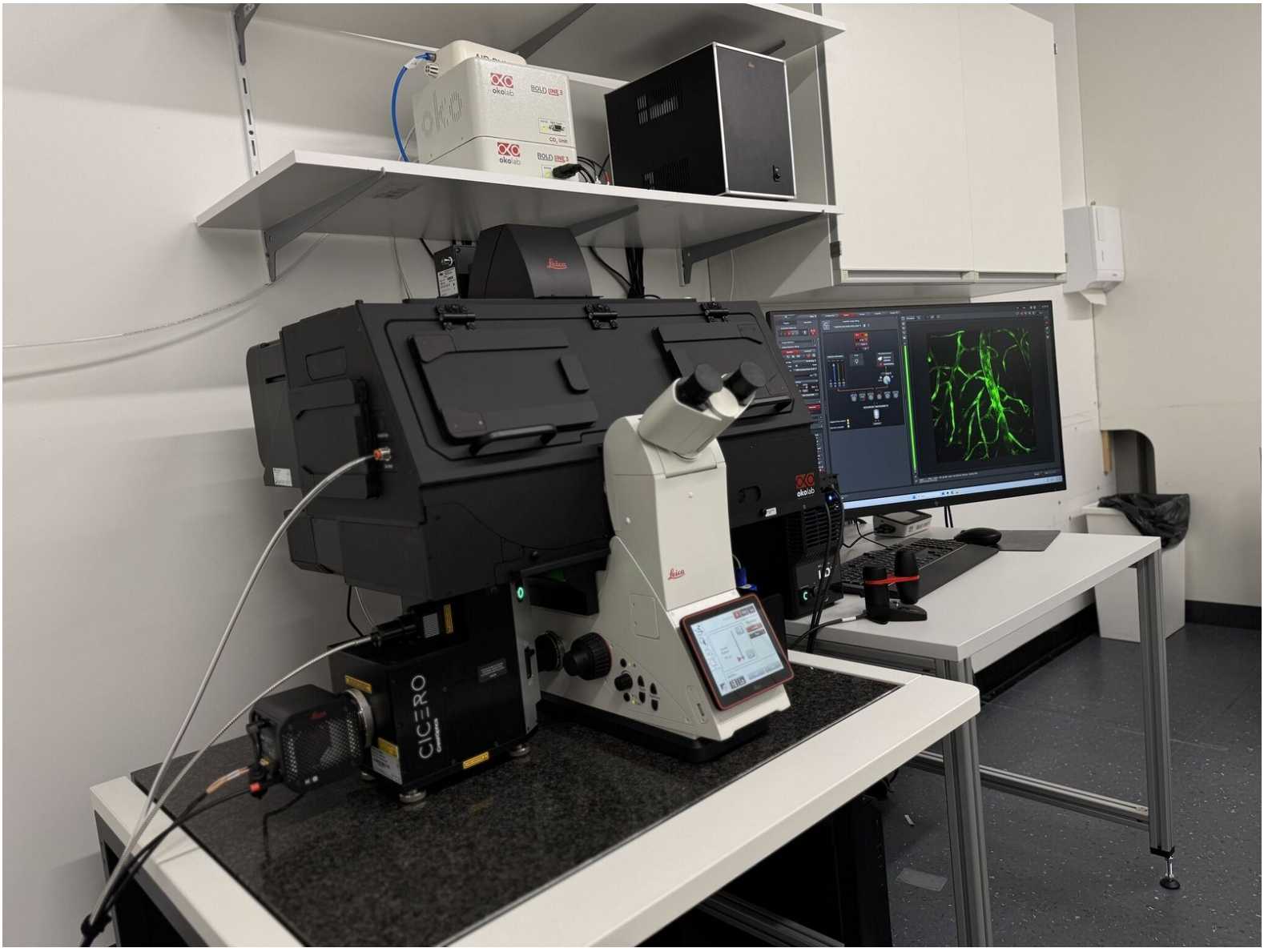
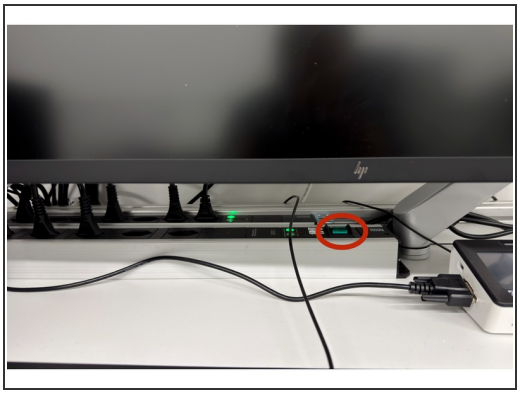
Leica CICERO SDC

Written By: z mbstaff



Step 1 — System Power-On and Hardware Check





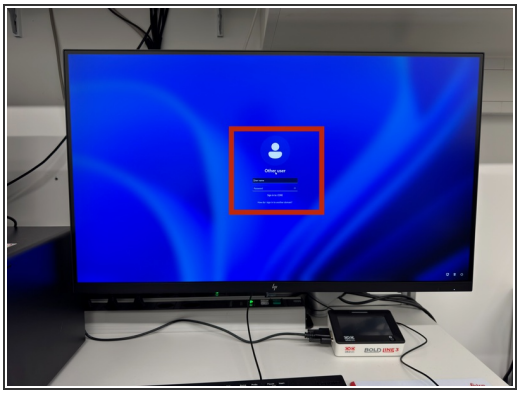


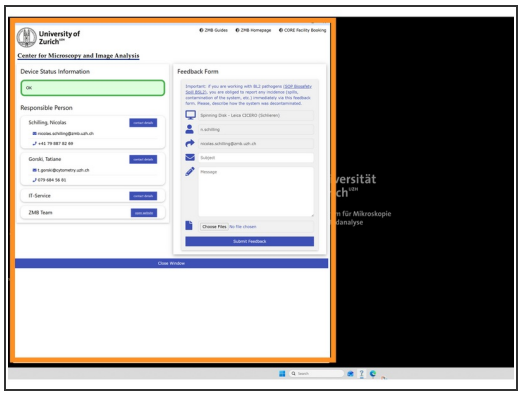
⚠ ***The Leica CICERO spinning disk system is normally powered on by default. Follow the steps below only if the hardware is switched off or was accidentally powered down by a user. Otherwise, simply verify that the following hardware components are powered on.***

- Switch on the front power strip.
- Press the Power/Status button briefly, then turn the laser key from OFF to ON.
  - Verify that the status LED ring turns green.
- Press and hold the button on the spinning disk unit for ~2 seconds.
  - Wait until the LED status ring turns steady green.

