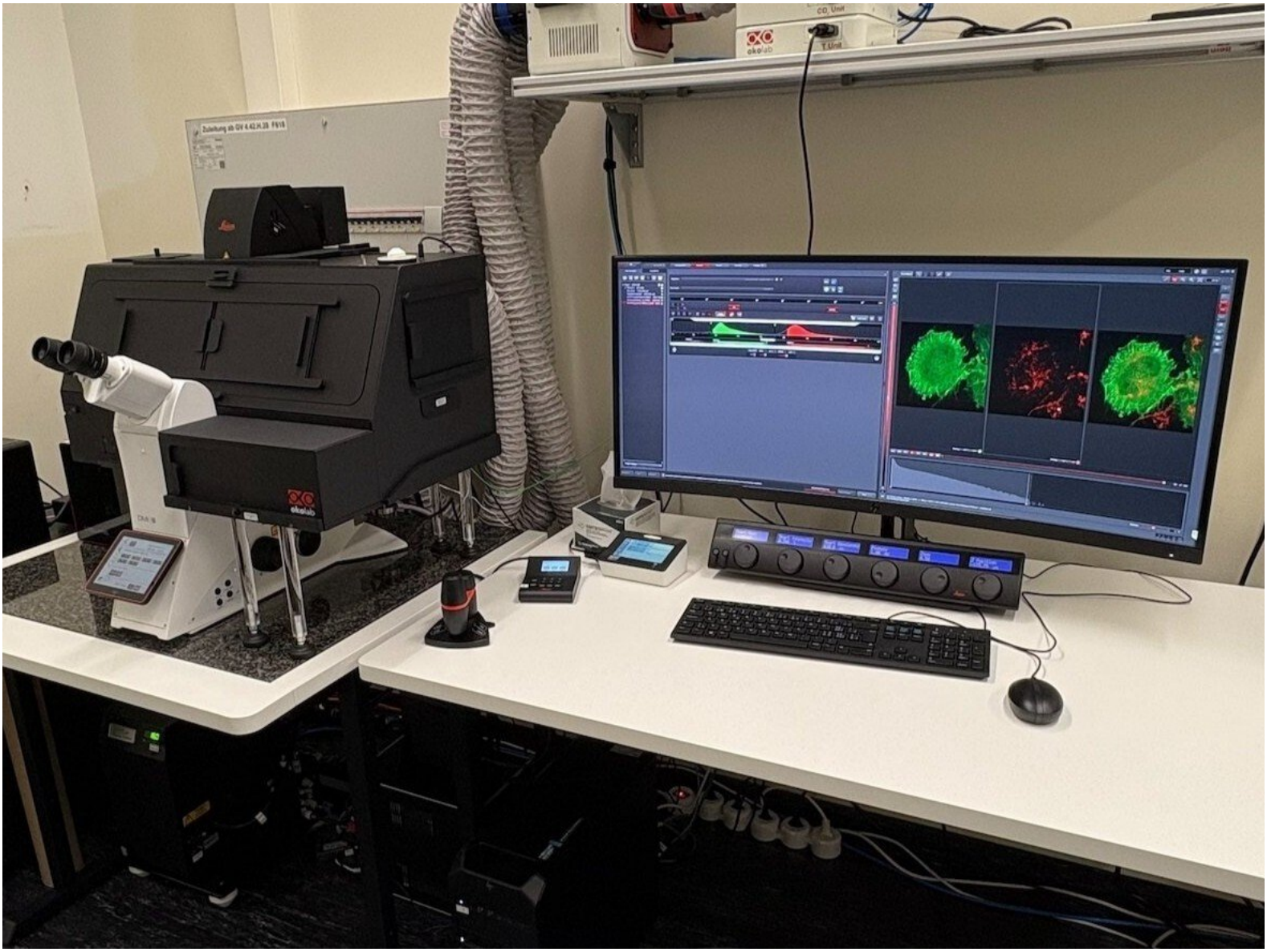
Leica Stellaris 2: Acquire an image in xy and z

How to acquire a xy image or xyz stack on the Leica Stellaris confocal laser scanning microscope.

