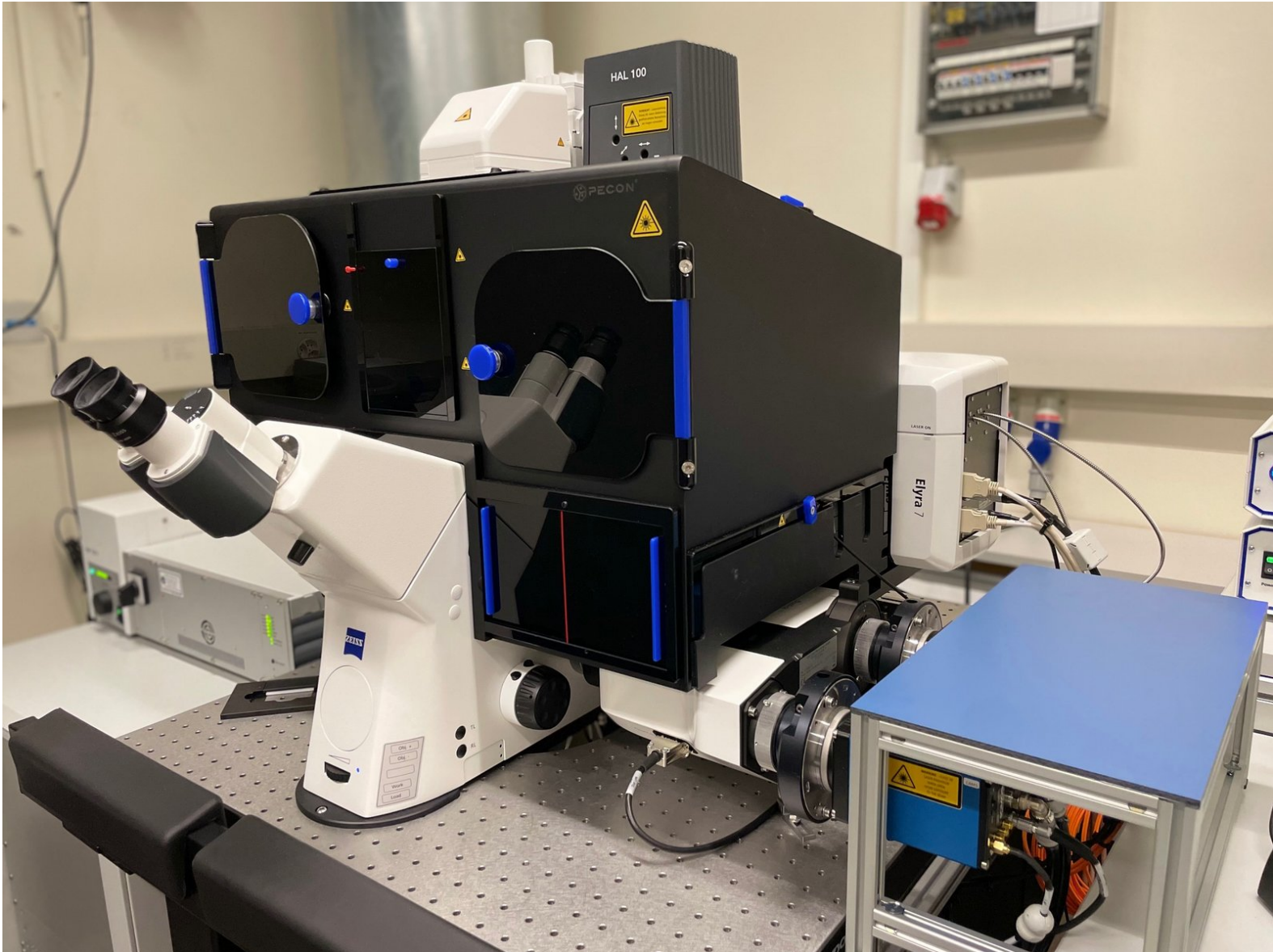
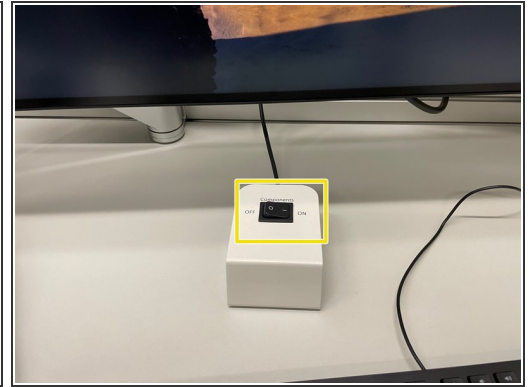


