

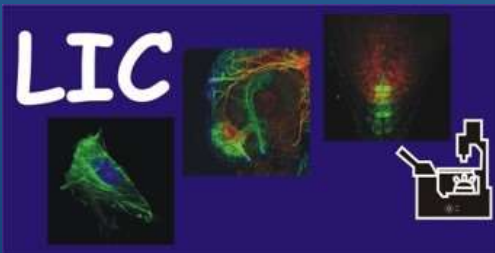


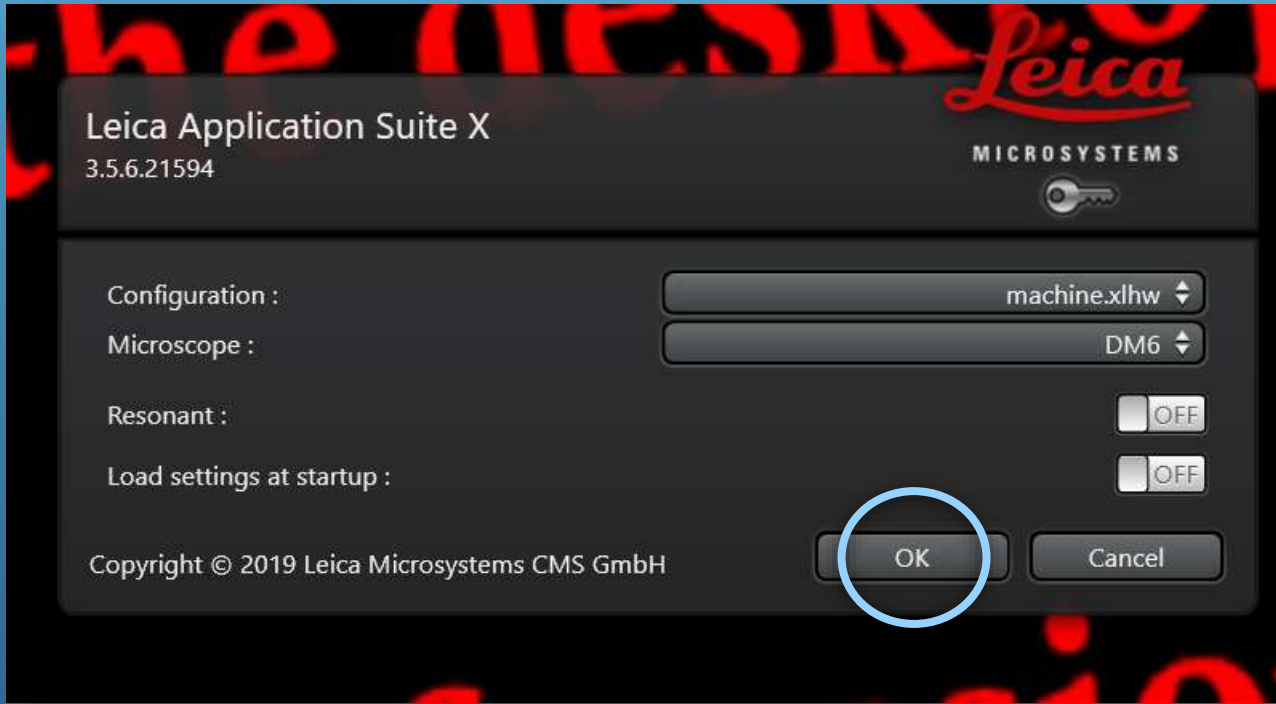
Video Tutorial - Basic

Leica Sp8-U-FLIM

room 00.017

Life Imaging Center 2020



