Ligthsheet 7 Basic Quick Guide

Turning ON

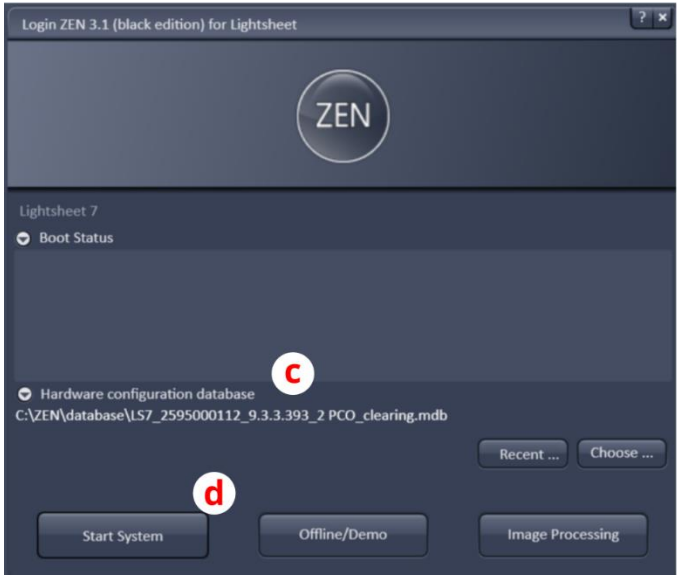
1. Turn on computer.
2. Switch the main switches on by one, wait 5 seconds between each switch.
3. Click on the ZEN Black icon (a) on the computer screen.
4. Expand the Login ZEN popup window Boot Status (b) and at Hardware configuration database (c) select the CORRECT database (".mdb" file) for the sample type you are using. Databases are found under C:/ZEN/Databases

OBS.: Available files are:

- LS7_2595000112_9.3.3.393_2 PCO_clearing.mdb
- LS7_2595000112_9.3.3.393_2 PCO_water_10x.mdb
- LS7_2595000112_9.3.3.393_2 PCO_water_20x.mdb

5. Start system (d).
6. If a popup window asks about **"attenuator"**, click OK.

OBS.: If error messages appear during startup, try restarting the system. If the errors persist, contact the SIF staff.



Objectives change

1. Chose the right objectives that fits for your experiment
2. Before installing the objectives make sure to adjust the collar ring to match the refractive index (RI) of the medium.
3. First CAREFULLY remove the illumination lenses then the detection lens.
4. Install the appropriate lenses beginning from the detection lens than the illumination.
5. On the ZEN Black click the tab Maintain (a) and click Objectives (b).
6. Go to Detection Optic (c) and Show/Edit Objective (d).
7. Go to Potential Objectives (e).
8. Choose from the list:

OBS.: The potential detection objectives available are:

- FLUAR 2.5x/0.12*
- EC Plan-NEOFLUAR 5x/0.16*
- EC PN 5x/0.16 foc
- Plan-APOCHROMAT 10x/0.5 (UV)-VIS-NIR
- W Plan-APOCHROMAT 20x/1.0 DIC Korr UV-VIS-IR W
- Clr Plan-NEOFLUAR 20x/1.0 Corr**

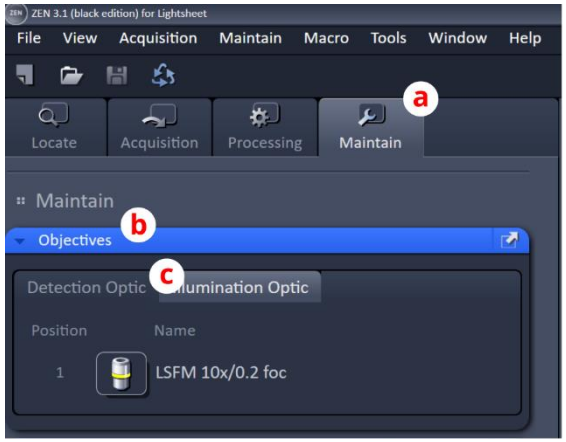
* Requires Mesoscale Imaging Systems (lens adaptors and chambers)

