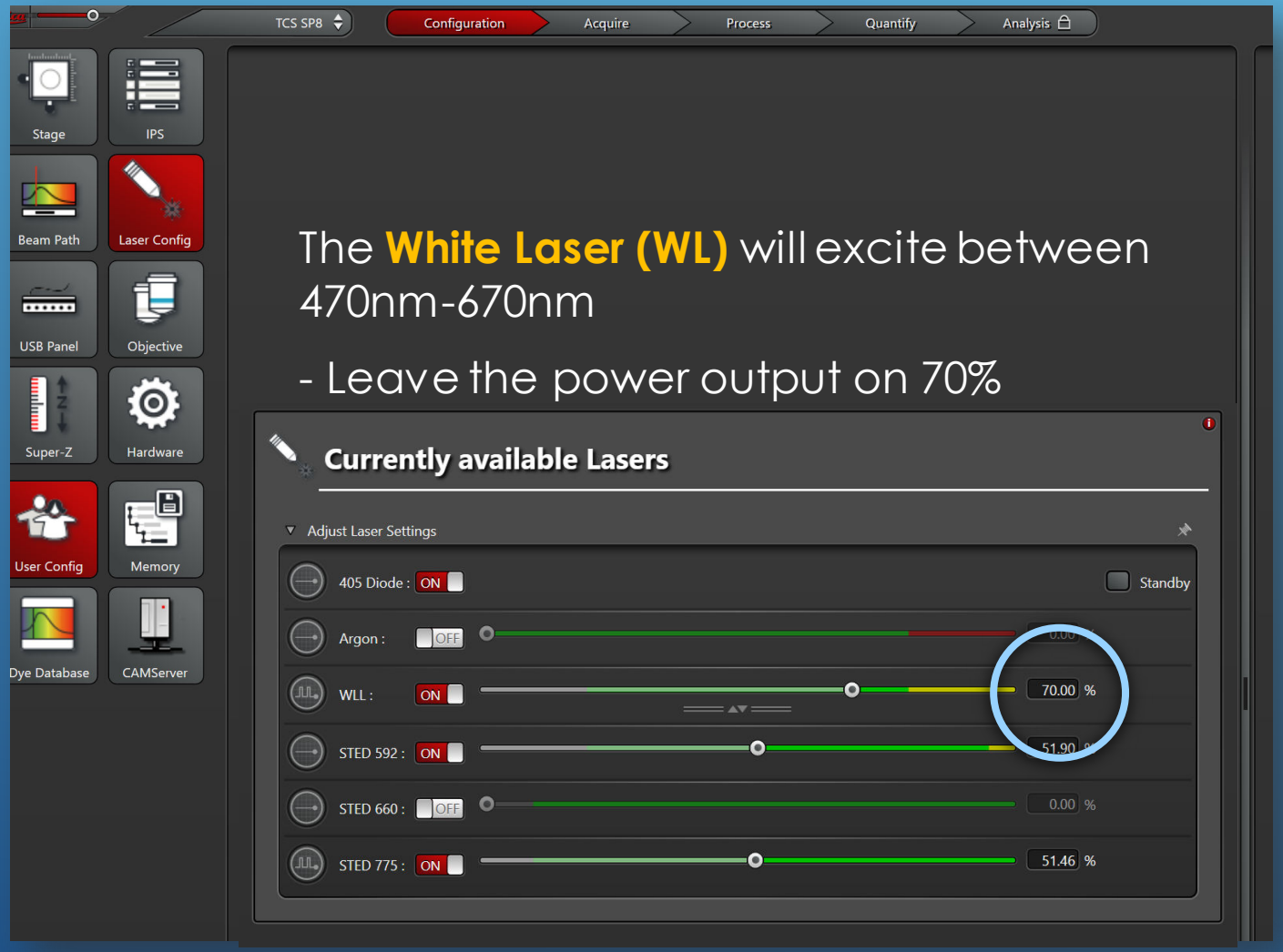
- **!!!** very **important sequence** when switching **on** and **off** laser lines **!!!**
- For STED always 592nm (for alignment)



