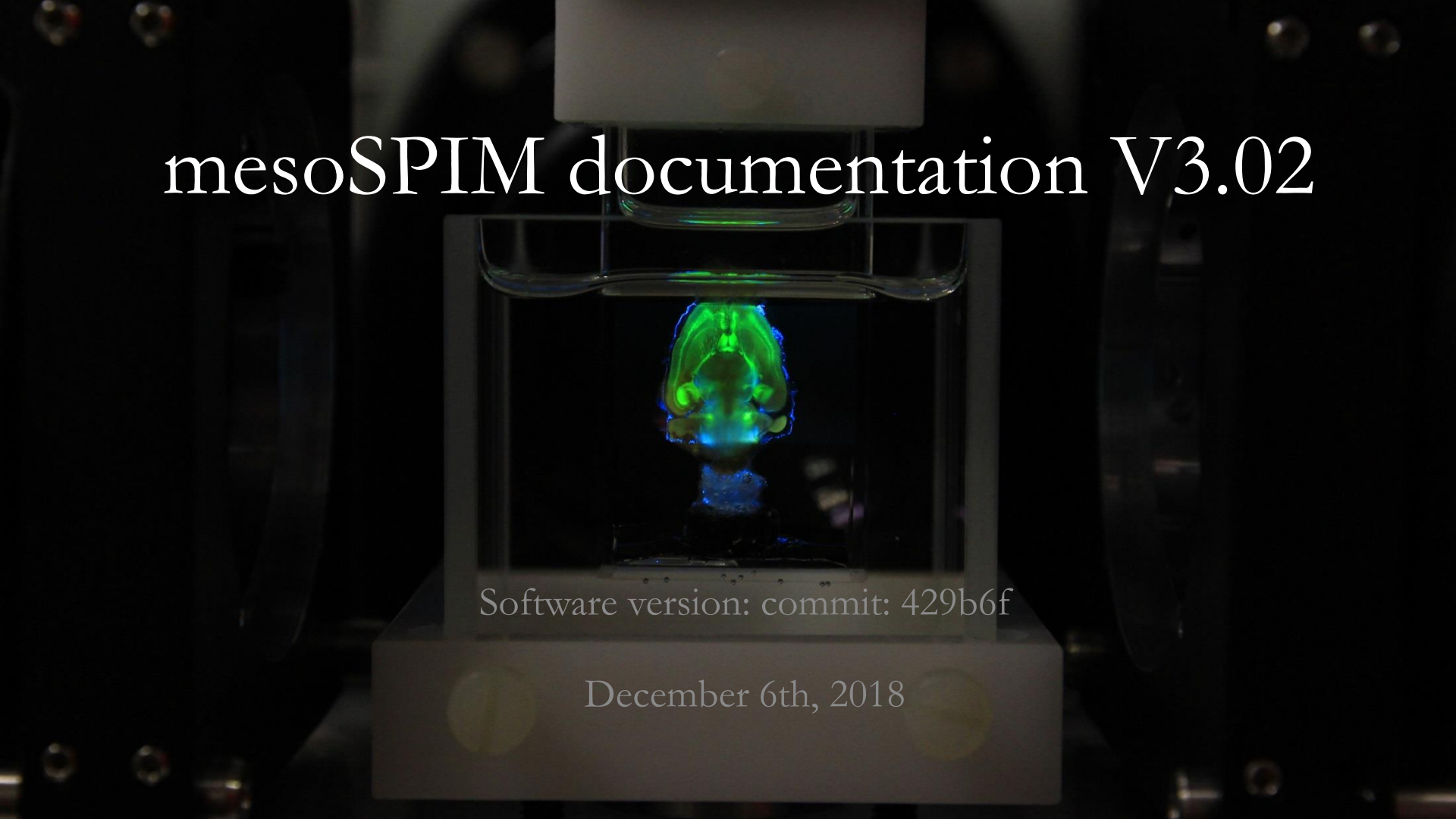
mesoSPIM documentation V3.02



Software version: commit: 429b6f

December 6th, 2018

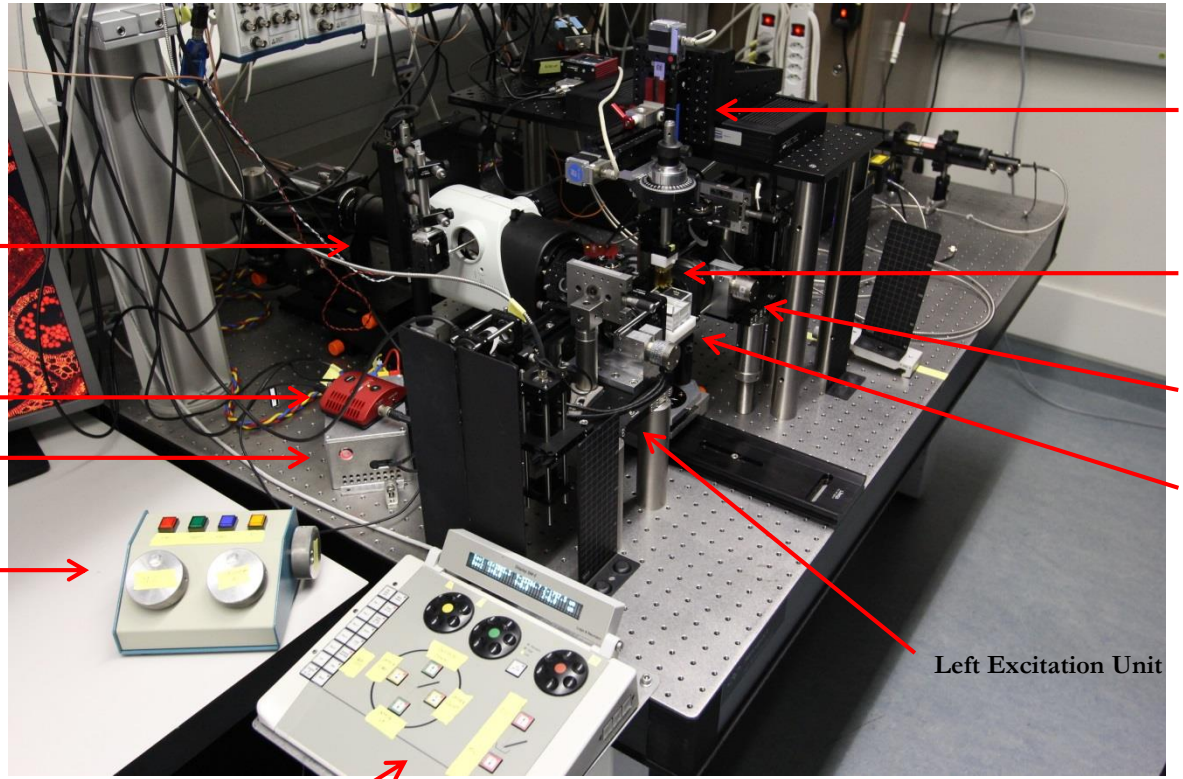
Part I: Specifications

FOV calibration

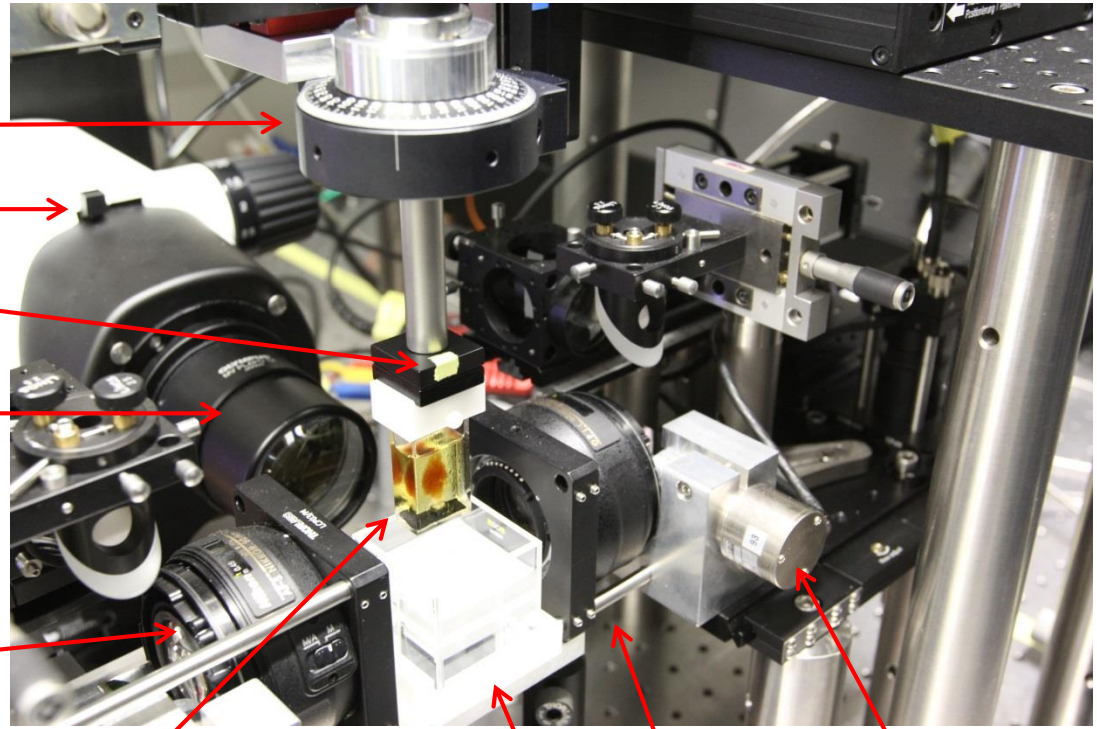
Objective	Zoom	FOV size (mm)	Pixel size (mm)
1x	0.63	21.56	0.0105263
1x	0.8	16.86	0.008234
1x	**1**	**13.42**	**0.006552**
1x	**1.25**	**10.79**	**0.0052666**
1x	1.6	8.36	0.0040844
1x	2	6.69	0.0032688
1x	2.5	5.34	0.0026059
1x	3.2	4.16	0.0020332
1x	**4**	**3.29**	**0.001606**
1x	5	2.62	0.0012788
1x	6.3	2.12	0.0010348