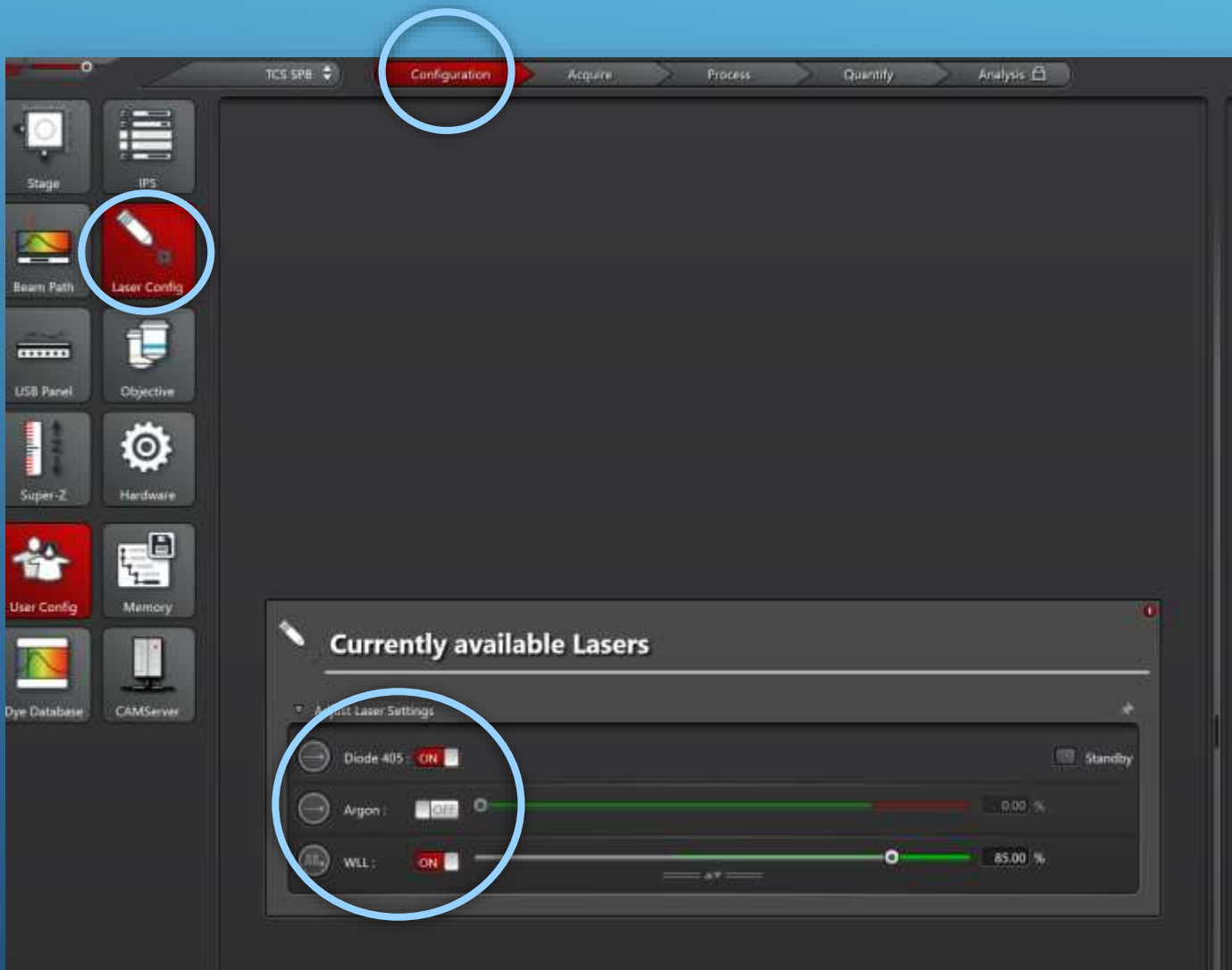


Start Software

Start **LASX** Software

Press **Ok**



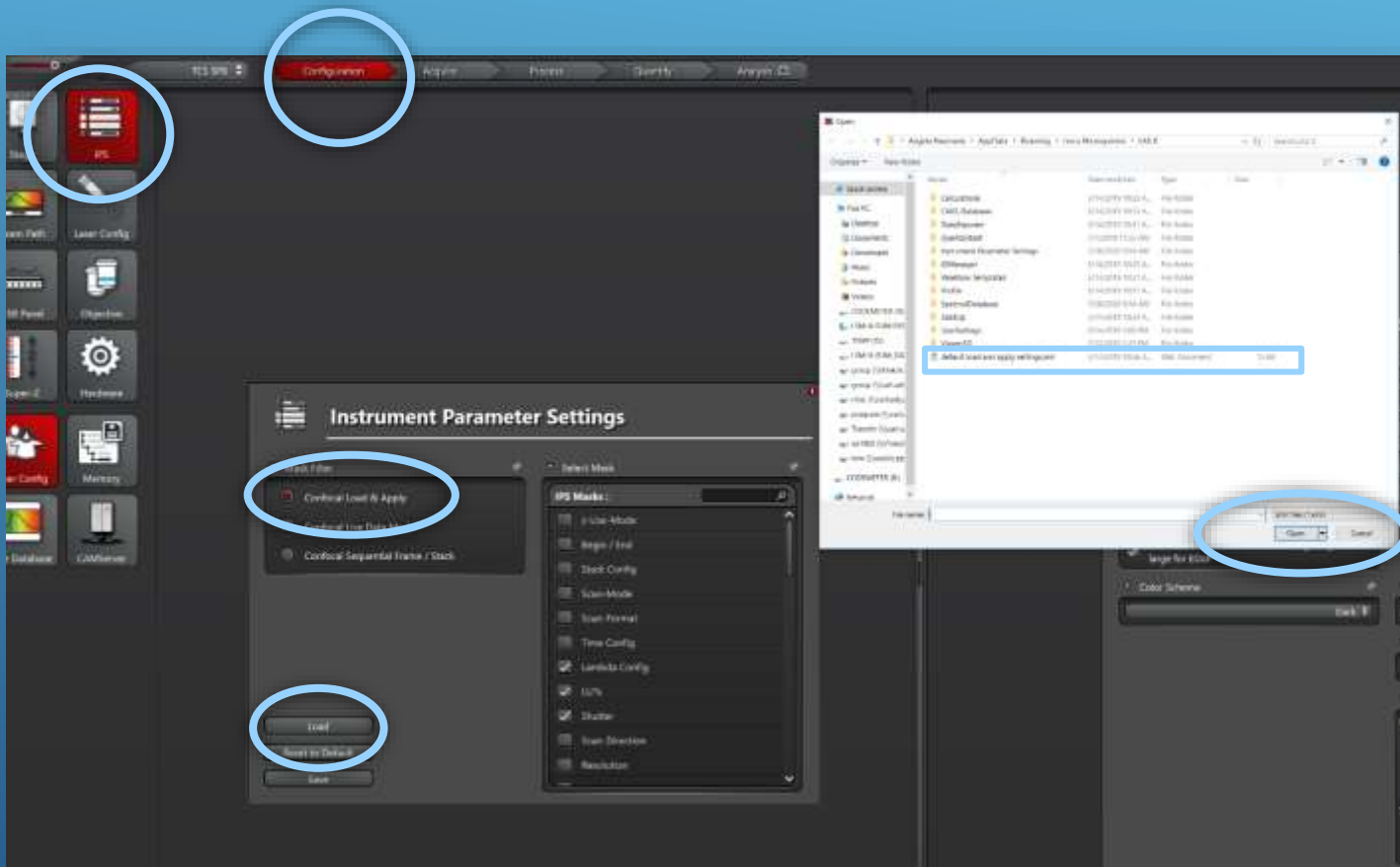


Start Laser

Open **Configuration**
Open **Laser Configuration**