Step 2 — Wait for Hardware Connection



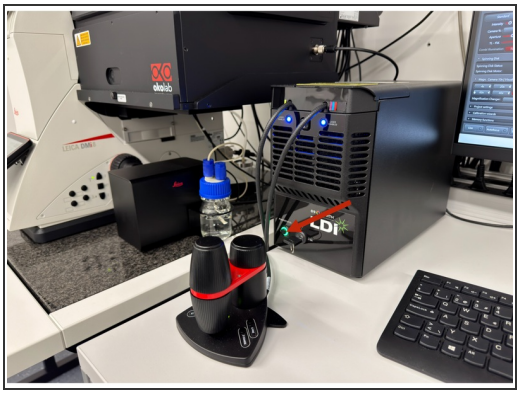
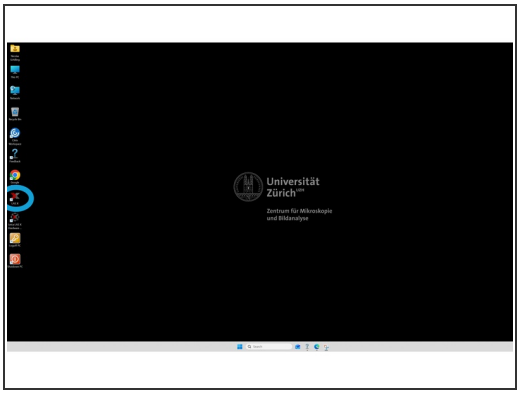


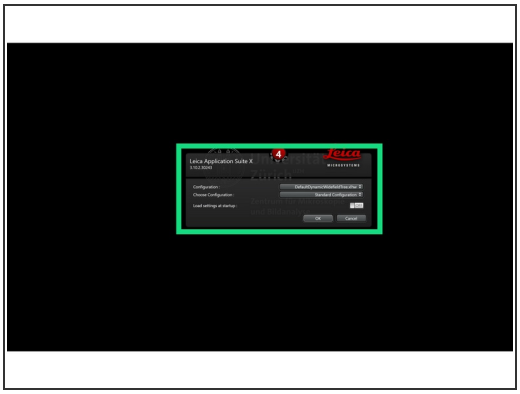


⚠ *The PC must remain powered on at all times. Image data are initially stored locally on the PC and are transferred later to the server via the **DataMover** into the user's data folder. **Do not shut down the PC**.*

- **Log in using your Core credentials.** If you have forgotten your password, follow this guide to reset it: Reset your Core account password
- **Wait for the feedback window that appears automatically after Windows login.** This allows the PC sufficient time to establish connections with the hardware and helps prevent communication issues between the PC and the microscope.

Step 3 — Verify Light Engine Status

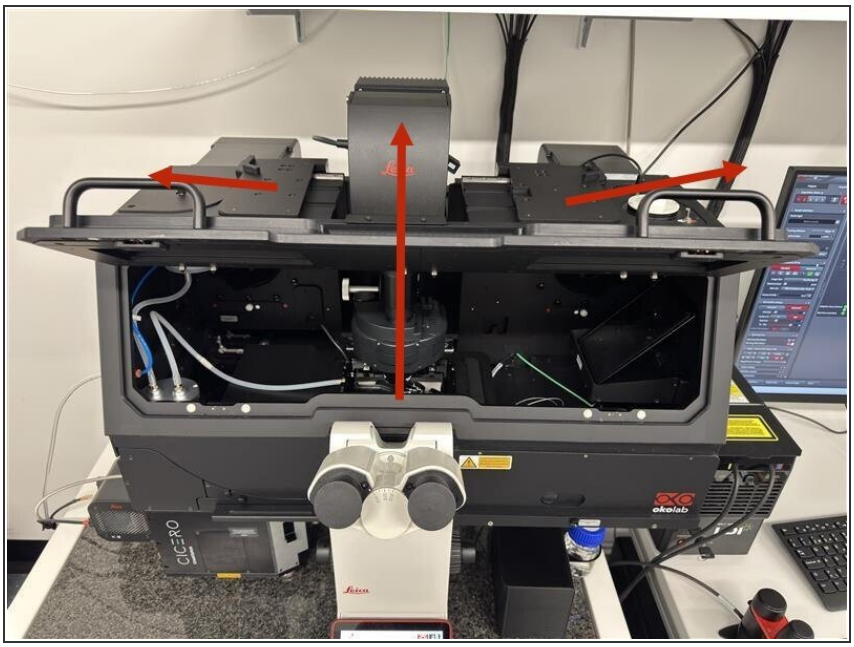





⚠ **Important:** Before opening **LAS X**, ensure that the **light engine LED status ring is green**. If the LED ring is not green, the **light source will not be controllable in the software** later.

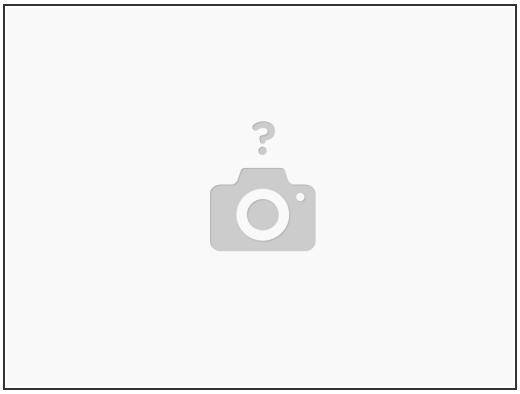
- **On the desktop, open LAS X.**
- In the pop-up window, keep the default settings: **DefaultDynamicWidefieldTree.xlhw** and **Standard Configuration**.

Step 4 — Load Your Sample



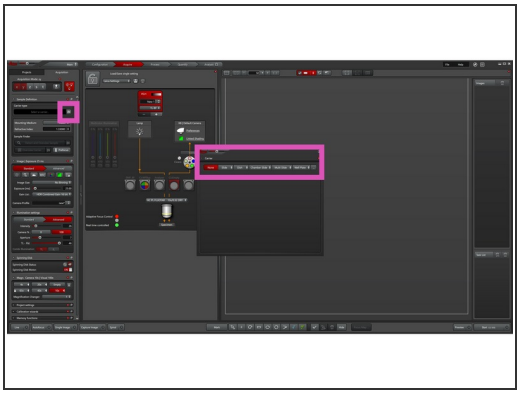
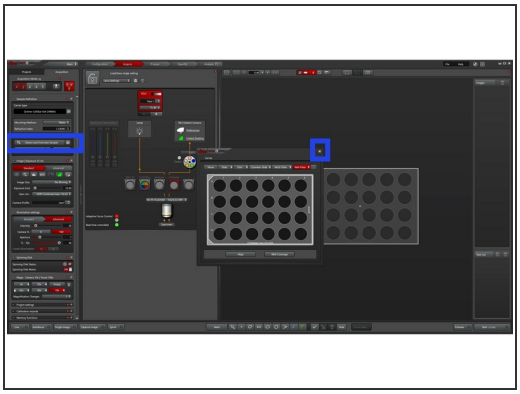
- Slide the **side panels** at the top of the **transmitted light arm** to the side, then fold the **transmitted light arm** backwards and open the **front flap** of the **Okolab incubator cage**.
- You can now load your sample onto the **stage**. The following **sample holders** are available:
  - **Universal slide holder**
  - **4-slide holder**
  - **Mixed slide holder** (1 slide + 2 × 35 mm dishes)
  - **Universal well plate holder**

Step 5



- **Ensure the universal frame is properly spring-locked**. As this is a **multi-user system**, always verify before imaging.
- available sample carriers.