Zeiss ELYRA 7 SIM² Guide

Written By: z mbstaff

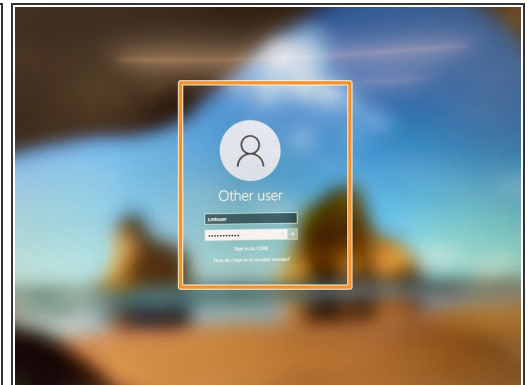



Step 1 — Start Up



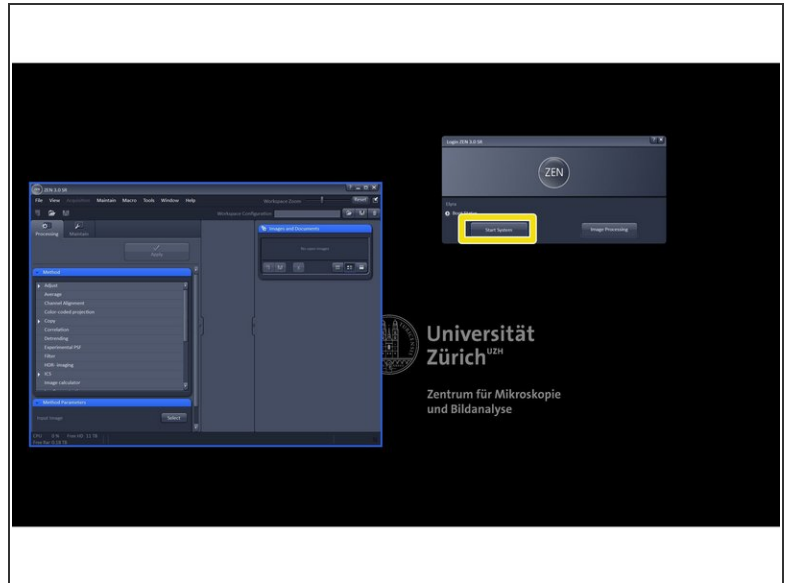
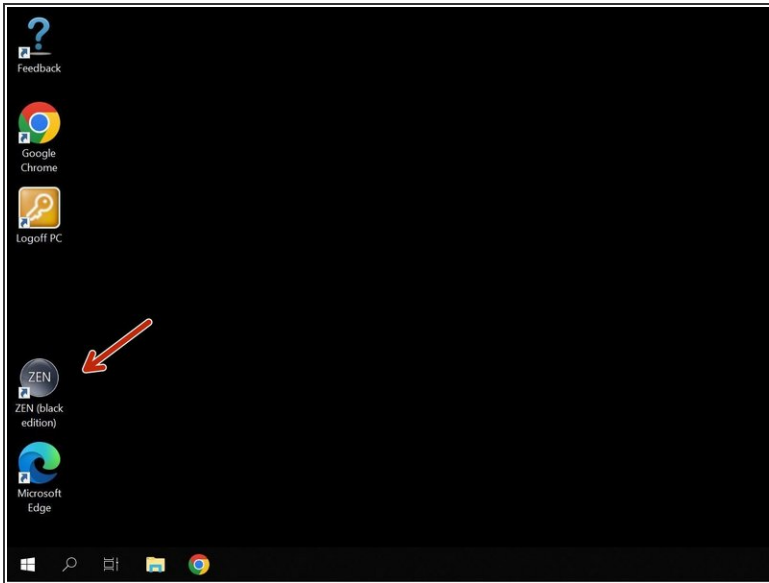
- Turn on the HXP lamp.
- Turn ON the "**Main Switch**" and also switch the laser key to "**ON**".
- Switch ON the "**Components**" on the table.

Step 2 — Turn on PC



- Turn "**ON**" the computer.
 - Sign-in with your ZMB core credentials.
-  *In case you forgot your password please follow this [link](#).*

Step 3 — Start the "ZEN" software



- Start the "ZEN (black edition)" software.
- Click "Start System".
- ❗ *The system will automatically initialize the hardware.*

Step 4 — Mount your sample



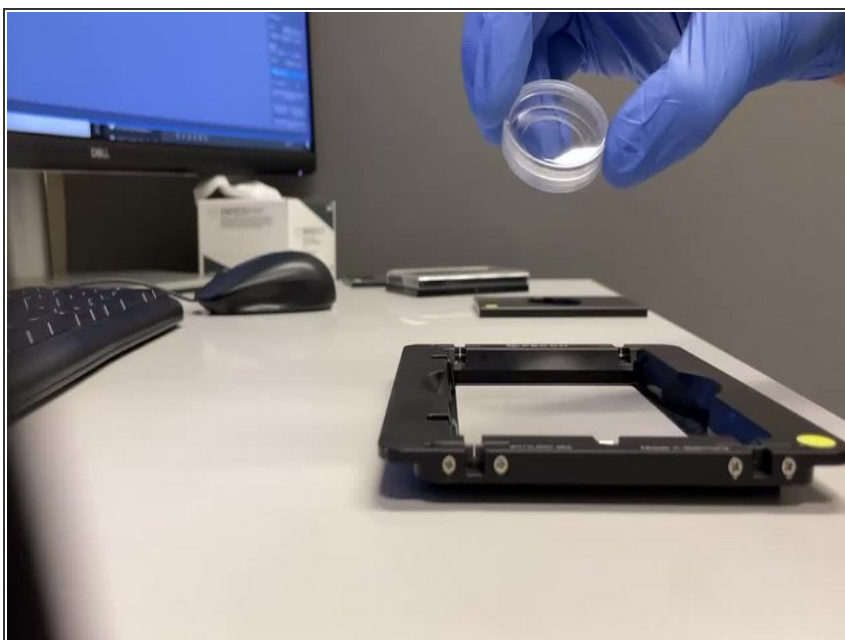
- Place you sample with the correspondin Click-In insert.
- *Make sure the frame locks securely into the stage completely flat.*

Step 5 — Mount your sample :Universal slide insert



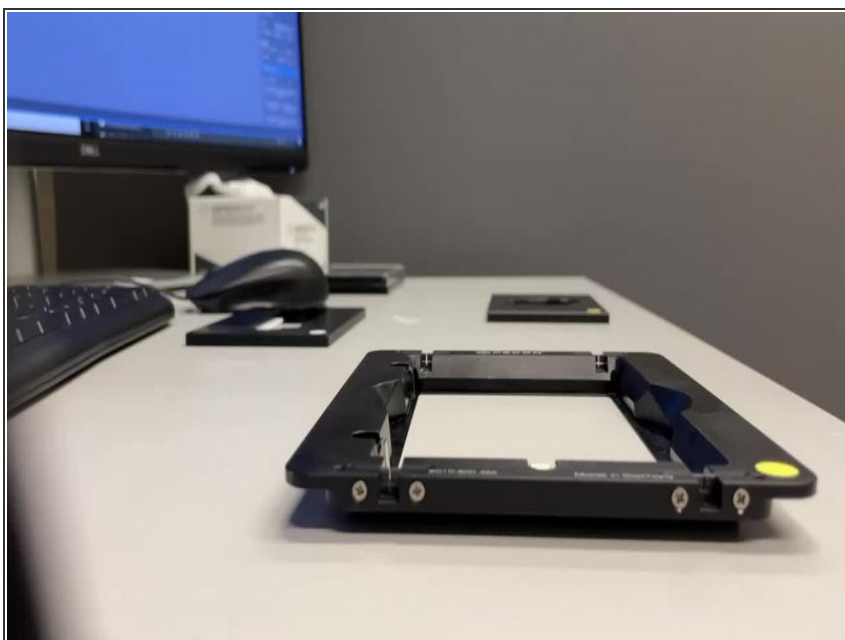
- *The slide insert is compatible with the following formats:*
- μ -Slides by Ibidi
- Nunc Lab-Tek™ Slides
- Chambers by Thermo Scientific
- Object Slides
- PDMS Chips based on 25x75mm slides

