

Zeiss Z.1 Lightsheet

Quick-Start Guide

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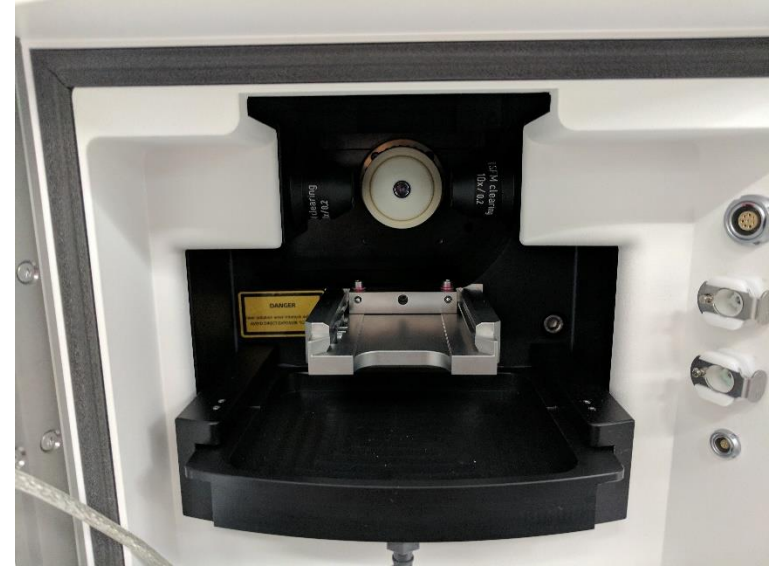


Start-up: Turn on the Microscope / Computers

- Turn on 3 switches on control panel
- Turn on both PCs.
- Log-in on both PCs using your ADS account name and password.
- For access to the network drive on storage PC, select Run and then type: \\microscopy-bignas1.nri.ucsb.edu
- Open Zen software on Lightsheet control PC.

Part 1: Chamber and Sample Preparation

- Assemble chamber
 - Windows and gaskets (correct for chosen objective)
 - 5 ports to plug
 - Temperature probe
 - Insert into chamber space in scope
 - Plug in water circulators, media port, temp probe
- Assemble sample holder
 - Capillary with agarose embedded sample
 - Sample stem with Teflon spacers
 - Place spacers with cut ends out
 - Insert into sample disk
- After software is loaded and sample load position is selected
 - Insert assembled sample holder