Switch Specific Laser **ON**

The **White Laser (WL)** will excite between 470nm-670nm.
Leave the poweroutput on 85%



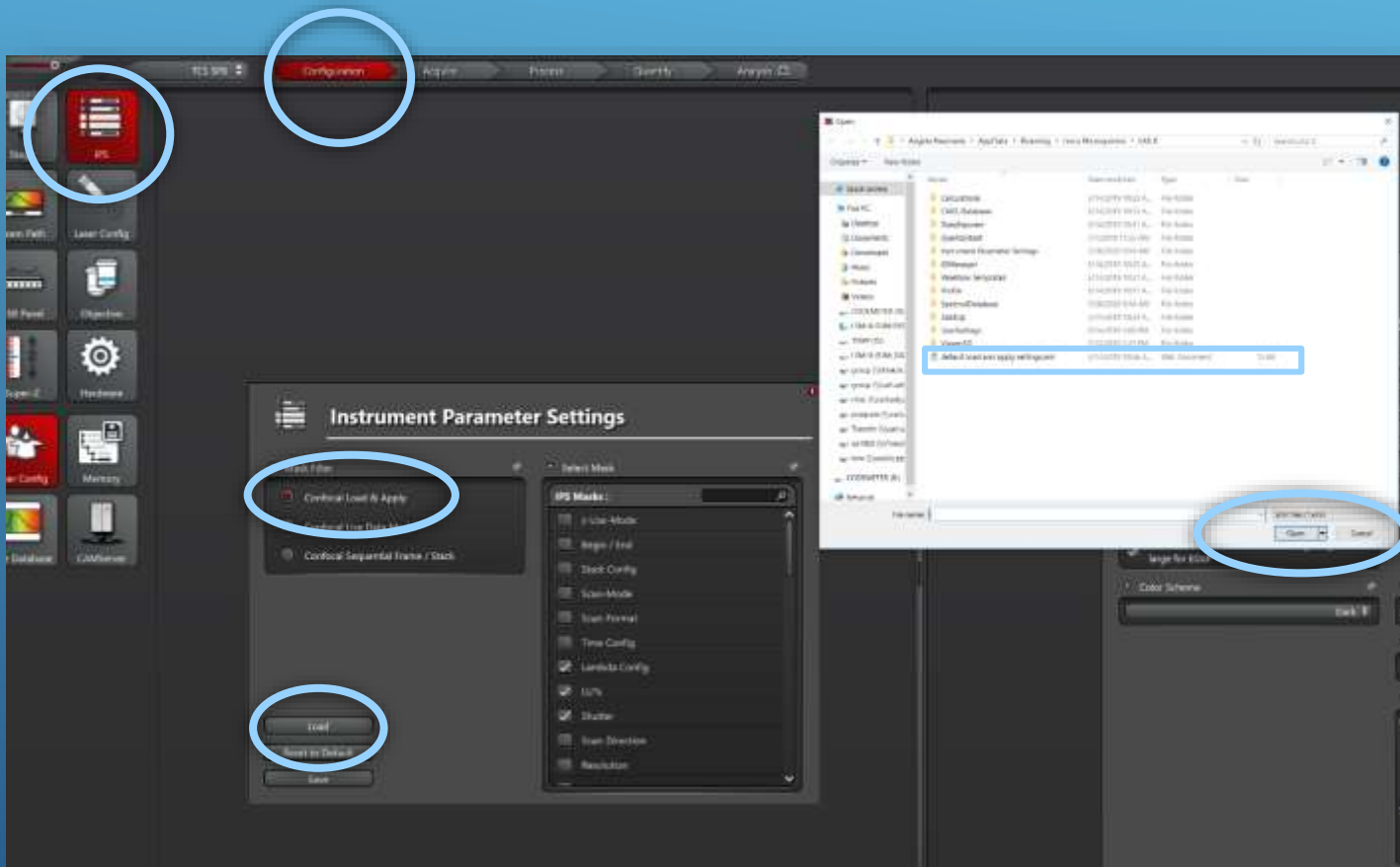
Instrument Parameter:

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

Select „Confocal Load and Apply“
Press **Load**

Window will open

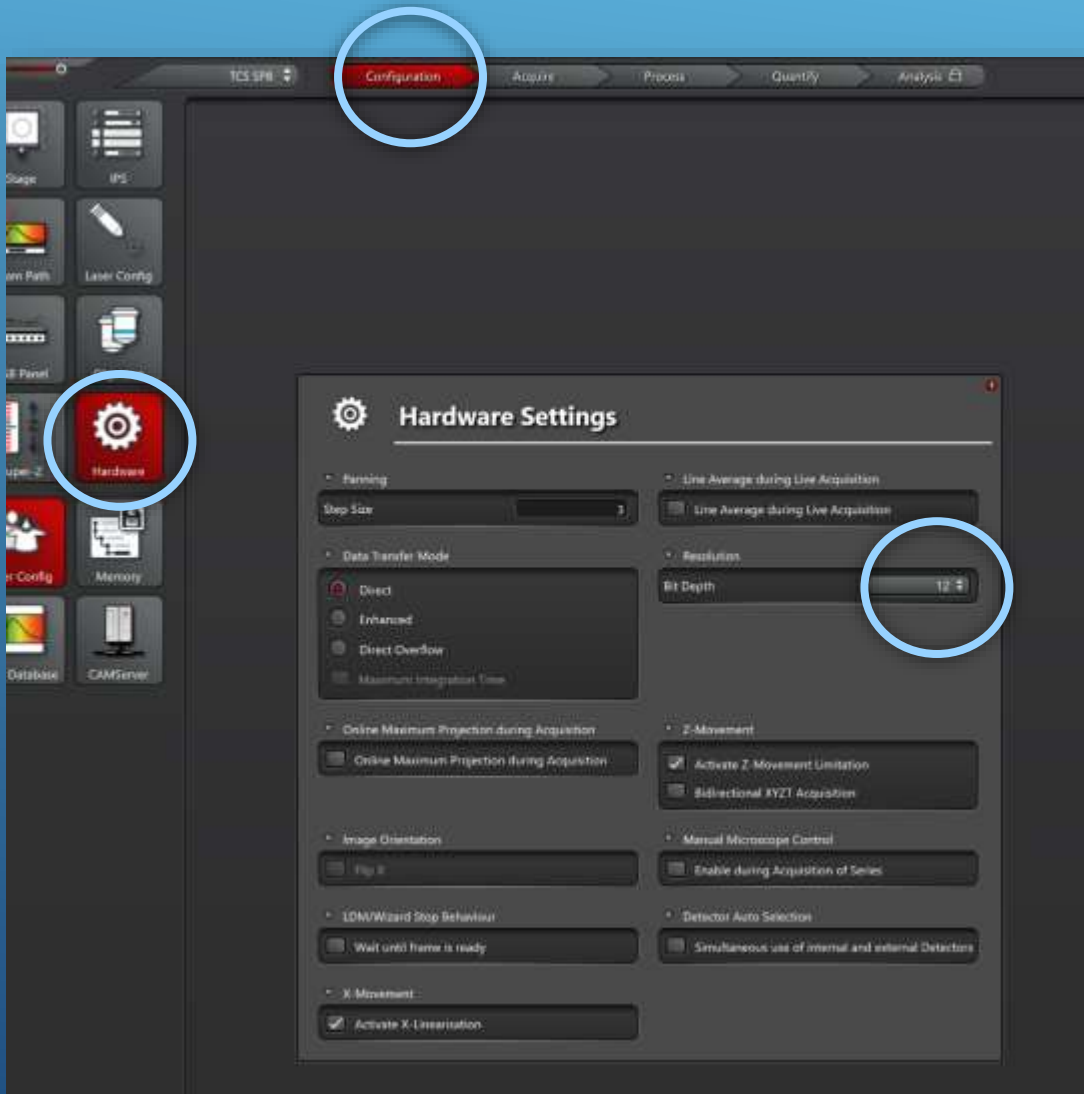
Select „Default Load and Apply“ file
Press **Open**



