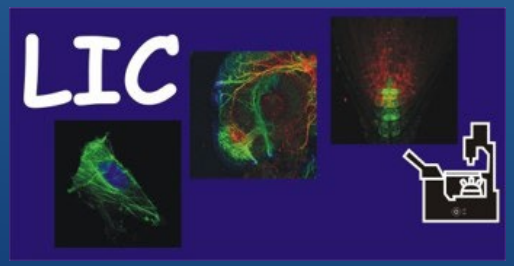
Video Tutorial - Basic

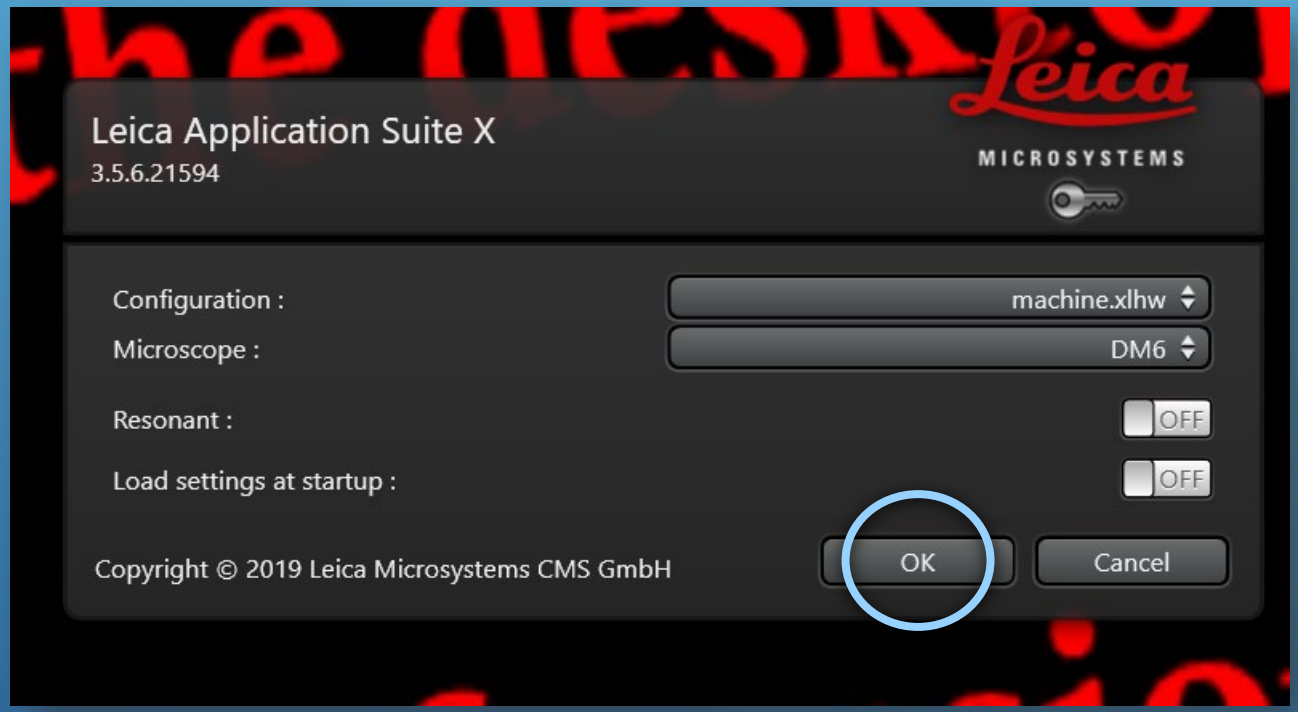
Leica Sp8-U-FLIM (confocal use)

