

- **Visual examination of the sample**
- On the microscope touchpad choose the illumination icon to open the illumination menu
- for transmitted light press the BF button and operate the TL shutter
- small intensity wheel is found on the left side of the microscope (TL indicator light is next to it)
- For fluorescent illumination, press FLUO button in the illumination menu of the microscope touchpad
- Choose filter cubes on the front panel: (DAPI, RHOD, LED\_470). Open the IL-shutter to see fluorescence
- small intensity wheel (same as for BF) on the left side of the microscope stand lets you change the LED intensity
- **FOCUS on your sample !!!**
- Visual illumination is disabled during scanning, the system is automatically changing to "CS" combi mode
  
- **Acquiring confocal images**

**Before you start:**

***Make sure that the Autosave option is switched off in Open Project !!!***

***CHECK IF THE HyD SMD 1 DETECTOR COOLING CONTROL LAMP IS GREEN. IF IT'S RED DON'T USE IT !!! CONTACT OUR BIF TEAM IMMEDIATELY !!!***

- **Acquisition mode: xyz**
- The easiest way to plan your confocal imaging is using the dye assistant (Fig.1). Open the menu and enter your fluorophores. Choose PMT or HyD detectors accordingly. Then choose offered imaging methods (simultaneous and/or sequential scanning according to your needs considering cross talk and imaging speed. Press "Apply"



**Fig.1**

- The system is turning on laser lines (tunes to the excitation maxima of selected dyes), activates detectors with appropriate bandwidth and shows the emission spectra of selected dyes.
- Switch on T-PMT if needed for transmitted light imaging.
- Press “Live” to get an image preview
- Laser power, detector bandwidths and Detector gain may be adjusted

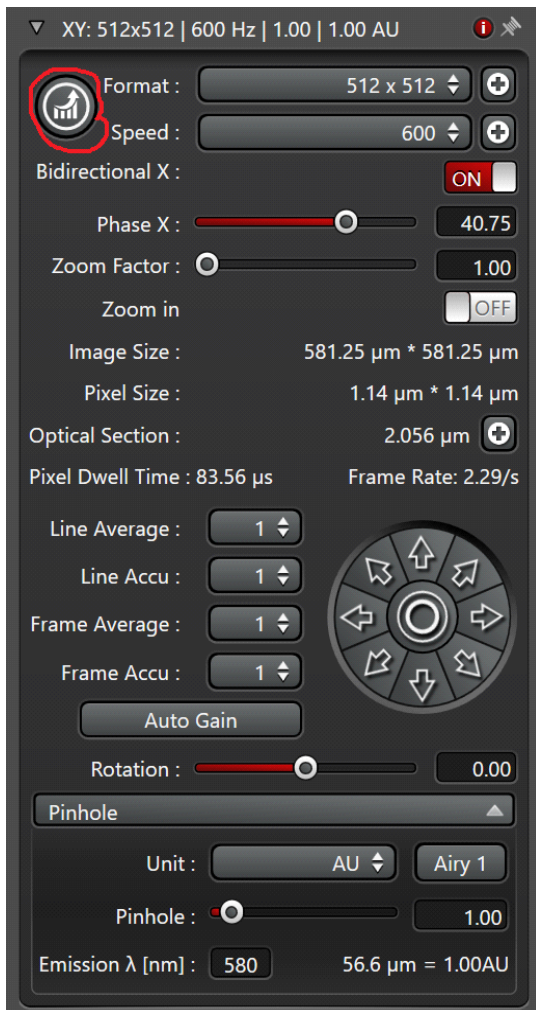


Fig.2

**Set your confocal image:**

!!! Note: gain, offset, scan field rotation, pinhole, zoom, z-position settings may be changed via the control panel in front of you

**Position your sample:**

change the zoom or rotate your image

**Adjust the image format:**

press Nyquist button for proper sampling

**Adjust the pinhole:**

Changes will alter the confocal section thickness

And the SNR of the image ; default is AU 1

**Adjust Signal to Noise ratio (SNR):**

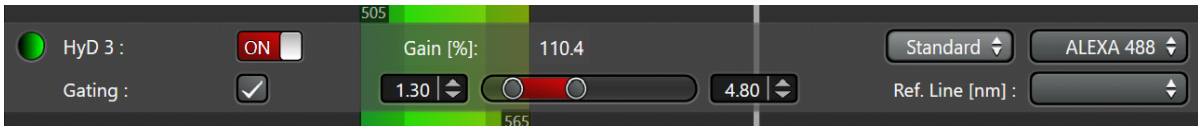
apply frame or line averaging, reduce the scan speed

check the pixel dwell time

- **Gated detection**

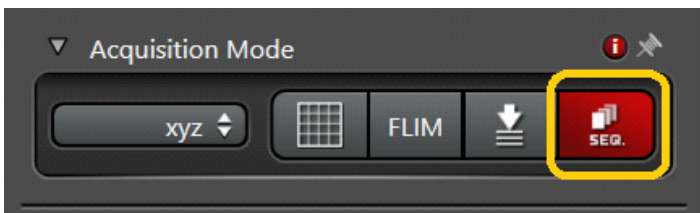
**Note: Only possible using HyD detectors!!!**