Step 6 — Mount your sample: Universal 35 mm petri dish insert



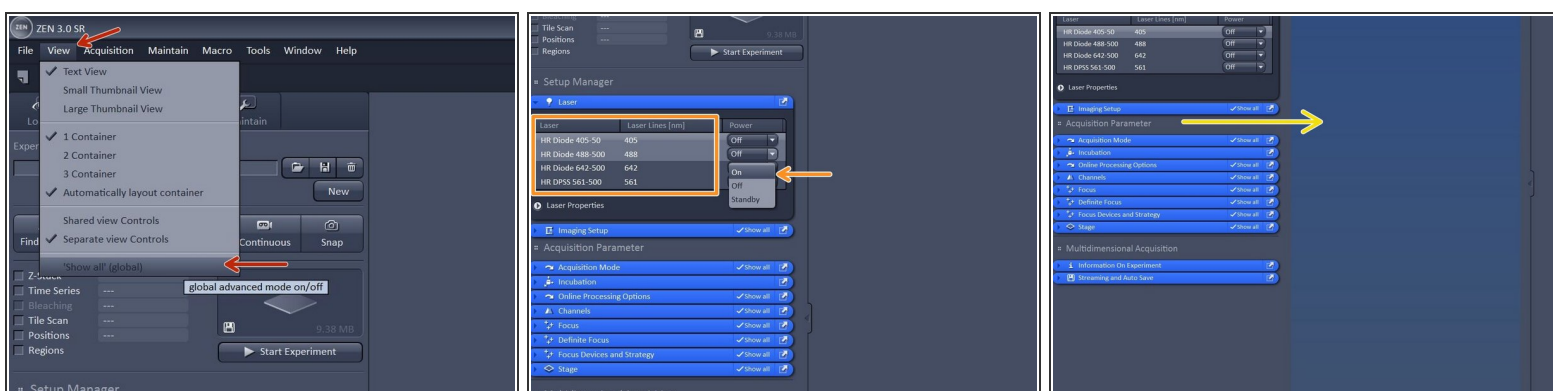
- Insert the 35 mm Petri dish into the KM Frame.

Step 7 — Mount your sample: Universal well plate insert



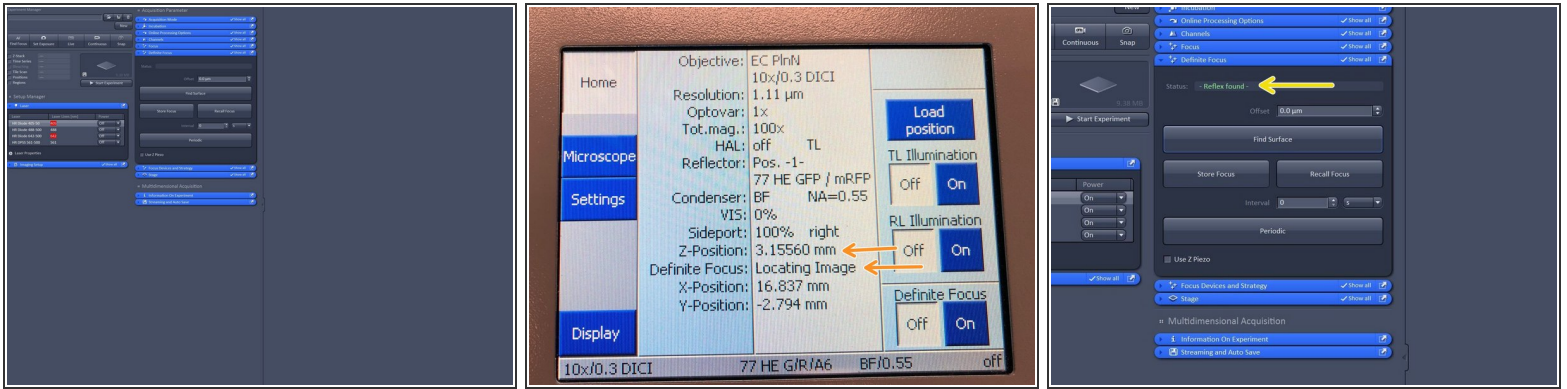
- *Compatible with all kind of well plate formats.*

Step 8 — Switch on lasers and get organized



- In acquisition tab: Click "View" - Show all global.
- Switch on all lasers needed for your experiment.
- ⓘ *Note: Red highlighting indicates that lasers are still in warm up phase.*
- Drag and drop the Acquisition parameters to the right in order to get more organized.

Step 9 — Get your mounted sample in focus



⚠ Make sure you have mounted your sample and the 10X objective lense is bellow your sample.

⚠ Not that the following feature is not available for the 2.5X objective.

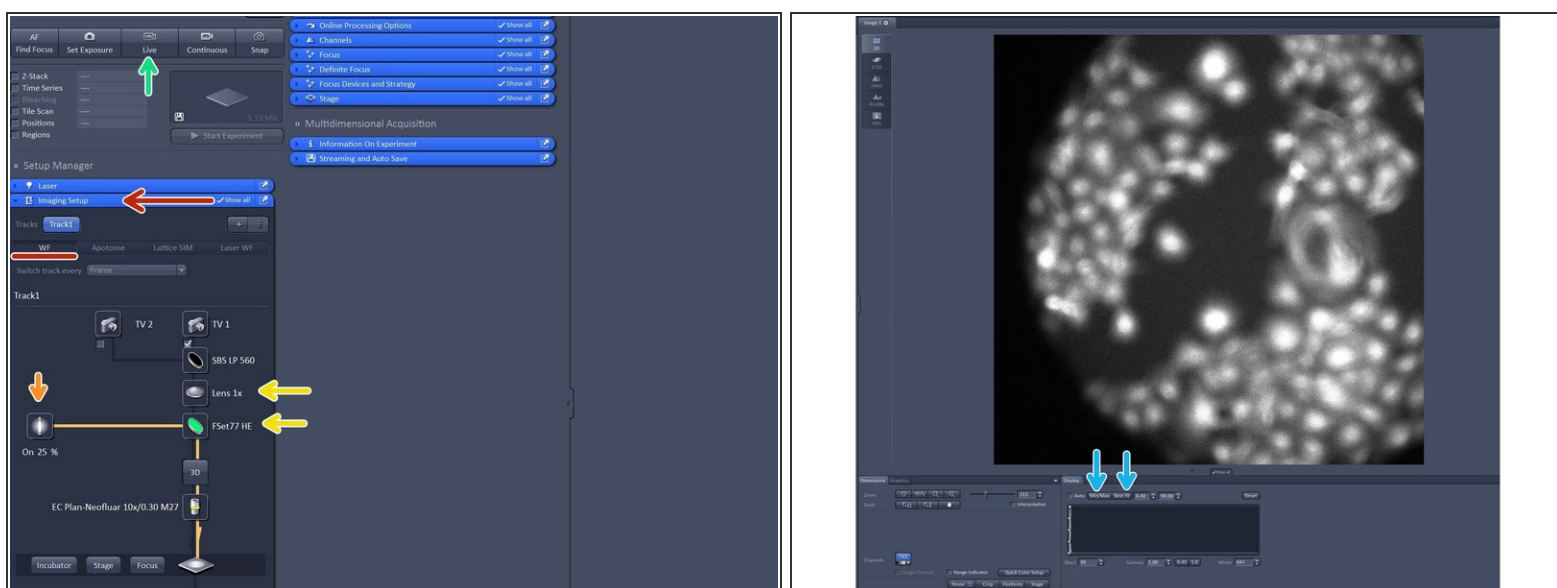
- Open "**Definite Focus tab**" and click "**Find Surface**".