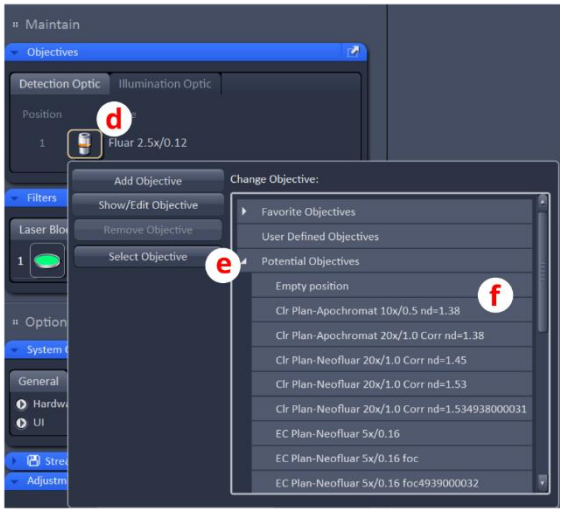
** Exclusively for cleared samples

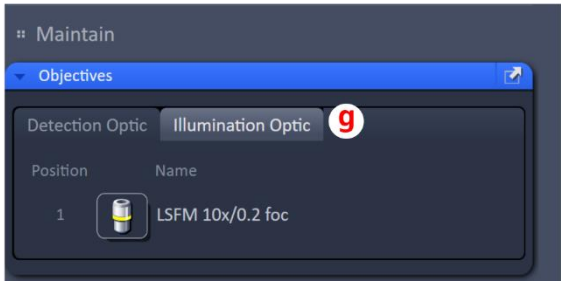
9. Hit Select Objective (f).
10. If necessary, repeat the procedure with Illumination optics (g):

OBS.: Acceptable combinations are:

- 5x LFSM for 5x and 2.5x Detection Optics
- 10x LFSM for 10x and 20x Detection Optics





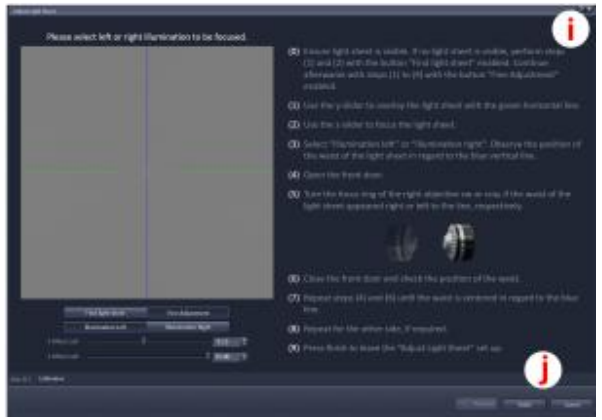


Aligning light sheet

1. Insert the chamber containing media into the LightSheet 7 (a).
2. On the ZEN Black click the tab Maintain (b) and click Adjustment (c) and click Adjust Light Sheet (d).
3. Follow the instructions in the popup window click on Verified (e) and then on Next (f).
4. Select a laser line (g), medium refraction index (h) and click next.

OBS.: In case of more than one laser line is going to be use closer to the middle of laser lines.

5. Make the adjustments as indicated in the wizard (i).
6. Hit Finish (j) to end up light sheet alignment.



Positioning sample into instrument and locating it

1. Have your sample holder ready including your sample.
2. In **ZEN Black** click the tab Locate (a).
3. Go to Specimen Navigator (b).
4. Click on Load Position (c) to bring stage into position for loading and open the upper door.
5. Check for the white mark on the sample holder. Alling it above the white mark on one of the ball bearings.
6. Carefully insert the sample holder and put it into place.
7. To locate the sample, press the Overview button (d).
8. Adjust the sample position using the Specimen Navigator (e) or the ErgoDrive operating panel.