Turning Off Laser

….after your session

always switch laser in this sequence **OFF**

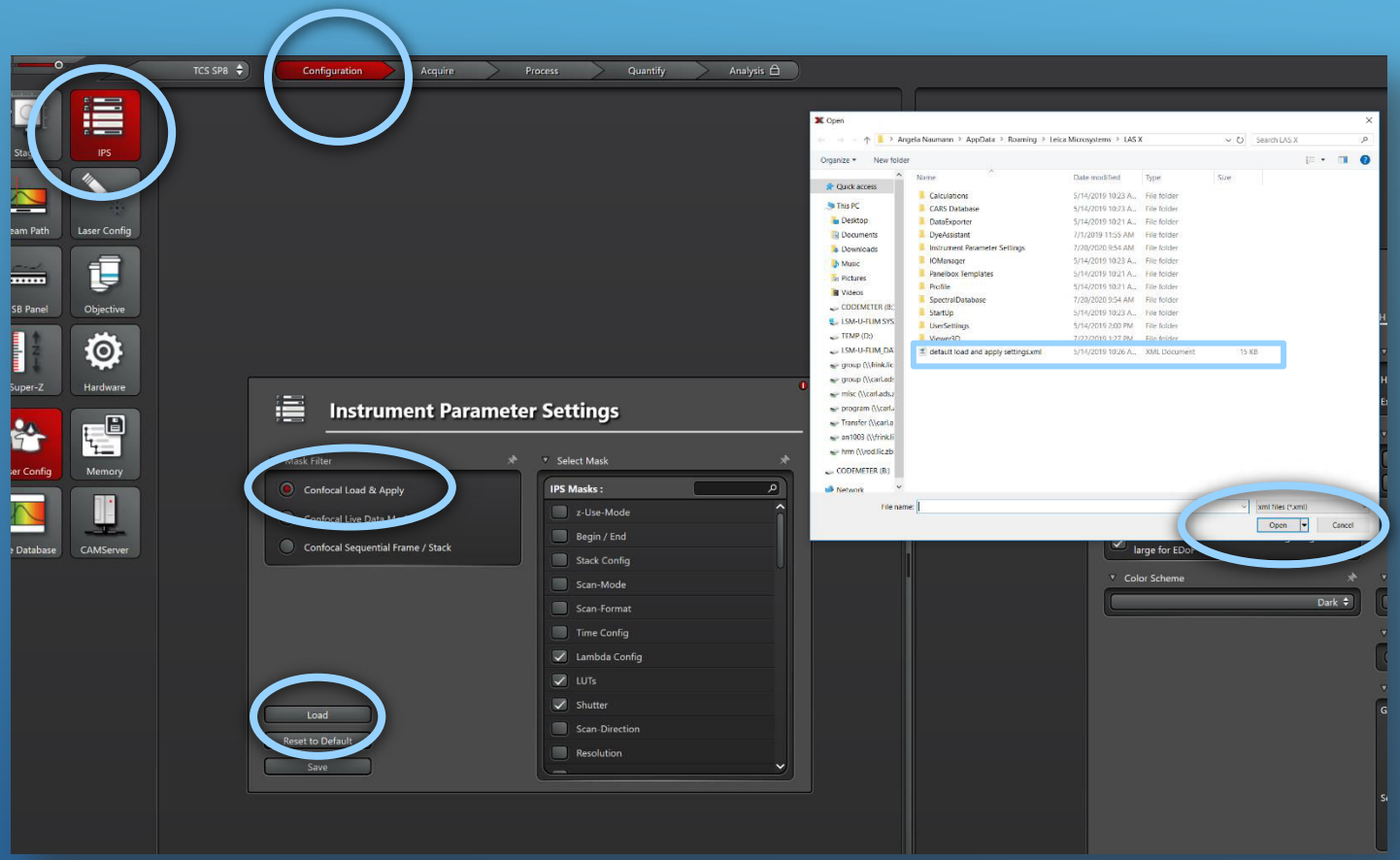