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

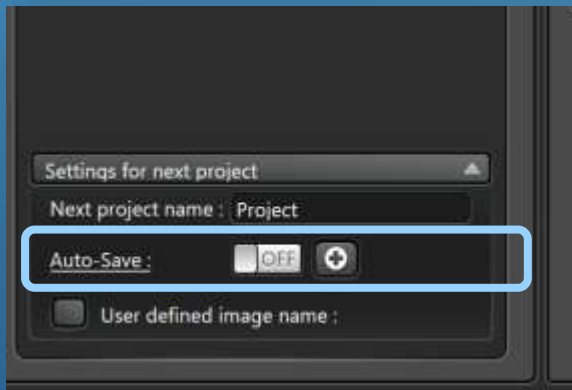
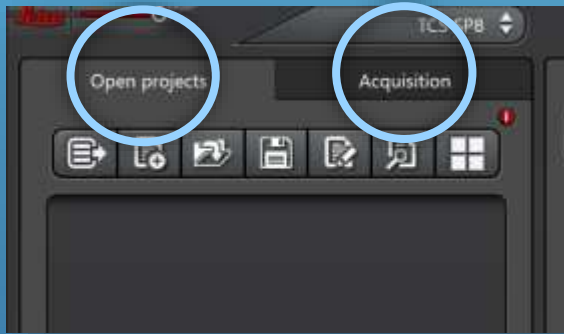


Change Bit Depth

- 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

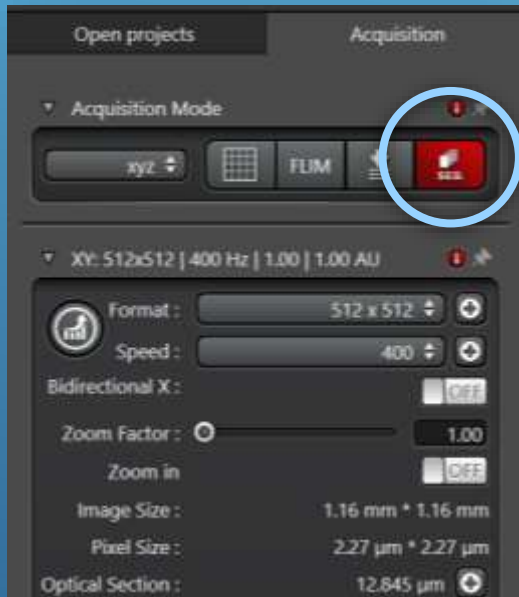


Projects/Acquisition pane

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

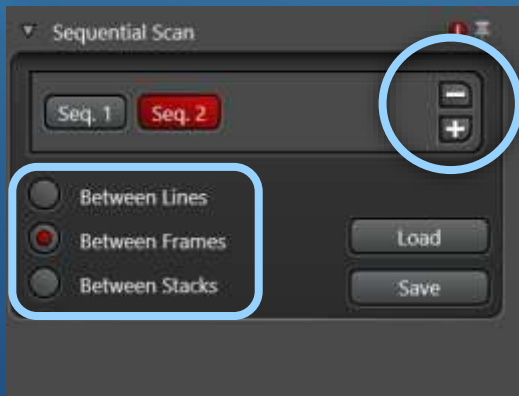
The **Open Projects tab** - which is the data management area.