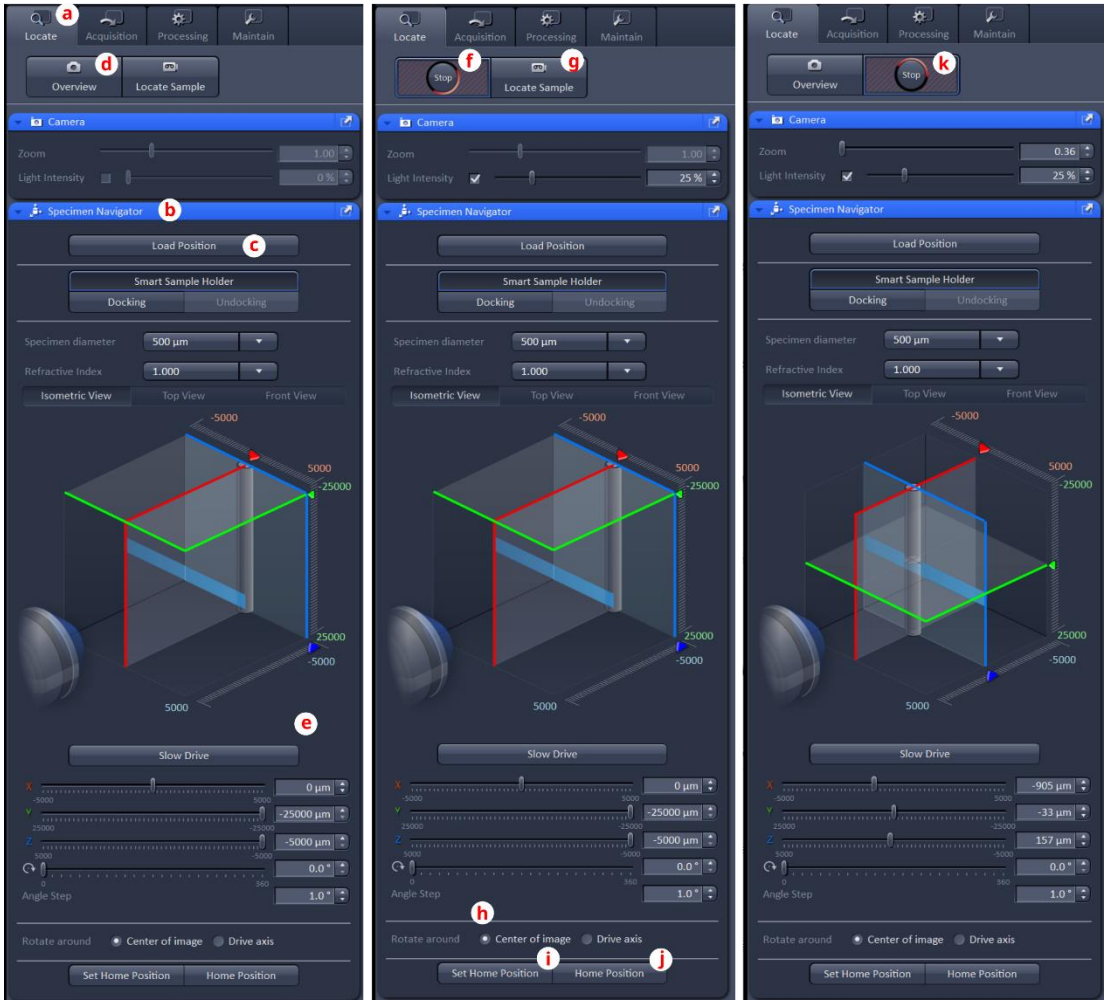
OBS.: It is advisable to give preference to the ErgoDrive to position your sample as it allows a smoother control.

9. Place your sample in the center of the detection optic lens.
10. Stop (f) overview, Click the Locate Sample (g) button to a fine adjustment of position, rotation and zoom.

OBS.: **(1)** Use the Rotate around Center (h) of image to automatically correct the X and Y position for rotational movement and keep the sample within the chosen field of view.

(2) Click on Set Home Position (i) to define a position you might what to return to during imaging. To return to return to home position click the button Home Position (j).

