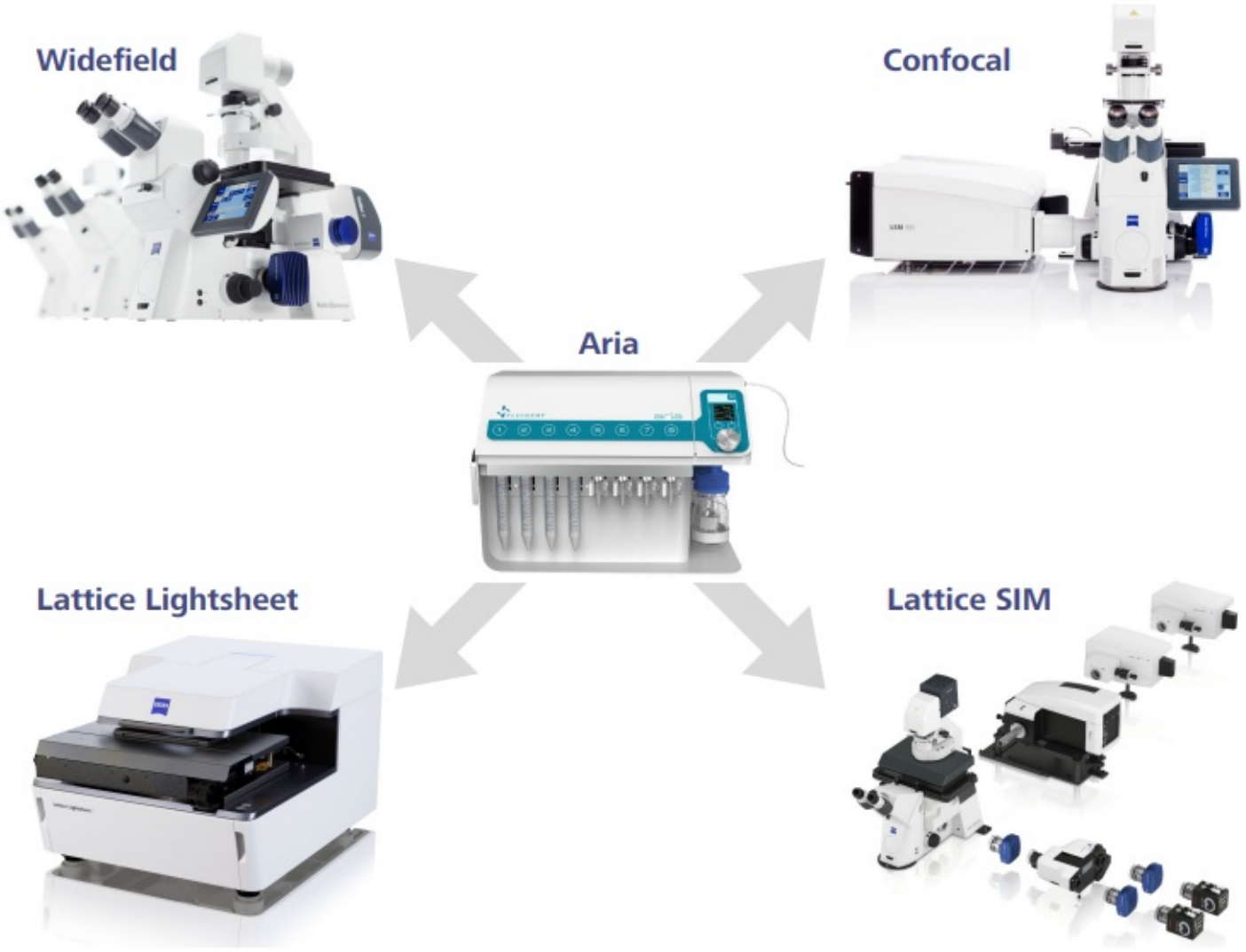
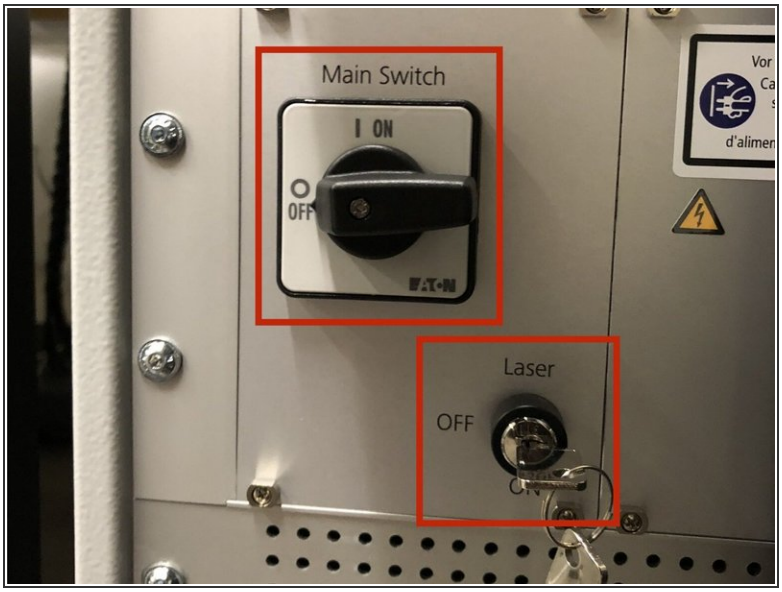
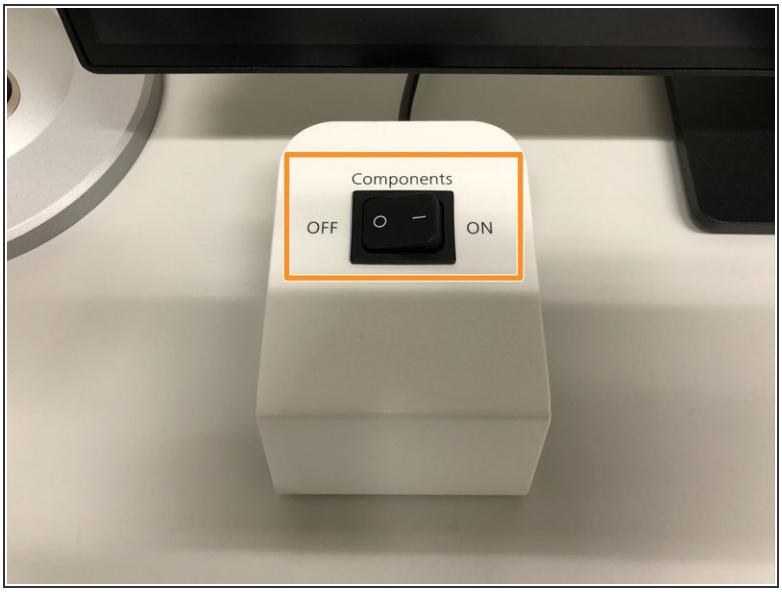
Fluigent Aria 3: Combine microfluidics with ZEISS microscopes