room 00.017

Life Imaging Center 2020



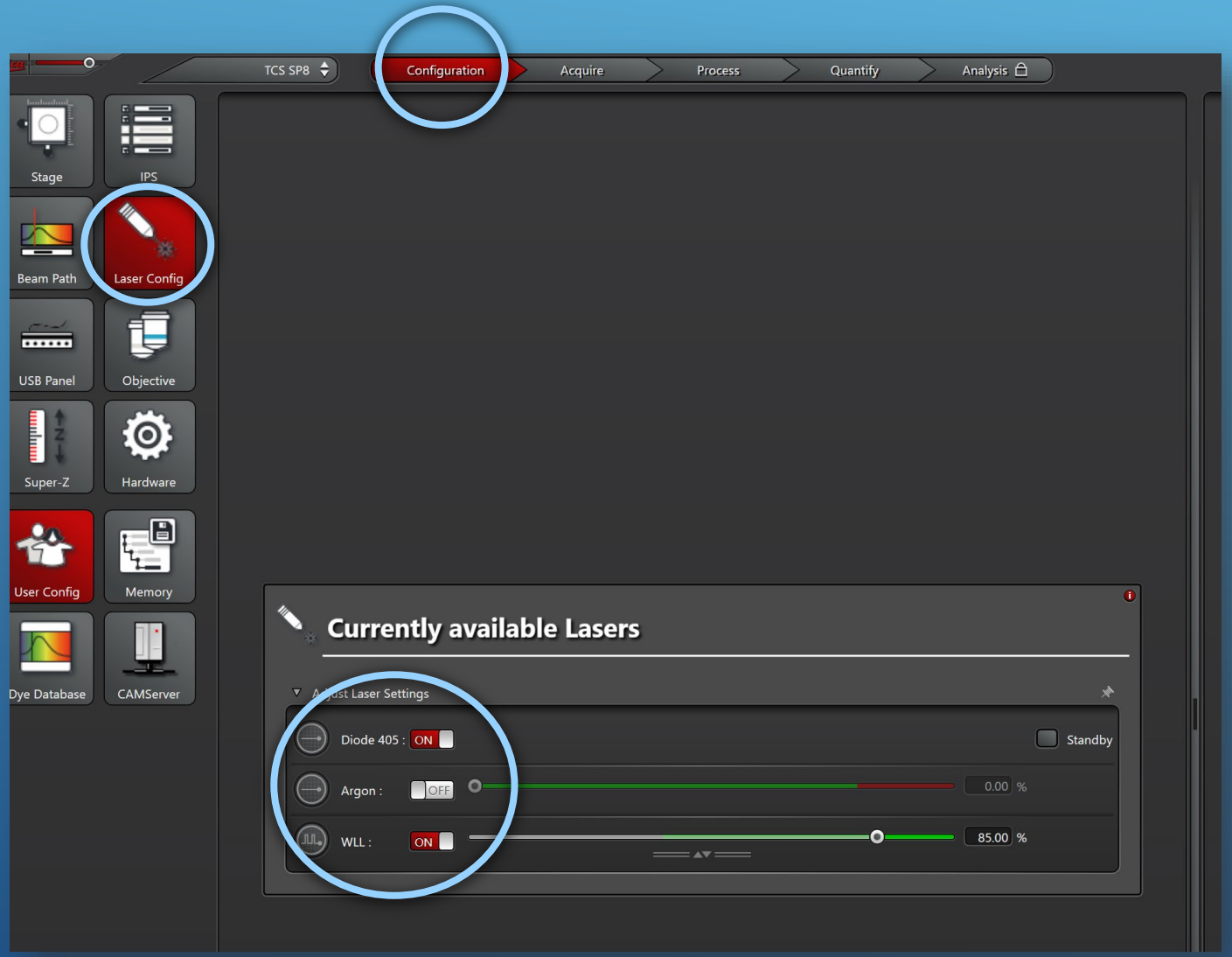
universität freiburg



Start Software

Start **LASX** Software

Press **Ok**

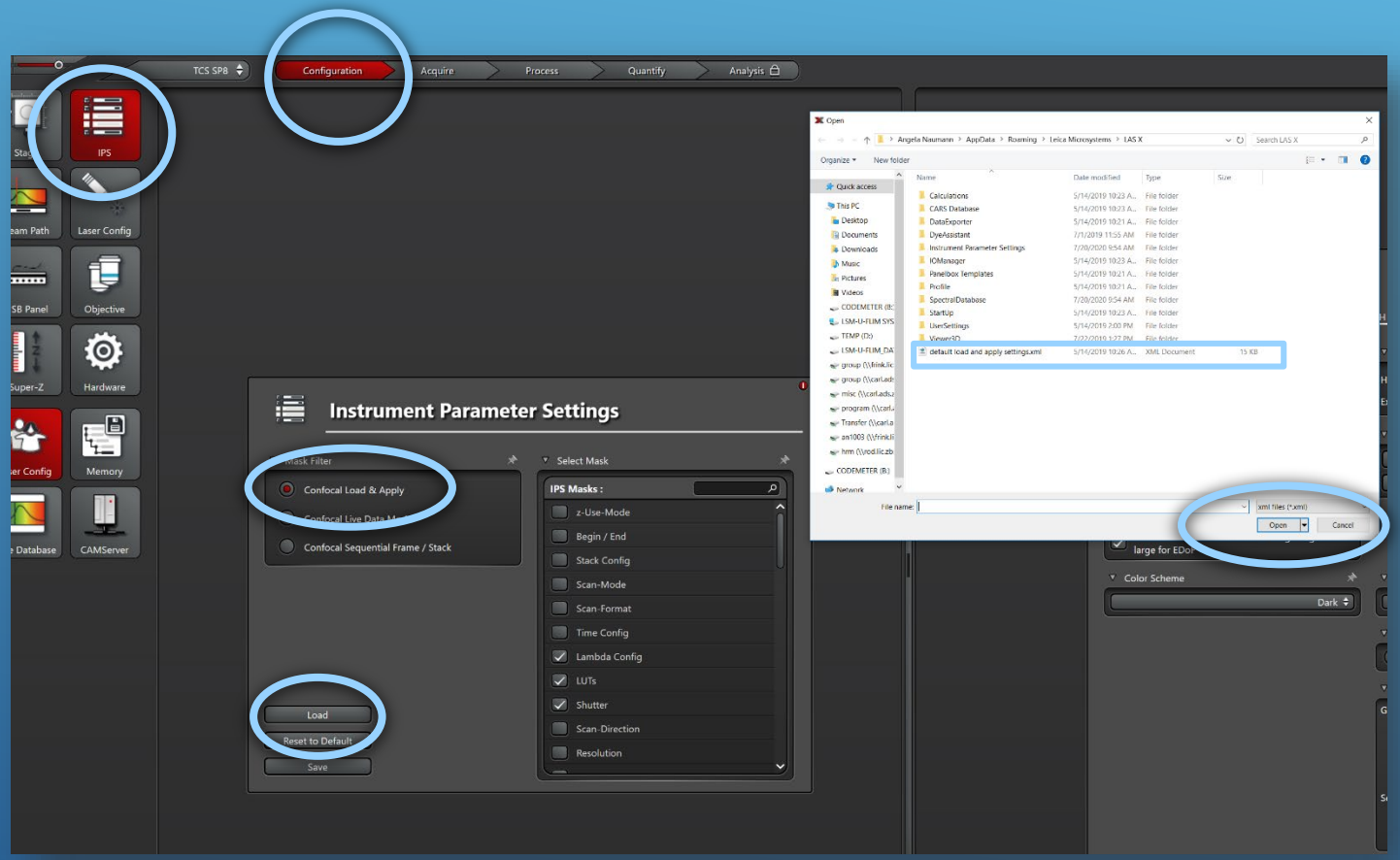


Start Laser