Part II: Hardware overview

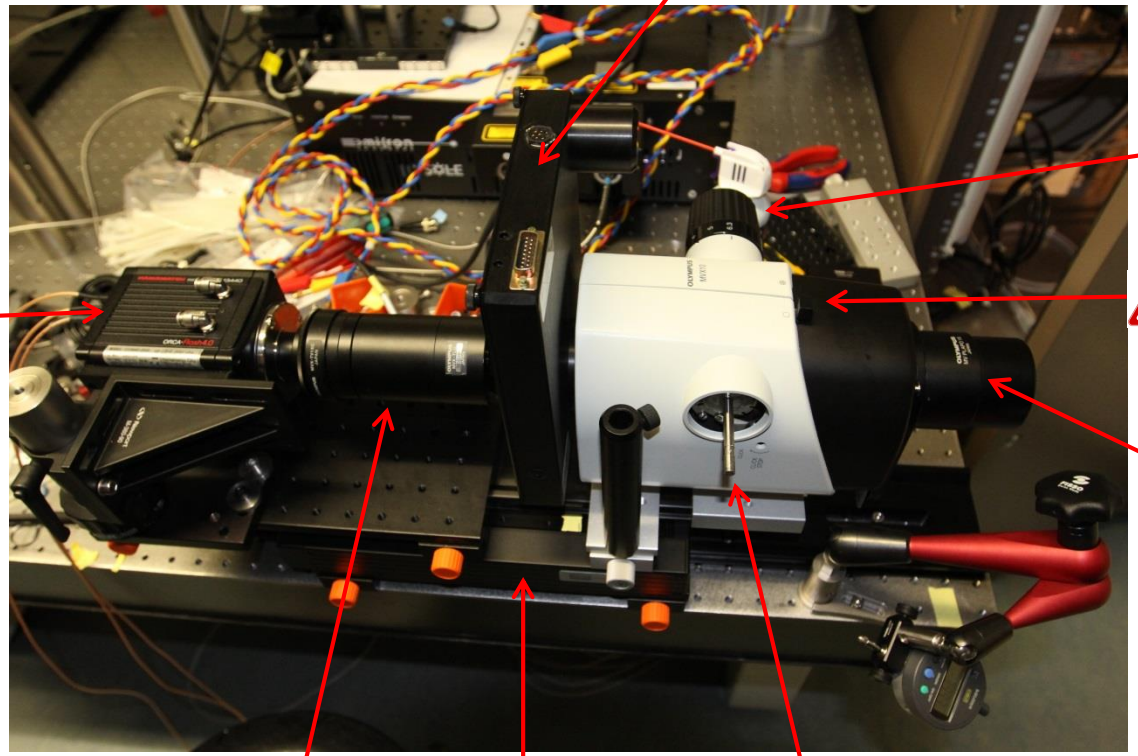
Microscope overview



Overview of the sample holder

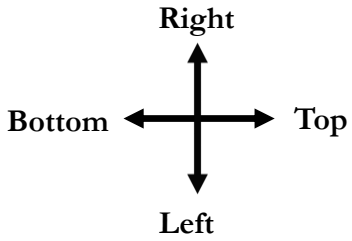


Overview of the detection system

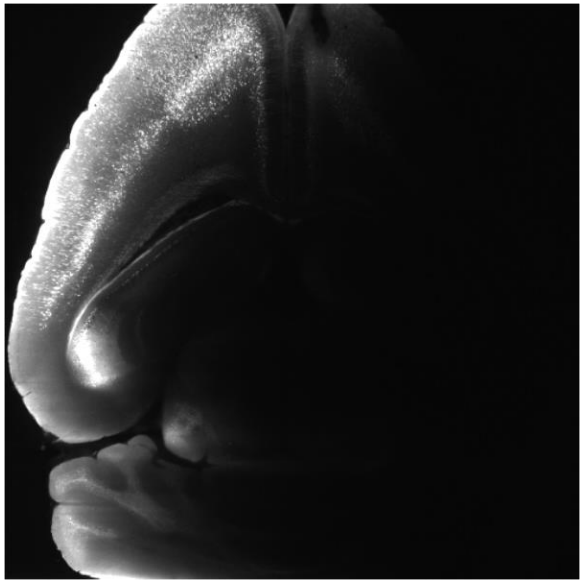


Part III: Learning to see with a light-sheet microscope

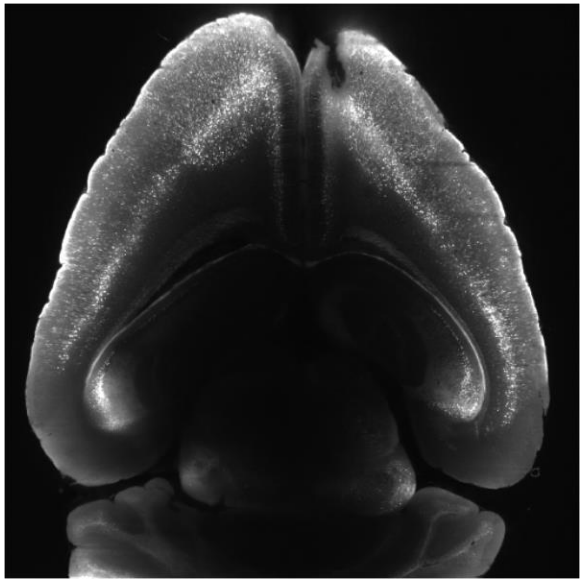
Light-sheet directions (@Zoom 1.25x)



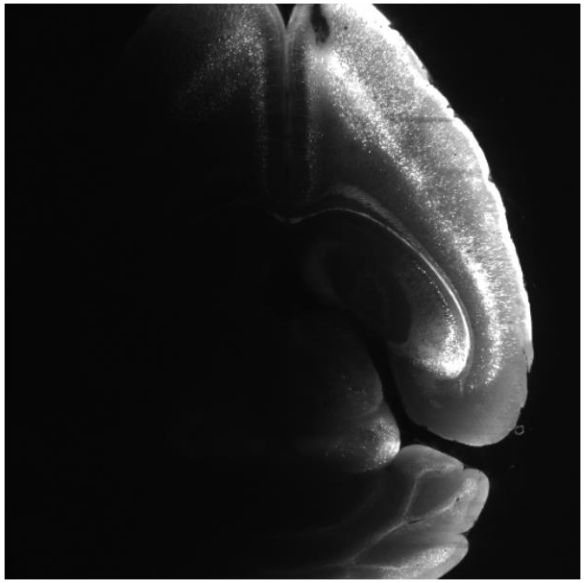
In order to allow the sweeping focus to be synchronized with the camera readout, the camera is rotated by 90º.
In not-so-well-cleared samples, the penetration direction is clearly visible:



Right Light-sheet



Both



Left Light-sheet

The perils of dual-sided excitation

Note Sep 11th, 2017: Coalignment depends on the position of the outer cuvette: Do Not move the outer cuvette after coalignment For now!

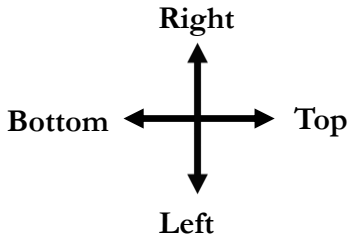
Lightsheet 1 →

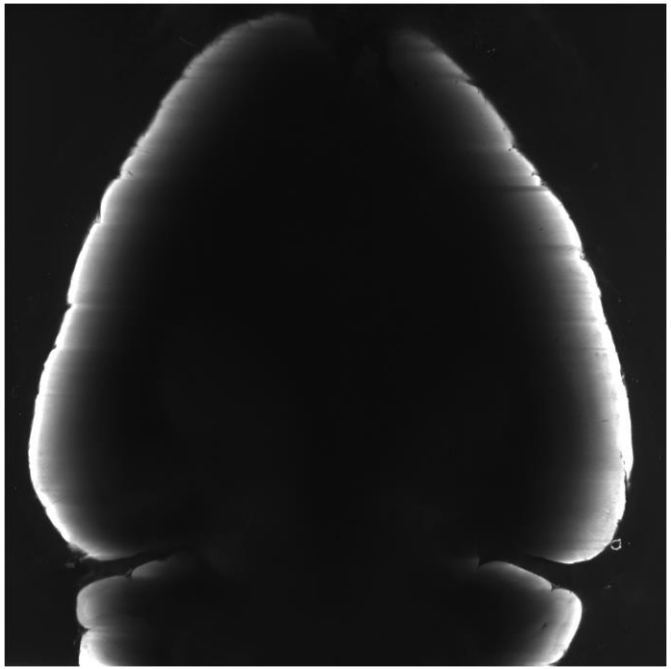
← Lightsheet 2

Z direction:
Acquisition movement of a stack

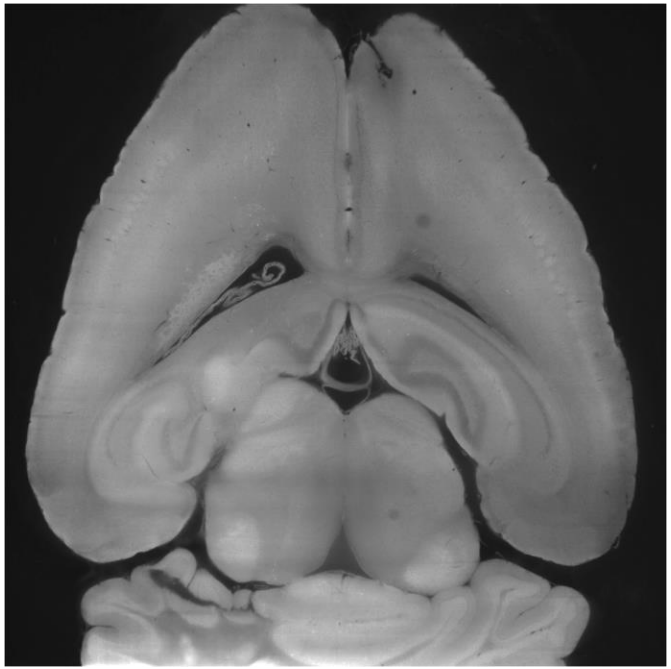
XZ reslice of a Thy1-YFP dataset
Notice the doubled cells in z because the two lightsheets can become locally misaligned to to index-mismatches inside the sample