❗ The microscope will perform a full range Z-scan and the Definite Focus will look for the strongest reflection signal.

- On the touch panel you can monitor that the system automatically tries to find the coverglass reflection while moving through Z.
- Once reflex is found the system will report "**Reflex found in green letters**"

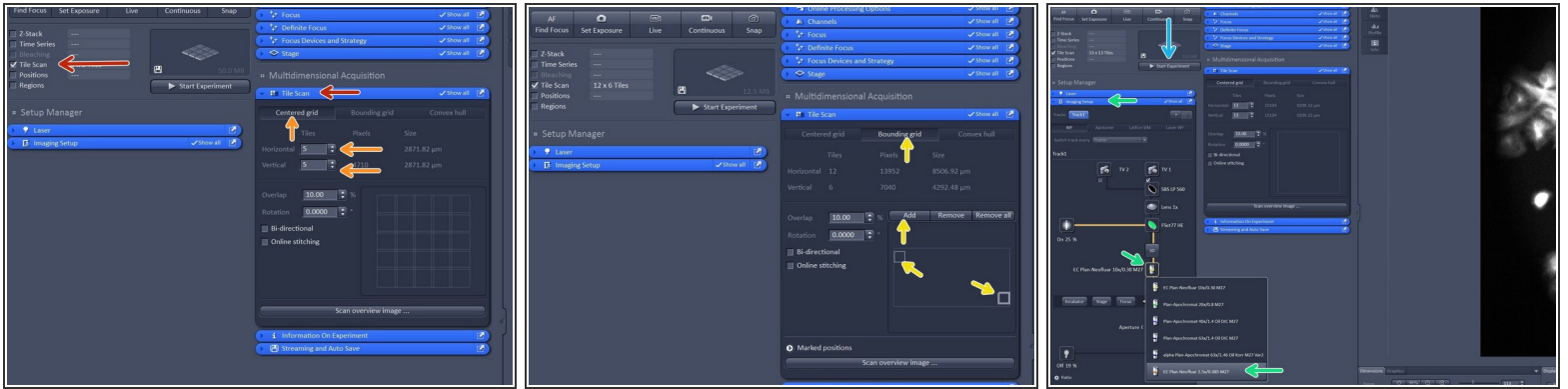
⚠ If the refractive index mismatch between the immersion medium and glass is low, a reflexion might be too weak to be detected.

Step 10 — Get in focus



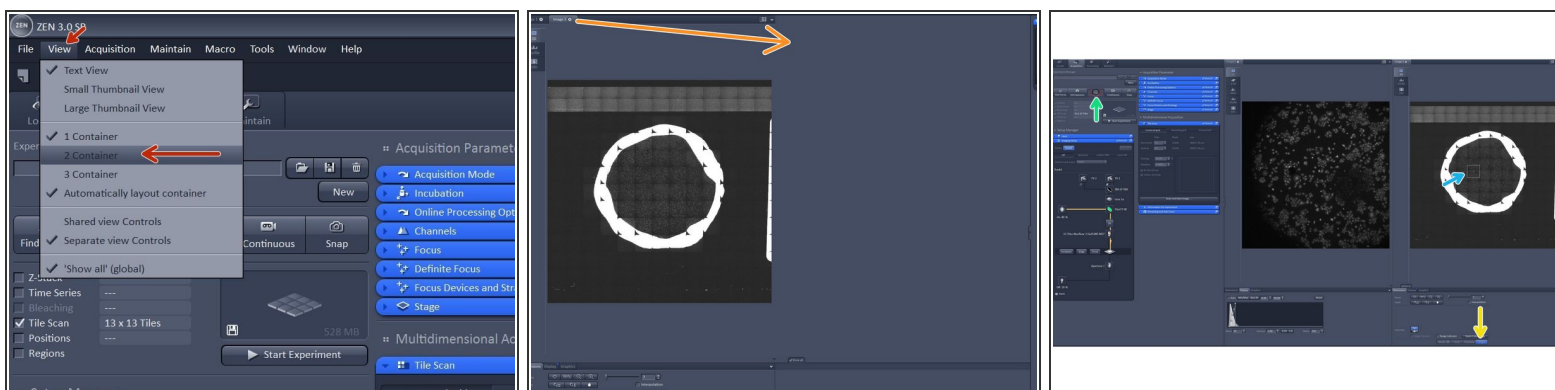
- Open the "**Imaging Setup tab**" and define a classic wide-field track by clicking "**WF**".
- Switch on the fluorescent lamp.
- Make sure that **FSe77HE** filter is selected and the **1X tube lense**.
 - ❗ FSet77HE is a triple band filter (Green-Red-FarRed) is the only filter for visual inspection.
- Click on "**live**".
- In case you have a black image click on "Min/Max" or "Best fit" in order to adjust the display of your image
- When a reflex is found, this does not necessarily mean that the sample is in focus. Therefore, fine tune your focus with the coarse hand wheel by turning towards you.