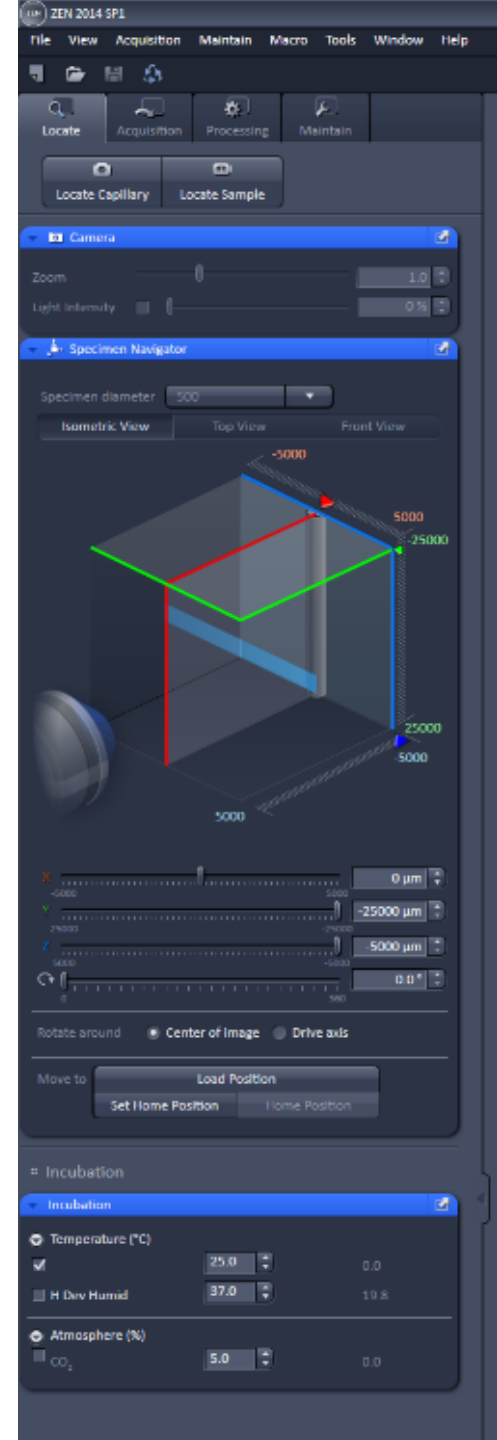


Part 2: Image acquisition and Software interface



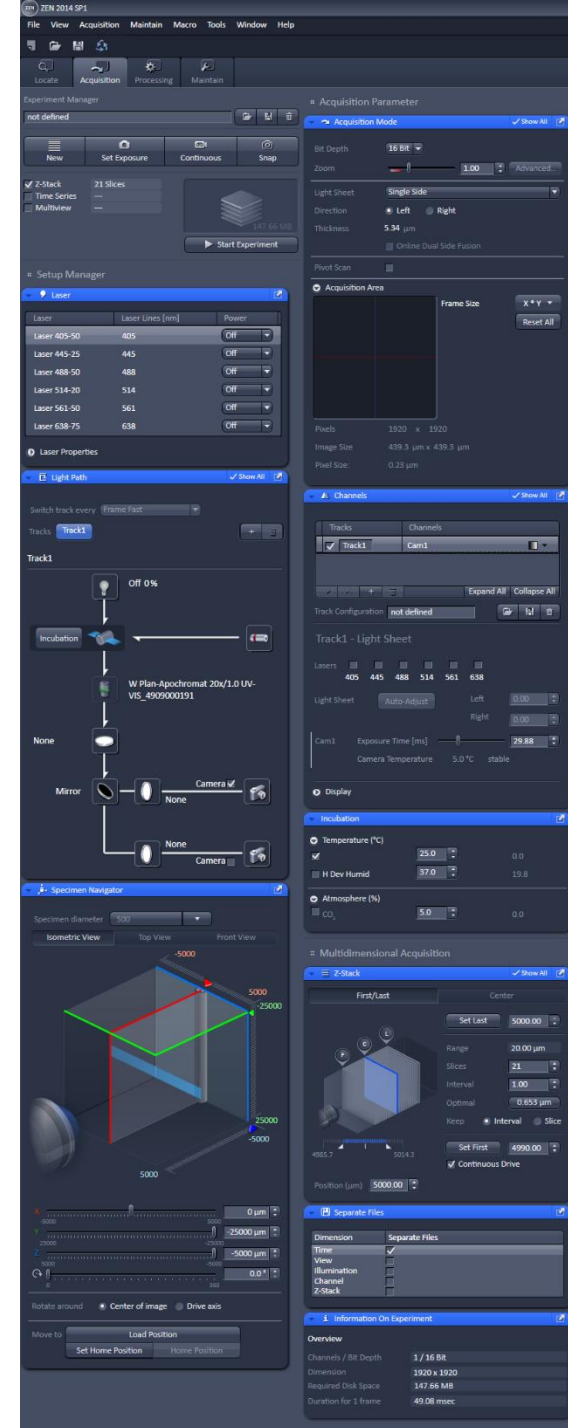
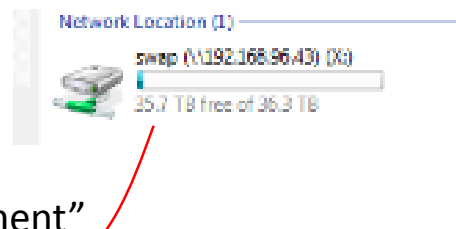
Part 2: Initial capillary position

- Click “Locate Capillary”
- In Specimen Navigator
 - Drag green plane down to blue line
 - Position capillary so that glass is just slightly above objective lens
 - Drag blue plane forward to blue line to bring capillary into focus
 - Push plunger down to place sample approximately in front of objective lens
- Click “Locate Sample”
 - Use controller to center, rotate, and focus your sample in the brightfield image
- Set incubation to desired temperature and CO₂ percentage
 - If using CO₂ be sure to open valve on CO₂ tank on wall
 - There are Teflon chamber covers that can be placed on the chamber to help retain CO₂.