- 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 - 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**

Light Path - Laser & Detector

Access to **UV** (405) **VIS** (458, 476, 488, 496, 514)
Wight-Light Laser – **WWL** (470 up to 670)

You can open the **Dye-Assistant**.

- Choose fluorochromes from the drop down list and detector type



Light Path - Laser & Detector

Photomultiplier – PMT

- Used with bright signals (like Dapi)
- Access to Gain and Offset

Hybrid Detector – HYD

- Used with weak signals
- Extremely light sensitive
- No access to Offset
- Standard Gain is 100%

Never turn laser (on) over an inactive or active detector



Hybrid Detector - Modi

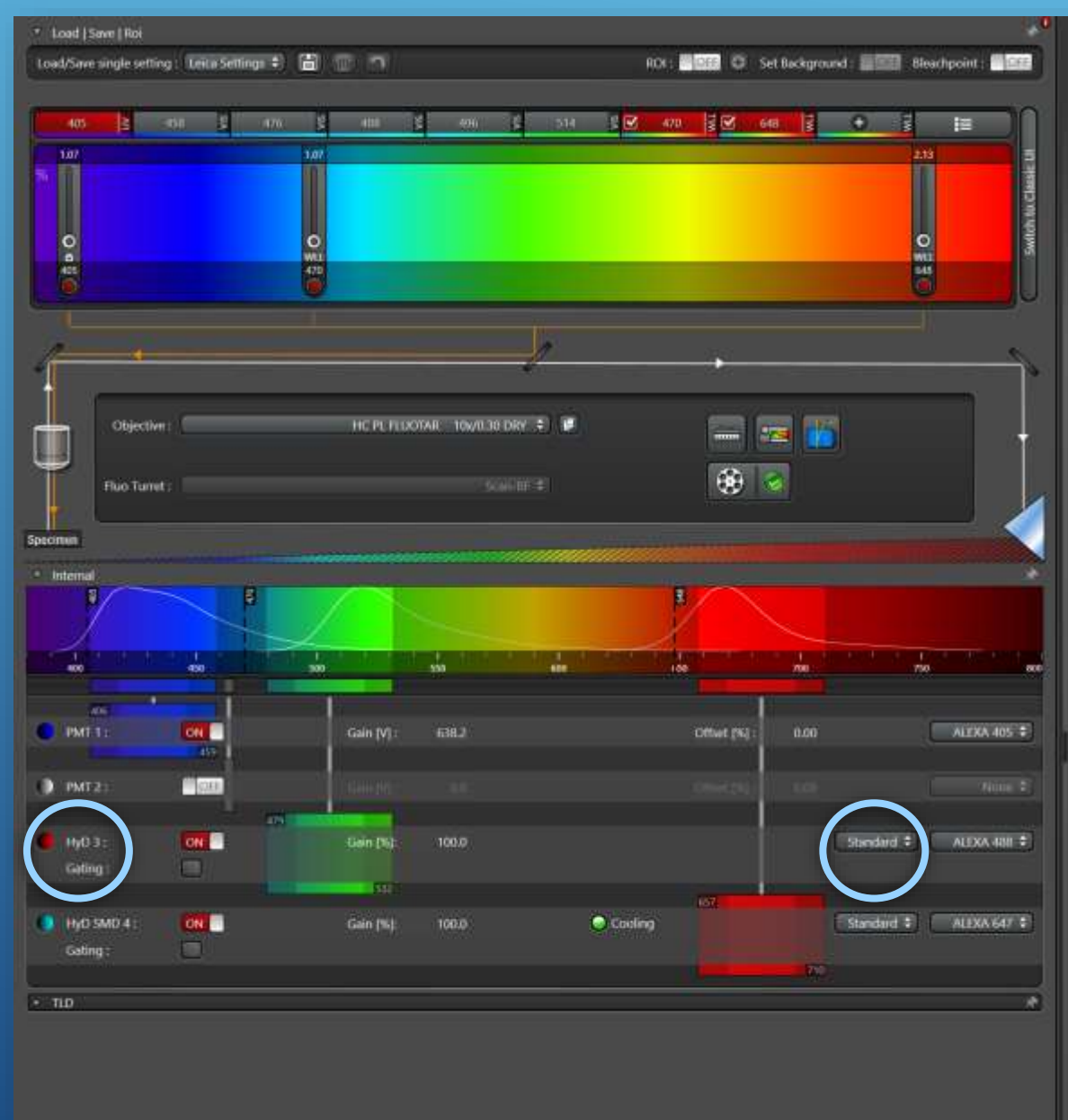
Standard mode used for default image acquisition. Start with gain on 100% and adjust the laser power

Counting mode will display the image based on the number of photons detected per pixel over a constant integration time

- used for ratio imaging and image correlation

BrightR mode makes it possible to display very bright and weakly fluorescent structures within an image.