Written By: z mbstaff



Step 1 — Switch ON the Hardware

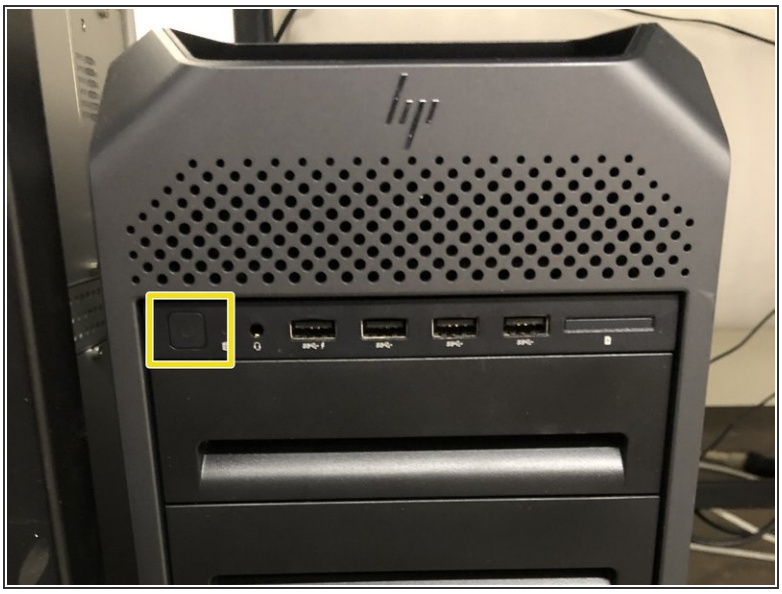




- Turn ON the **"Main Switch"** and also switch the **laser key** to "ON".
- Switch ON the **"Components"** on the table.

Step 2 — Switch ON the Hardware





- Switch ON the **fluorescence lamp**.

  ⓘ *Once turned on, the lamp should remain on for a minimum of 30 min.*

- Turn ON the **computer**.

Step 3 — Sign in

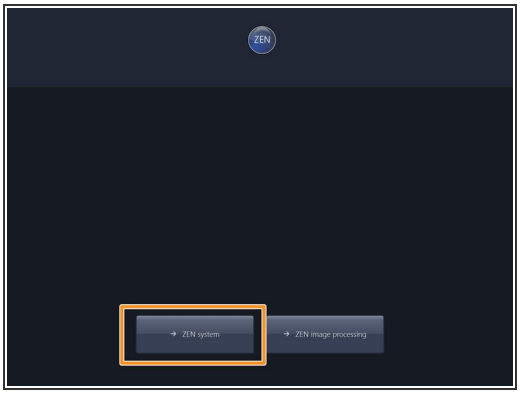


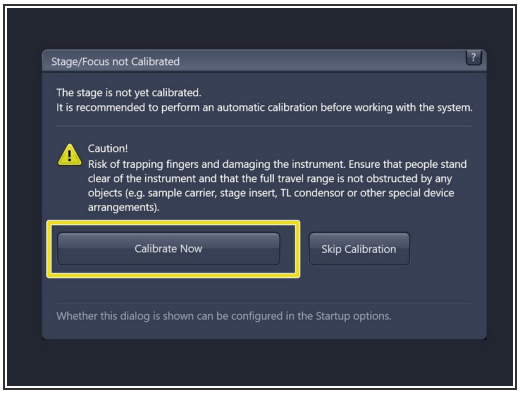
- **Sign-in** with your **ZMB core** credentials.