- used to reduce auto fluorescence or reflection with shorter lifetimes than actual fluorescence



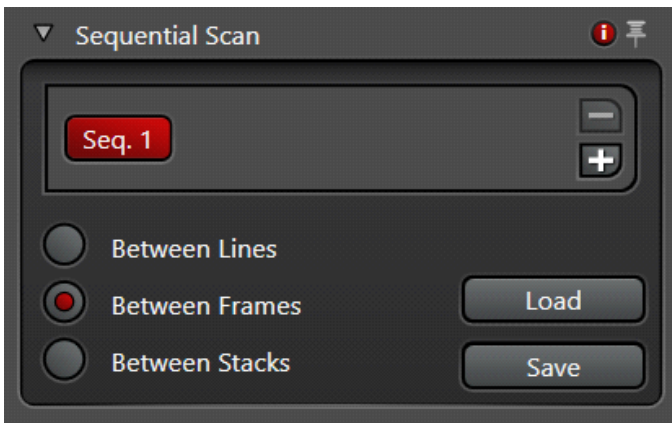
- activate gating **Fig.3**
- choose the detection window in ns for your fluorophore, adjust during live scan

- **Setting up sequential scan**



**Fig.4**

- Select 'seq' symbol in the 'acquisition mode' menu



**Fig. 5**

- select line, frame or stack sequential
- Set up first configuration
- **ATTENTION!!! in line sequential only PMTs and Lasers are switched on/off between the lines (laser shutters, detector bandwidth remain the same, NO HARDWARE MOVEMENT between lines is possible !!!**
- Press '+'. Set up next configuration, and so on. Set optimal imaging conditions for each configuration.

- You can save "sequential files" in your folder and load them again.
- **Setting up a Z-stack**
  - only use 'Z position' knob on the control panel in front of you!!!
  - **Acquisition mode xyz**



**Fig.6**

Select 'Z-galvo' or z-Wide in the 'Z-stack' menu

Start scanning with 'live'.

Use 'Z position' on the control panel, select the first slice.

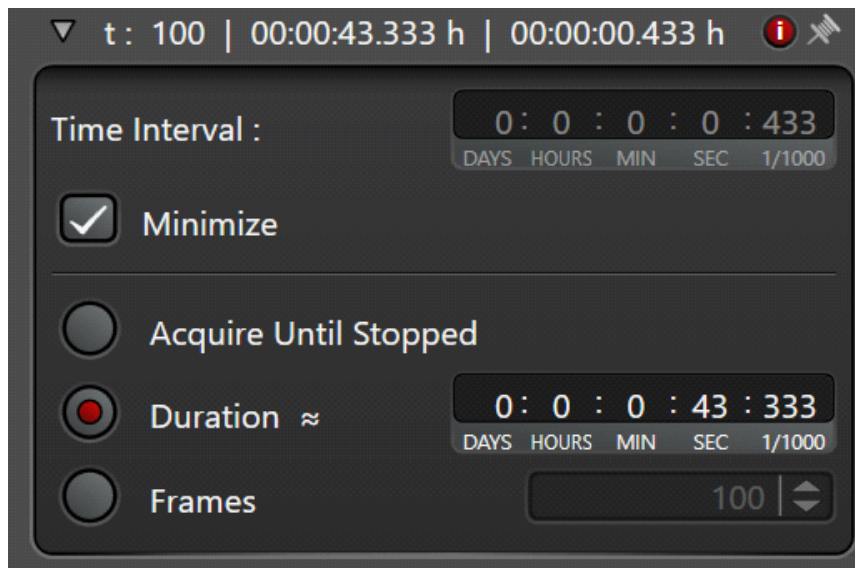
Press 'begin' in the Z-stack menu. Move focus up with 'Z position', select the most superficial slice, press 'end'.

Set up Z-step size according to your needs

activate system optimized for Nyquist settings according to the objective and pinhole settings used

to capture Z-stack, press 'start'. The Z-stack will be stored in projects

- **Setting up time series:**
- **Acquisition mode xyzt or xyzt**



**Fig.7**

- To estimate minimal interval, activate 'minimize' in 't' menu
- Or set your own time-lapse interval (should exceed the minimal)
- Select experiment duration or number of frames
- Press 'Apply'
- To start time series, press 'start'