Light-sheet penetration is stongly wavelength-dependent





405 nm + Quadrupleband

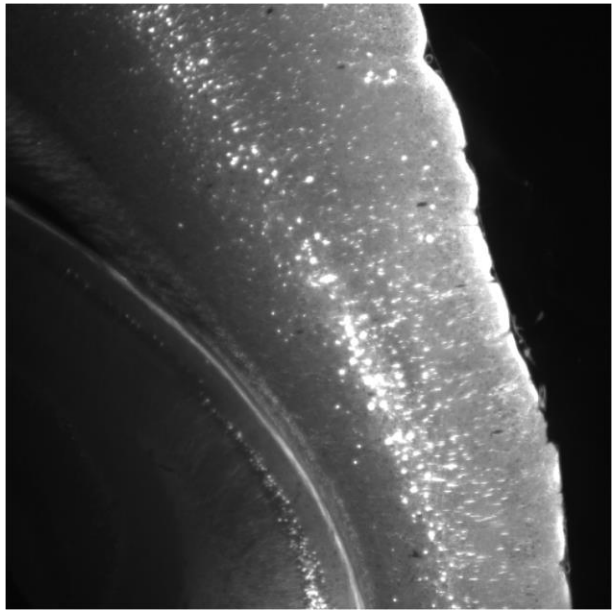


647 nm + Quadrupleband

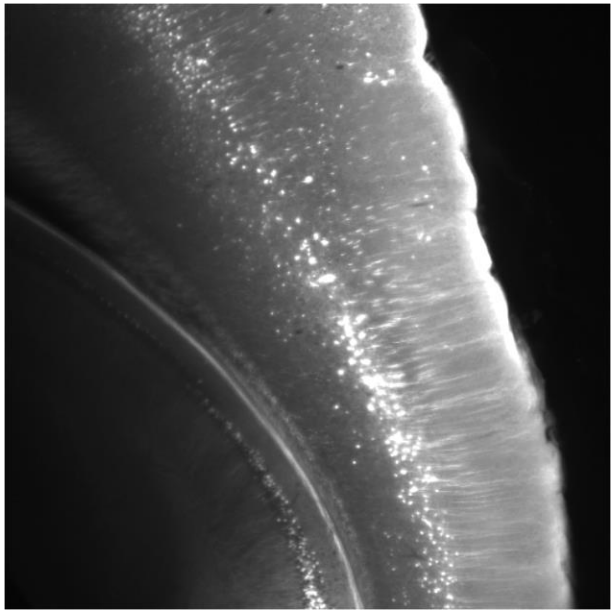
What happens if the tunable lens parameters are incorrect?

A thicker light-sheet leads to more features being visible at the expense of contrast

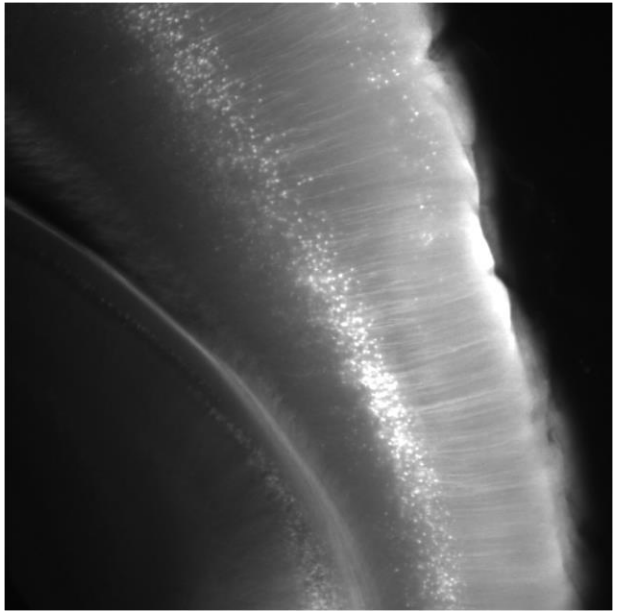
Thy1-YFP (H-line), 488 nm excitation, zoom 4



ETL offset = 2.47 V
ETL amplitude = 0.17 V



ETL offset = 2.47 V
ETL amplitude = 0.0 V

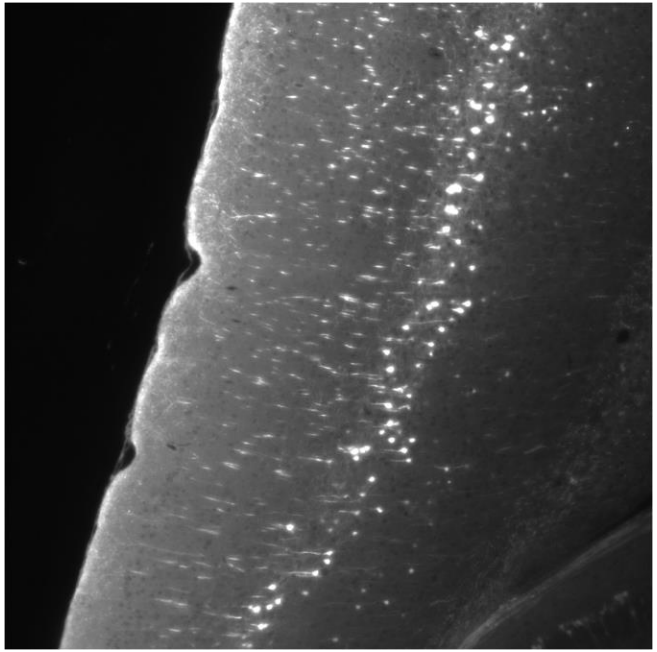


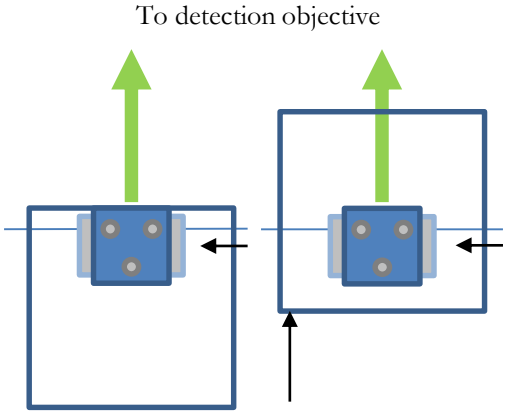
ETL offset = 2.2 V
ETL amplitude = 0.0 V

Counterstained cell-bodies and blood-vessel cross-sections are usually good structures to judge this

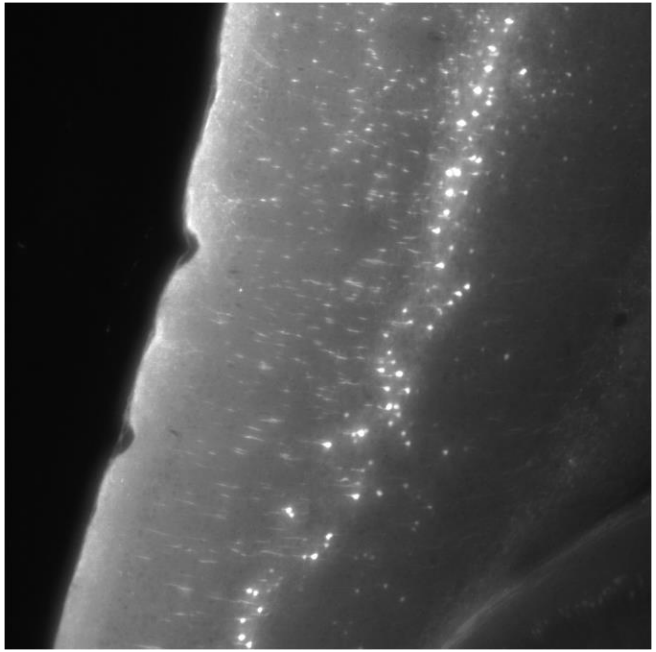
What happens if the path length is to large?