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Step 4 — Start the Zen Software

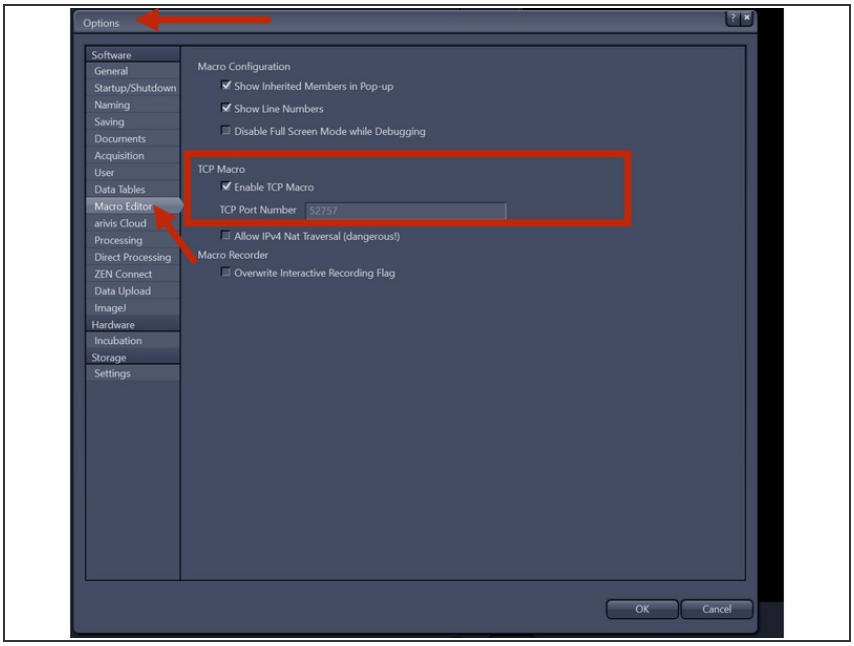






- Start the **"ZEN 3.x (blue edition)"** software.
- Choose **"ZEN system"**.
- Make sure there is **no sample** on the microscope stage and calibrate the stage by clicking **"Calibrate Now"**.

Step 5 — Set up the pump



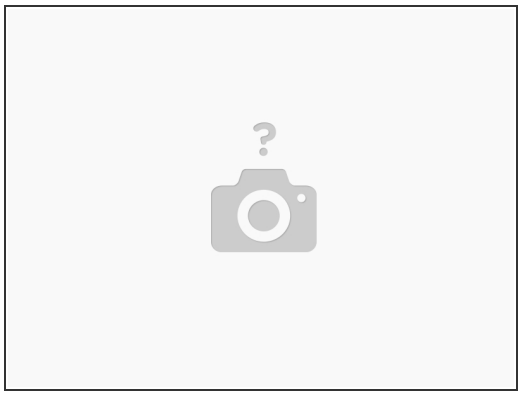
- Set up the Aria pump according to this guide.
- Claim TCP license. In Zen. Tools. Options with wrench symbol. Macro Editor
- 1. connect Aria via USB to microscope PC

- 2. Open Tools -> Module manager -> make sure the Floating licence (FLic Developer Kit) here called Macro Enivrionment is active meaning consumed/checkmarked
  2. Open Tools -> Options -> TCP

Macro -> enable TCP macro / TCP Port Number 52757 (may vary from system to system)

- 3. Open Aria software -> open parameters (settings) -> make sure TCP port number matches the one in ZEISS ZEN
- 4. In ZEN define your experiment and save as czexp file in Z:\home\n.schilling\Carl Zeiss\ZEN\Documents\Experiment Setups (make sure matching path in Aria)
- 5. create the Aria protocol as shown in example screenshot.

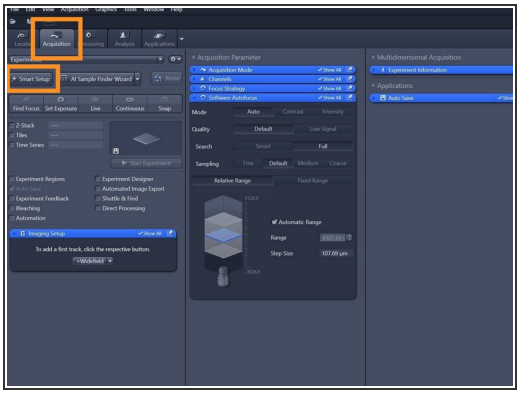
Step 6 — Connect the slide to the Aria pump