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INTRODUCTION

In this guide of the Center for Microscopy and Image Analysis we describe the major steps/aspects required for image acquisition on the NEW Leica Stellaris confocal laser scanning microscopes.

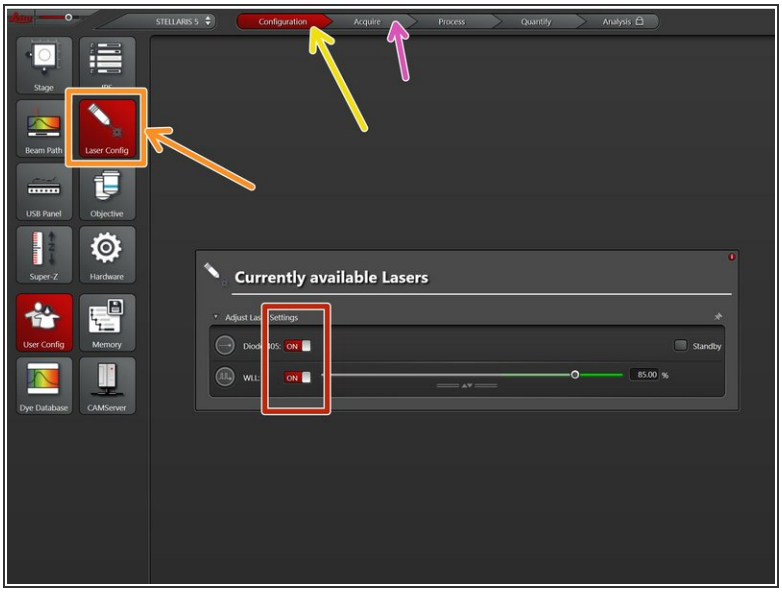
The most relevant new features are:

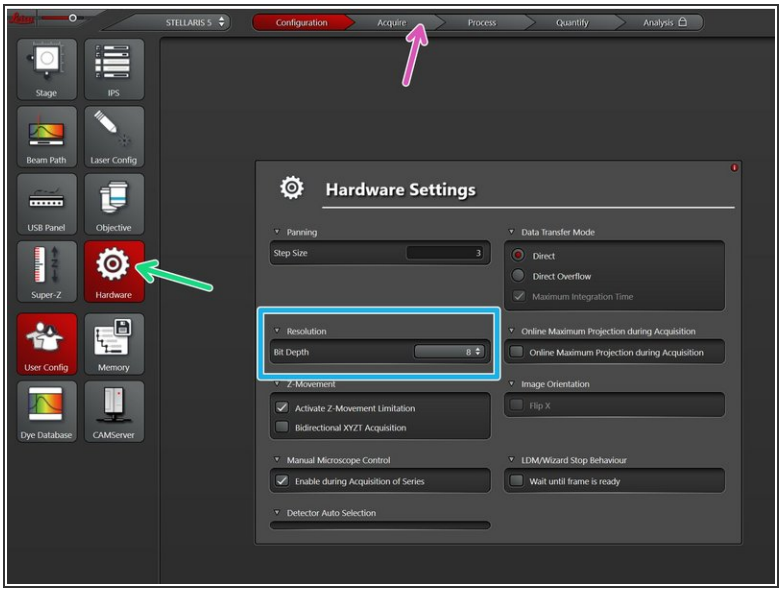
- Spectrally flexible white light laser (WLL) with an extended spectral output on the red and near-infrared spectrum (440 nm up to 790 nm).
- NEW Power HyD S, X and R detector.
- TauSense technology, which gives access to lifetime-based information, delivering additional insights to your experiments.
- Possibility of switching between resonant and non-resonant scanner directly within the software

Please find detailed information about the available systems here:

Leica Stellaris (Irchel)

Step 1 — Laser and hardware configuration





- Go to **"Configuration"**.
- Select **"Laser Config"**.
- **Switch "ON"** the lasers you need.
  - ⓘ The **WLL** is set to **85%** by default and allows selection of excitation wavelengths from 440 nm to 780 nm.
- Go to "**Hardware**".
  - Here you can change the **image "Bit Depth"** if necessary.
- Go back to **"Acquire"**.