11. Click Stop (k). Your specimen is properly oriented.

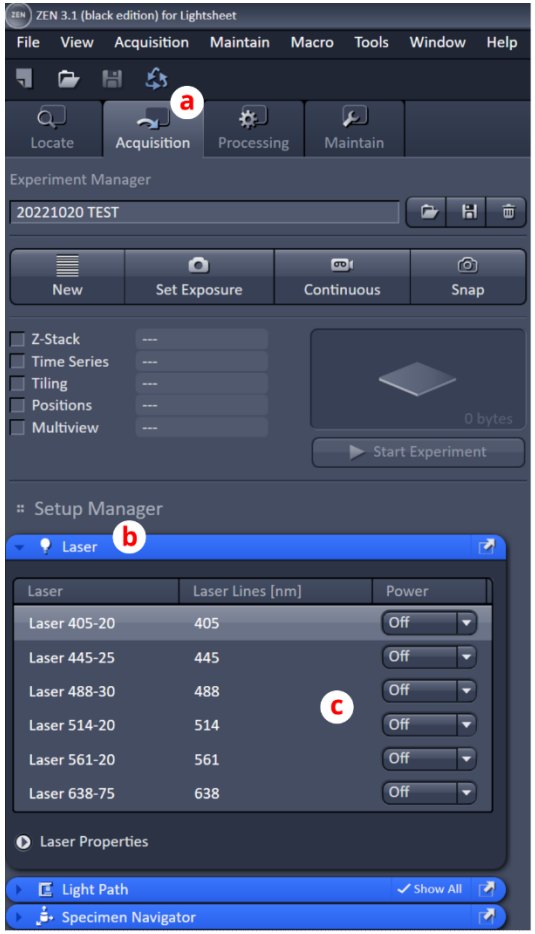


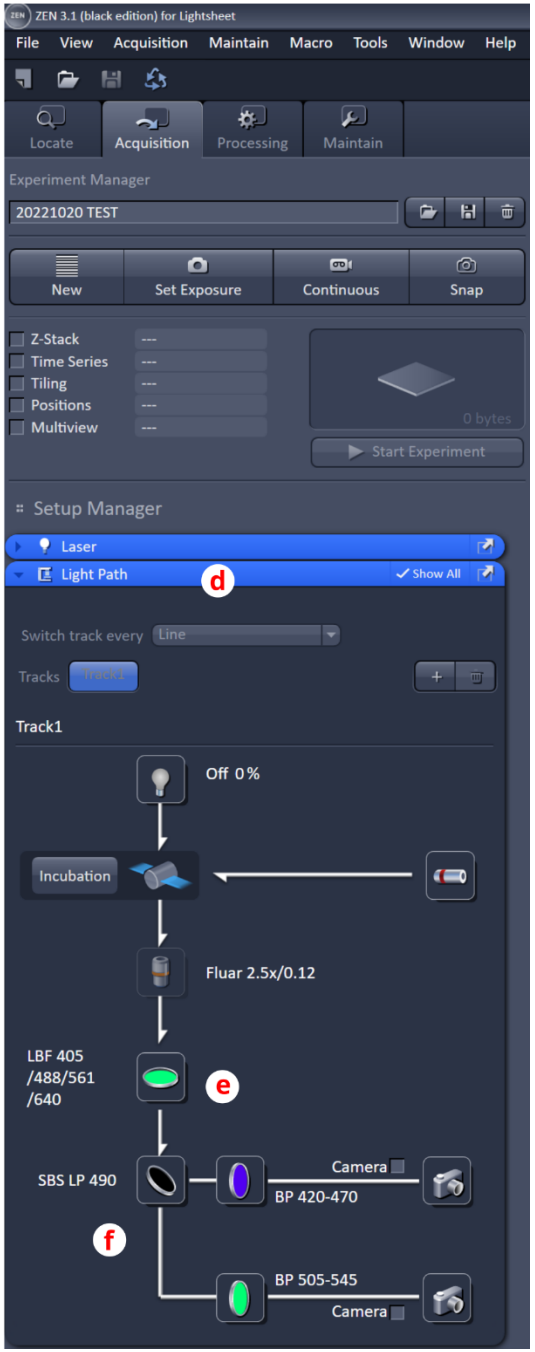
Light path configuration

1. Click Acquisition (a) to access the functions for setting up the multidimensional imaging.
2. Click Laser (b) and activate lasers you want to use (c).
3. Click Light Path (d).
4. Select suitable Laser Bloking Filter (LBF)(e).
5. Select suitable Emission Filter Cube (f).