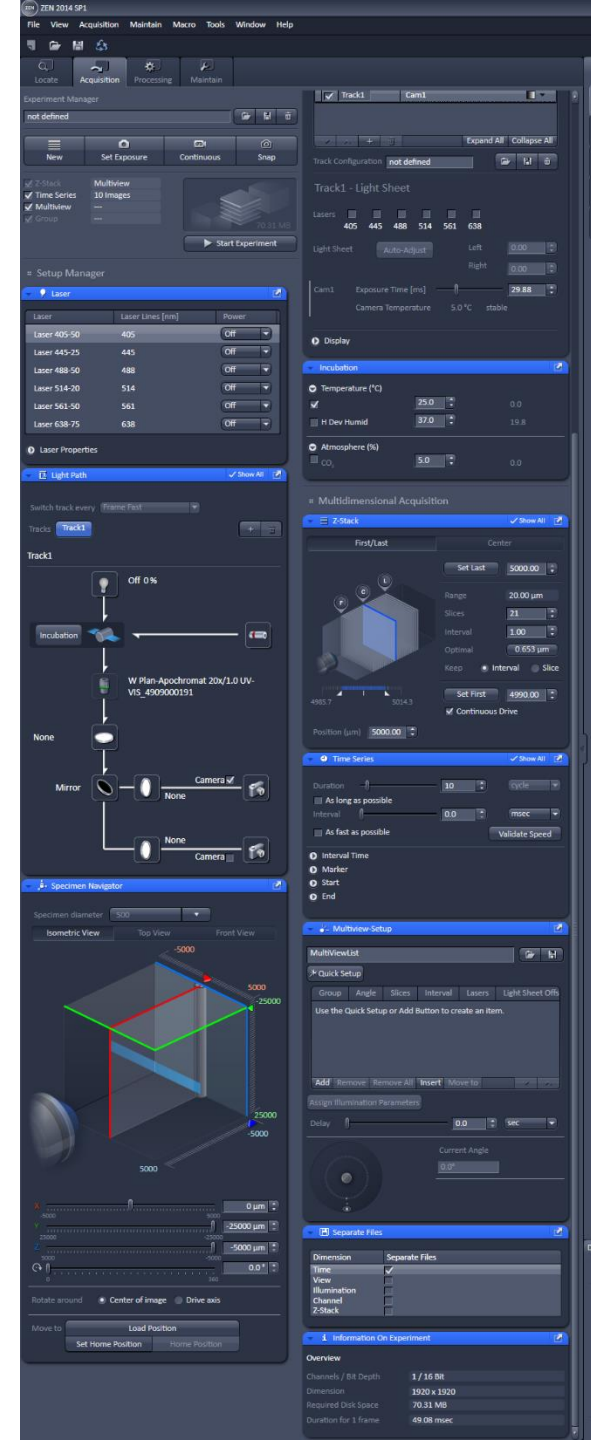
Part 2: Setup Acquisition

- Choose checkboxes for Z-stack, time series, and/or Multiview
- Turn on desired lasers
- In Light Path
 - Choose laser(s) and objective
 - Choose laser blocking filter
 - Choose filter set
 - BP 490 – DAPI-GFP (420-470, 505-545)
 - BP 510 – CFP-YFP (460-500, 525-560)
 - LP 560 – GFP-mCherry (505-545, LP 585)
 - LP 560 – GFP-DRAQ5 (505-545, LP 660)
 - LP 640 – Cy3-DRAQ5 (575-615, LP 660)
- In Acquisition mode
 - Choose zoom amount
 - Choose single side or dual side illumination
 - Turn on “Online Dual Side Fusion” if desired
 - Turn on pivot scan
 - Set frame size
- In Channels
 - Choose laser output amount
 - Adjust lightsheet position left and right
 - Set Camera exposure time
- Set Z-stack
 - Set Last and First
 - Set slice size (usually optimal)
- Once everything is set click “Start Experiment”
 - Save file to “Swap X:” drive



Part 2: Time series and Multiview

- Time series
 - Choose duration (cycles, second, minutes, or hours)
 - Click “Validate Speed” to determine how long one cycle takes
 - Choose interval time
- Multiview Setup
 - Choose positions for desired Multiview
 - These can be entirely different locations or rotations of the same field-of-view
 - Combining rotations into a single image happens as a postprocessing step in Zen



Tips

- File sizes can be huge so consider how much data you really need.
 - Use online dual-side fusion
 - Crop image to reduce black space
 - Use appropriate time intervals
 - Crop in xyz and t in postprocessing to remove anything unnecessary.
- If you plan on using Imaris you will need to save timelapses as one large file. This can be done before or after image acquisition.
- You can use ImarisFileConverter to convert your .CZI to the Imaris file format. This will reduce file size and run smoother in Imaris.
- Remember that you can rotate your sample around the y-axis. Use rotation to find the best view of your sample.
- If your immersion media is expensive there are 5 clean nickels you can put in the bottom of the sample chamber to reduce the media volume needed. You can also include some volume of air in the syringe so that you can push all of the media through the connecting tube.

Shut-Down Procedure

- Put sample into load position
- Use syringe to pull all media out of chamber
- Remove sample holder and capillary
- Remove chamber, rinse with 70% ethanol, rinse with distilled water
- Rinse imaging objective lens with distilled water, gently pat dry
- Transfer data to network drive
- Shut off the computer
- Turn off the 3 switches on the control panel
- Adjust your online reservation end-time if you finished early or late

Specifications

- 3 objectives
 - 5x/0.16 air, CLARITY compatible
 - 20x/0.7 water
 - 40x/1.0 water
- 6 laser lines (405, 445, 488, 514, 561, 638)
- Two sCMOS cameras
 - 7.5 ms minimum exposure time (130 frames per second)
 - Cooled to 5°C to reduce noise
 - 1920x1920 pixels