

Lightsheet Z.1 User Protocol

Code for laser interlock on door: 1111A

Start-up:

On pad to the left of the microscope, switch on System, PC and Incubation. As soon as Incubation is switched on CO₂ will flow through the system. Therefore do not switch Incubation on if you don't intend to use it and untick CO₂ in the Incubation window in ZEN (see below) if you do not need it. Power up the computer on the right side under the desk and the computer on the left.

Click on Lightsheet

Double-click on ZEN

To check how boot-up is going click on boot status arrow.

Start System

Load your sample only when start up is complete! Keep meniscus just in the field of view in Locate Sample.

Locate or Acquisition:

Incubation:

Tick channel 3 and channel 4 and set to desired T

Tick CO₂ and set to desired CO₂ conc.

The whole system needs at least 30 min to warm up, 1 hour is best.

Acquisition:

Laser:

Turn on laser lines you want to use (405 nm, 445 nm, 488 nm, 514 nm, 561 nm, 638 nm). Lasers will be ready in a few minutes.

Light Path:

Make sure the correct objective is showing (5x NA 0.16 air objective, 20x NA 1.0 and 63x NA 1.0 water immersion objectives).

For brightfield to camera 1 choose LSM none and mirror.

For brightfield to camera 2 choose plate and SBS LP 560 in position 4.

For 488 nm alone choose LBF 405/488/561, Mirror.

For 488 nm and 561 nm lasers choose LBF 405/488/561 and SBS LP 560 in position 4. This will give you green on camera 1 and red on camera 2.

Locate:

Specimen Navigator:

Shows you where the capillary is in relation to the lightsheet and the detection objective. Allows you to move the stage through the software.

Move to Load Position (at the bottom) then load the sample

Click Home Position (this should get you close to a reasonable start position if people don't change it to something mad)

Click Set Home Position once you have located your specimen

Locate Capillary:

This gives you a view of the capillary and allows you to position your sample in front of the objective.

Locate Sample:

This gives you a close-up of the sample in brightfield and allows you to find the right part of the sample.

Camera:

Choose Zoom and Light Intensity.

Acquisition:

Tick the appropriate functions out of Z-Stack, Time Series, Multiview, otherwise these windows will not appear.

Acquisition Mode:

Only use 16 Bit, 8-bit files look horrendous.

Light Sheet: single or dual side as appropriate. Dual side will slow down image collection as the two sides are imaged sequentially.

Direction: as appropriate

Online Dual Side Fusion will make image collection much slower and the two files can be fused after acquisition.

Tick Pivot Scan if appropriate – it does not always improve your view but will reduce intensity.

Choose smallest possible acquisition area to reduce file size and speed up acquisition.

Channels:

For sequential 2-channel acquisition create a separate track for each camera. For simultaneous 2- channel acquisition have both cameras in the same track. The latter may lead to some bleed-through, which can be removed post acquisition through linear unmixing, and is twice as fast.

The lightsheet needs to be calibrated for each channel before you start your experiment. This calibration will only run through both illumination objectives if you choose Dual Side. Run through the Auto-Adjust function for each laser with your sample moved out of the way while still imaging through agarose. Move a fair way out of any stripes and bright blotches during Auto-Adjust. You can also rotate the sample get those features out of the way. *When the calibration is finished the microscope will automatically move your sample back into view.* Then adjust manually to see if you can further improve the image. This has to be done separately for each side. On the 20x objective up to +/- 2 is acceptable, on the 5x objective up to +/- 6 is acceptable for the automatic alignment. For manual adjustment you need to focus through the sample while you change the values. Minimum exposure time is 7.5 ms. Maximum speed is 20 f/s at full frame. The current rate is shown at the bottom of the window. Speed depends on frame size, exposure time and on the number of cameras if not in one track. A small frame size in y increases frame size.

Z-Stack:

Choose First/Last and define first and last section (direction does not matter) or Center and define number of sections. There is a bug in the display, which marks First as Last and vice versa. *Use the optimal interval by clicking Optimal.* Using between Optimal and 4-5 times Optimal is ok if speed is important. Keep Interval rather than Slice. You can either use continuous Drive or stepped Z. The former is faster but might have an impact on image quality if your exposure time is long.

Time-Series:

Duration can only be defined in cycles or As Long As Possible. *Do not choose As Fast As Possible as acquisition times become erratic.* Exposure times up to 30 ms do not have any influence on acquisition times per time point. Exposure times above 30 ms will increase acquisition times per time point. The software

will not warn you if your time settings cannot be met! Validate Speed works with z-stack or multi-view to tell you how long an experiment will take to acquire.

Multiview-Setup:

If you do it manually you can define a different z-stack (different number of slices but same intervals!) for each view. Type in each angle in **Specimen Navigator** and rotate around Center of image. Click Add for each new angle including the first. You can also create several groups with different locations and different laser power and different lightsheet alignment.

Quick Setup is exactly that but will keep the number of slices the same for each angle.

Moving between Acquisition and Locate

If you go back from **Acquisition** to **Locate Sample** you first need to change the light path set-up on Acquisition to a brightfield setting. For some reason the correct filters / mirror for brightfield imaging does not always work if you return from **Acquisition** to **Locate Sample**.

When you have defined your experiment parameters click Start Experiment. This will open a window that allows you to save the experiment preferably as a czi file. Please save directly onto the left computer as follows:

Computer

Network Location

SWAP

Any data saved on the acquisition computer will be periodically deleted without warning. From the analysis computer data can be moved to Micron1.

Shut down:

Move capillary to the Load Position and remove sample holder.

Switch lasers off in software.

Untick incubation settings (very important, otherwise the last used settings will be activated automatically when the next user starts ZEN!).

Exit software.

On pad to the left of the microscope, switch off System, PC and Incubation. Rinse chamber 3 to 6 times with demin. water depending on the buffer strength used for the experiment. Leave chamber filled with demin. water.