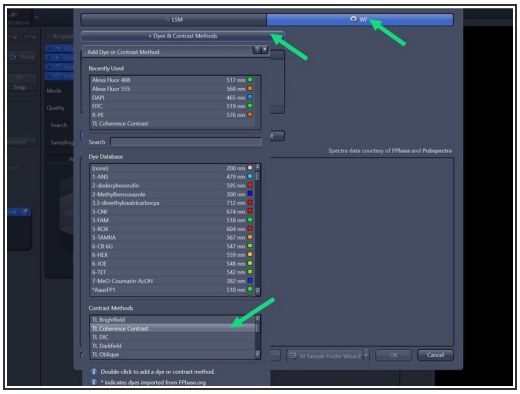


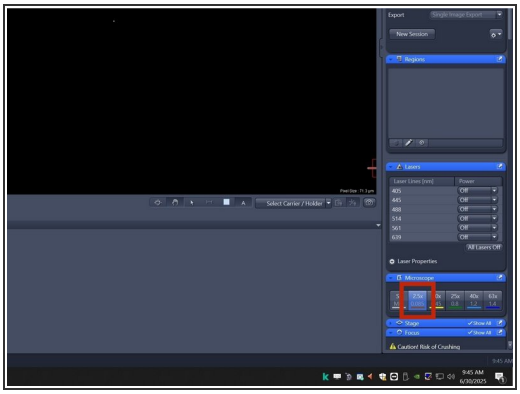
- Place the slide in an appropriate sample holder.
- Place the sample in the sample holder into the microscope.

⚠ Connect first the waste and then the input tubing to your ibidi slide.

Step 7 — Create an overview of your sample



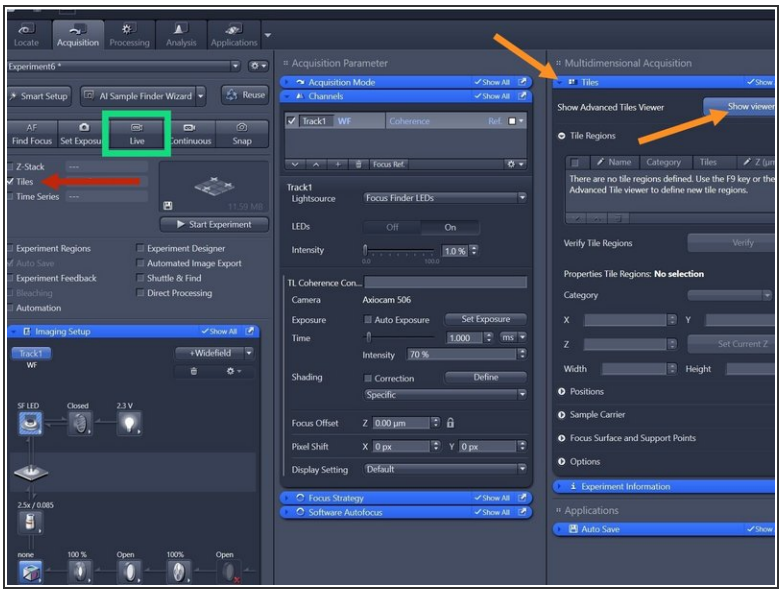


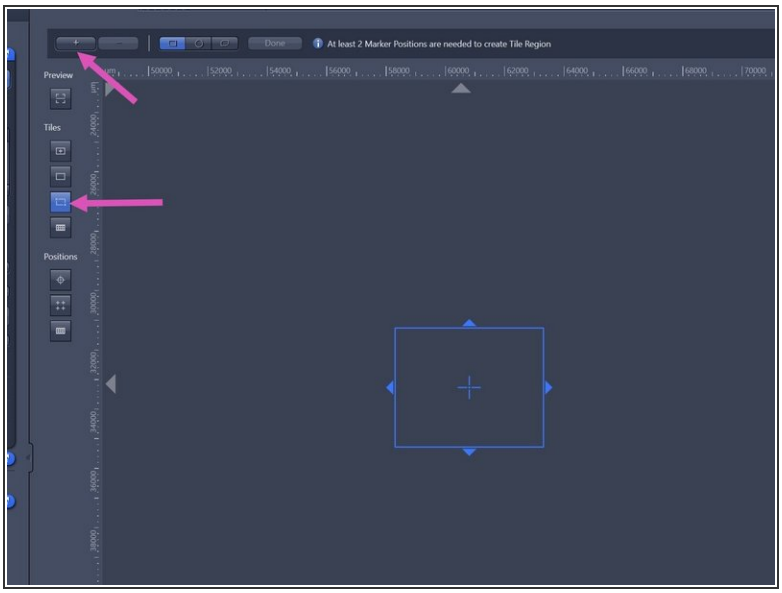


- Open the **"Aquisition"** tab and press **"Smart Setup"**.
- Select **"Widefield"**, press **"Dyes and Contrasts"** double-click**"Coherence Contrast"**
- Make sure the 2.5x objective is selected.

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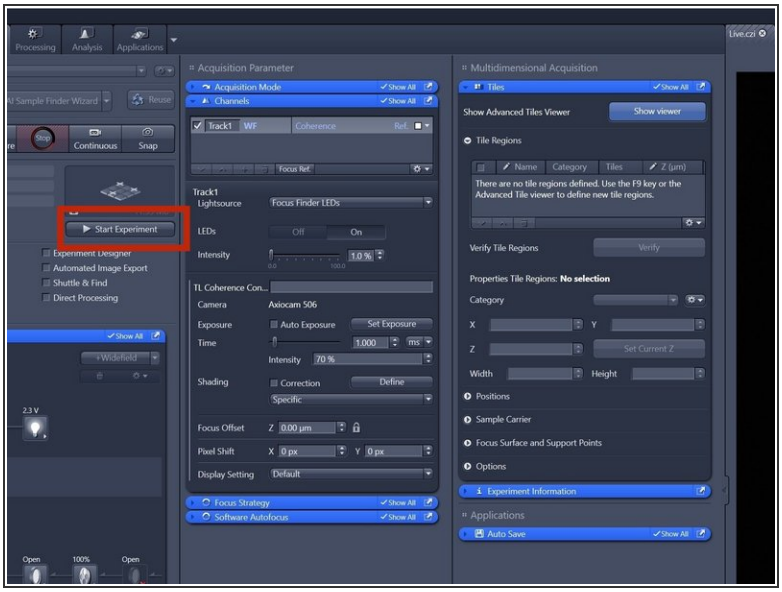
Step 8 — Create and overview of your sample