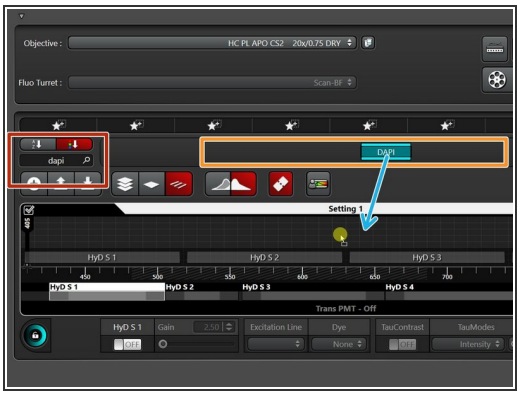
Step 2 — "LAS X" software



Make sure your sample is correctly placed and focused. Check the appropriate Start-up guide for more info.

- Identify the following sections in the **"Acquire" panel**:
  - Make sure you are in the **"xyz" scan mode**.
  - Settings for **"XY"** ("Format", "Speed" e.g. adjustments).
  - Settings for z ("Z-stack" option).
  - Excitation settings.
  - **Objectives.**
  - **Detectors** and emission settings.

Step 3 — Imaging settings

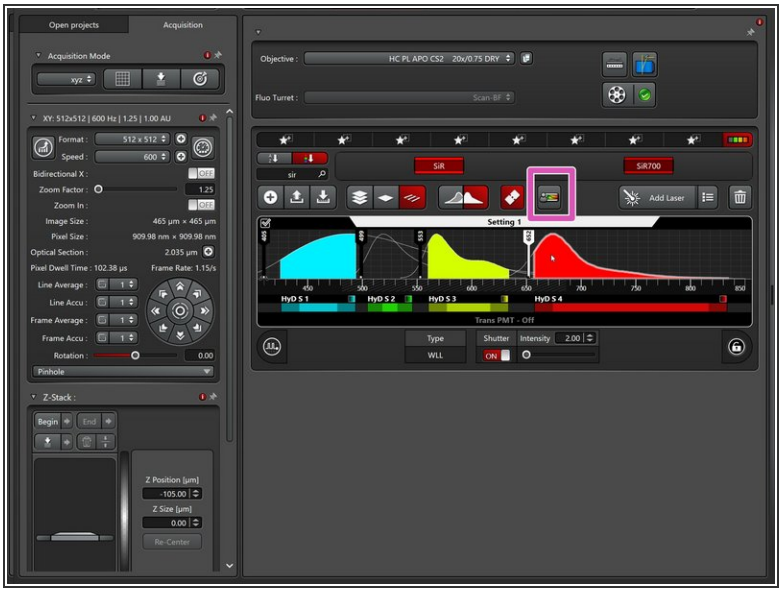






- Type to **search** for your **fluorophore/dye** of interest.
- Available results will be displayed here.
- **Drag** the suitable one into the empty panel ("Setting 1").
- The **corresponding laser line** will be automatically added.
- **Repeat** the same procedure for the remaining fluorophores by adding them to the **same panel** ("Setting 1").