Thy1-YFP (H-line), 488 nm excitation, zoom 6.3x





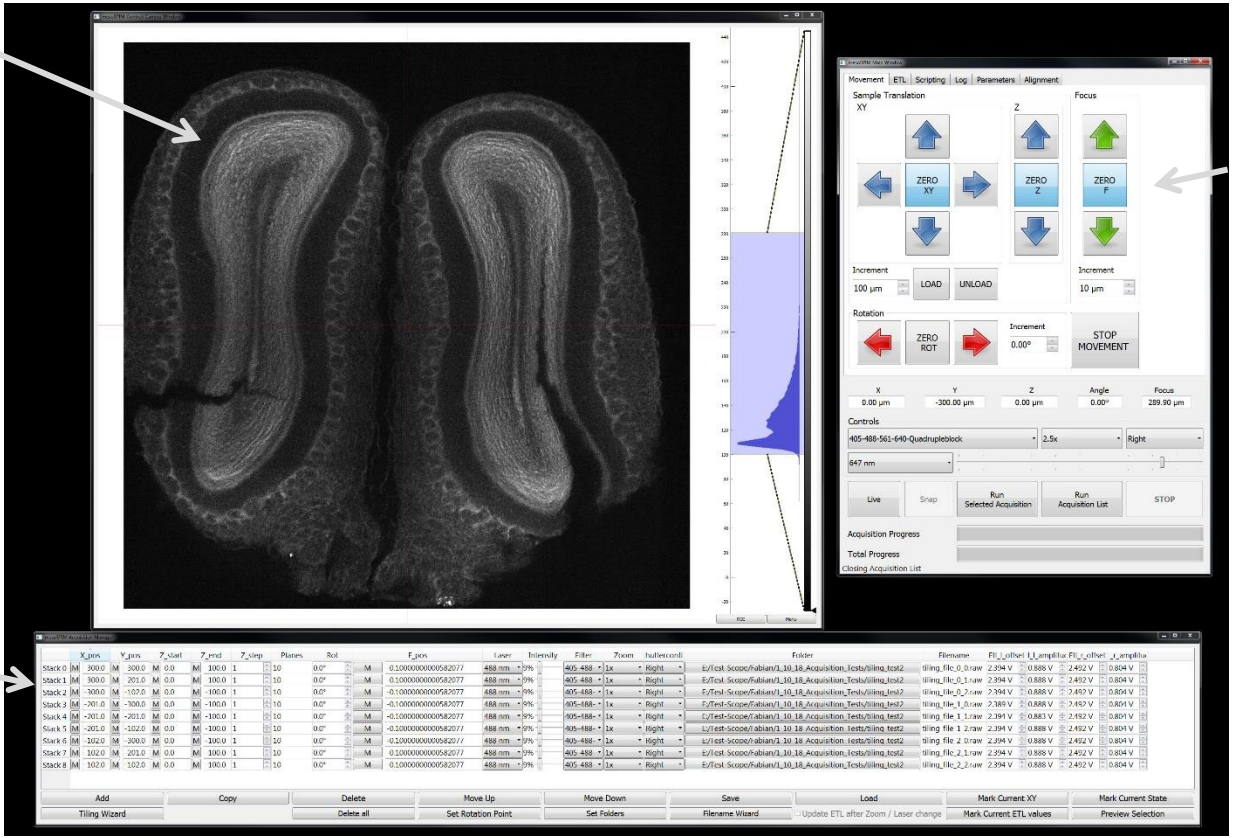
Thy1-YFP (H-line), 488 nm excitation, zoom 6.3x



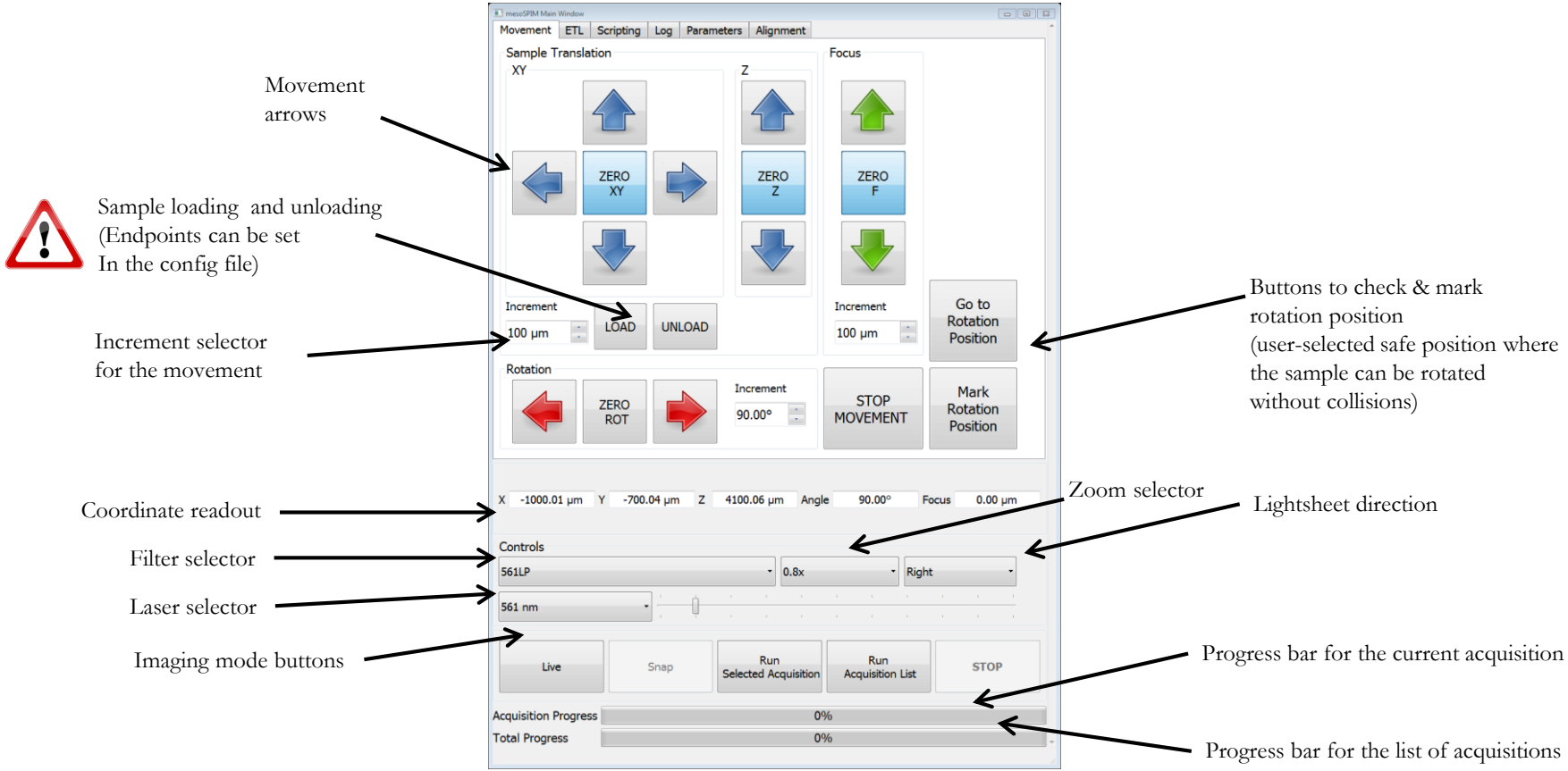
Select a position of the outer cuvette that minimizes spherical aberration while offering enough space for sample movement during stacks & rotations