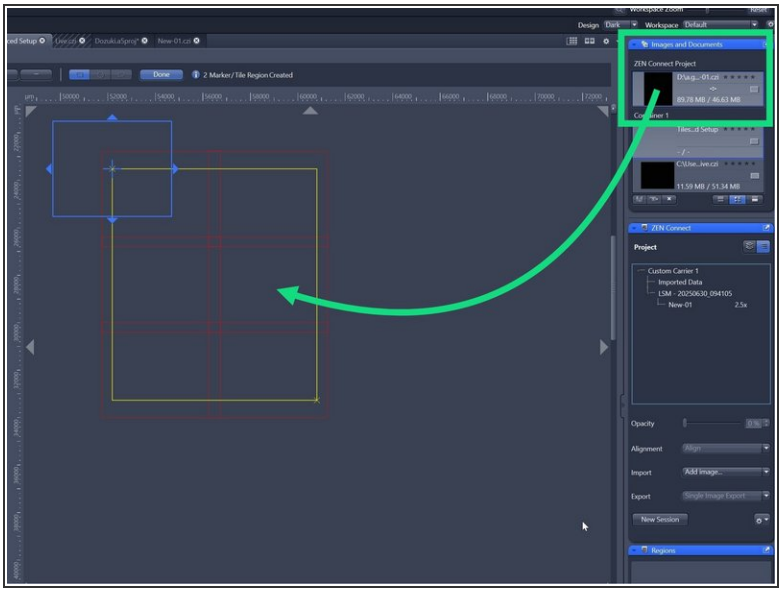




- Check **"Tiles"**
- Open the **"Tiles"** tab and click on **"Show viewer"**
- Press **"Live"**
- Navigate the crosshair to the top left corner of your sample , choose the **"Marker-position mode"** and press **"+"**
- Repeat this step for the lower right corner of your sample

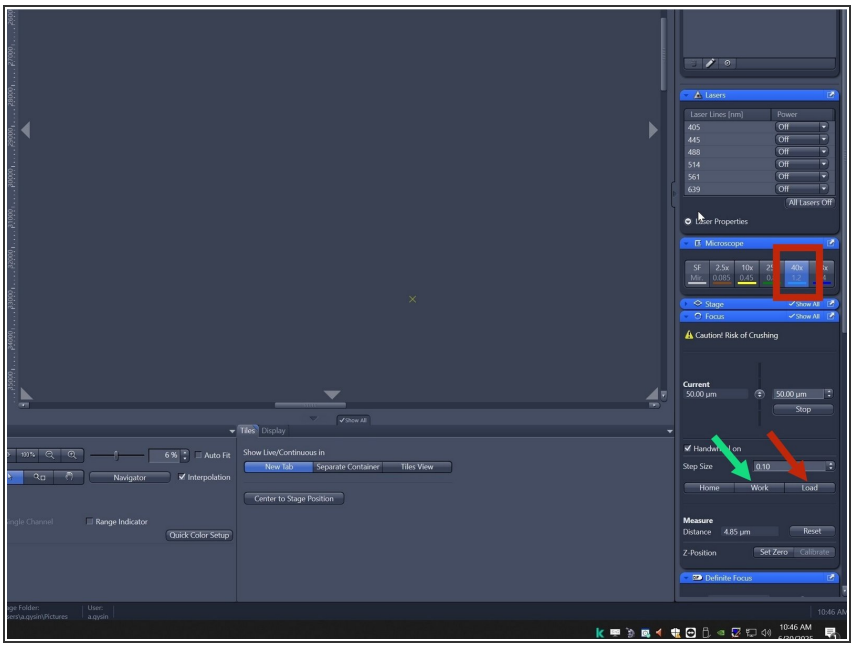
Step 9 — Create an overview of your sample