Step 4 — Open dye assistant



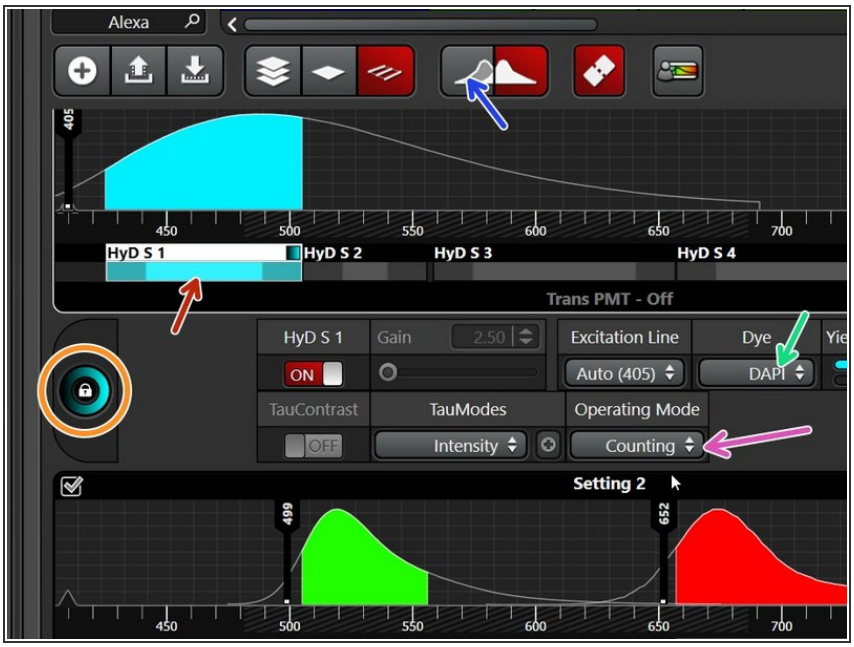


- Click the **"Open Dye Assistant"** button.
- "HyD S" Detectors are used for regular acquisition.
- Choose the most appropriate configuration from the different options - avoid having crosstalk!

  ⓘ *"Line sequential" acquisition is faster compared to "Frame sequential".*

- For TauSense acquisition see the appropriate guide.

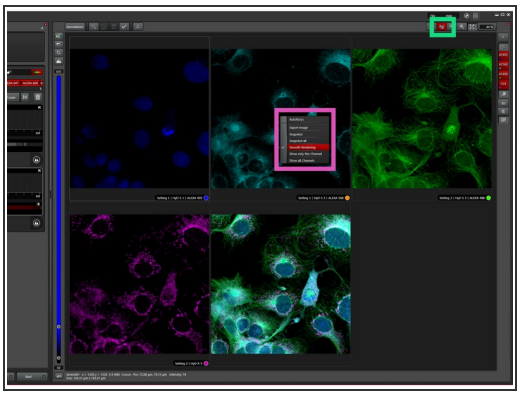
Step 5 — Adjusting detectors



- Click on the different **detectors** to adjust imaging parameters.
  - Check that Detectors are in **"Counting"** "Operating Mode".
- Here you can also select your dye to visualize the **emission spectra**.
- Toggle to activate/deactivate the **excitation spectra** as well.
- Double click here to change **look-up table (LUT)** for each active detector.

Step 6 — "Fast Live"





- You can use the **"Fast Live" button** to quickly adjust focus or zoom.
  - ⓘ *Only images from the active sequence will be shown while live.*
- Click the clock wheel to adjust "Fast Live" settings (each scanner has independent settings).
- Adjust **laser intensity** by double clicking and typing in.
  - ⓘ *Hovering over the laser bar and rolling the mouse wheel also allows intensity adjustment.*

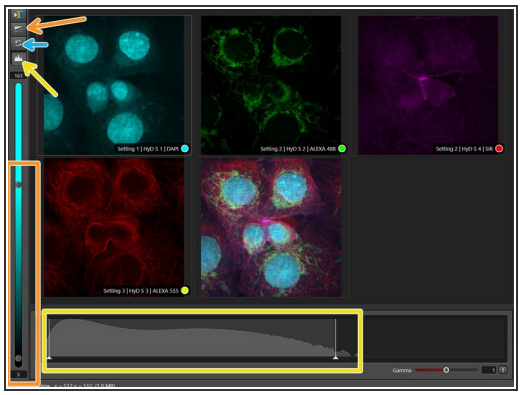
⚠ These settings are **only** used to allow a fast screening / localization of your sample. You will **need to readjust** proper imaging setting under **"Live"** view for image acquisition.

- Activate the **mouse option** - This will allow you to use a mouse to move around in your sample and navigate directly in the SW.
- **BE AWARE OF SMOOTH RENDERING:** When right-click somewhere on the image, you can select to **Disable smooth rendering** - this is how your real image looks like and will look like, when open in another SW.