Step 11 — Create an overview



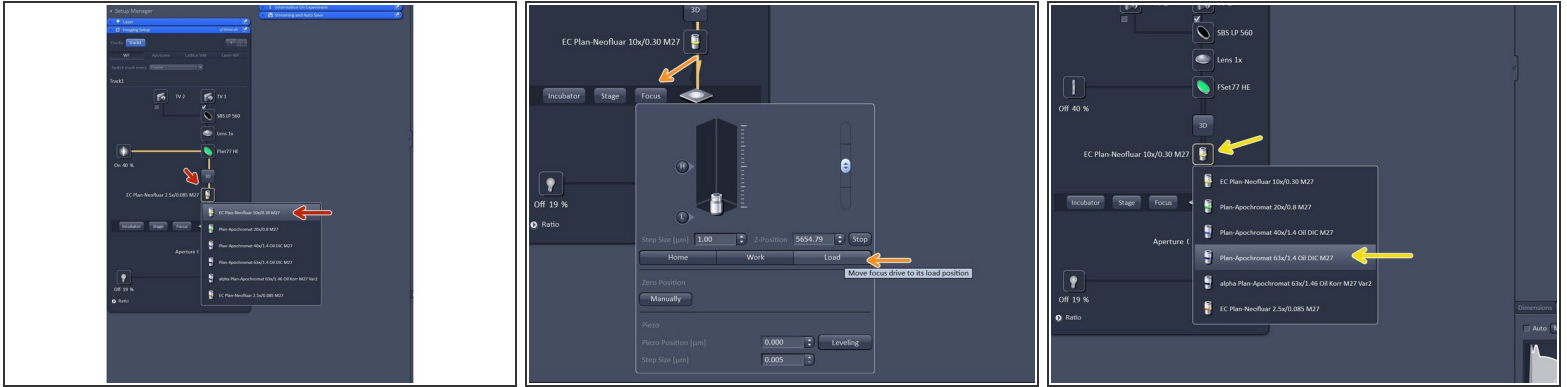
- In order to create an overview - activate the "**Tile Scan**" option.
- For a coverslip or 35 mm petri dish choose "**Centered grid**" and adjust the tiles in horizontal and vertical direction (13x13 is usually enough for a 18 mm coverslip).
- ❗ *Make sure your objective lens is roughly in below the center of your sample.*
- For a chamber slide or a well plate choose "**Bounding grid**"
 - Here you manually move the stage via the joystick to the very top left corner of your sample carrier and then click "**add**" then you move to the very right bottom corner of your sample carrier and click "**add**".
- In "**Imaging Setup tab**" change the objective lens to the **2.5X lense** as it has a larger field of view in order to create a quick overview.
- In order to start your overview click "**Start Experiment**".
- ⚠ *Make sure that the fluorescence lamp is switched on before starting the overview.*

Step 12 — Navigate within your overview



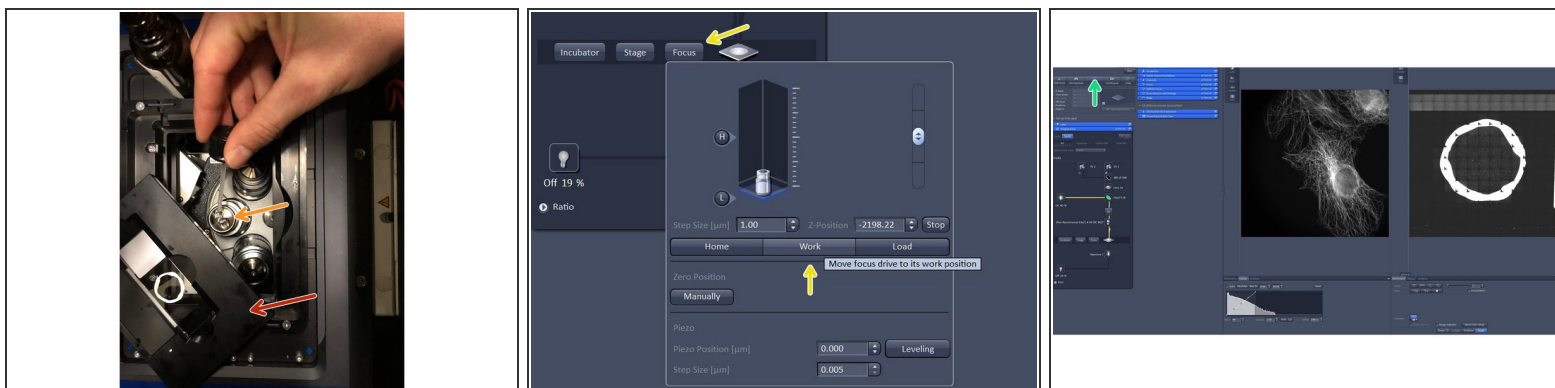
- In order to make use of your overview click "**View**" and then click "**2 containers**".
- Drag and drop the overview image to the second container.
- Activate the "**Stage tool**".
- Click "**Live**".
- You can now move the stage by clicking in your overview image. The stage position is indicated by the white rectangle.
- *You can create these overviews even with higher magnification in order to see more details.*
- *You can create these overviews even with transmitted light, SIM, Apotome or laser WF.*
- *When done with the overview do not forget to deactivate the "**Tile Scan**" option.*

Step 13 — Changing to an immersion objective lens



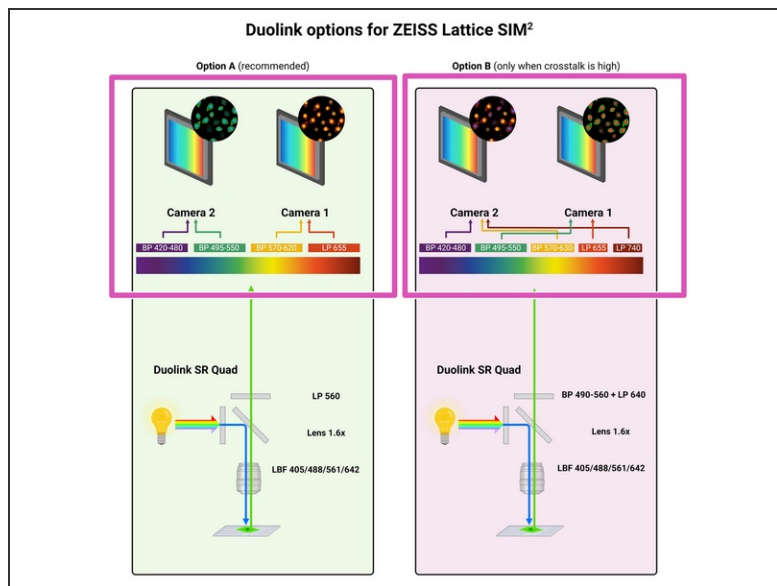
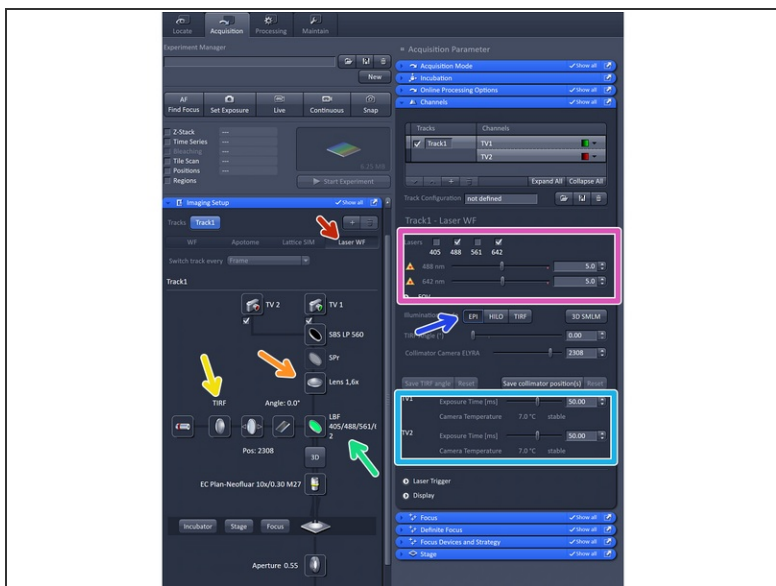
- Switch to the 10x objective and check that you are in focus.
- In "**Imaging Setup tab**" click "**Focus**" and click "**Load**" - in order to lower the objective.
- Change to the desired objective via the drop down menu.
- ❗ *Objectives suited for a certain imaging mode will be highlighted, but every objective can be selected.*
- ❗ *Note that for SIM the Plan-Apochromat 63X 1.4 oil and for TIRF/SMLM the alpha Plan-Apochromat 63X 1.46 oil are the objectives of choice due to their optimized optics.*