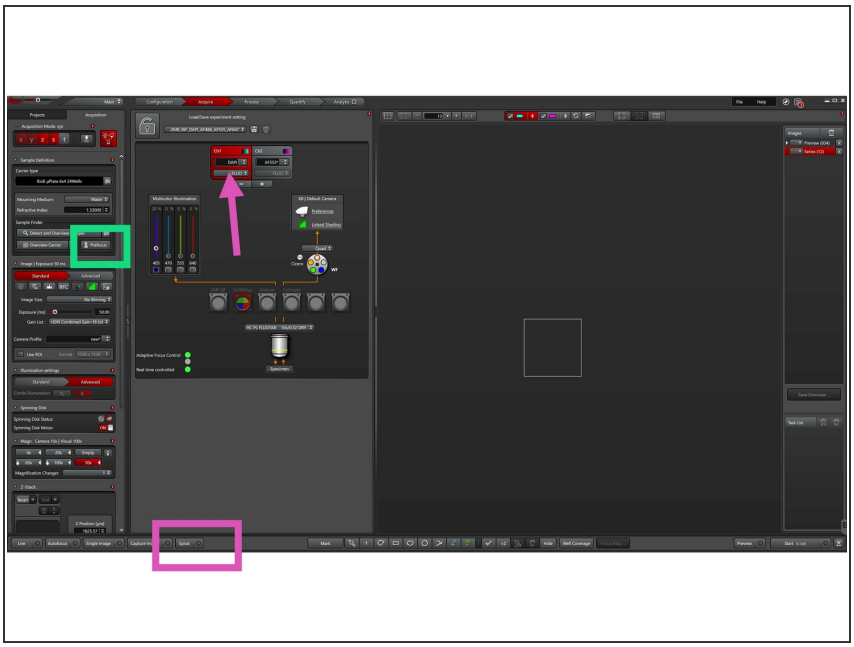
Step 6 — Automatic Carrier Detection and Overview Scan

- Click this **icon** to display the **complete beam path**. This view will then be **loaded by default** during your next imaging session.
- Click the **Define Carrier** icon and, in the pop-up window, define your **sample type** (e.g., slide, well plate, etc.).
- Close the pop-up window, then click **Detect and Overview Sample**.

ⓘ *The system now automatically performs the following steps: Using the **10× objective** and **AFC (Automatic Focus Control)**, it detects and aligns the **bottom of the imaging carrier**. The system then switches to the **4× objective** and acquires a **transmitted light overview image** of your sample for later navigation.*

Step 7 — Optional: Alternative overview generation with Spiral scan



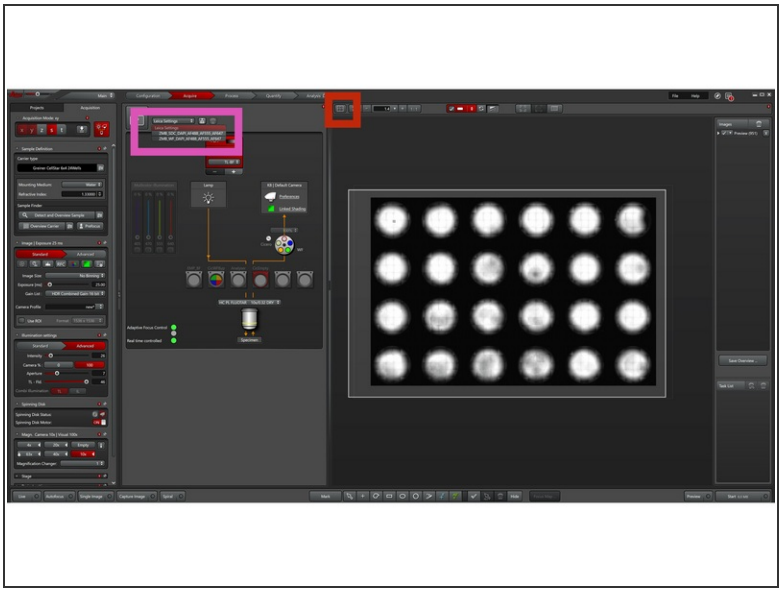
⚠ **Skip this step if automatic overview detection is used**

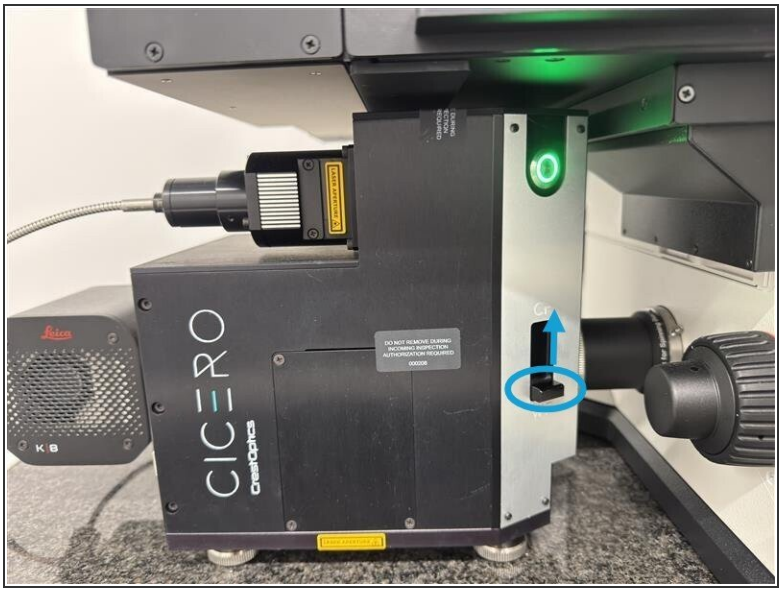
ⓘ *Not all **sample carriers** are supported by automatic overview detection. If **"Detect and Overview Sample"** is greyed out, use the alternative method below with a **spiral scan**.*

Move your sample above the **10× objective** while visually observing the **stage** with the **incubator cage open** to guide positioning.

- Click **"Prefocus"** to use **hardware focus** to approximate the focus position by detecting the **coverslip reflection**. Works best with the **10× objective** and **glass-bottom samples**. Then fine tune focus with SmartMove joystick in "**Live**" mode.
- Click **"Spiral"** to start generating an **overview image** in a spiral scan using the **active channel**. We recommend using **DAPI** or **transmitted light** for most samples.