OBS.: Available filters are:

- BP 420-470 (DAPI) + BP 505-545 (GFP)
- BP 420-470 (DAPI) + BP 575-615 (RFP)
- BP 505-545 (GFP) + LP 585 (mCherry)
- BP 505-545 (GFP) + LP 660 (Draq5(toto3))
- BP 575-615 (RFP) + LP 660 (Draq5(toto3))





Adjusting acquisition parameters

1. Click Continuous (a) for a “live view” of all active tracks.
2. Set parameters under the Acquisition Mode tab (b)
3. In acquisition Mode, adjust bit depth (c) (stick to 16)
4. Adjust Zoom (d) as need. It is recommended to use zoom > 0.7 to optimize quality towards edges.
5. Click Channels (e) to adjust detectors, illuminations settings and display options.
6. Adjust laser power (f) for each laser line. Change here percentage of total output. Keep it as low as possible.
7. Adjust Exposure time using slider or input box (g).
8. On Display (h) use the histogram to change contrast and brightness in seen image without changing image data. Use best fit/autoadjust to star with.
9. Adjust hardware zoom by optics

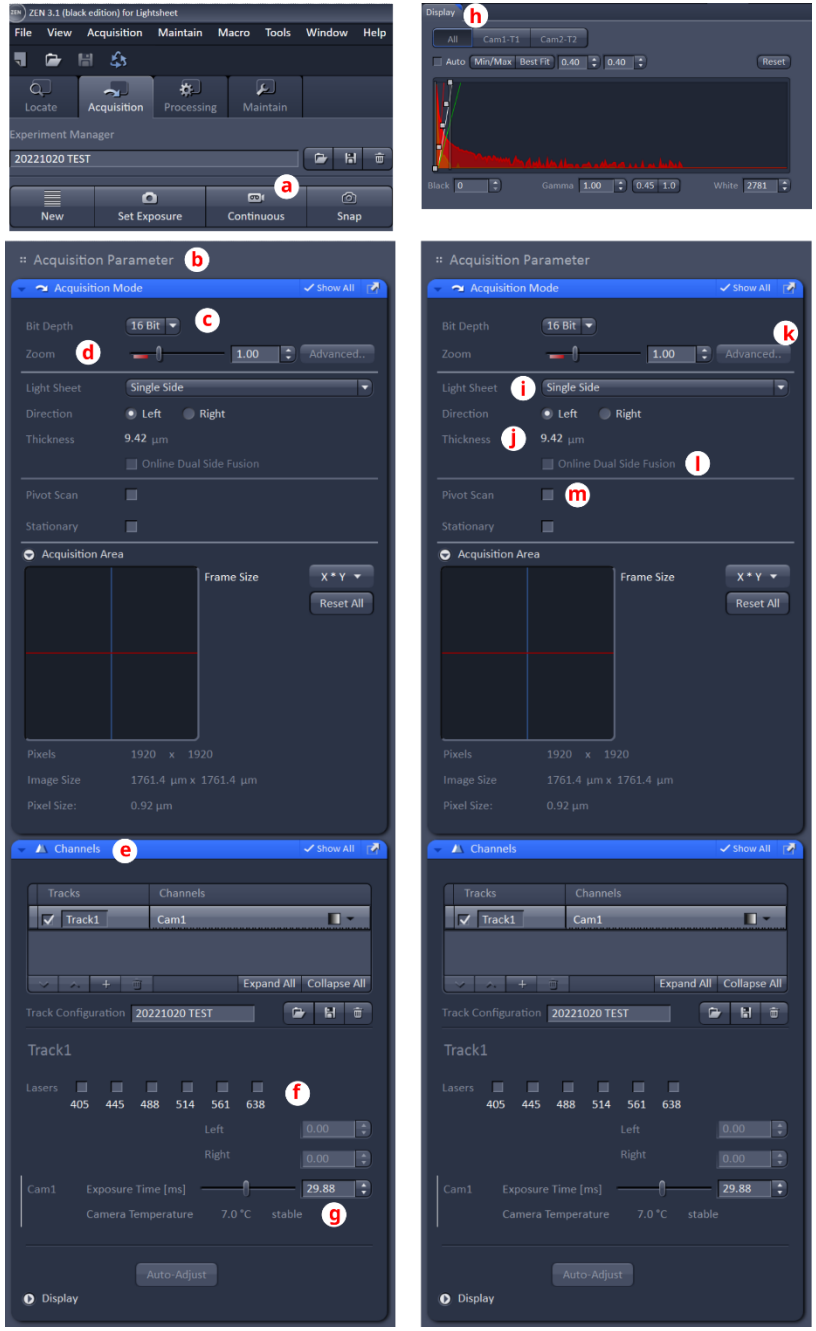
OBS.: **(1)** Zoom range 0.36 to 0.7 image quality can decrease towards edges.