Step 14 — Changing to an immersion objective lens



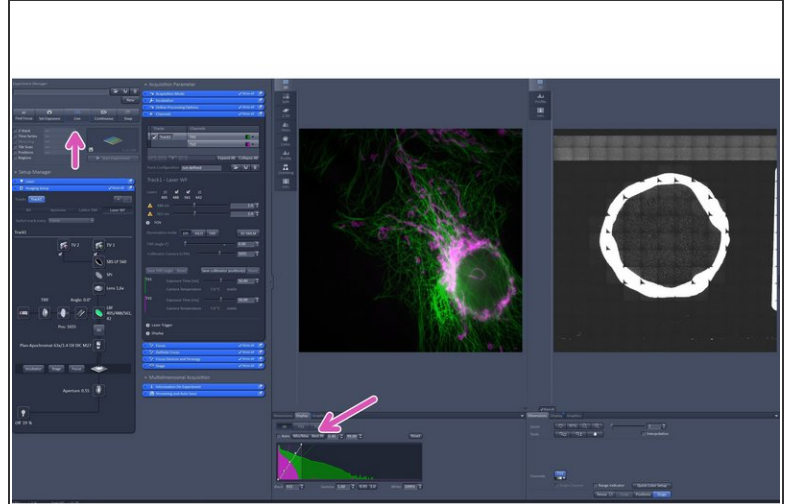
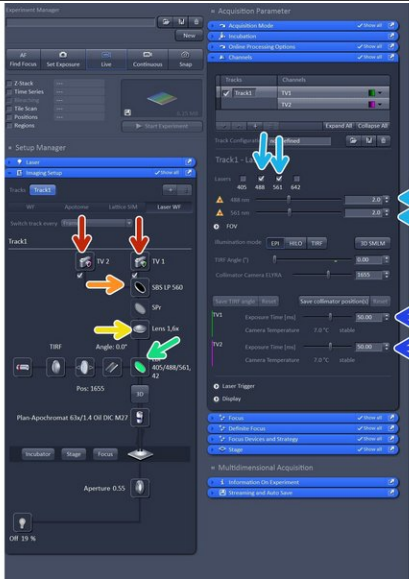
- Remove the sample carrier plate.
- ⚠ *Do not touch your sample within the carrier plate as this will disalign the overview.*
- Add a drop of immersion oil onto the objective lens.
- Place the sample carrier plate again back in the frame and close the incubator box of the microscope.
- Bring sample in focus by clicking on "**work**".
- Click "**live**" and fine-tune your focus with the hand-wheel.
- ⓘ *Try to use the coarse hand-wheel and turn it slowly in your direction.*

Step 15 — Setting up a laser wide-field track



- Select "**Laser WF**".
- Choose the camera (TV2 or TV1) depending on your fluorophores used.
- Make sure 1.6 X is selected.
- ❗ For WF imaging mode, Lens 1x is appropriate, for Laser WF, SIM Apotome and SIM imaging modes Lens 1.6x should be used.
- Make sure you have "**TIRF**" mode activated.
- Select via the reflector button the "**LBF**" filter.
- Activate and adjust your **laser lines**.
- Adjust your required **exposure time**.

Step 16 — Setting up a dual colour track



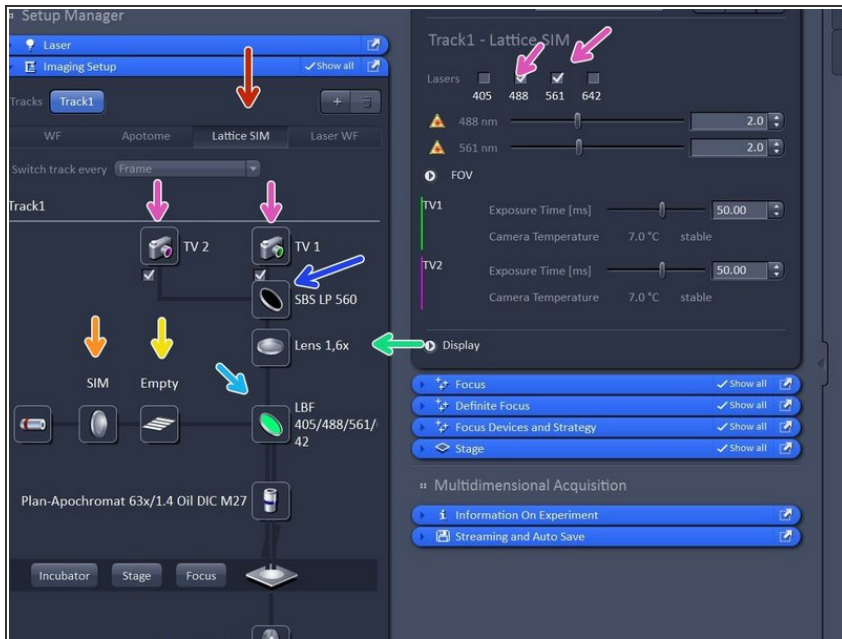
- Activate both cameras.
- Choose your **SBS** configuration.
- Make sure tube lense is **1.6X**.
- Select your **filter** of choice. We recommend the **LBF**.
- Activate two compatible **laser lines** based on **SBS** configuration.
- Adjust **exposure time**. Note that when using dual link that exposure time is the same for both cameras.
- Click **live** and and adjust the display by **adjusting the histogram**.