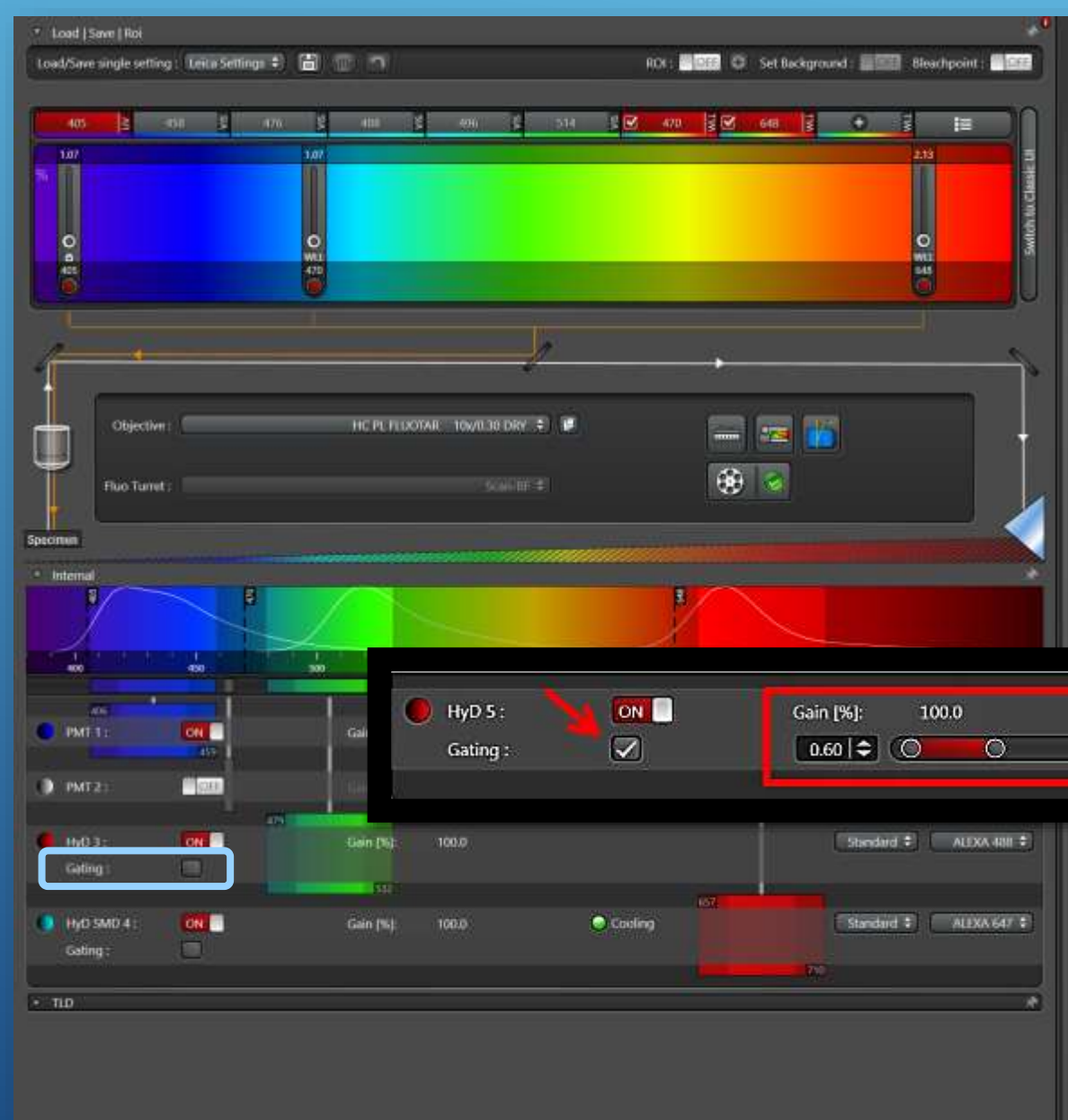
- used for ratio imaging and image correlation



Hybrid Detector - Gating

Gating defines **Start and End** of image acquisition in relation to the laser pulse by using the slider