(2) CHANGING ZOOM REQUIRES A NEW LIGHT SHEET ADJUSTMENT.

10. Choose from Single side or Dual side (i).

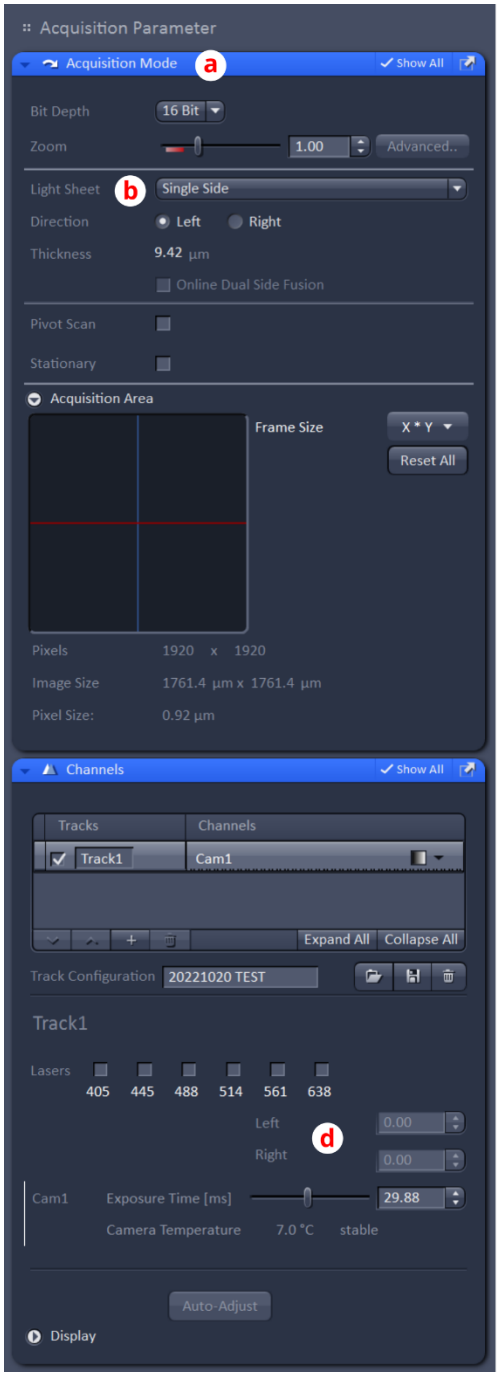
OBS.: When Single side is chosen, select Left or Right direction of the light sheet.

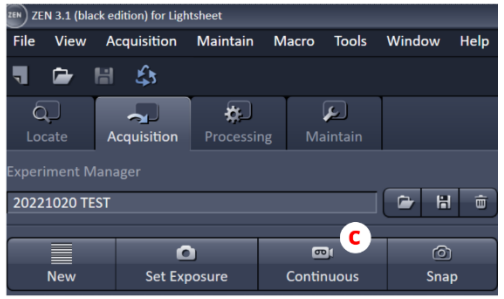
11. Light sheet thickness (j) in µm, change it via Advance in Zoom (k)
12. Online dual side fusion fuses the left and right illuminated images (l).
13. Use Pivot Scan (m) to reduce shadows cast by optically dense structures in the sample.