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

Switch Specific Laser **ON**

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



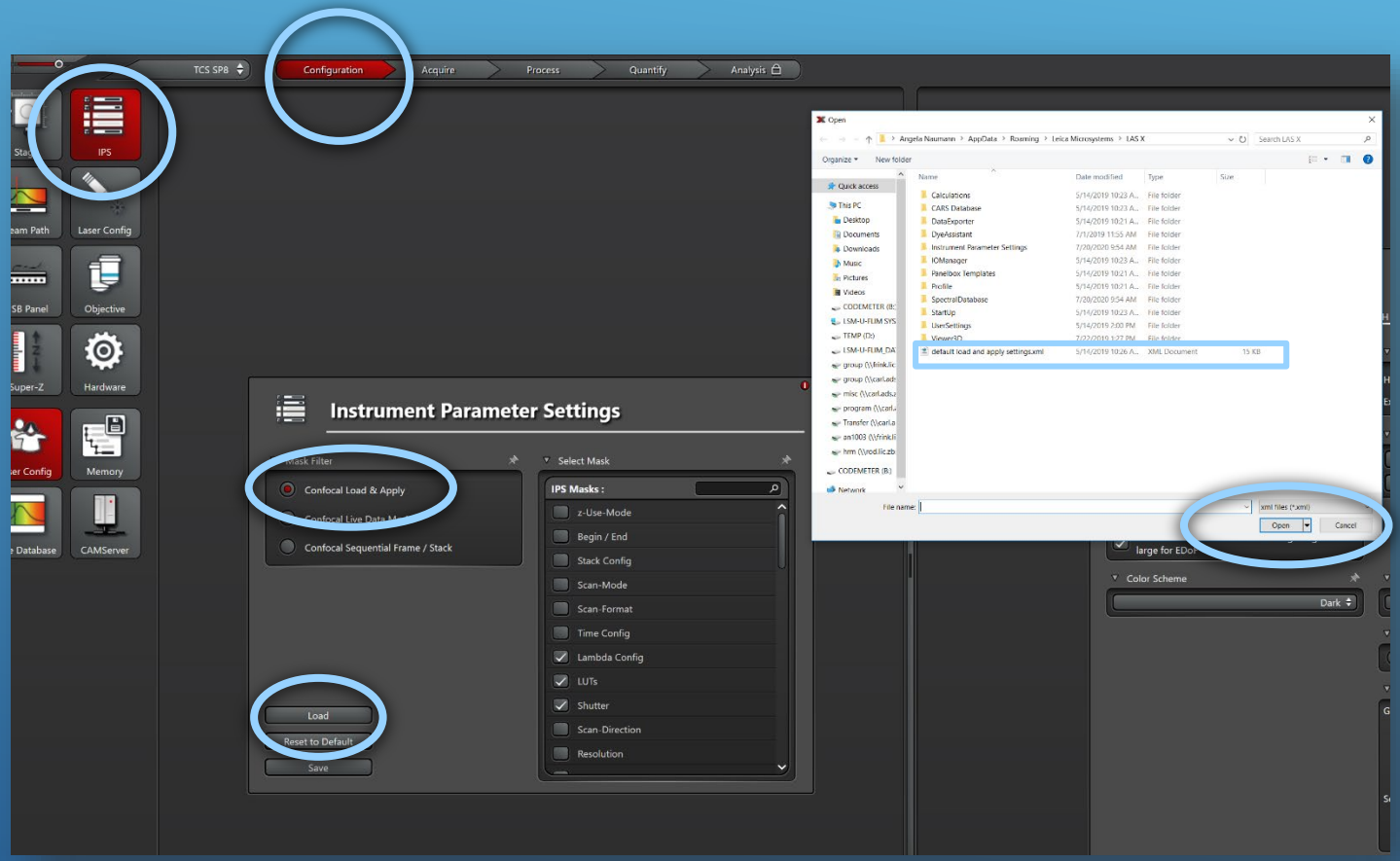
Instrument Parameter:

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

Select „Confocal Load and Apply“
Press Load

Window will open

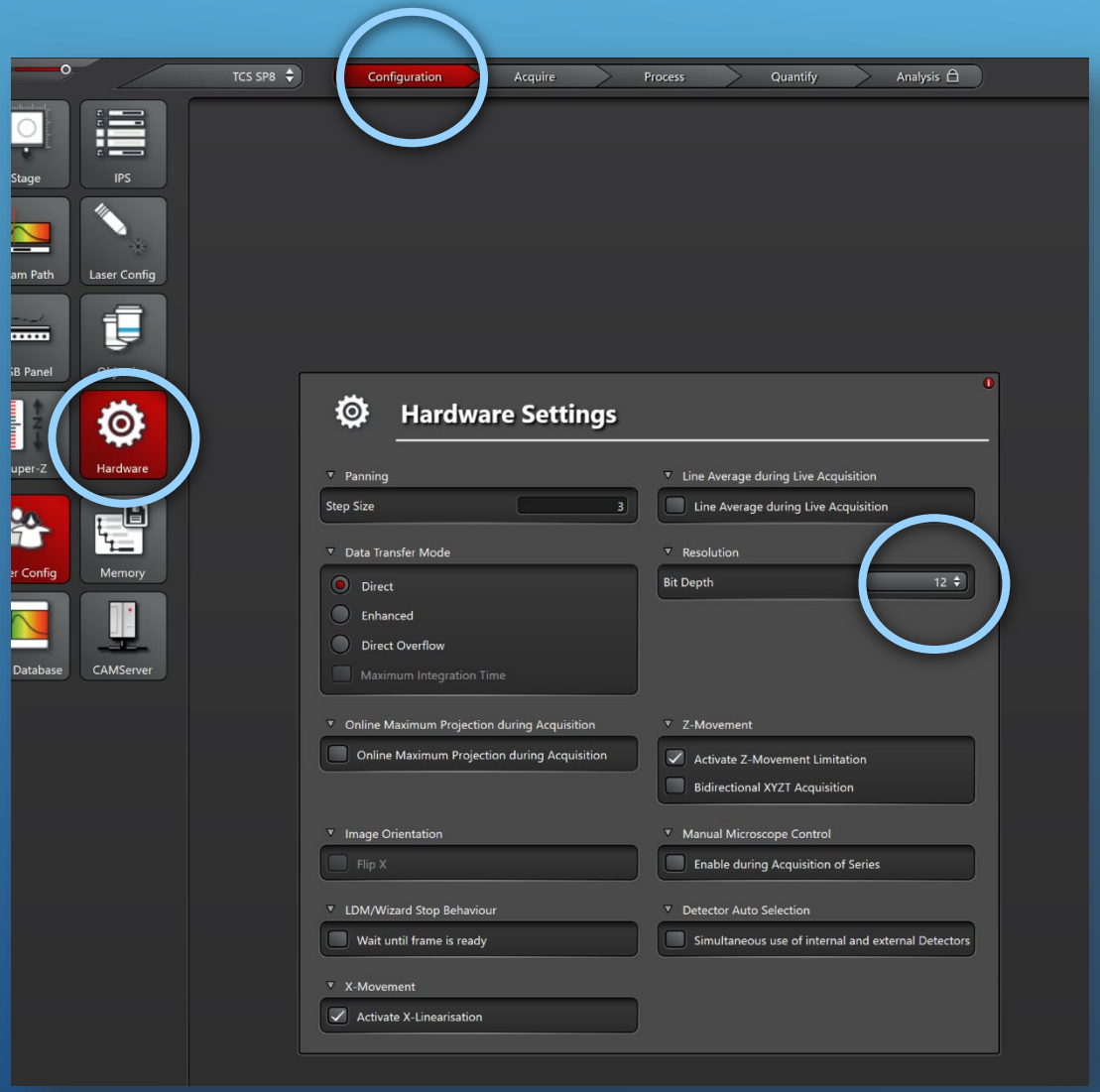
Select „Default Load and Apply“ file
Press Open