Laser Power

Turn up the power

WL 70%

STED for warming up to 50%



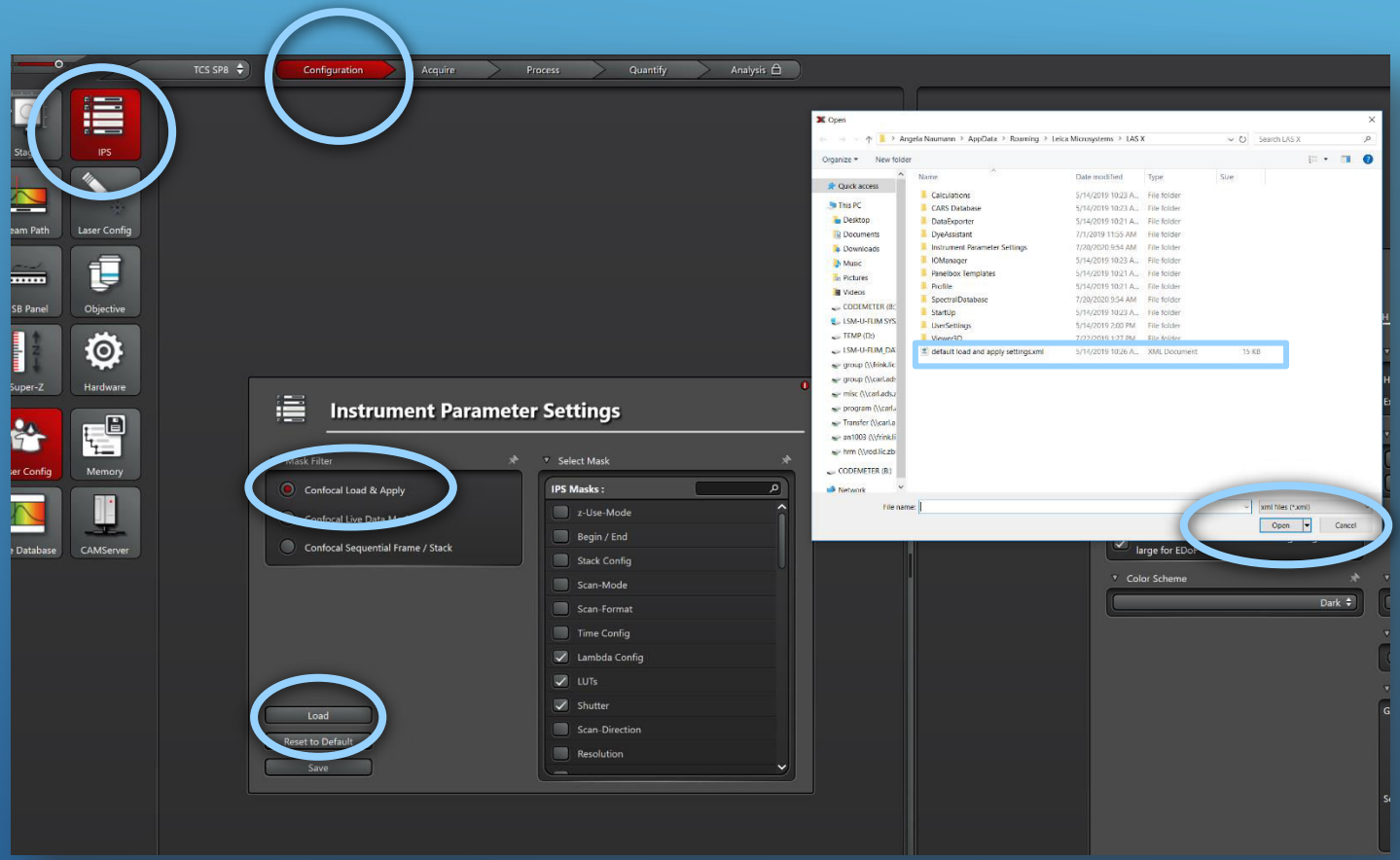
Instrument Parameter:

Open **Configuration**
Open **IPS**

Select „Confocal Load and Apply“
Press Load

Window will open

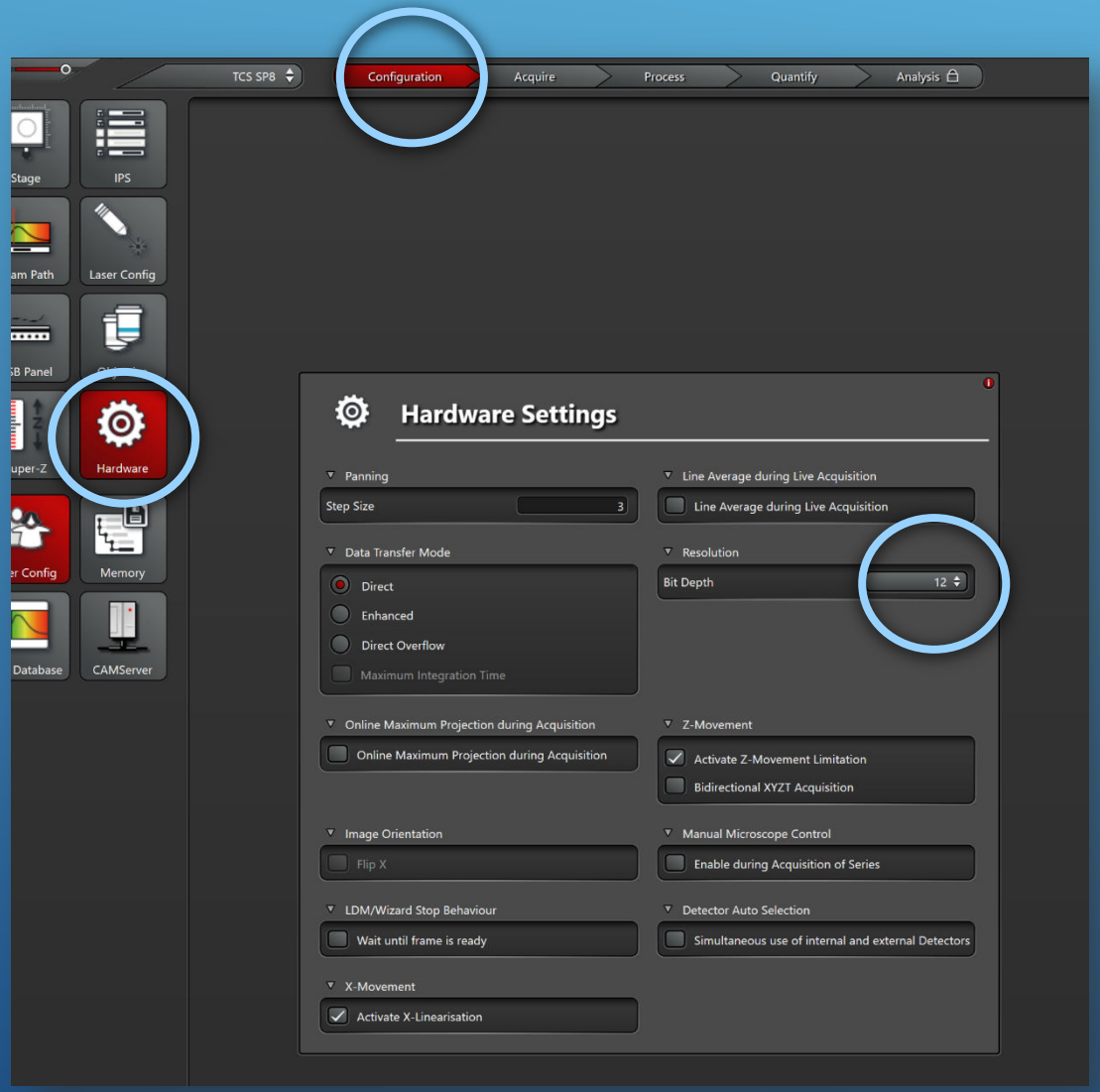
Select „Default Load and Apply“ file
Press Open



Instrument Parameter:

Instrument Parameter defines specific settings, which should be transferred later from an already recorded image using "Apply settings" to the hardware:

ie. Resolution - scan format - channel set up and so on

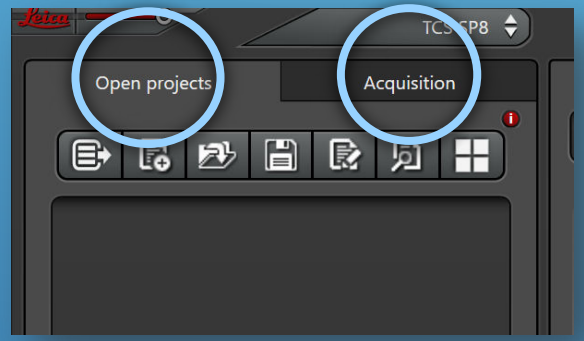


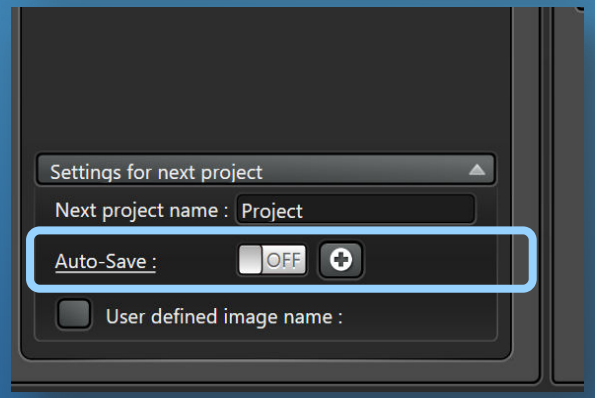
Change Bit Depth

8 bit 256 Grey Shades
12 bit 4095 Grey Shades
16 bit 65536 Grey Shades

i.e. grayscale intensity stored as an 8-bit integer is giving **256** possible different shades of gray from black to white

12 and 16bit are recommended





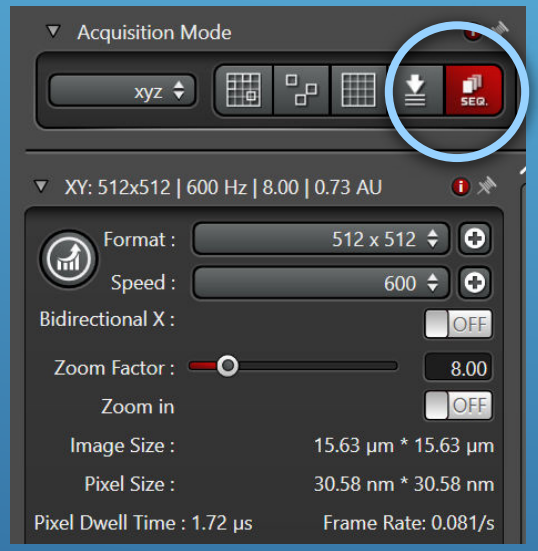
Projects/Acquisition pane

The **Acquisition tab** – which controls the microscope

The **Open Projects tab** - which is the data management area.

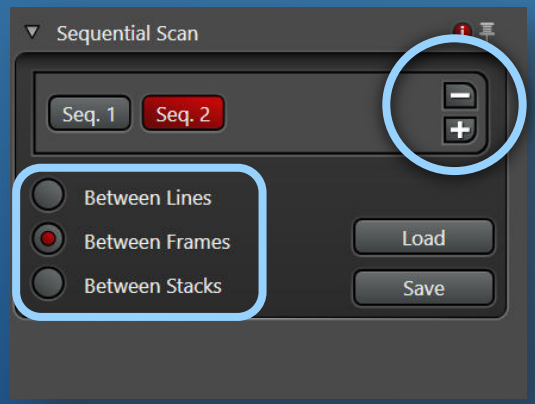
- As image data is captured (using *Capture* image or *Start* - Button in the *Acquisition tab*) it is **temporary** stored under this tab until the user tells the software to save the project

- Please **do not turn** on the **Auto-save function** (bottom of the Project tab)



Acquisition pane – Sequential Scan

The SEQ will indicate that you are scanning sequentially



Plus + and Minus – you can duplicate or remove sequences

- For **Live** Imaging use Between Lines
- For **Fixed** sample use Between Frames
- For **Fixed** sample and **Z-stack** use Between Stacks

Objective for STED

Select the appropiate STED objective inside the software :

- **HC PL APO CS2 93x/1.3 GLY**

Make sure you match the immersion media with the mounting media or use the objective correction ring to adjust in xzy mode.