Manual light sheet adjustment

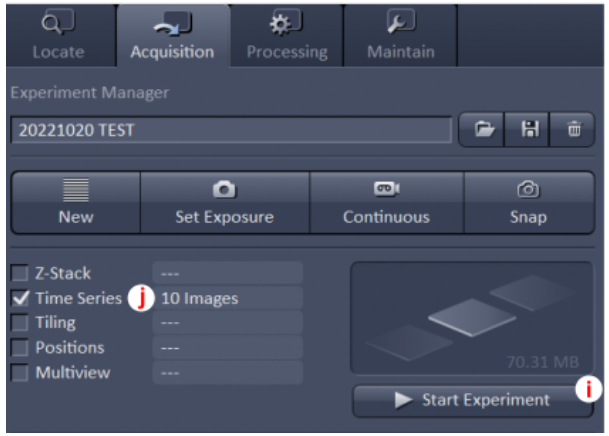
1. Chose a part of the sample where a dual illumination is expected to give the same image.
2. Under the Acquisition tab (a), set the Light Sheet (b) to “single side illumination”.
3. Chose Left or Right Direction (c) and click Continuous button (d) for live view.
4. Using the laser position boxes (e), change light sheet position to find the best quality for your imaging.
5. Change the laser side, find the best laser setting match the other side illumination.
6. If needed, repeat the procedure with opposite side illumination for fine adjustment.
7. Stop (c) live view.
8. Set the Light Sheet (f) to “double side illumination”.
9. Click again on Continuous button (c) to live view.
10. The image from the two lighting sides should overlap, this is noticeable by a slight flickering. If this flickering is too high, it means that the lighting alignment needs to be improved.
11. Once adjustments are done, stop (c) live view, click on Online Dual Side Fusion (g).
12. Click on Pivot Scan to reduce shadows cast by optically dense structures in the sample (h).
13. Now you can start imaging. (**REMEMBER!!** CHANGING ZOOM REQUIRES A NEW LIGHT SHEET ADJUSTMENT).