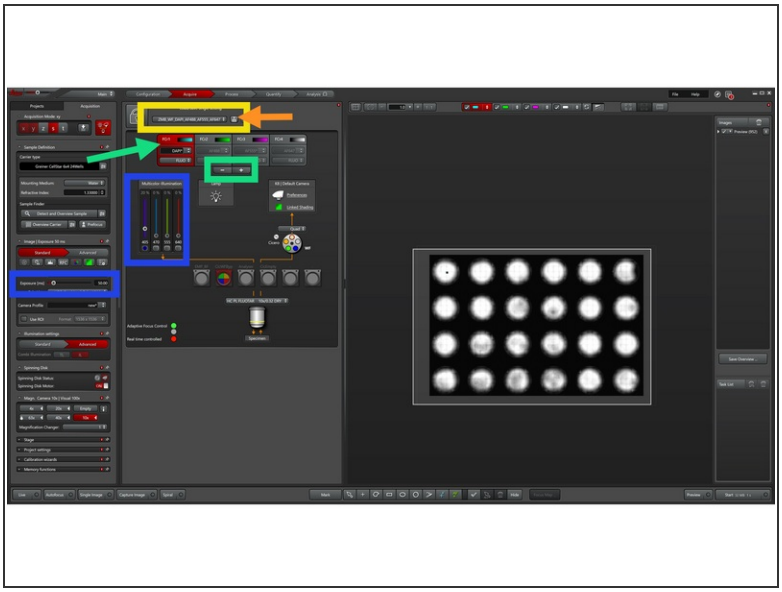
Step 8 — Load Default Fluorescence Settings

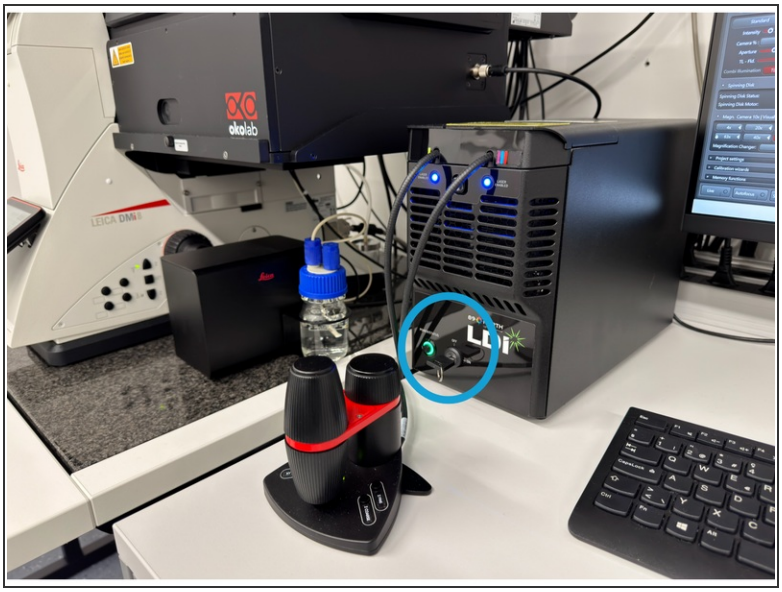




- By default, you are in the **Leica Navigator** (see this icon).
  - *The **Leica Navigator** allows you to **navigate your sample**, define **multiposition experiments**, or set up **tiling experiments**.*
- You can now load the **default fluorescence settings** from this **dropdown menu**:
  - **WF** for widefield
  - **SDC** for spinning disk confocal.
    - SDC always requires to physically insert Spinning disk ("**CF**") into beampath.

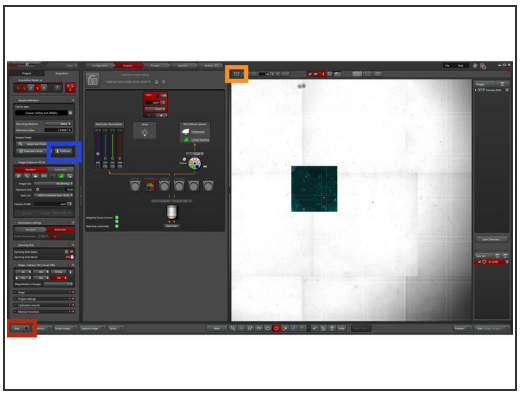
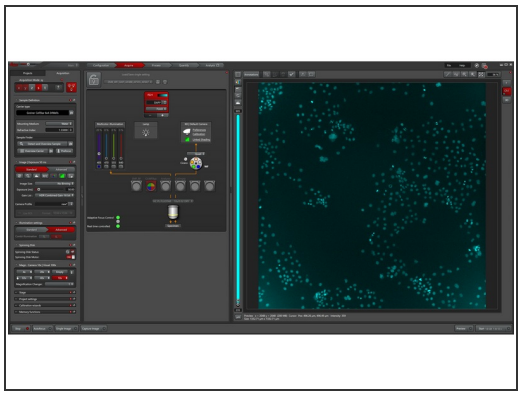
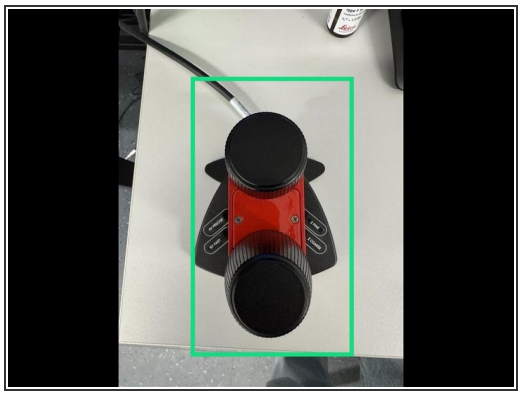
Step 9 — Verify Light Engine Status Before Live Imaging





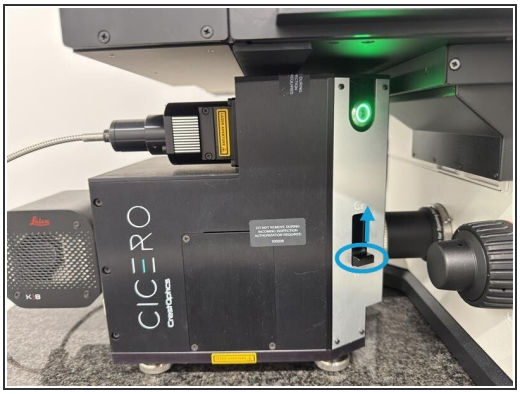
- We recommend using the **WF setting** for initial focusing, as it collects **emission signal from all planes**, making it easier to find the **correct focus**.
- The default settings use **20% illumination power** and **50 ms exposure time**. Depending on the sample, these parameters must be **adjusted experimentally**.
- Remove any **default channels** you do not need using the **“–” icon**. "**Red-Box**" highlights active channel.
- You can reload the **default settings** at any time or save your **own settings** for your personal use.
- With **LAS X 3.11**, the **light engine** should switch automatically from **red** to **green** when **Live** is started after a laser interlock event. If this does not happen, check that the **incubator cage is fully closed**.

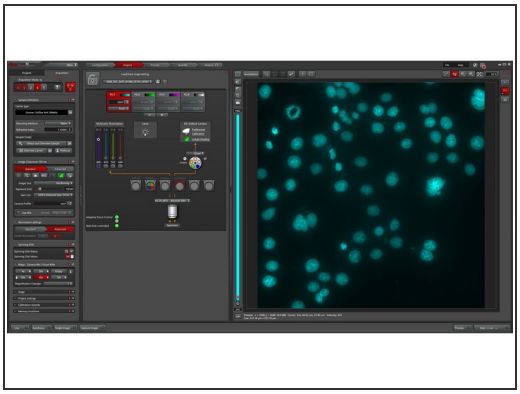
Step 10 — Find focus

- Click "**Live**"
- Click the **"Multichannel Viewer" icon** to display the **live view** in a larger window.
- Click **"Prefocus"** to use **hardware focus** to approximate the focus position by detecting the **coverslip reflection**. Works best with the **10× objective** and **glass-bottom samples**.
- Focus your sample using the **Smart Move controller**. **Z-axis** control **XY** stage movement You can switch between **coarse** and **fine (precise)** movement for all axes.