Part IV: User Interfaces

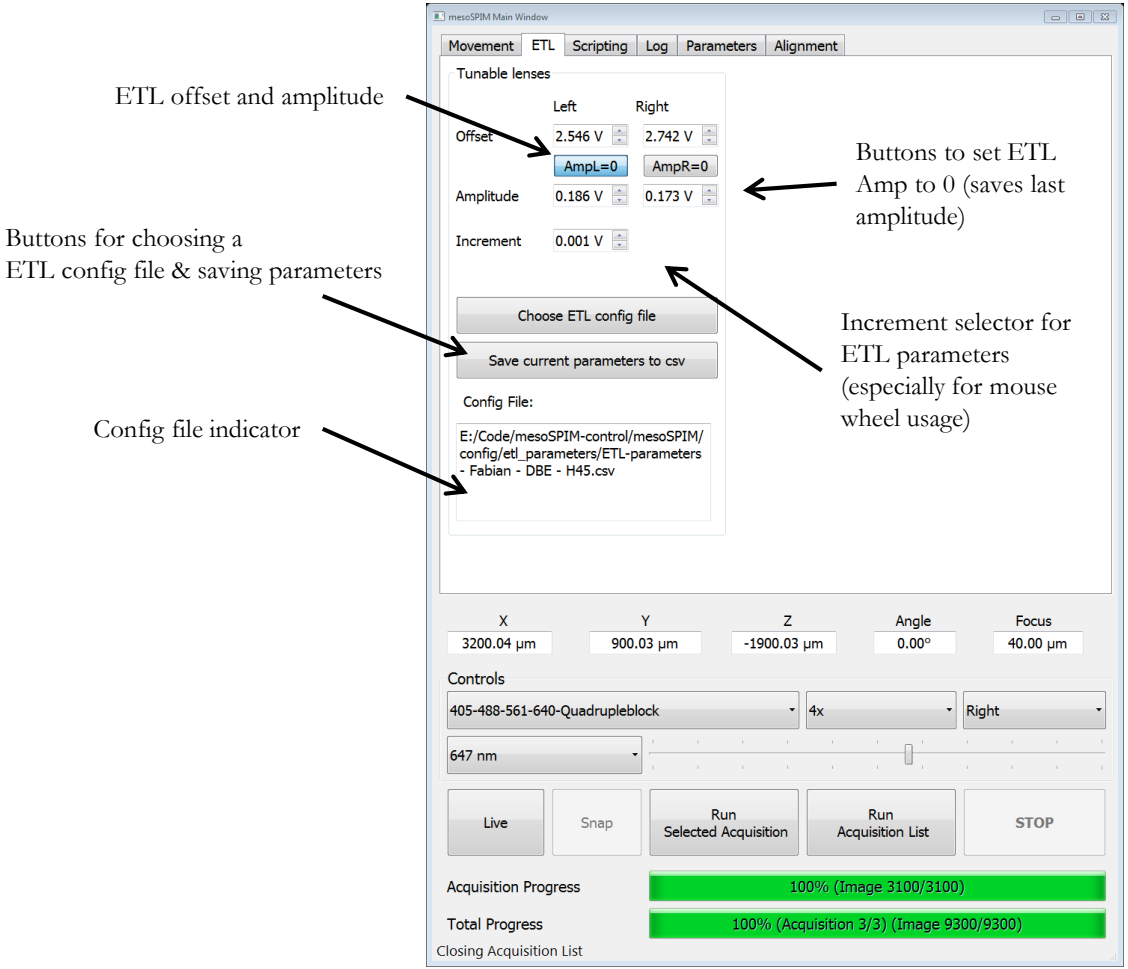
mesoSPIM-control: Overview



mesoSPIM-control: Main window: Movement tab

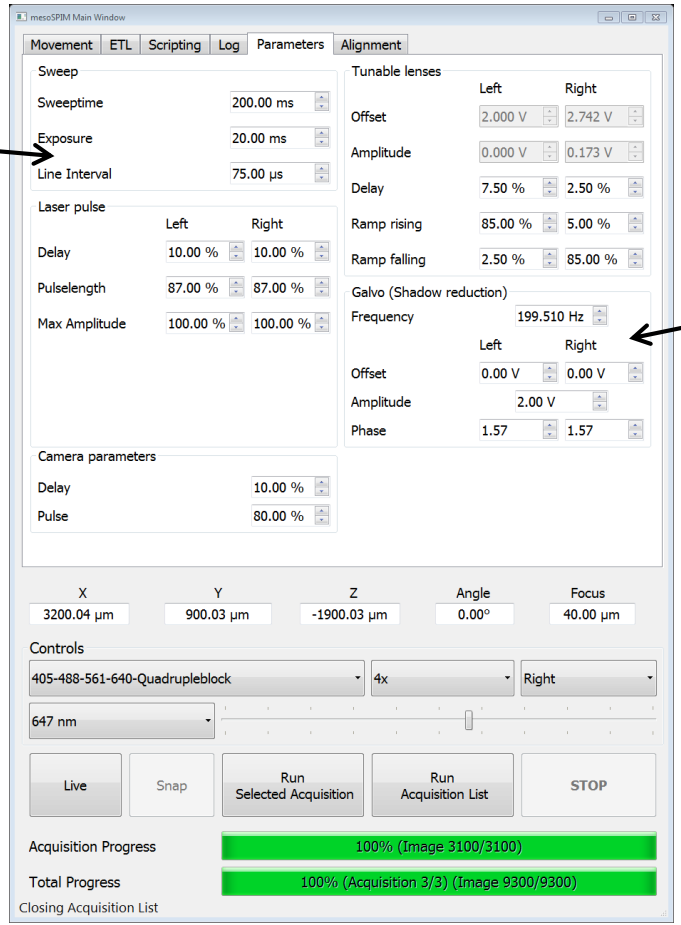


mesoSPIM-control: Main window: ETL tab



mesoSPIM-control: Main window: Parameters tab

Camera exposure &
Line interval



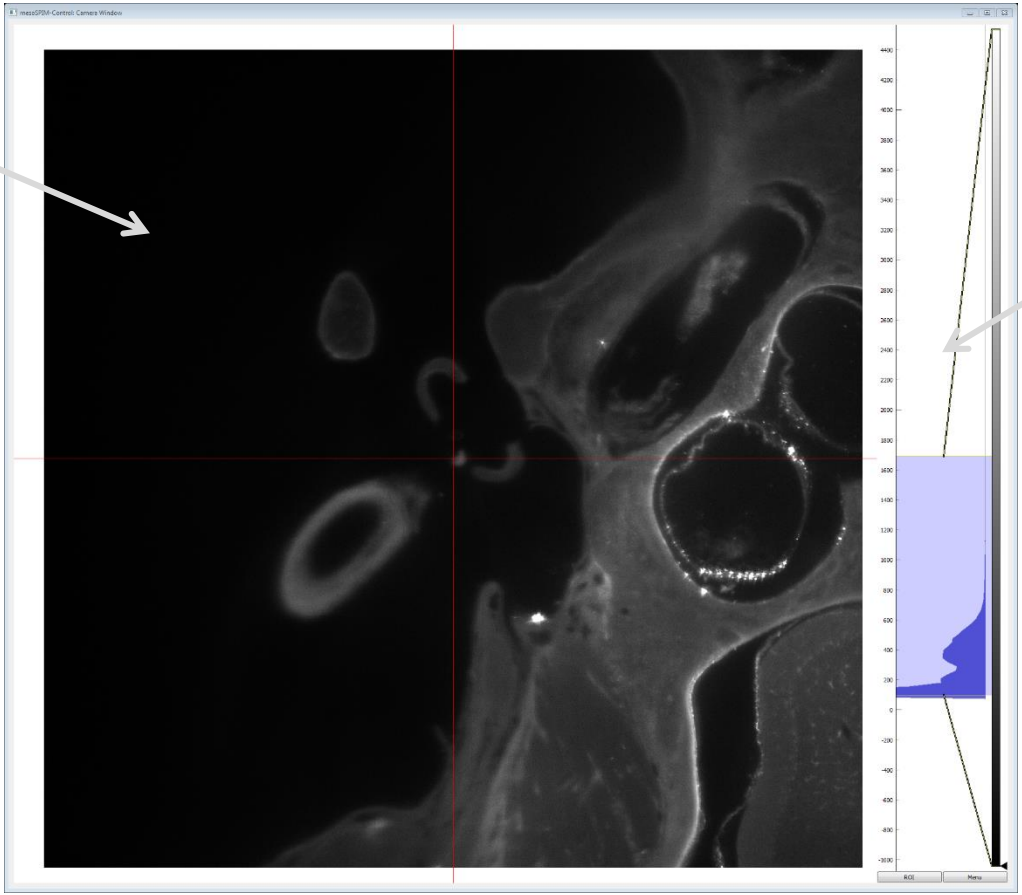
Galvo parameters

mesoSPIM-control: Camera window

Main camera window
With crosshairs for
ETL alignment

Mouse drag: Moves
View

Mousewheel: Zoom in/out
(not the motorized zoom)



Histogram:

Mousewheel zooms

Right click on the histogram:
Set range / autorange

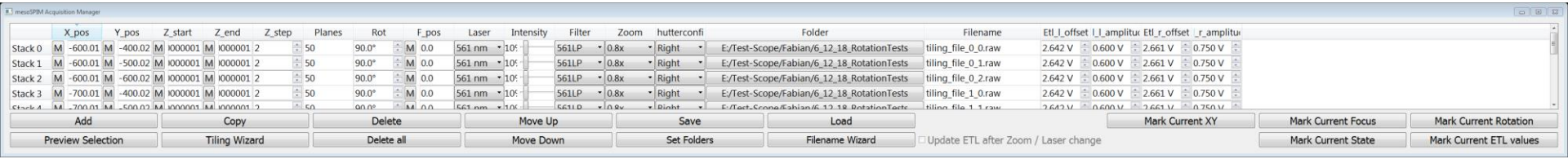
Right click on the colorbar:
Select lookup table

mesoSPIM-control: Acquisition Manager

The acquisition manager replaces the old stack tab in the main window
A single row in this table describes an entire stack, the microscope acquires them one-by-one from top to bottom

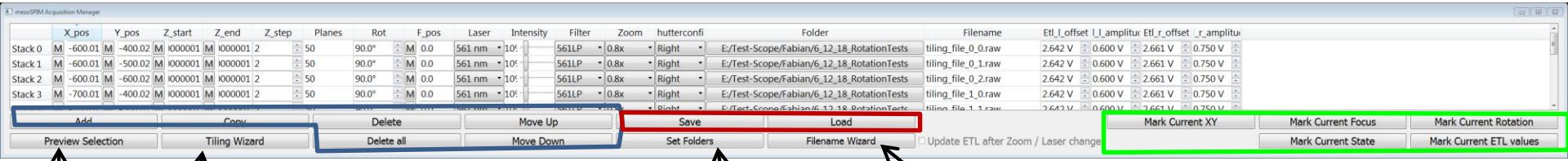
The parameters shown in the table are the ones used for the respective acquisition, not the ones shown in the main window

When specifiying acquisitions, the stages should only be zeroed once, so that different stacks refer to the same coordinate system!



mesoSPIM-control: Acquisition Manager

Save & load tables



Controls for table entries

Update parts of a selected table entry & preview

Preview Selection:
Move to start position & set up the microscope state accordingly