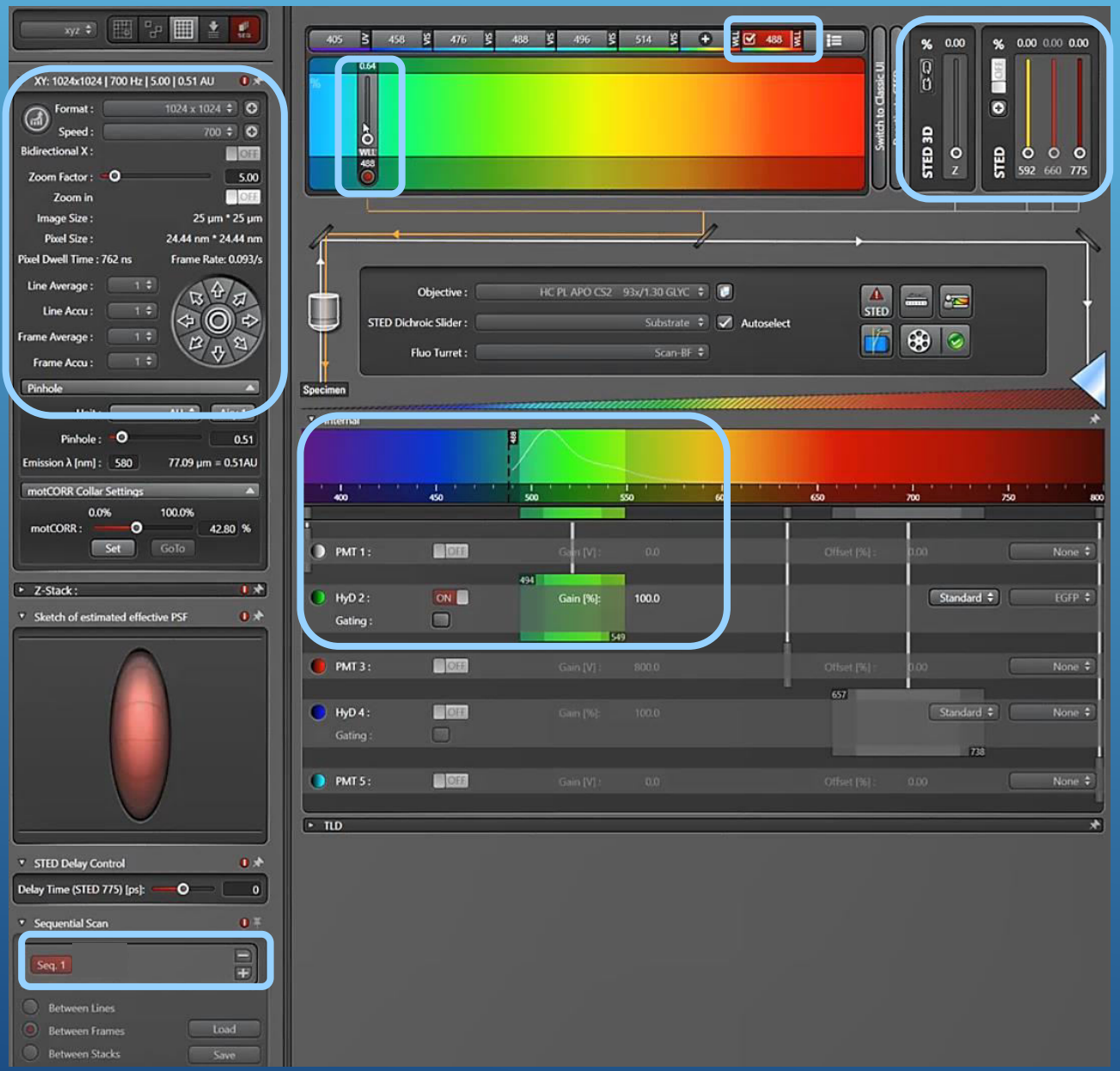
Mismatch will lead to loss of intensity and resolution and STED effect

Light Path - Laser & Detector

Activate **Seq** scan tool

To control whether STED works, we will first set up a confocal and in addition a STED channel,

- Later you work only with a STED channel.



Light Path - Laser & Detector

Set up now Seq 1 as confocal channel

Use for **excitation** of the fluorophores the **WWL** (470 up to 670) and setup specific wavelength - here 488

Activate **HYD** and adjust detection range according to the emission of your fluorophore

Adjust - using ZOOM and FORMAT -**the pixelsize** to 20-30 nm range – here 24nm

Open **STED panel**, but leave laser **OFF**

Light Path - Laser & Detector

To set up the STED channel - **duplicate Seq 1**

Go to **Configuration tab** and **laser window** and increase the during the experiment used STED laser power to ~ **85% -** here the 592nm

Go back to **Acquisition tab** and configure Seq 2 as STED Channel

Light Path - Laser & Detector

STED % switch **ON**

- Select the **appropriate depletion laser** for your fluorophore.