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

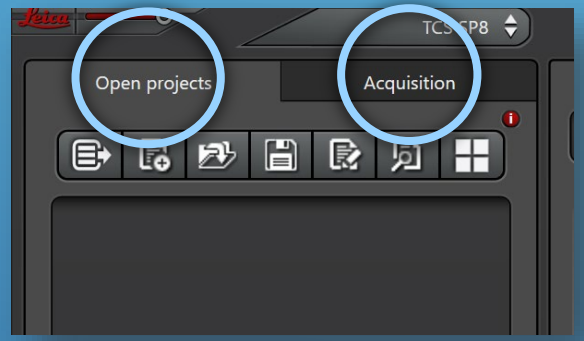


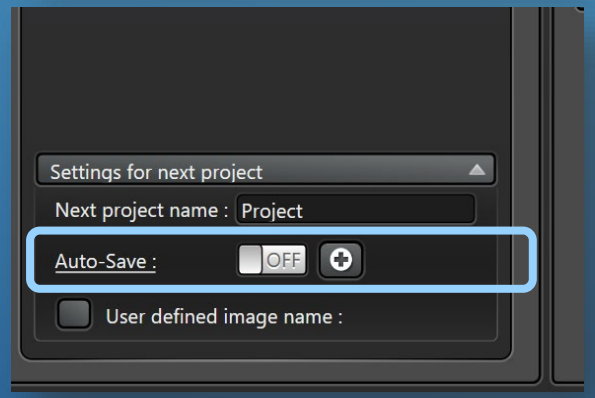
Change Bit Depth

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

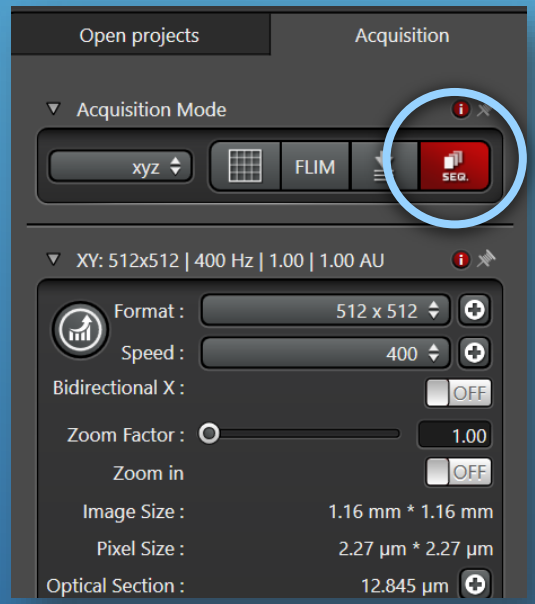
The **Acquisition tab** – which controls the microscope

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

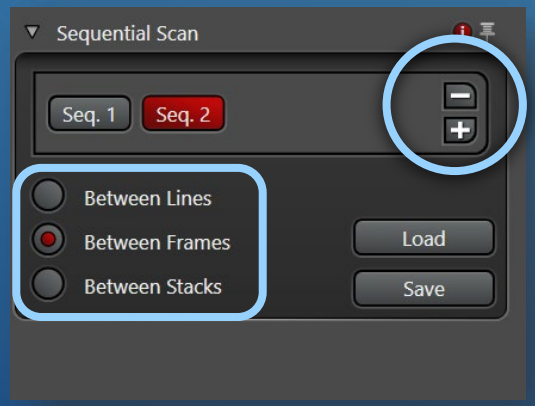
- As image data is captured (using *Capture* image or *Start* - Button in the *Acquisition tab*) it is **temporary** stored under this tab until the user tells the software to save the project