Step 7 — Live acquisition - Image adjustment





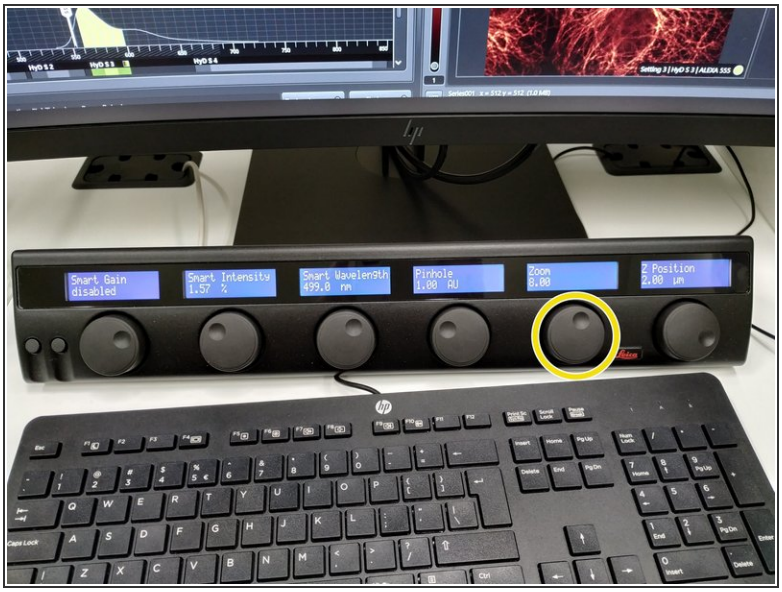


- Start adjusting your image sequences/settings.
  - Click **"Live"**.
  - Adjust **laser power**.
- Adjust the **image contrast** by using the slider or activate automatic contrast adjustment (**"Auto Contrast"**).
  - Click here to reset the contrast adjustment to full scale.
- Activate the **image histogram** to inspect the intensity distribution and to optimize your signal. Avoid under- and overexposure.

For **counting mode** adjust to **approx. 80 counts** / **usec pixel dwell time** (keep an eye on the pixel dwell time - *depending on the set format and scan speed*).

Step 8 — "Zoom" and "Format"

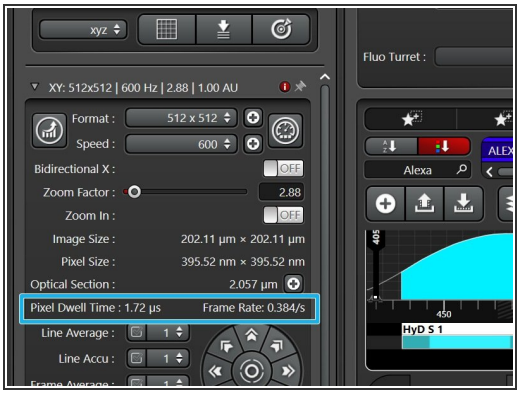


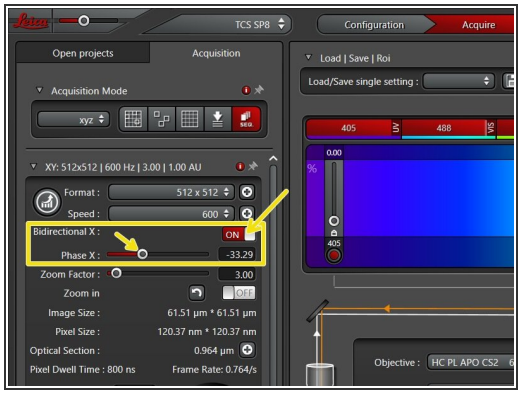


- Proper setting of the **xy sampling** (pixel size) is crucial for acquiring optimal images.
- Change your field of view by using the **"Zoom"**. You can also use the knob in the external control panel.
- **"Format"** defines the number of pixels in one scan area.
  - One can choose preset formats via the drop down menu.
  - By clicking the **"+"** every other format can be chosen.
- To adjust for the correct pixel size/optimal sampling you can either use the online calculator such as the SVI Nyquist Calculator or the auto button for an estimate.