Step 11 — Load Spinning Disk settings



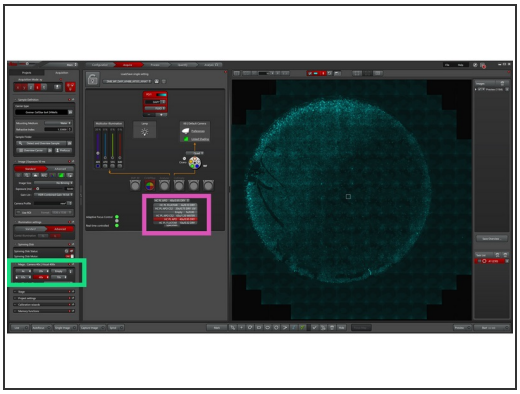
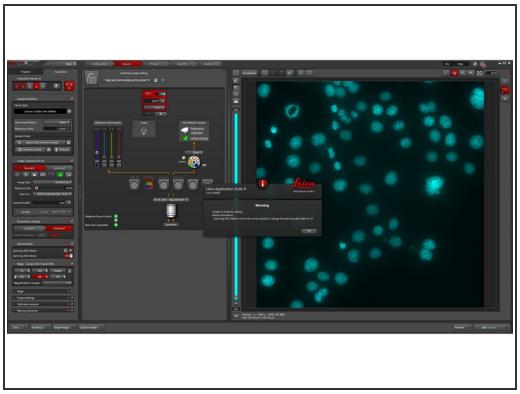
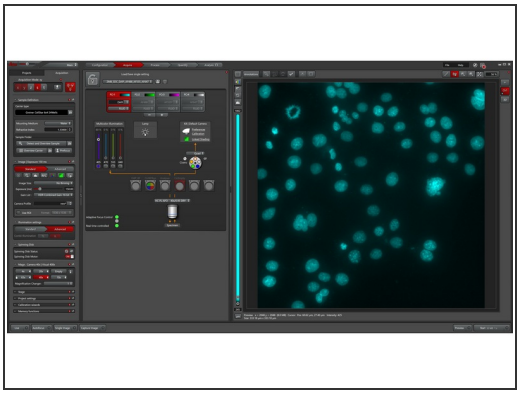
- SDC always requires to physically insert Spinning disk ("**CF**") into beampath.

ⓘ The default settings (SDC) use **80% illumination power** and **150 ms exposure time**. Depending on the sample, these parameters must be **adjusted experimentally**.

ⓘ **Compensation of light losses:** In spinning disk confocal microscopy, only a small fraction of the excitation light passes through the pinholes (we loose up to 95% of light). To ensure sufficient signal for high-quality imaging, a **high initial light intensity** is required.

- The warning pop-up appears only if **SDC settings** are loaded while the **spinning disk slider** is not in **"CF" position**. If this occurs, move the slider to **"CF"** and reload the **SDC settings** from the dropdown menu.

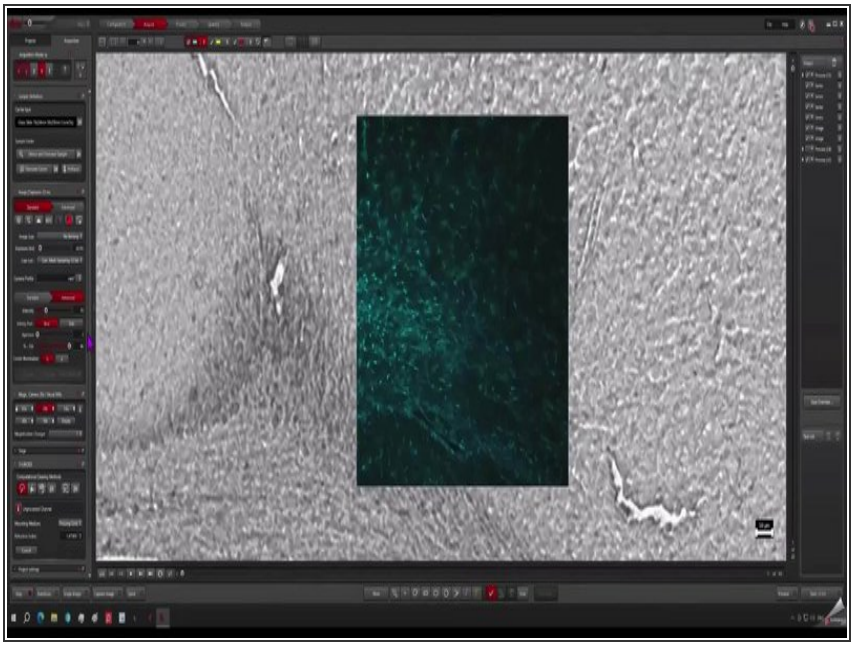
Step 12 — Change of objectives

You can change objectives in 2 different ways:

- *While **not** in "**Live**" mode*
- While in "**Live**" mode

Step 13 — Using the 63× Water Immersion Objective (Auto Immersion)



⚠ Optional Step: **Skip this step when you are not using 63x 1.2 objective.**

- For long-term experiments (live-cell, multiposition etc.), the system uses **Adaptive Immersion** to automatically maintain the **water droplet**.
- The system will **automatically apply and maintain immersion water** during imaging.
- When switching objectives or moving to the loading position, the **water droplet is removed automatically**.
- **Note:** The system is factory-installed and works automatically. No manual intervention is required during normal operation.

Step 14 — Using SmartCORR (motCORR) with 63x 1.2 Objective



⚠ Optional Step: **Skip this step when you are not using 63x 1.2 objective.**

ⓘ In conventional high-resolution microscopy, the **correction collar** must be adjusted manually to match the **coverslip thickness** or the **refractive index** of the sample.

ⓘ **SmartCORR** automates this step by correcting **spherical aberrations** caused by optical mismatch. It reduces **spherical aberrations** and ensures optimal **resolution and contrast**, especially for **deep imaging** in 3D samples.