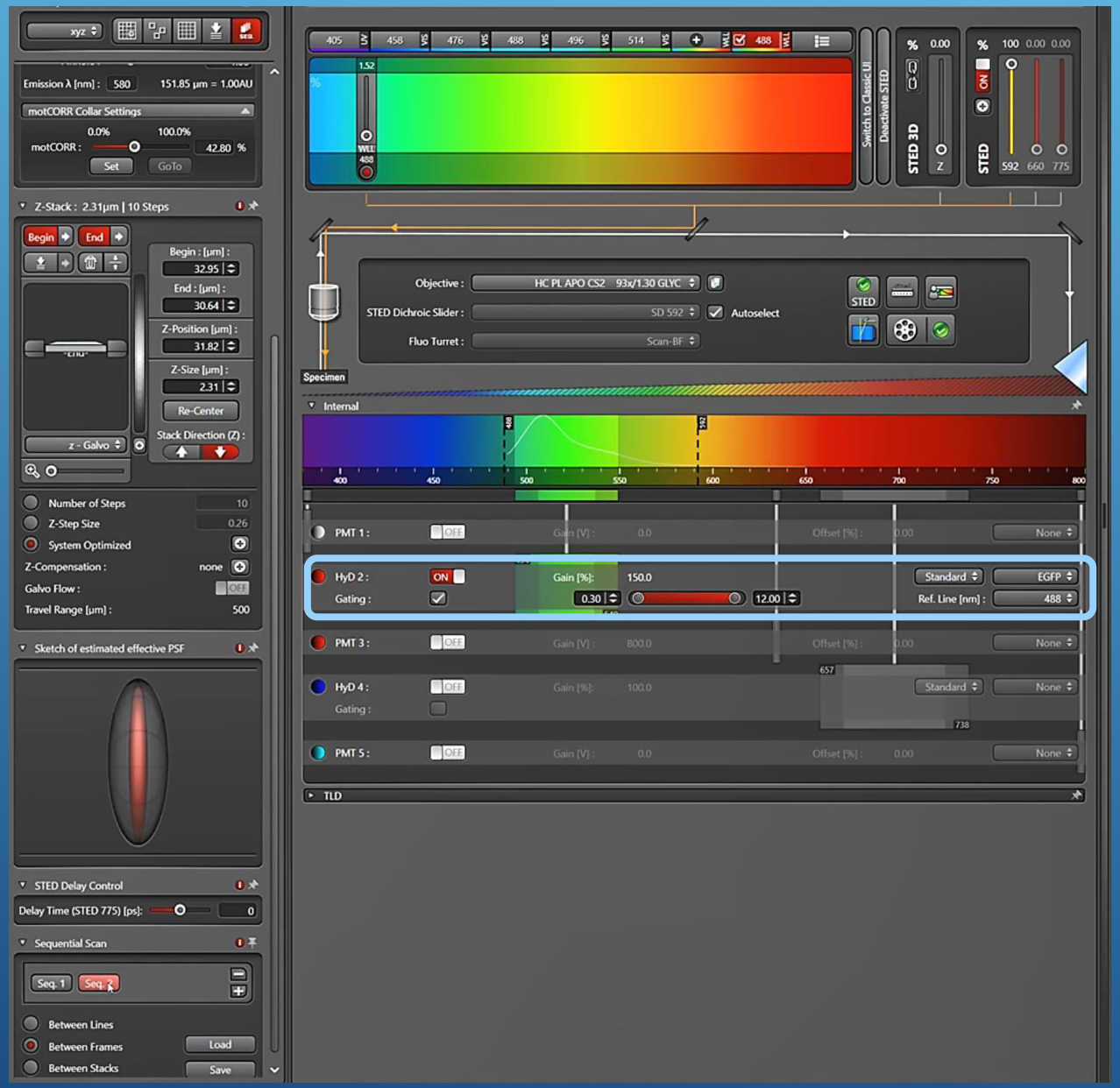
Leave STED 3D % - **OFF**

Note that no inactive detector may be positioned below this laser-line.

Light Path - Laser & Detector

Altering here **% Power** changes STED effect, i.e. the resolution in XY, see visualization of the PSF.

However remember, bleaching also increases with higher STED % power.



Light Path - Laser & Detector

To achieve even better effects, activate **Gating**

With **Gating** you define the start and end of your image acquisition in relation to your laser pulse (ref. line):

- exclude background of laser refection
- increase STED resolution
- decrease intensity (need higher laser %)

Thanks for viewing