Tiling wizard

Set folder for saving data for all rows

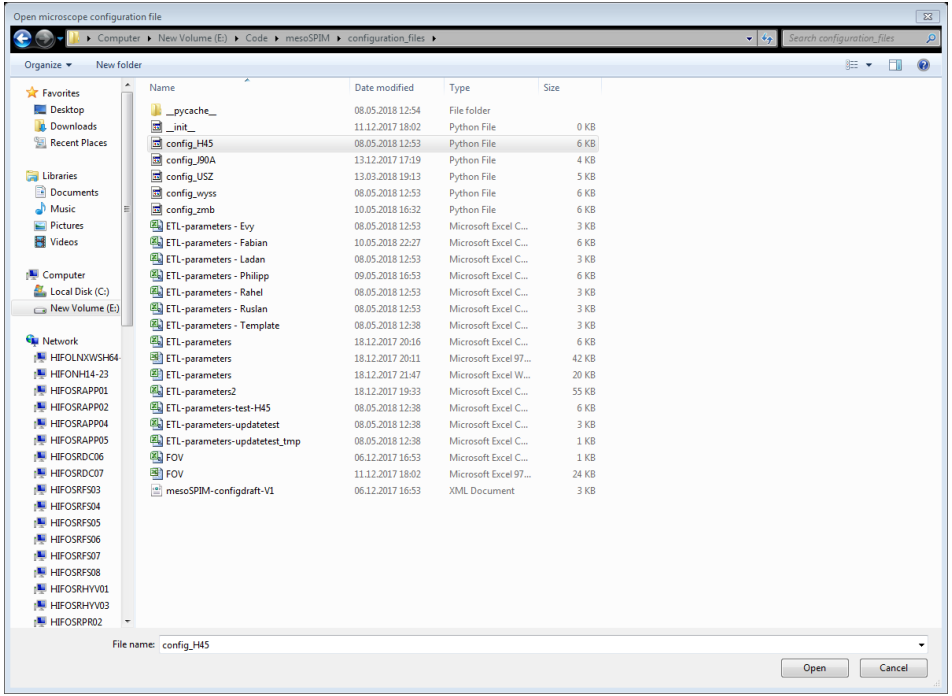
Autogenerate filenames

Part V: Startup

Startup procedure

- Switch L+R Shutters & Galvos on & Activate Galvos! (long press on the enable button till the second light blinks shortly)
- Switch Laser + Camera on
- Start mesoSPIM-control.py (in a console: «python mesoSPIM-control.py»)

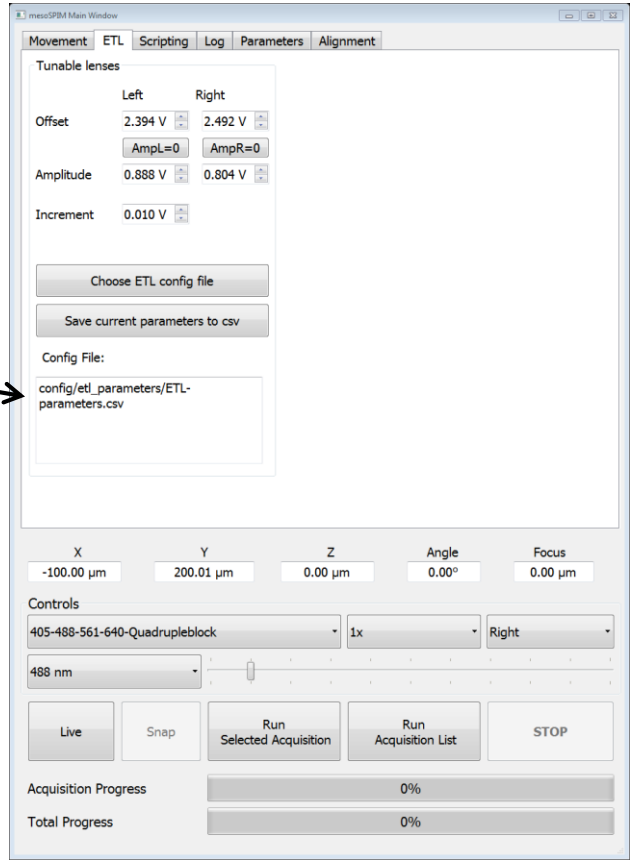
mesoSPIM-control: Startup I



1. Choose config file according to your setup. This is a .py file containing the microscope configuration, NOT a ETL-parameter file.
2. The software takes 10-15s to start while performing a reference move with the focusing stage

mesoSPIM-control: Startup II

3. Select your ETL parameter file



Part VI: Joystick

Lasers 1-6
(or 1-4)

5: Unload

10: Load

11 & 12: Increase & decrease Filter
13 & 14: Increase & decrease Zoom
15: Zero XYZ
16: Zero Focus

17: Both Shutters
28: Both Shutters

19: Left Shutter
20: Right Shutter

21: Live/Stop Live

Stop Motion

Laser
intensity

Stack controls currently deactivated

BLUE:
Joystick X (Left-Right): Sample X
Joystick Y (Top-Down): Sample Y
RED:
Joystick X (Left-Right): Focus
Joystick Y (Top-Down): Sample Z
Joystick Twist: Fine Focus

Part VII: Typical acquisitions

Live Mode

1. Click Live in the mesoSPIM Control software
2. Stop Live in the mesoSPIM Control software

When using different lasers/filters, refocusing is often necessary

Be aware of bleaching – in light-sheet microscopy, you bleach a plane into your sample

Setting ETL parameters

1. Go to ETL tab, go to Live mode, select desired lightsheet.
2. Select the correct user-ETL-config file.
3. Toggle the AmpL or AmpR=0 button to see the waist location
4. Using the scrollwheel of the mouse, scroll the offset value so that the waist is in the center of the FOV (on the red crosshair line in HCImage if the crosshair is switched on). It the value changes to quickly, reduce the increment setting.
5. Toggle the AmpL or AmpR-button again. If desired, the amplitude can be optimized by scrolling and selecting the thinnest light-sheet (fewest sample features visible)
6. If desired, save the settings for this zoom & wavelength

ETL parameters depend on the mounting medium, its temperature, zoom, and excitation wavelength

The ETL config file provides reasonable presets, but its accuracy should be checked in each sample

When changing the excitation laser or zoom, the ETL parameters will update from the config file. If the chosen values should be retained, save them.

Acquiring stacks / lists of acquisitions

1. Zero the stages (XY, Z, Focus) in live view in an appropriate location

When creating an acquisition list, never zero different rows at different locations!

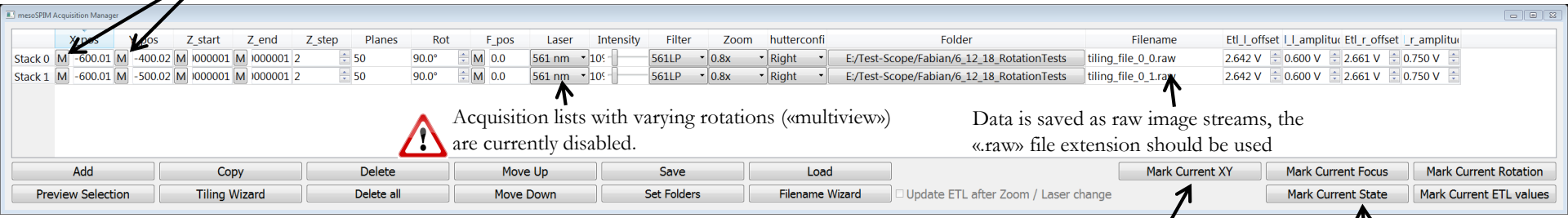
2. Using the mark buttons, select the X,Y,Focus, Z_start and Z_end positions

Mark buttons («M») = copy current coordinate

When using different lasers/filters, refocusing is often necessary

The ETL values for each row are the ones used for this stack, not the ones in the Main Window ETL tab.

To stop an acquisition, click «STOP» in the main window



Acquisition lists with varying rotations («multiview») are currently disabled.

Data is saved as raw image streams, the «.raw» file extension should be used

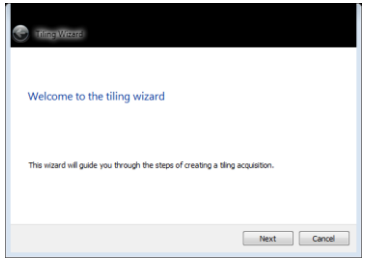
Mark X + Y position
For a selected row

Mark state:
Copy filter, laser, intensity, shutter & zoom from Main GUI

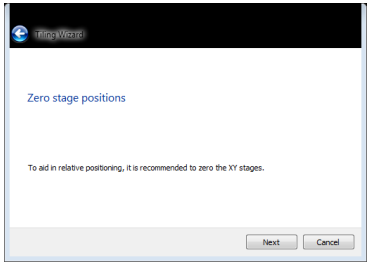
3. Using the mark buttons, select the X,Y,Focus, Z_start and Z_end positions
4. Select, laser, intensity, filter, shutter for a row
5. Select a folder for the stack to be saved in by clicking on the button in the folder column. The current folder will be displayed. Using the «Set Folders» button, the folders for the entire table can be changed at once. You can autocreate filenames using the filename wizard.
6. In Live view, you can change your ETL values and then copy them to a selected row using «Mark current ETL values»
7. Using «Mark current state», you can copy the current laser, filter, intensity, zoom and shutterconfiguration from the main window
8. If you click «Preview selection», the microscope will move to position in the selected row and set up filter, zoom, laser, ETLs etc
9. Using «Run Selected Acquisition» in the main window, you can run a single stack. Using «Run Acuquisition List» allows to run the entire table

Tiling Wizard

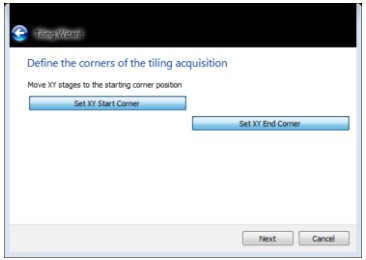
1. Start the Tiling Wizard



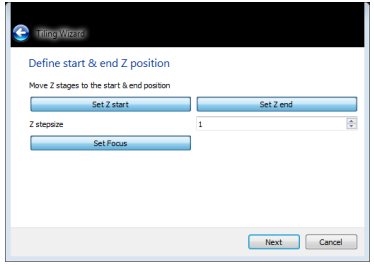
2. If desired, zero the stages using the buttons in the main user interface