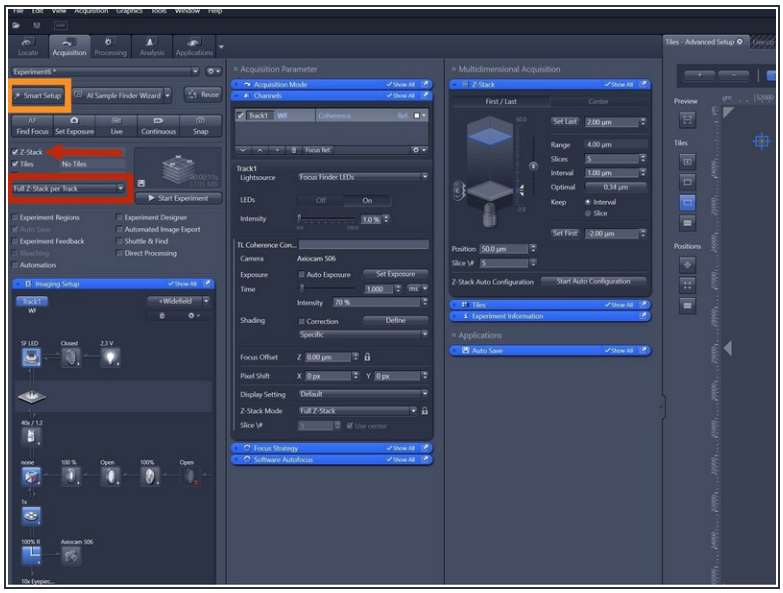
- Press **"Start Experiment"**
- Drag and drop the image from the **"Images and Documents"** Tab onto the **"Tile Viewer"**
- ⓘ You can now use the Tile Viewer to navigate within your sample

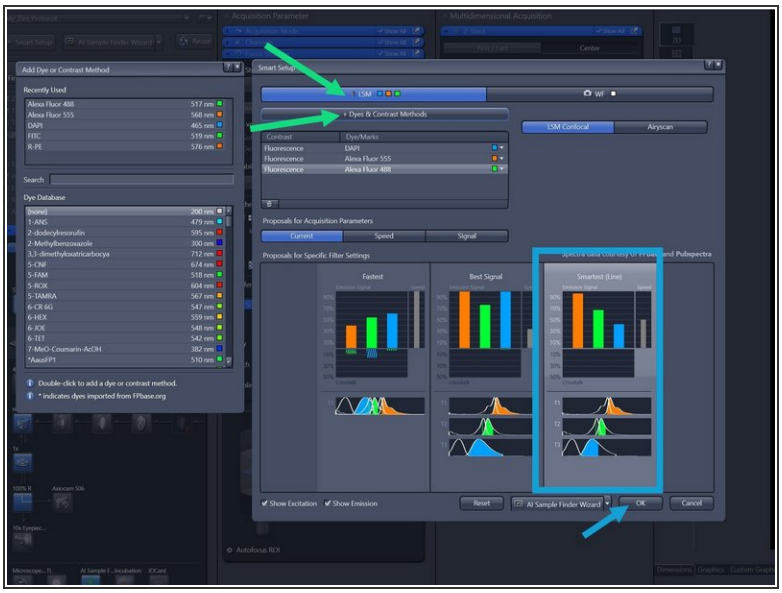
Step 10 — Change to the 40x objective



- Select the **"40x"** and press **"Load"**
- Remove the **entire** sample holder and set it down within the microscope **without** moving the sample in the sample holder or disconecting the tubing
  - ⓘ Not moving the sample within the sample holder avoids large discrepancies between the 2.5x overview and the later acquired 40x images.
- Apply a generous amount of oil on the objective
- Carefully place the sample holder back
- Press **"Work"** in the Focus tab