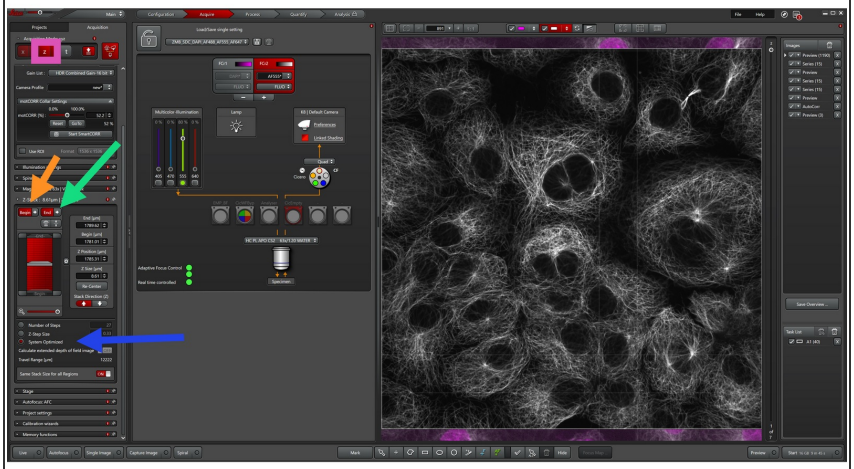
Click **"Start SmartCORR"** to automatically determine the optimal correction. The system acquires an **image series** and sets the **optimal CORR position**.

- Requirements:
  - **Sample must be** in focus **and** fluorescent

  ⚠ Correct **refractive index** and **embedding medium** must be defined. In sample definition tab.

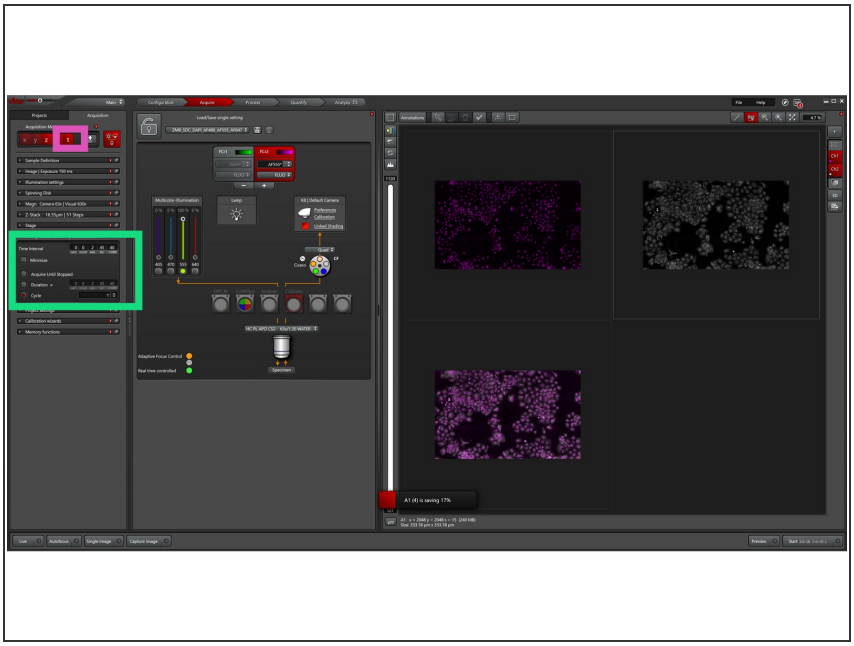
  - Available only in fluorescence (WF and SDC) mode with **63x 1.2 objective**. **Note:** The objective is optimized for **~21 °C** and **140–180 µm coverslips**.

Step 15 — Acquire a Z-Stack



- Enable "**Z-Stacks**".
- Define your **z-stack** by setting the upper and lower boundaries of your sample.
- Move to the desired start position and click **"Begin"**
- Move to the end position and click **"End"**
- Define the **Z-step size** or enable **"System Optimized"**
- ***"System Optimized"*** *automatically calculates the* ***optimal Z-step size*** *and* ***number of steps*** *based on the optical settings. This ensures proper* ***sampling (Nyquist criterion)*** *for accurate 3D reconstruction without unnecessary data.*
- The system will acquire images across the defined **z-range**.

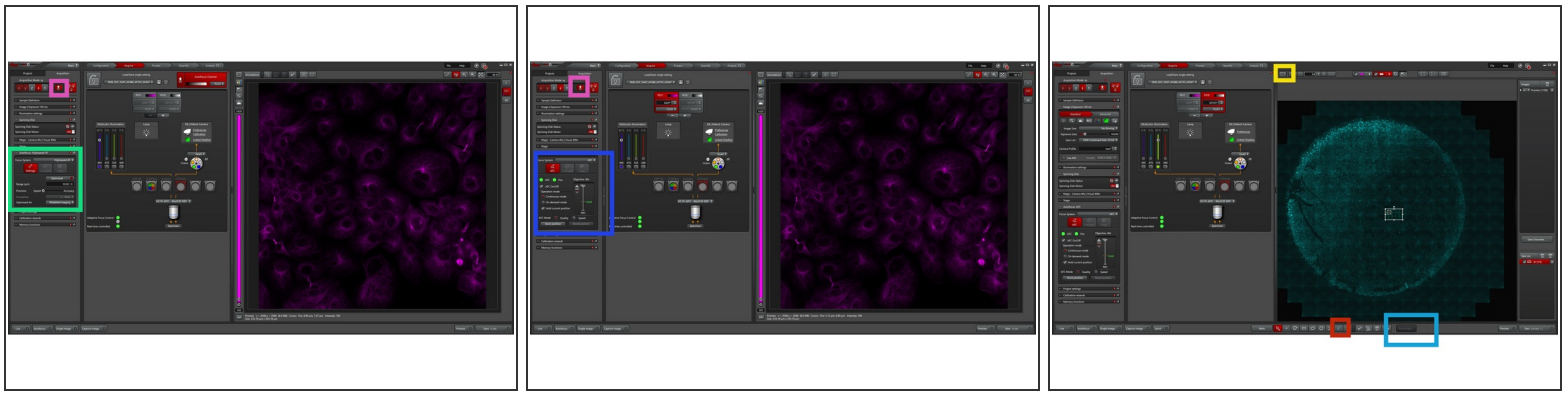
Step 16 — Acquire a Time-Lapse



- Enable "TimeLapse" to define how your sample is imaged over time.
  - Set the **Time Interval** between acquisitions
  - Enable **"Minimize"** for maximum acquisition speed (optional)
  - Set the **number of cycles** or total **duration**
  - (Optional) Enable **"Acquire Until Stopped"** for continuous imaging

ⓘ The system will acquire images at the defined **time intervals**.