- 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

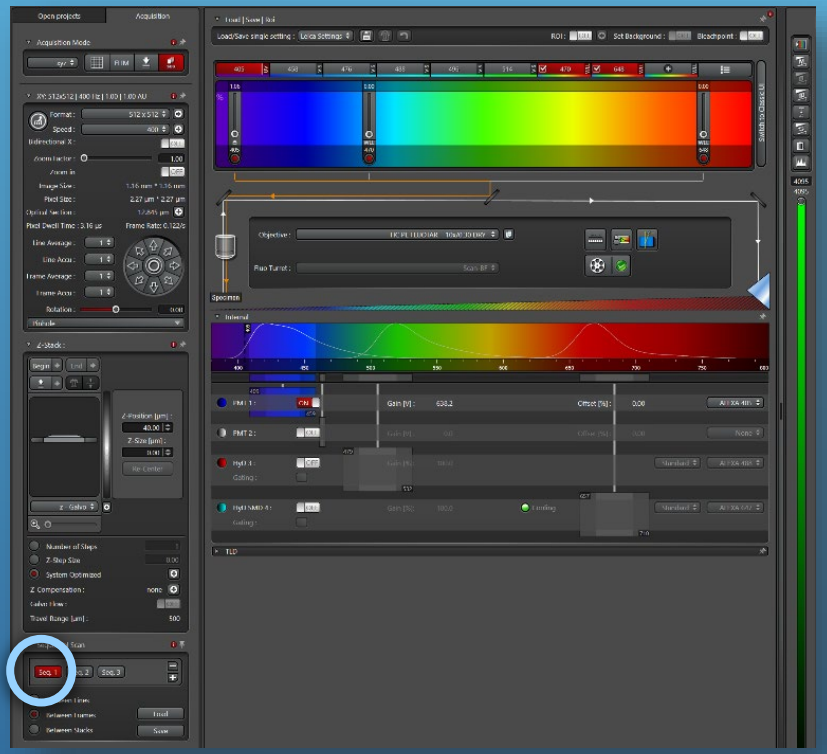
- Could be used to exclude the background generated sometimes by excitation light

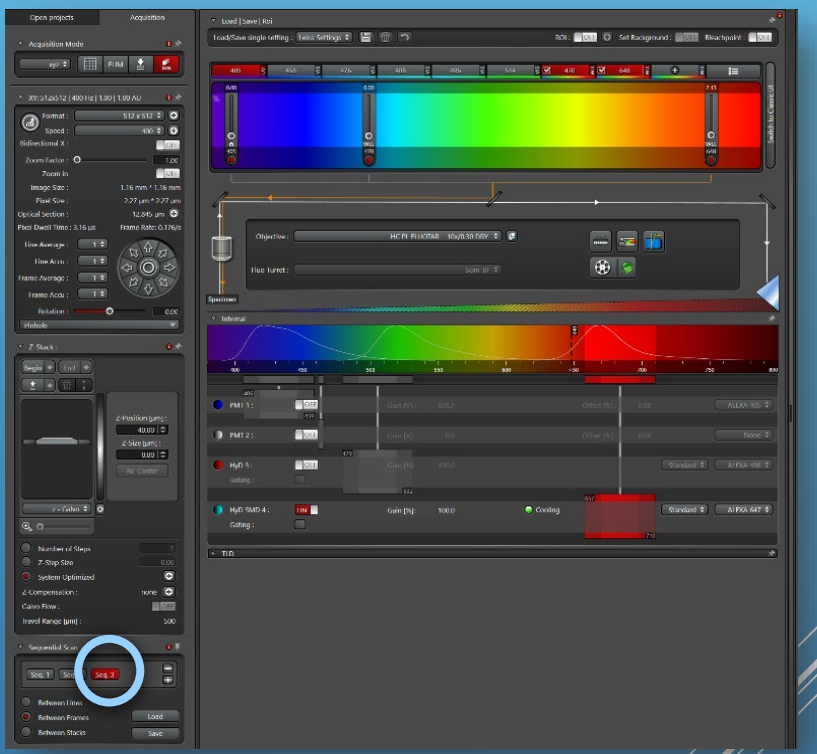
Light Path - Laser & Detector

Simultan - Scanning

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

However we recommend : sequential scan

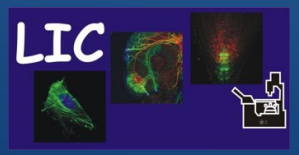




Light Path - Laser & Detector

Sequential - Scanning

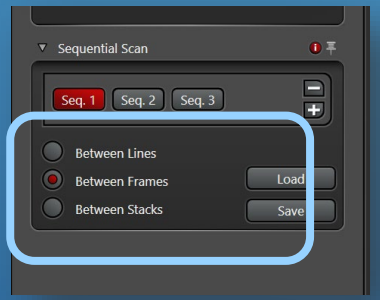
- Example: here **sequential** scan with three laser lines:
  - Reduced risk of blead through
  - Slower than simultan scanning