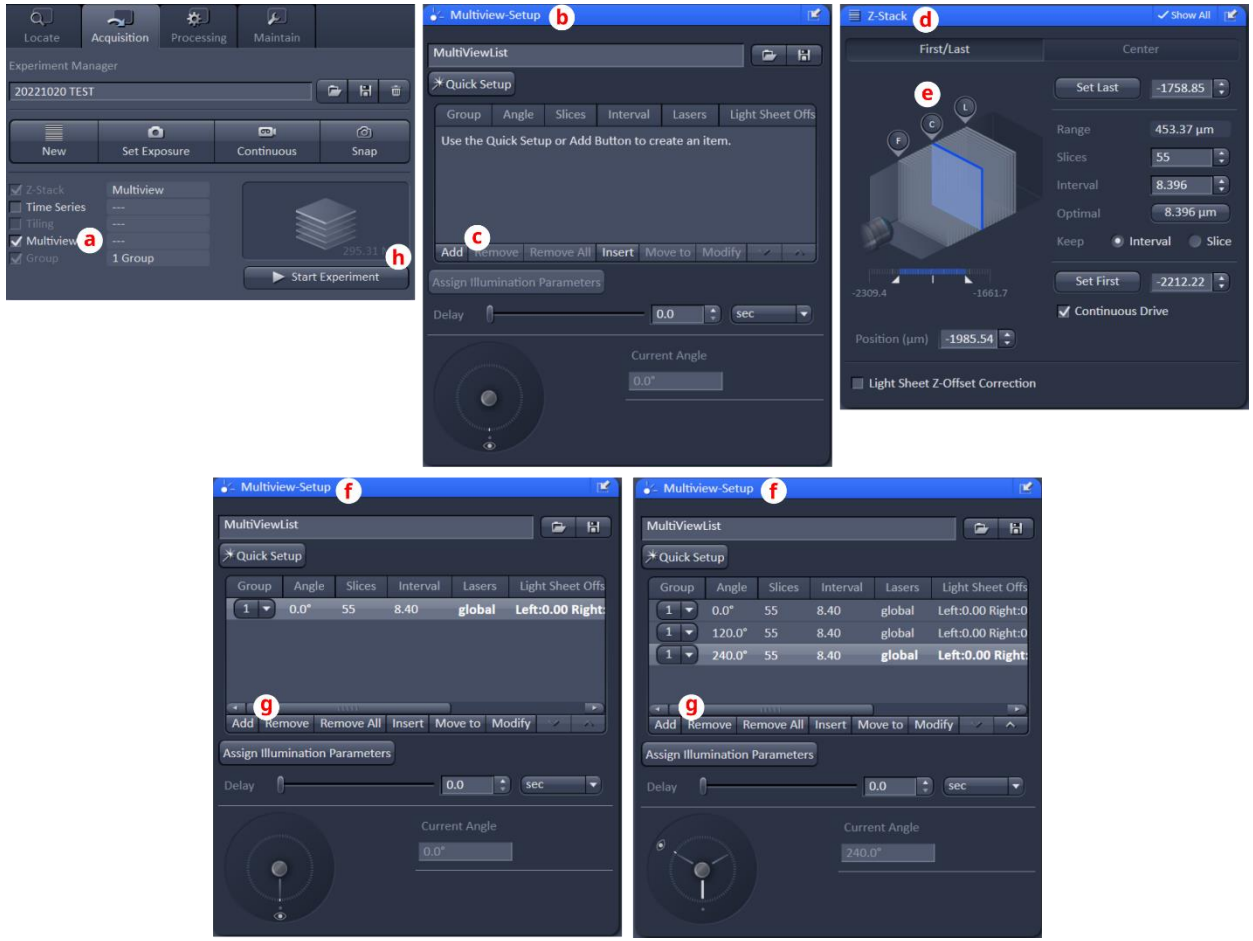


Imaging acquisition

1. For **one single** image using all active channels and tracks click SNAP (a).
2. For **Z-stack acquisition**, click the Z-stack checkbox (b) in multidimensional acquisition field. Expand the Z-stack Toll bar to see the panel (c).
3. Click Continuous (d) to activate live imaging and position sample in XYZ/rotation.
4. Move Z position for the first image you ante to acquire for Z-stack and click Set First (e), repeat procedure for the last image and Click Set Last (f).
5. Click on Optimal button (g) to set slice number o match optimal Z interval with two adjacent slices overlapping to 50%.
6. Check for Continuous Drive (h) to speed up Z-stack acquisition.
7. For image acquisition, click Start Experiment (i). You will be asked to indicate the name and the path of the file.
8. For **time series**, activate the Time Series (j) and expand the Time Series tool tab (k).
9. Set Duration (l) (cycle, msec, sec, min, h, days).
10. Set Interval (m) (msec, sec, min …) to separate beginning of two cycles.
11. Click Validate Speed (n) to perform acquisition check on all parameters active (Z, Multiview, channels, tracks…) to calculate the time needed to finish on cycle.
12. Once set, click Start Experiment (i).



13. For **multiview** imaging, click Multiview (a) and expand Multiview Setup (b) to access to tools (Z-stack is simultaneously activated).
14. Make the Z-stack as previously described (steps 2 to 6), click on Add (c) on Multiview Setup to create this view with this angle.
15. On Z-stack Tool (d) move to the center of the z-stack by clicking Center (e).
16. Chose a new view by changing the angle by Specimen Navigator or the ErgoDrive operating panel.
17. Make a new Z-stack then move to the center of the z-stack by clicking Center (e) on Z-stack Tool (d), on Multiview-Setup (f) click on Add (g) to create a new view with this angle.
18. Repeat steps 14 to 16 to create new views if needed.
19. Click Start Experiment (h).



Turning OFF

1. Take out you sample
2. Exit ZEN
3. Shut down the computer
4. Switch of the main switches in the inverse order.
5. CARFULLY unplug and **EMPTY** chamber.
6. Take out and clean chamber.
7. Place all small pieces disassembled back into its appropriate place/box.
8. Make your notes in the logbook and report any error/problem.

More tutorials on Ligthsheet 7 can be found on: https://www.zeiss.com/microscopy/us/local/zen-knowledge-base-home/zen-knowledge-base-lightsheet.html