3. Set the corner positions (go to live mode & set positions)

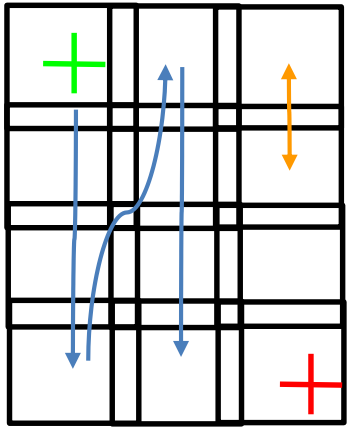


4. Define Start & End Position & Focus



For some reason, screenshots have a «black bar» on top (not visible in the window during usage)

The first FOV is centered on the start position



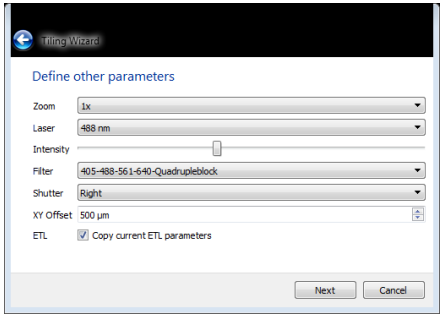
«Offset» is the mechanical FOV-to-FOV (ΔX or ΔY) movement distance

- You have to calculate offset depending on your FOV

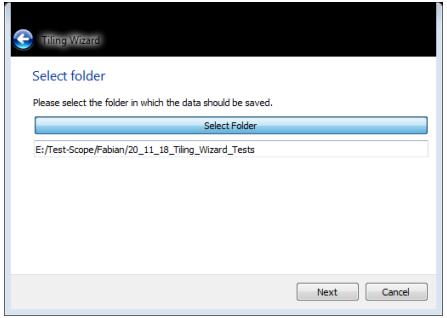
The last FOV contains the End position (not centered)

Tiling Wizard

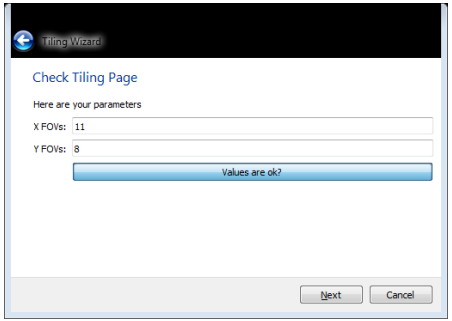
5. Define other imaging parameters



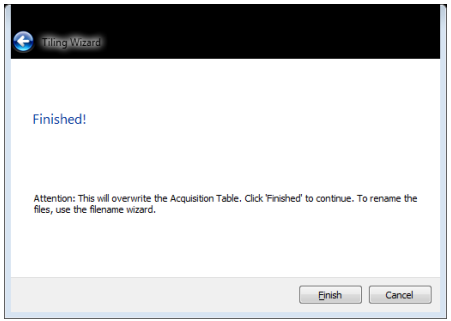
6. Define Folder



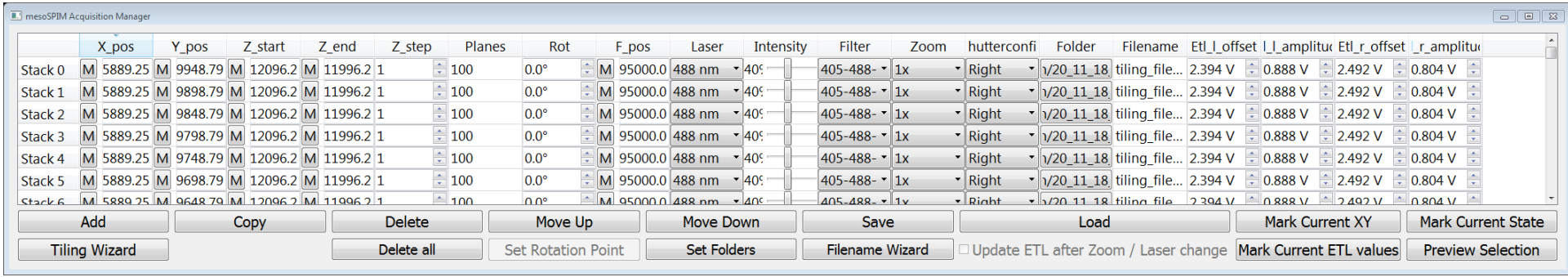
7. Check FOV counts:



8. Finished!

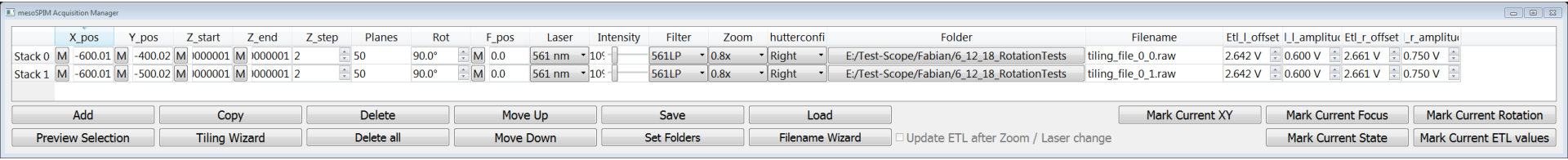


9. Enjoy your table – if**, necessary, change filenames using the file wizard**



10. Modify your table before scanning, for example by checking ETL parameters

Acquisition checklist



General

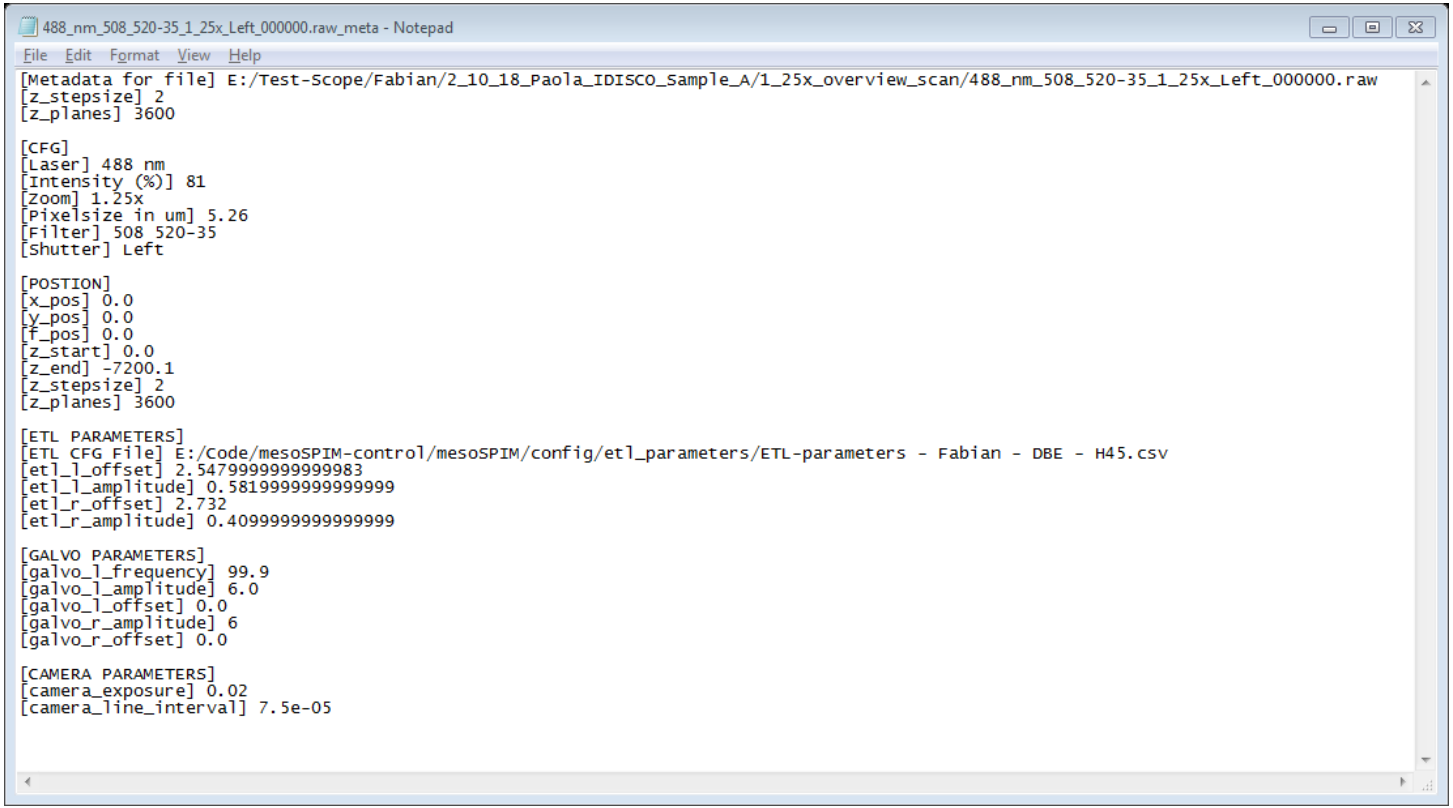
- Sample does not collide at the end positions?
- XYZ Zeroed properly?
- Focus zeroed?
- Noted down the absolute coordinates of the origin (to zero properly before crashing)
- X/Y/Z coordinates make senses?

Last minute

- No last-minute manual focus changes?
- No last-minute changes in Laser, Filter, Intensity, Zoom, Shutterconfig (Left/Right)
- Noted down the absolute coordinates of the origin (to zero properly before crashing)
- Number of Planes are as desired? No varying z-steps?
- No rotations in the list (currently, the software does not accept varying rotations)
- Folder selection makes sense?
- Filenames are correct?
- All ETL values set properly? No default ETL values left?