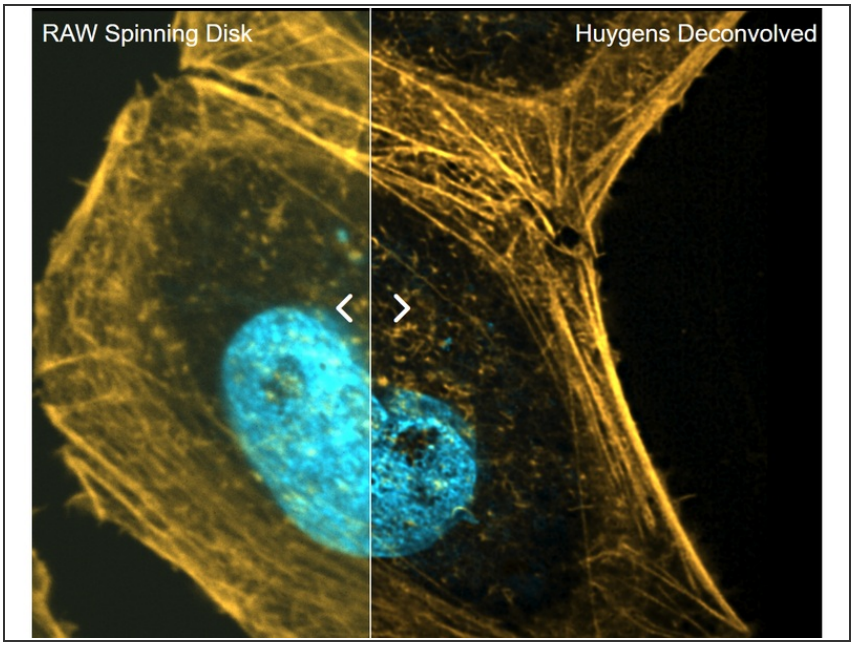
Step 17 — Focus strategies



Leica systems typically offer three focus strategies to keep images sharp either by mapping the sample surface, computing focus from image contrast, or actively locking focus with dedicated hardware.

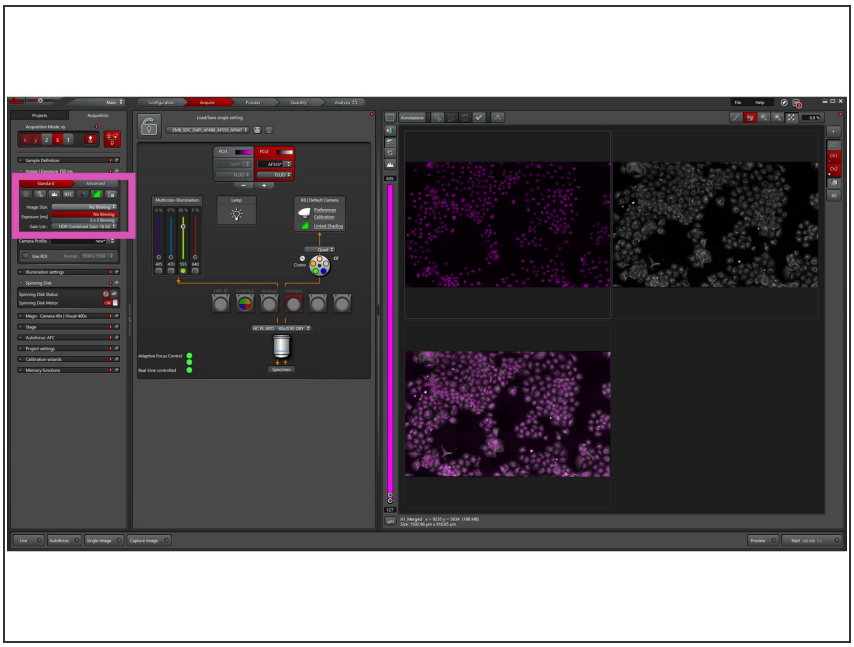
- Click on Autofocus Panel to be able to see the different strategies:
  - **Software Focus**: Image-based autofocus using a contrast/sharpness metric. Runs a short Z-search and selects best focus. Flexible but slower; good for occasional refocus when no reliable reflective plane exists.
  - **AFC (hardware focus)**: Optical focus lock using reflection (typically at coverslip–medium interface) to track and correct Z-drift in real time. Very fast and stable for long time-lapse/live imaging.
- In order to generate a "**Focus Map**" make sure you are in "**Navigator**" interface.
  - **Focus Map**: Define focus at several XY points to model sample tilt/curvature. The system interpolates Z between points during acquisition to maintain focus across tiles/wells, ideal for uneven samples and large area scans.
  - Distribute **focus map points** across your **tile region**. Use at least **four points**, depending on sample complexity.
  - Select the first **focal point** in the image or table, then define its **focal plane** manually with **"Set Z"** or automatically with **"Set by Autofocus"**. Use **"Find all by Autofocus"** to detect all remaining focal planes automatically.

Step 18 — Improve Image Quality with Deconvolution



ⓘ While **spinning disk confocal imaging** already provides good **optical sectioning**, additional **deconvolution** can further reduce residual out-of-focus blur and improve **contrast** and **resolution**.
See also: Image Deconvolution using SVI Huygens

Step 19 — Adjust Binning Settings



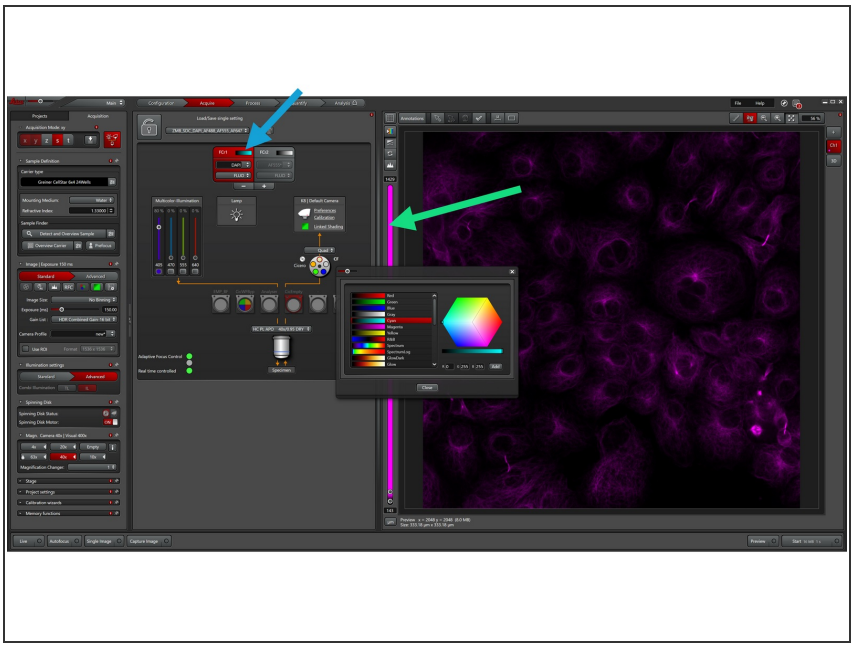
- **Binning** combines neighboring camera pixels into one larger pixel. For example, with **2 × 2 binning**, four pixels are merged into one.

⚠ This increases **signal per pixel** and reduces the **data volume**, allowing faster acquisition, but at the cost of **spatial resolution**.