Step 17 — Dual Link alignment



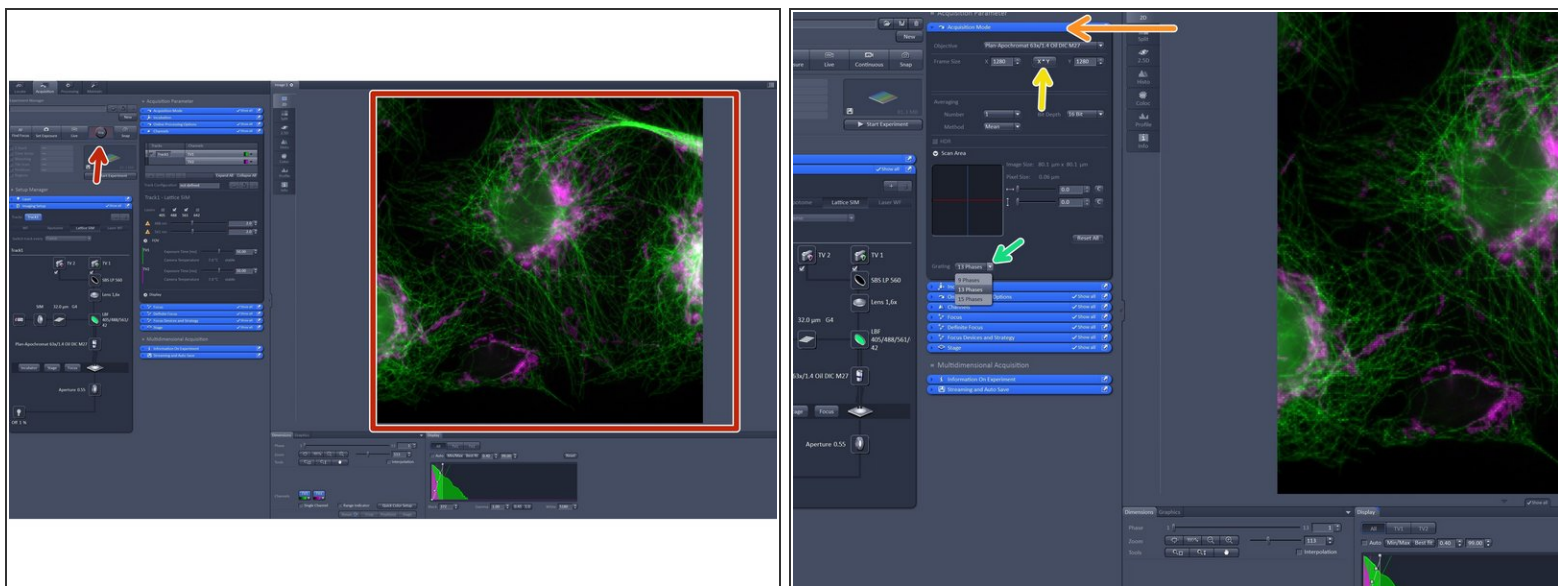
- Change to the "**maintain tab**".
- Insert "**Calibration pattern**" by activating it.
- Click "**XY**" and wait for finishing.
- Click "**XRot**" and wait for finishing.
- Click "**Z**" and wait for finishing.
- Note that the alignment should only be judged in the center as at the edges it will be off due to lense distortion.
- When done with the alignment remove the calibration pattern by deactivating it. And go back to the "Acquisition tab".
- Please note that the alignment via the calibration pattern is only rough, in case you need a precise alignment please it manually with multi-spec beads.

Step 18 — Setting up lattice SIM2



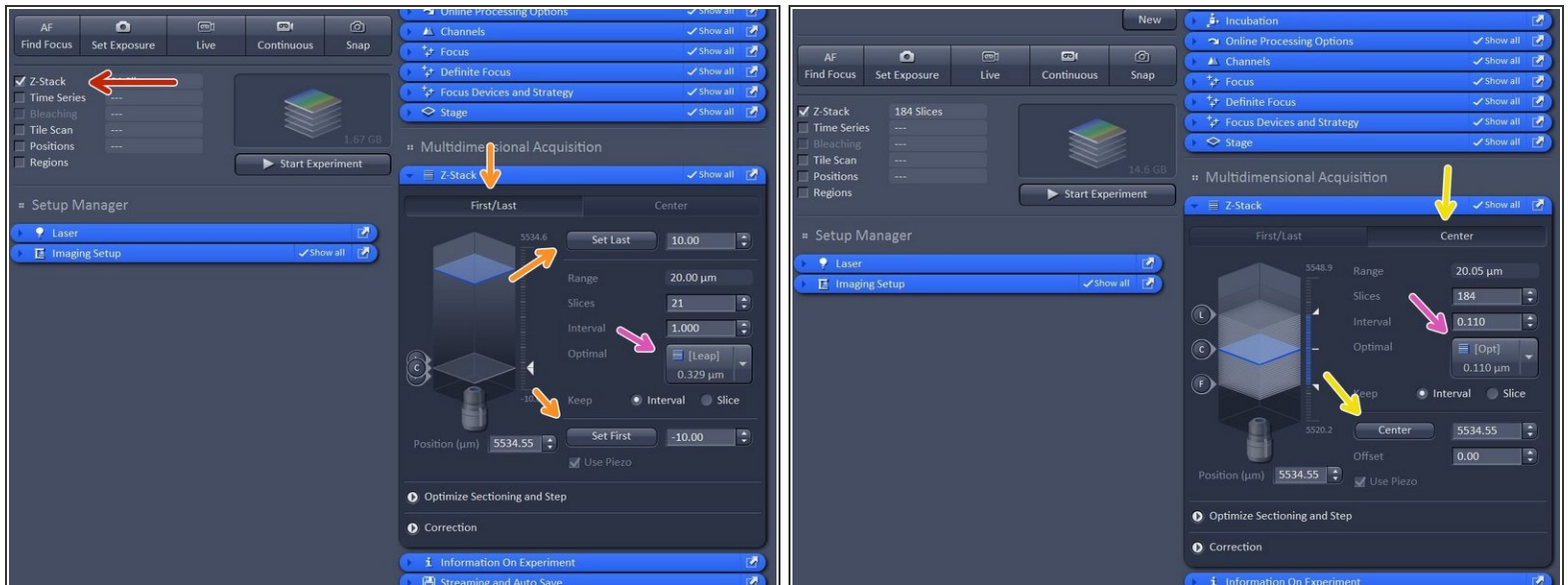
- Activate "**Lattice SIM**".
 - Check that "**SIM**" is selected.
 - Check that tube lense is **1.6X**.
 - Choose your **filter of choice**.
 - Choose your **SBS** configuration based on your experiment.
 - Choose **cameras** and **lasers** accordingly.
 - Click "**Grating**" button to open the **Grating selection**. The system should automatically load the grating for optimal resolution enhancement, if this is not the case switch on and off lasers.
- ① You can choose between an **Empty** position and 5 grid (**G1-G5**) with increasing spacing. The grating depends on laser line and objective used. Not that the SIM grid pattern will always be adjusted to the higher wavelength when using dual camera.*

Step 19 — Acquisition Mode



- Click "Continuous" in order to apply all imaging parameters to see SIM modulation contrast on your sample.
- In "Acquisition Mode":
 - You can adjust your **frame size**.
 - You can adjust your **grating**. More phases will result in more modulation information meaning and better resolution.