Step 11 — Set up a Z-Stack

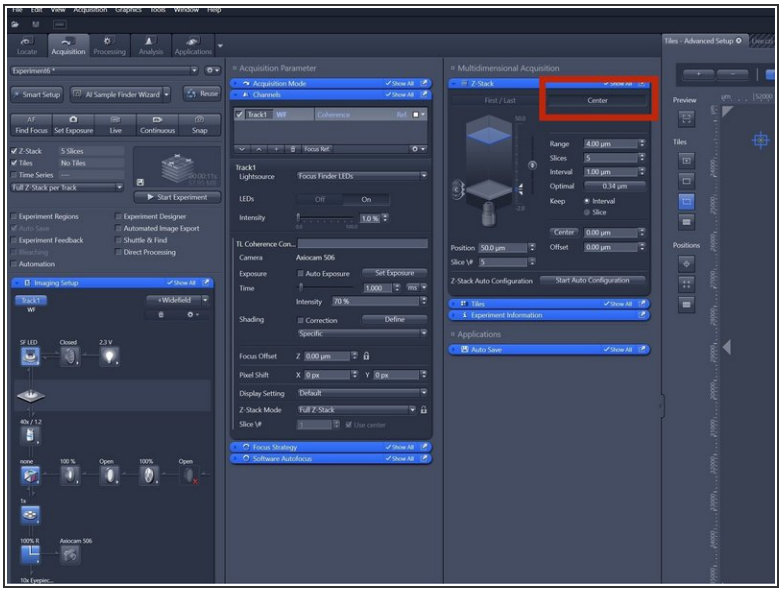


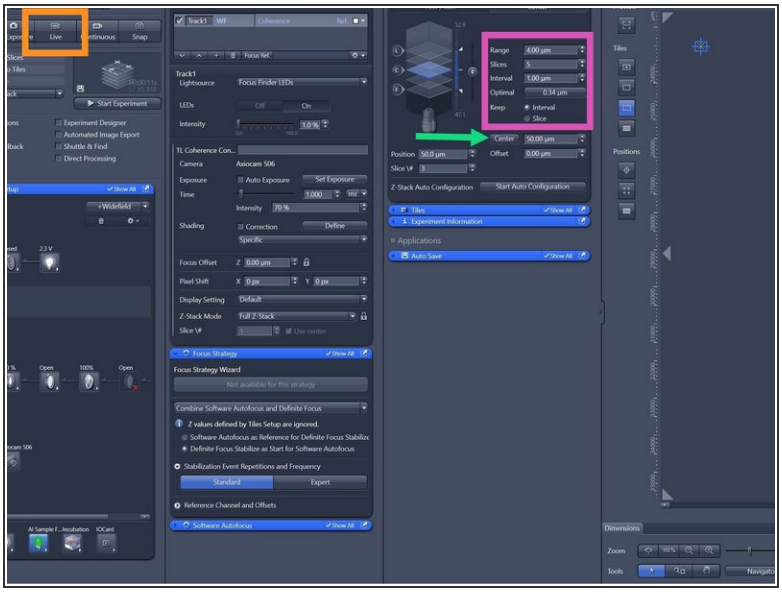


- Check **"Z-Stack"** and choose **"Full Z-Stack per Track"**.
- Open **"Smart Setup"**.
- Press **"LSM"** , press **"Dyes and Contrast"** and select your dyes.
- Select **"Smartest (Line)"** and press **"OK"**.

ⓘ Alternatively select **"Best Signal"**

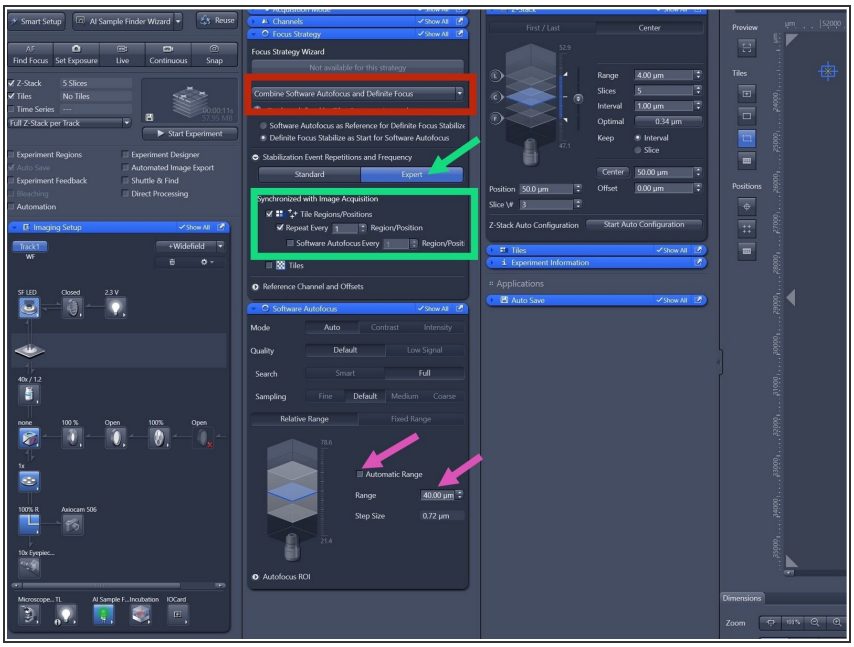
Step 12 — Set up a Z-Stack





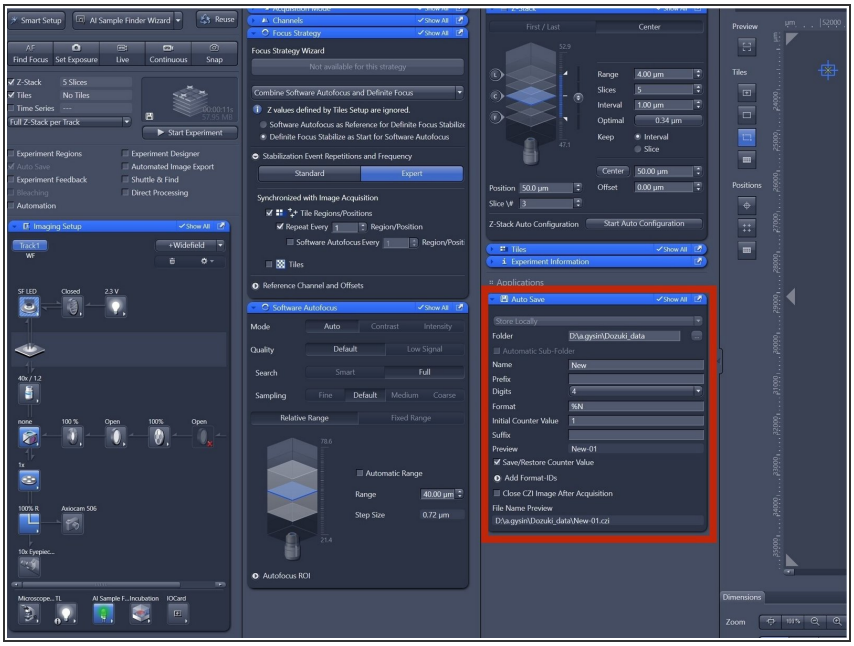
- Go to the **"Z-Stack"** tab and select **"Center"**.
- Press **"Live"** and focus by either using the focus wheel or by pressing Ctrl and using the mouse wheel.
- Once you have focused, press **"Center"**.
- Choose the size of your Z-Stack and of the steps.
  - ⓘ Press **"Optimal"** for optimal step size

Step 13 — Focus Strategy



- Go to the **"Focus Strategy"** Tab and select **"Combine Software Autofocus and Definite Focus"**.
- Press **"Expert"**, check **"Tile Regions/Positions"** and check **"Repeat Every 1 Region/position"**.
- Go to the **"Software Autofocus"** tab, uncheck **"Automatic Range"** and set **"Range"** to 40 micro meters.