- Could be used to exclude the background generated sometimes by excitation light



Light Path - Laser & Detector

Simultan - Scanning

- Example: here simultan scan with three laser lines:
 - Risk of bleed through
 - But fast

However we recommend : **sequential scan**





Light Path - Laser & Detector

Sequential - Scanning

- Example: here sequential scan with three laser lines:
 - Reduced risk of bleed through
 - Slower than simultan scanning

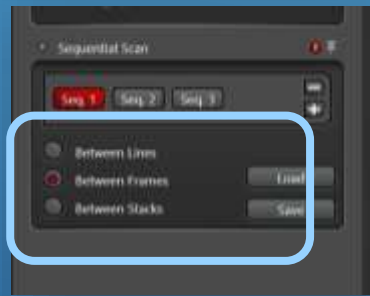




«**Live**» button [lower left corner] is used for a preview scanning

«**Capture Image**» will acquire just the active channel as image, no z-stack

«**Start**» button used to scan all channel, z-stack – start whole experiment



Switch Seq. between each

- **Line** - used for live sample,
- **Frame** or **Stack** used for fixed sample



Scan speed can be adjusted individually

Scan speed is measured in Hz (lines per second) between 10 -1800Hz

- Lower speed – higher signal to noise ratio (SNR), risk of bleaching
- Faster speed – lower signal to noise ratio (SNR), low risk of bleaching

We recommend as default speed: 600





Bi-directional

Bidirectional scanning will double the speed as pixels are recorded in both directions!



If you encounter mismatch in the phase, you can correct this with the control panel (phasecorrection).



Average Function (arithmetic mean as final pixel value)
Used to improve image quality

- **Line** - recommended - especially for live sample
- **Frame** - used for weak and strong bleaching dyes

Rotation used to orientate the sample





Scan Format

Preview

- For preview scanning use 512x512 pixel as default

Acquisition

- For optimal resolution, use the **«optimal»** button.
- This will take into account the NA of the objective and the active channel wavelength and **set up the recommend pixelsize**
- Therefore always optimize with the same Seq. active to avoid differences in your experiment





Acquisition Z-stack

Make sure that you are in XYZ - Mode

Define begin and end

Press «**Live**» and move to the **top** of sample region of interest using the right z-position knob of the Leica panel tool in front of you – **click « begin »**

Move through the sample to the **bottom** of the region of interest– **click « end »**

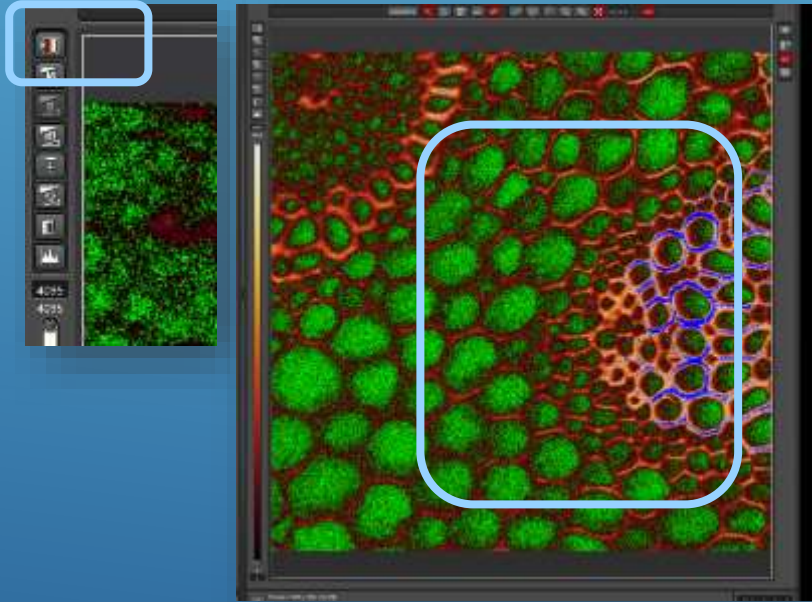
Define the z-step size

- manually (Z-step size) or
- choose « **optimized** », which calculates the best resolution of system and set the size to 50% overlap. This is needed for high resolution and 3D reconstruction. **Recommended**

Additional Tips - Range indicator

- «**Range indicator**» button
- top left at the image container
- used to avoid **over-and under-saturated pixel**

- « **Blue pixels** » saturated
- « **Green pixels** » undersaturated
- « **Black-red-yellow-white** » = good signal



Thanks for viewing

Additional Information

- Video Tutorial: Navigator Leica Sp8-U-FLIM



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