Step 9 — "Speed"

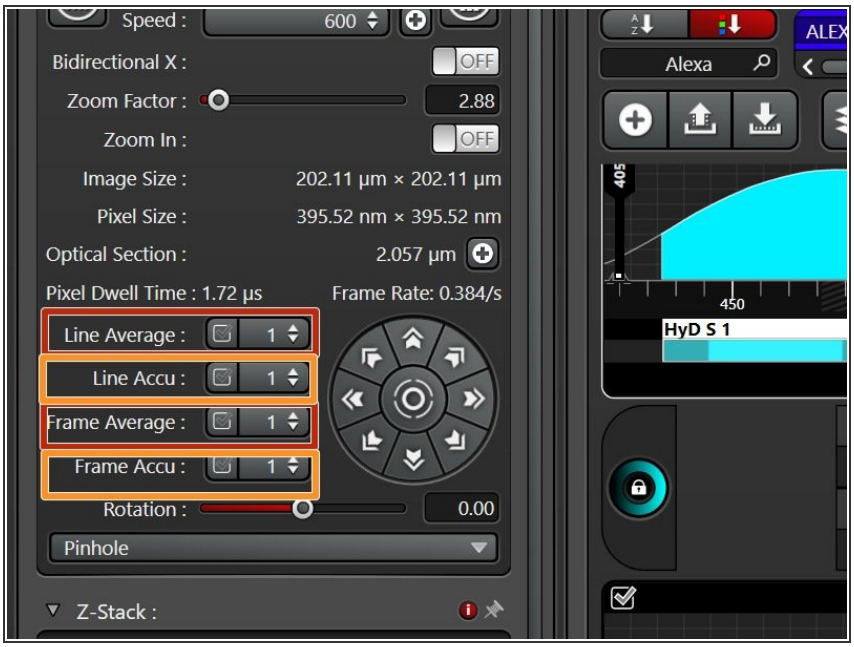






- Here you can change the **scanning speed**:
  - via the drop down menu (presets) or via **"+"** for every other scan speed.
  - ⓘ *Use slower scan speed to increase the **pixel dwell time** and thus collect more light.*
  - ⓘ *Increased scan speed leads to faster imaging, lower photo-damage and bleaching, but gives more noise and allows a smaller field of view.*
- Toggle between the **resonant** and **galvo scanner** here. It might take a few seconds to adjust after switching.
- You can also activate **"Bi-directional"** scanning to speed up acquisition.
  - ⚠ Please make sure the **phase** is properly adjusted. Usually, there is no need for adjustments. Best is asset on know structure (e.g. nuclei). Nuclei should be rounded - when you see doubling of that structure, this might be a sign of not corrected phase.

Step 10 — Averaging/Accumulation



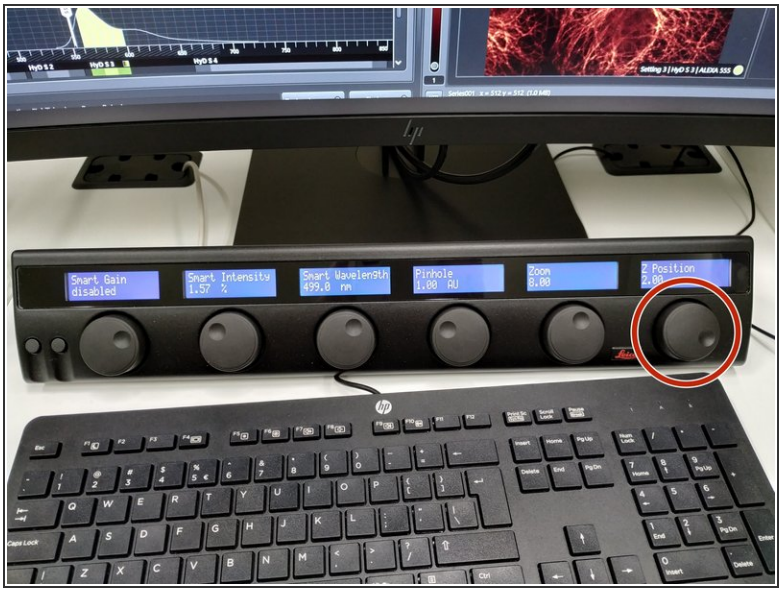
- If you are limited by the laser power but still need to increase the signal (or reduce noise) use:
  - **Accumulation (by line or by frame)**: Useful when using detectors in counting mode or for very weak signals.
  - **Averaging (by line or by frame):** may be used to remove noise.

If applied the acquisition time will increase.