Metadata

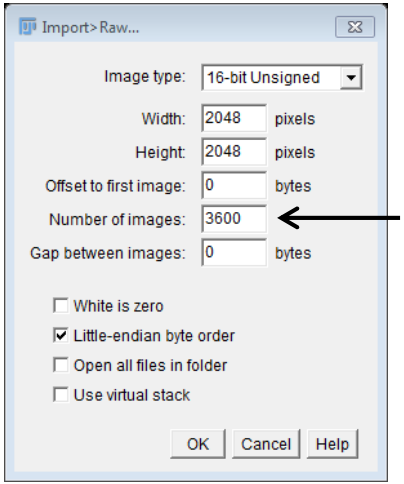
For each acquisition, a metadata text file is saved as well which contains the most important parameters



Opening stacks/acquisitions in Fiji

1. In Fiji, select «File» → «Import» → «Raw»
2. A window opens, enter the following parameters:

Height:	2048 pixels
Width:	2048 pixels
Offset:	0 bytes
#Images:	according to metadata / larger than acquired
Gap:	0 bytes
Little endian byte order	

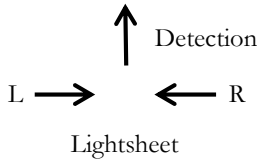


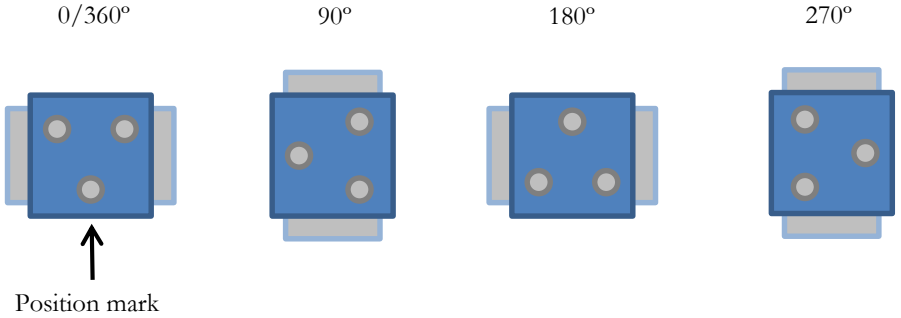
Number of images can
be larger than the number of acquired images,
the import will work normally

3. The stack will open in the center

Controlling sample rotation

Rotations of a 20 mm cuvette:



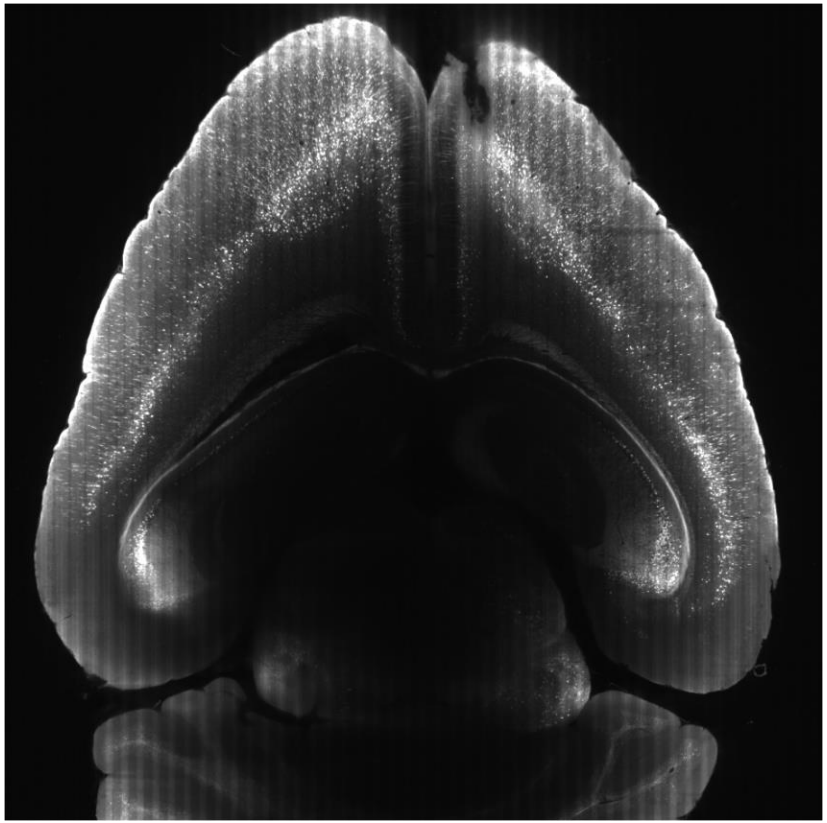


Viewed from the top,
positive rotations are oriented
clock-wise

Especially when using 20 mm cuvettes, watch out for collisions with the outer cuvette!

Part VI: Troubleshooting

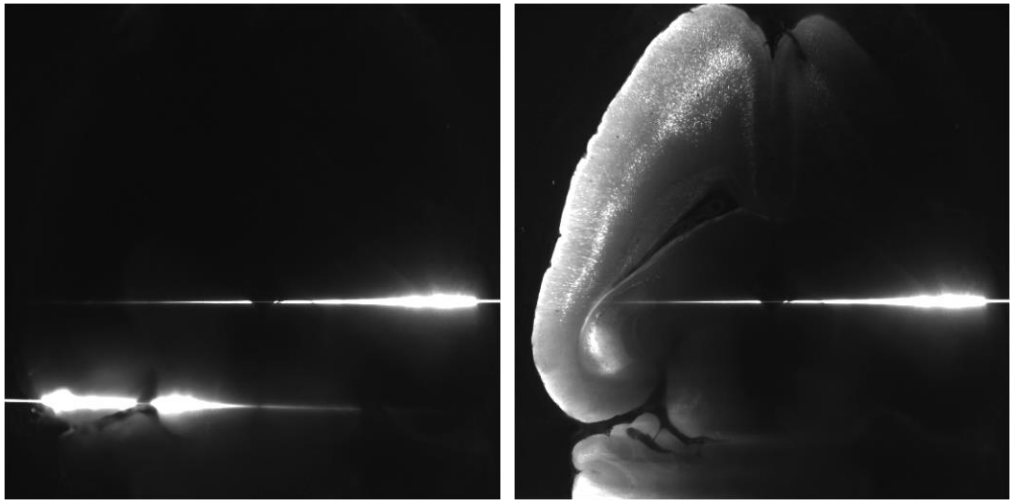
Horizontal Stripes in the image



Stripes indicate that the Galvo
Frequency is not averaged out by the exposure.
Possible reasons:

- Wrong Galvo Frequency
- Wrong Exposure
- Wrong Line interval

Vertical bright line(s) in the image



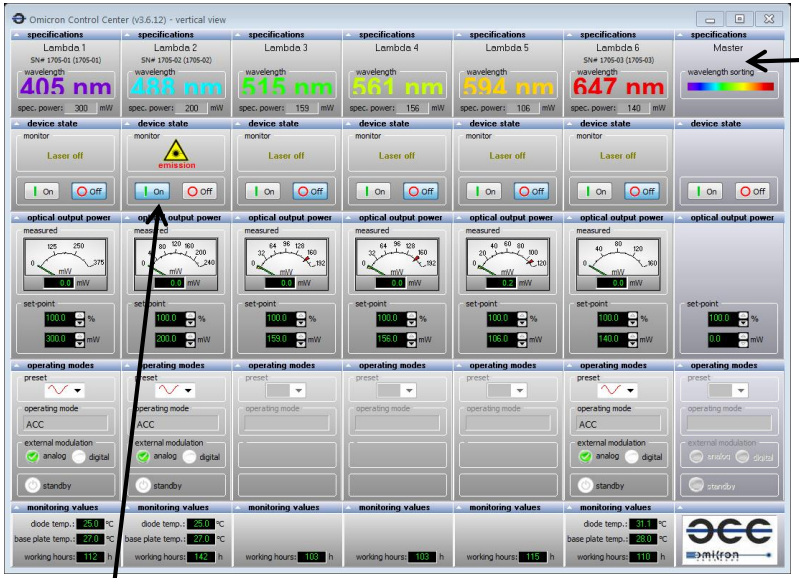
One or two vertical lines in the image:

- Galvos are switched off
- Galvos switched off due to overheating (usually at amplitudes >3.7 V)

Depending on the laser power, this might leave bleached lines in the sample

→ Turn scanner off and back on (box with red power light)

Omicron Control Center: Controlling the lasers (enable/disable)



All six lasers should be displayed, if not, restart the software and possibly the laser itself (the power switch is on the rear of the laser, left side)

The 515, 561, and 594 nm lasers require some time after enabling them to warm-up properly .

Enable single wavelengths – ideally, only a single wavelength is enabled for each stack to avoid co-excitation
During acquisitions, the intensity is controlled via Labview, but currently, Labview cannot disable a laser completely, so manual

Labview: Galvo Control (Galvo-Sawtooth-6259-OUT.vi)

The Galvos create the lightsheet out of a round beam by scanning vertically. They have to run during all acquisitions.

Start Button (Right arrow)

Central frequency:

@Zoom <1
Frequency 99.5 Hz
Amplitude 5V

@Zoom 1.25
Freq 199 HZ
Amplitude 3.67 V

@Zoom 4
199 Hz
Amplitde 1.4 V

Galvo Parameters:
defaults for Zoom 1.25x and higher, do not need to be changed