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

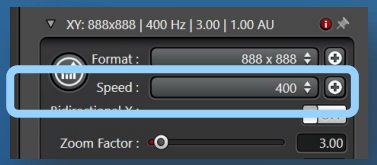
«**Capture Image**» will acquire just the active channel as image, no z-stack

«**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

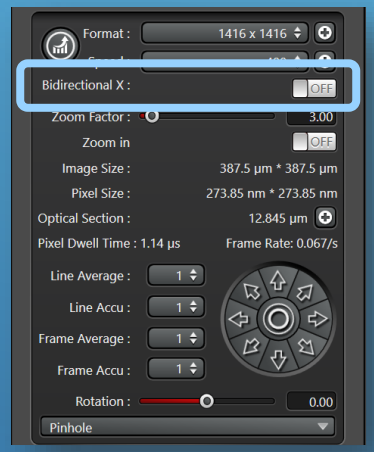


Scan speed can be adjusted individually

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 ration (SNR), low risk of bleaching

We recommend as default speed: 600



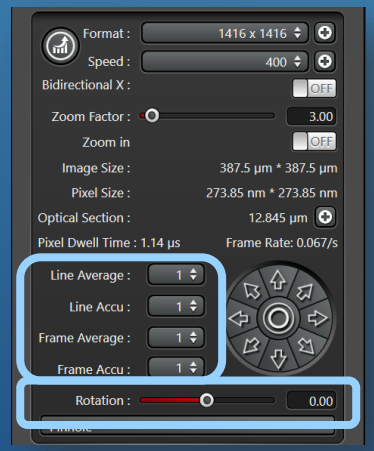
Bi-directionl

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

UNI -

Bi-directional

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