⚠ In Frame sequential mode, number of Accum/Averaging can differ per phase. In Line sequential mode, the same setting applies to all.

Step 11 — Optional - Define "Z-stack"



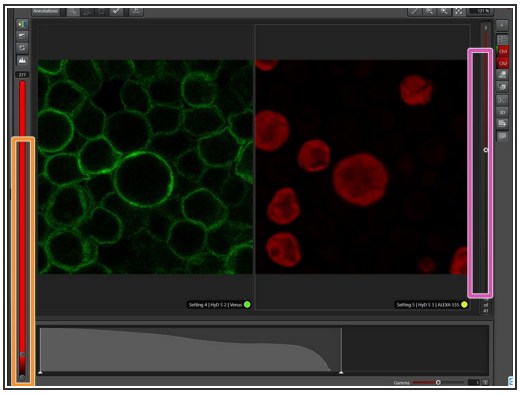


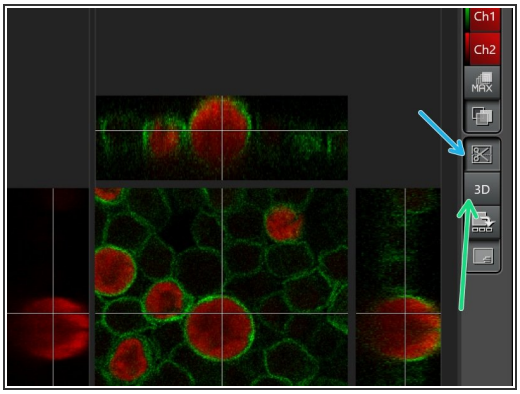
- Use the z-drive controller (**"Z-Position"** on the **"control panel"** to define the limits with **"Begin"** and the **"End"** of your z-stack.
- Define the appropriate **"Z-Step size"** or go for optimal z sampling by choosing "System Optimized". The "Number of Steps" will be automatically calculated.

You should refer to the [SVI Nyquist Calculator] if you plan to deconvolve your image as a post-processing step.

Step 12 — Start acquisition



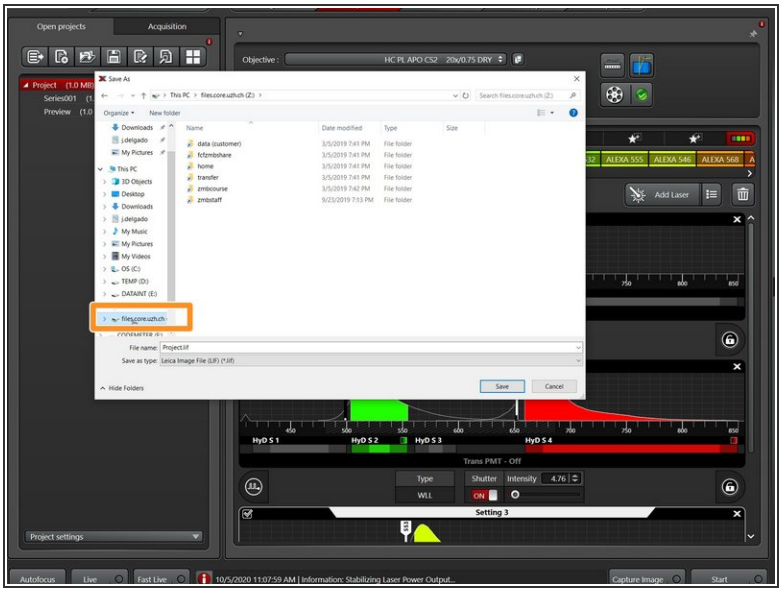




- Press **"Start"** to begin acquisition.
- You can adjust contrast by using the slider.
- Go through the different z planes here.
- Use the orthogonal viewer to inspect your z stack.
- You can also use the 3D viewer here.

Step 13 — Save your data





- Go to the **"Open projects"** tab.
- Click on your data file and give it a meaningful FAIR name.
- Click on the disk button to save the data. Alternatively right click and choose "Save as..." from the drop down menu.

  ⚠ During long sessions, save your data regularly to avoid losing everything in case of a crash or unexpected issue
- Save the data to your **zmb-data network folder**.