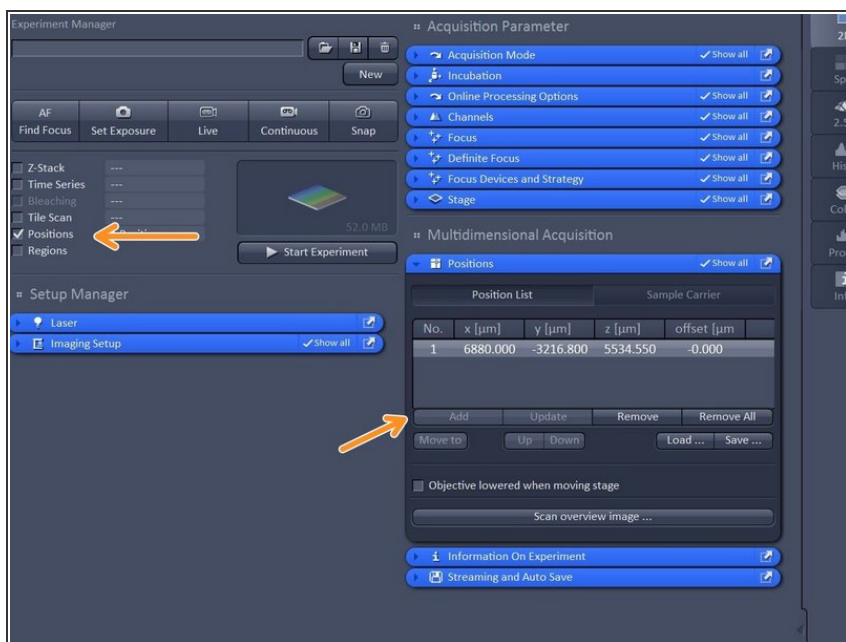
Step 20 — Setting up Z-stack



- Activate "**Z-stack**".
 - In case "**First/Last**" option is selected, move to the start of your Z-stack with the hand-wheel and click "**Set First**", then move to the end of your Z-stack and click "**Set Last**".
 - In case "**Center**" option is selected, move to the center of your Z-stack with the hand-wheel and click "**Center**".
- Click "**Optimized**" to have ideal sampling for your Z-dimension.
 - ① *Optimal sets interval to match the Nyquist criteria (2 fold over sampling) and accordingly adjusts the slices to keep the range within the limits defined.*
 - ① *Leap sets the spacing 3 fold broader than Nyquist.*

⚠ Note: For SIM it is important to define your Z-stack in the range of the modulation contrast otherwise SIM processing will be less effective.

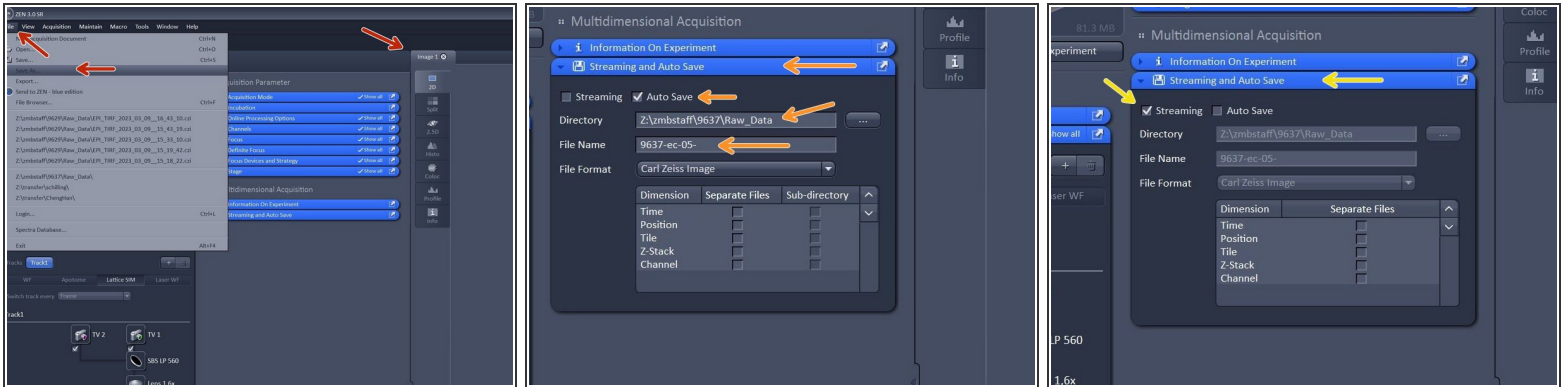
Step 21 — Multi-positioning



- Activate "Positions".
- Select **ROI** by navigating in your overview and click "**add**" to add a position.

ⓘ *Note: Multipositioning can be combined with **Time Series** and/ or **Z-Stacks**.*

Step 22 — Save your data



There are several way in order to save your data.

- **Manually:** Activate your image of choice Click "**File**" - "**Save As...**"
- Open "**Streaming and Auto Save**" and activate "**Auto Save**" - **define Directory** and define your **file name**.
 - Your file will be saved as soon as the acquisition is completed. Note that Auto Save might slow down performance.*
- Open "**Streaming and Auto Save**" and activate "**Streaming**". When Streaming is activated, pressing "**Start Experiment**" will open a Windows Explorer window - Here the file name and location can be defined as to where the experiment will be saved during the acquisition.
 - Streaming will activate the function that data is saved and automatically written to the hard drive during the acquisition process, so when image acquisition is disrupted the data up to this point will be available.*

Step 23 — Leaving system



- Click "**Focus**" and click "**Home**".
- Remove your sample.
- Clean immersion-objective with **lens paper** and **100% EtOH**.
- Change to the **10X air objective**.
- Make sure your data is saved.
- Check booking system if anyone booked the system within the next 2 hours:
 - If yes just log off.
 - If not shut down the computer, then switch OFF the "**Components**" on the table, then turn OFF the "**Main Switch**" and finally turn OFF the **HXP lamp**.