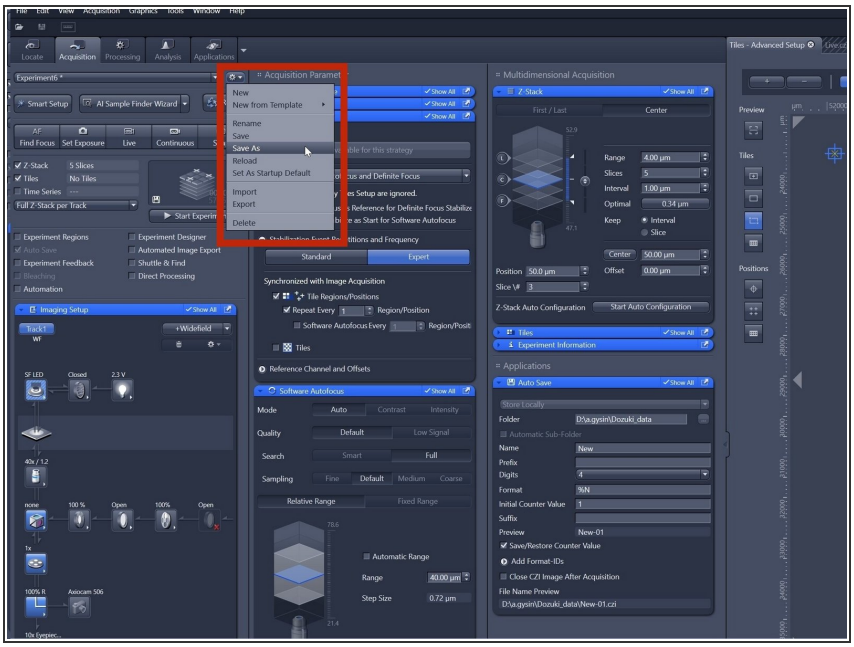
Step 14 — Auto Save



- Go to the **"Auto Save"** tab and choose a naming strategy.

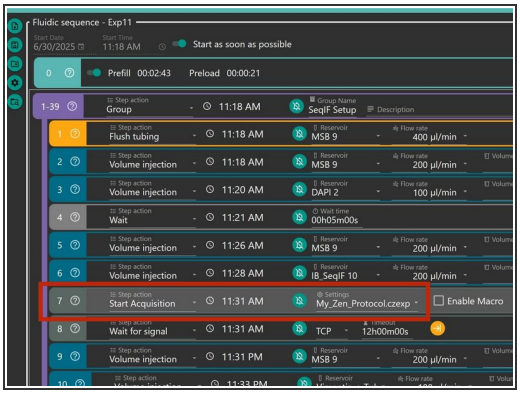
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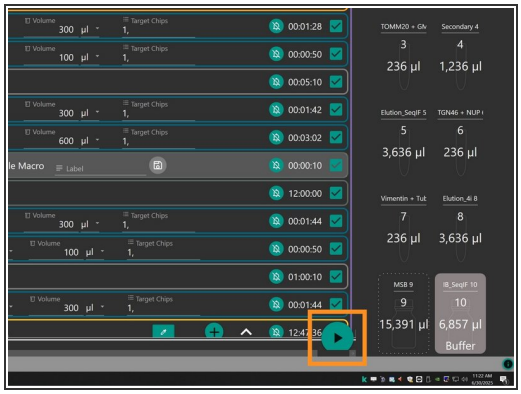
Step 15 — Save the Protocol

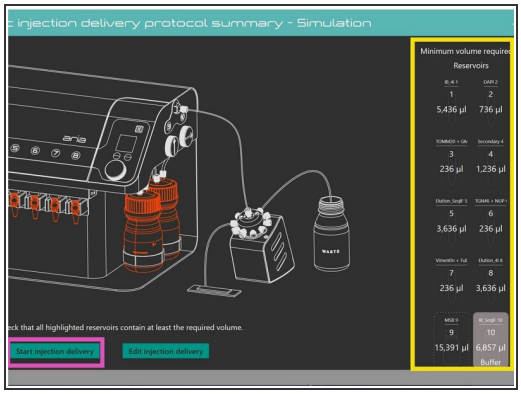


- Press the **Gear icon**, select **"Save As"**.
- Choose a name for your Zen Protocol and press **Enter** on the keyboard.

Step 16 — Insert the Zen Protocol into the Aria Sequence and start the sequence







- Open the Aria Software and load a sequence using [this guide].
- For **ALL** "Start Acquisition" steps make sure to select the correct Zen Protocol
- Press the **Start Button** in the lower right corner.
- Check if you have enough volume in all reservoirs
- To start the sequence press **"Start injection delivery"**