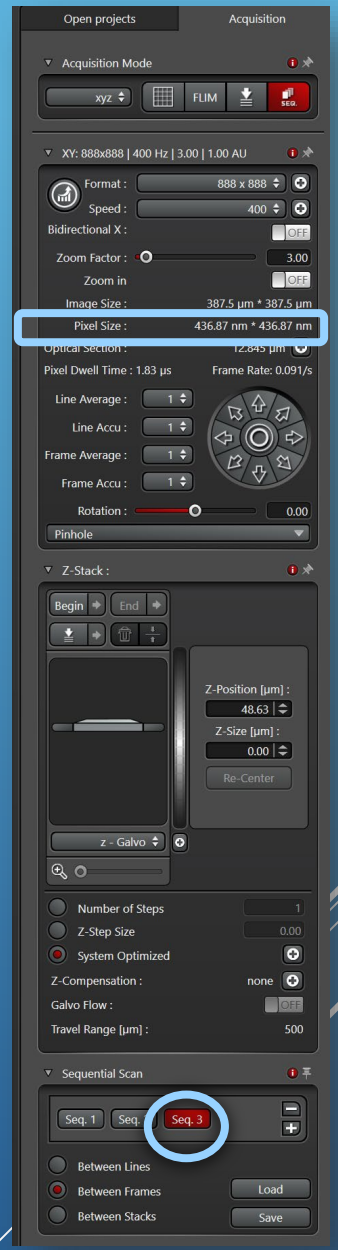
Scan Format

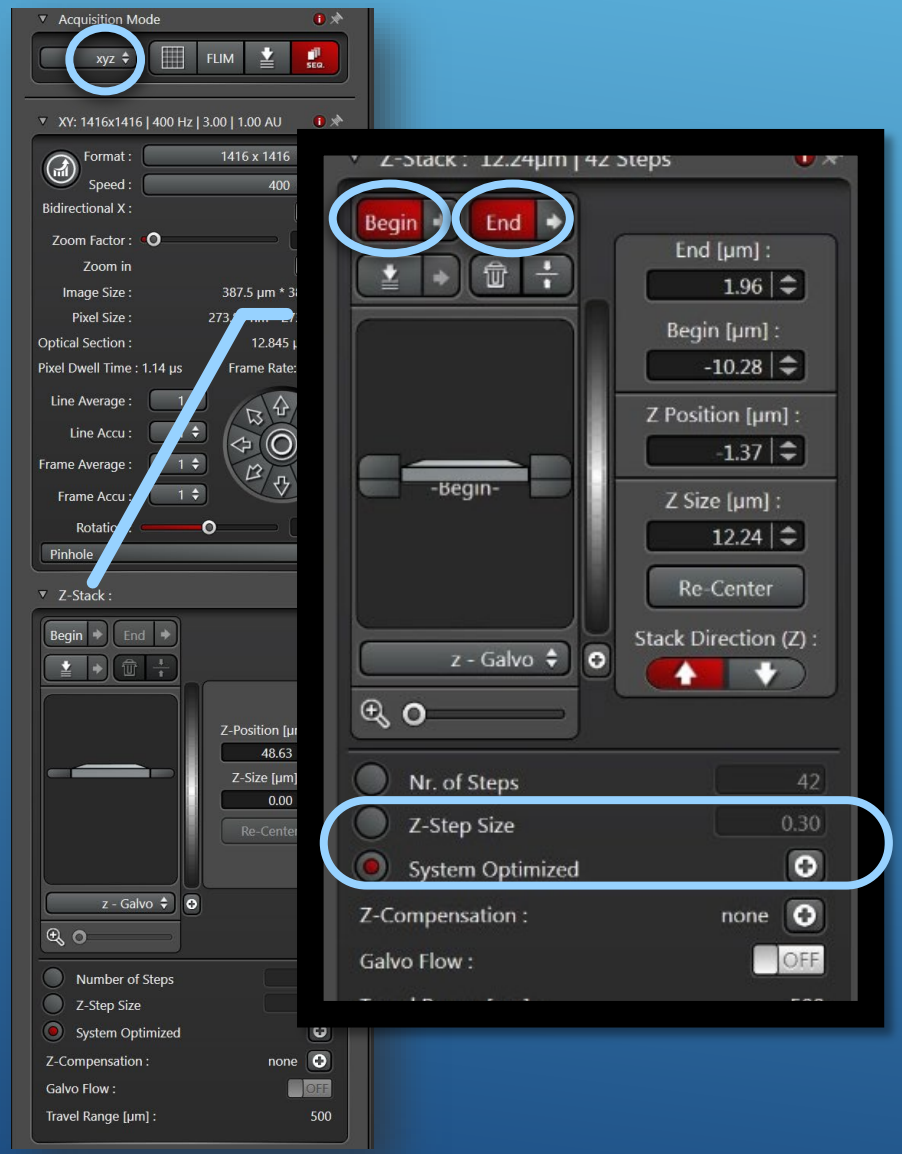
Preview

- 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**

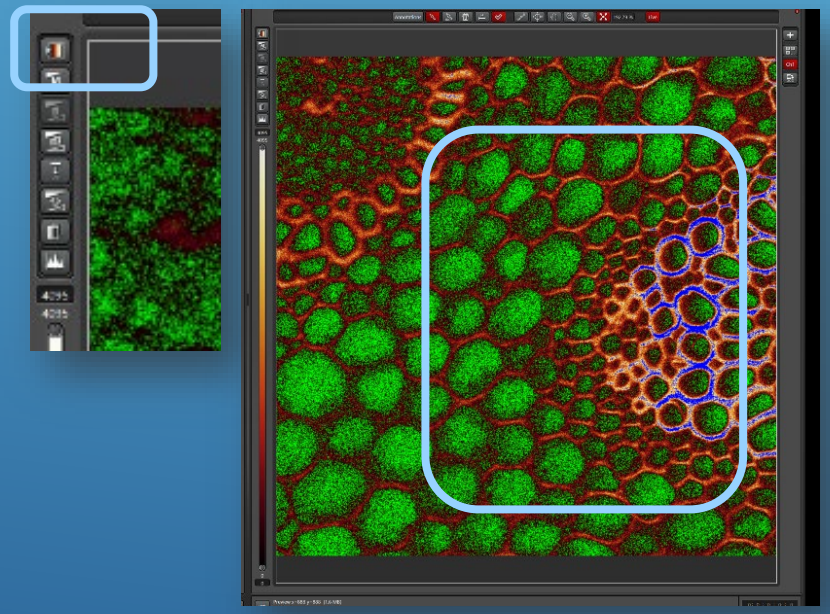
Press «**Live**» and move to the **top** of sample region of interest using the right z-position knob of the Leica panel tool in front of you – **click** « **begin** »

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 t system and set the size to 50% overlap. This is needed for high resolution and 3D reconstruction. **Recommened**

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