ⓘ You could use **binning** when signal is low or speed is critical:

- ⓘ **Live-cell imaging** to reduce exposure time and phototoxicity
- ⓘ **Fast dynamics** where high frame rates are required
- ⓘ **Low signal samples** to improve signal-to-noise
- ⓘ **Overview scans** where maximum resolution is not needed

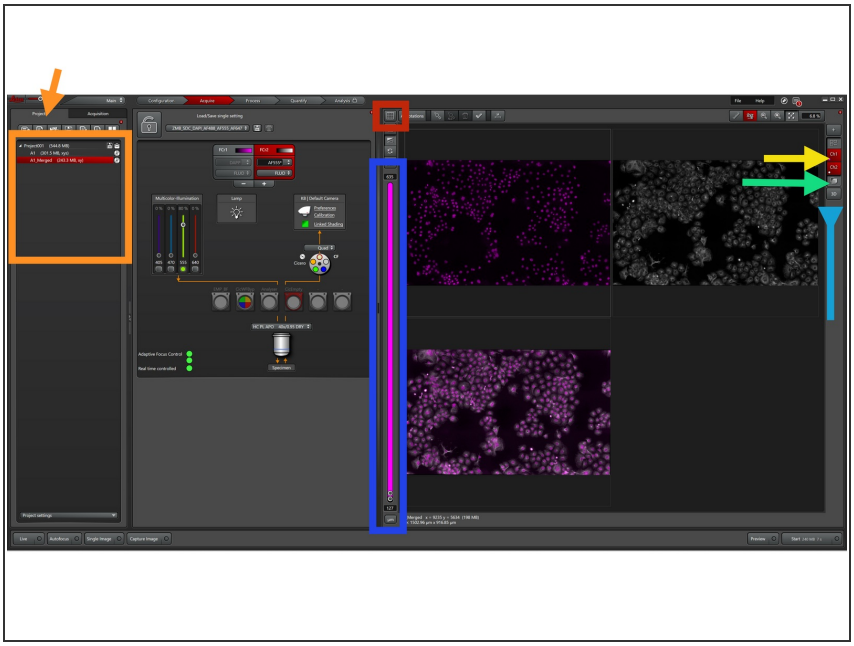
Step 20 — Adjust Look-Up Table (LUT)



ⓘ *In general, the selection of a suitable look-up table for a specific use depends on the user's own judgment and needs.*

- You can assign a specific **LUT** to a channel by double-clicking here.
- You can temporarily assign a specific **LUT** to an acquired channel by double-clicking on the **histogram bar**.

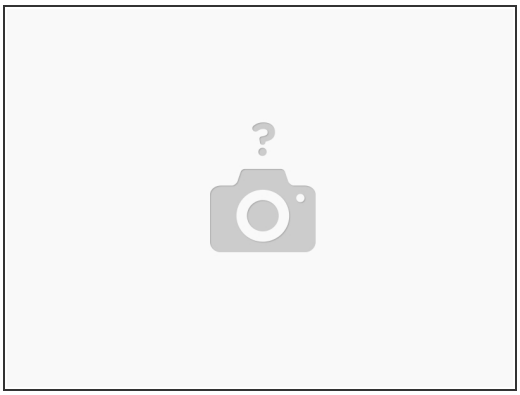
Step 21 — Review Acquired Data and Stitched Tile Scans



- To review acquired images, ensure you are in the **"Multichannel Viewer"** interface.
- Open the **"Project"** tab and select the dataset you want to view in the **Image** tab.
- Select which **channels** to display.
- Click **"MIP"** to generate a temporary **maximum intensity projection**.
- Click **"3D Viewer"** to visualize your data in **3D**.
- Adjust the **histogram** and modify the **LUT** to optimize image display.

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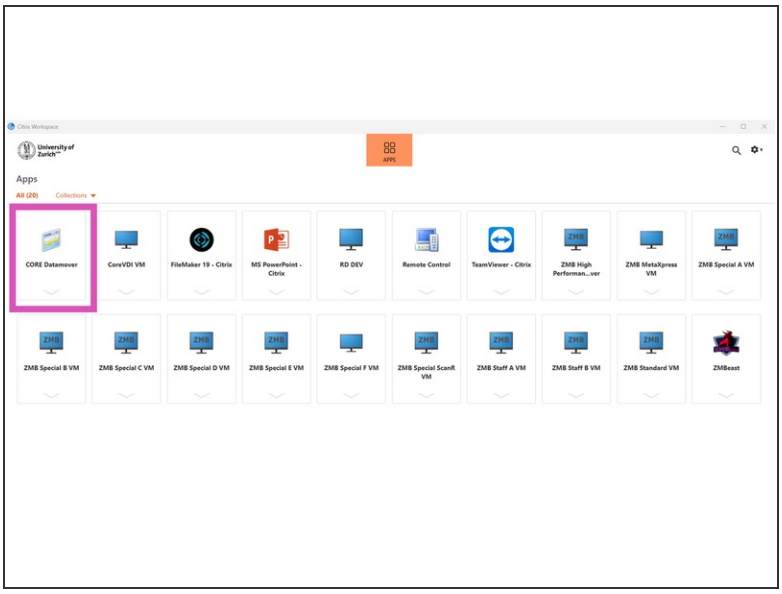
Step 22 — Optional: Auto-Save and How to Handle the Resulting Files

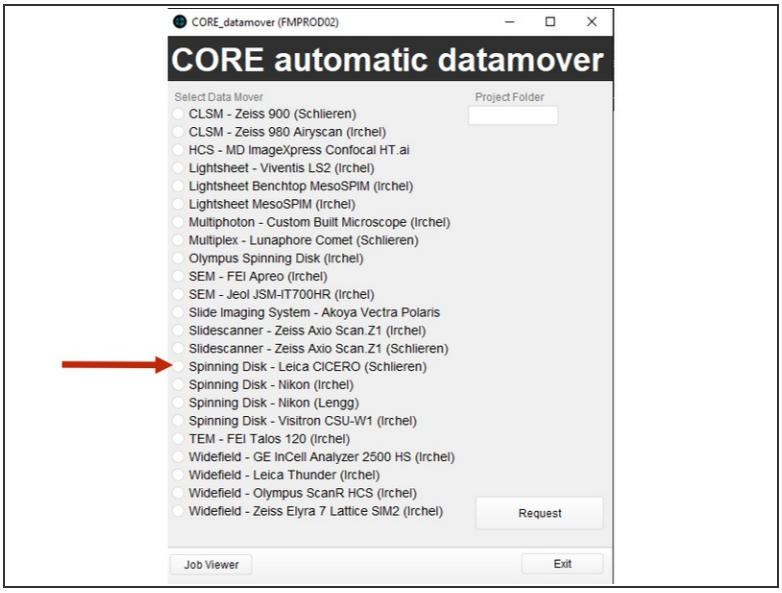


By using auto-save, there is less risk of data loss during an extended image acquisition session and long file saving times after an extended imaging session can be avoided. ***Since auto-save does not generate standard Leica .lif files, we also show how the resulting files can be handled and visualized.***

- Follow this Guide

Step 23 — data saving and data mover export





- Save data to the **E:\automatic_transfer\files\n.schilling: drive**. The network can be **slow and unstable**, especially for **large 3D datasets** and **live-cell experiments**.
- In the **automatic transfer folder**, create a subfolder named with your **Core username** (e.g., n.schilling).
- After exporting, close the **software** and **log off**.

⚠ Leave all other **hardware powered on**. Do not shut down any additional components.

- Use **Citrix** to initiate data transfer via the **ZMB DataMover** from the **local CICERO PC** to the **server infrastructure**.
- Access **Citrix** via the **app** or through the browser: vdi.core.uzh.ch
- Select in Datamover **Leica CICERO SDC**