

Zurich mesoSPIM documentation

V3.01

Software version: commit: 407344

3.10.18

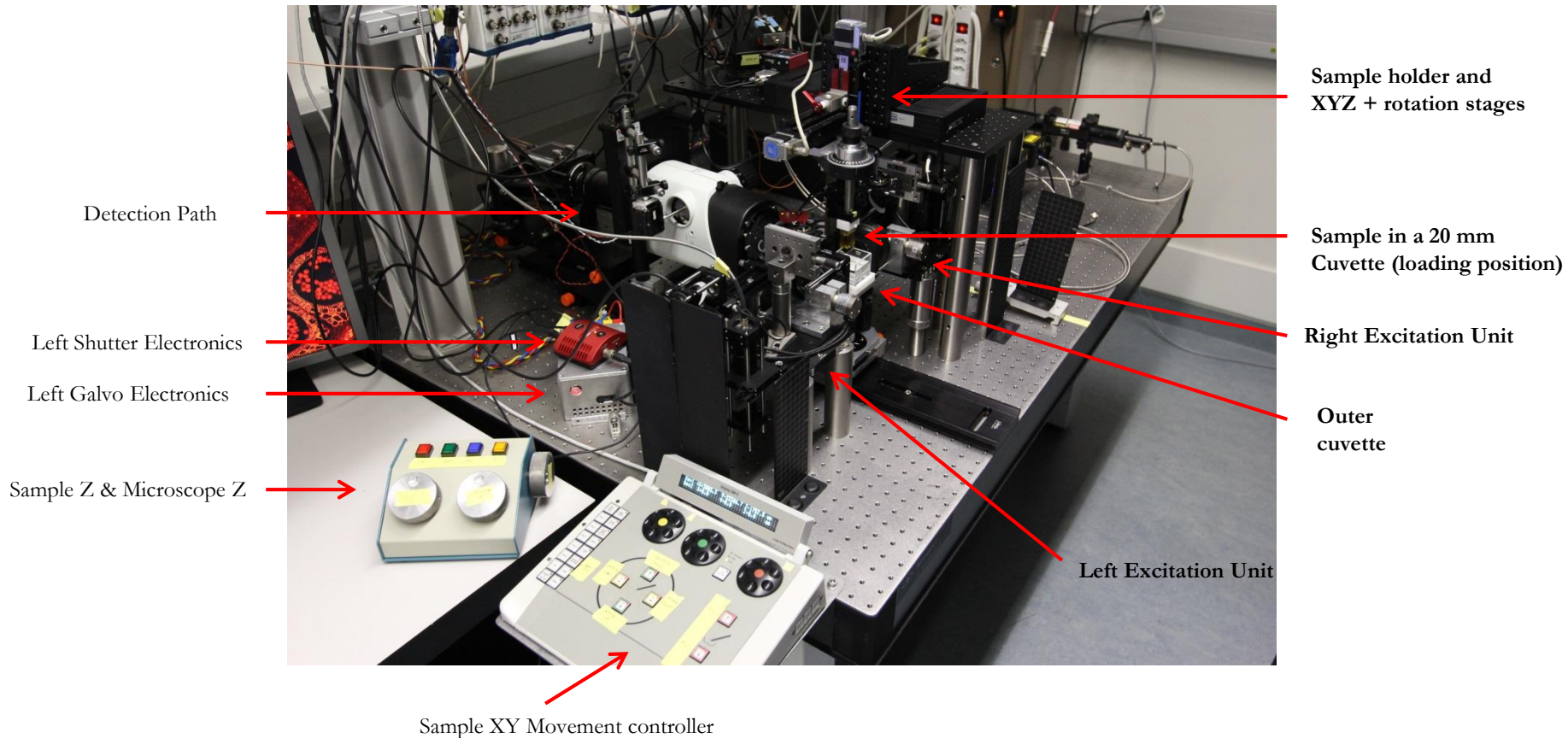
Part I: Specifications

FOV calibration

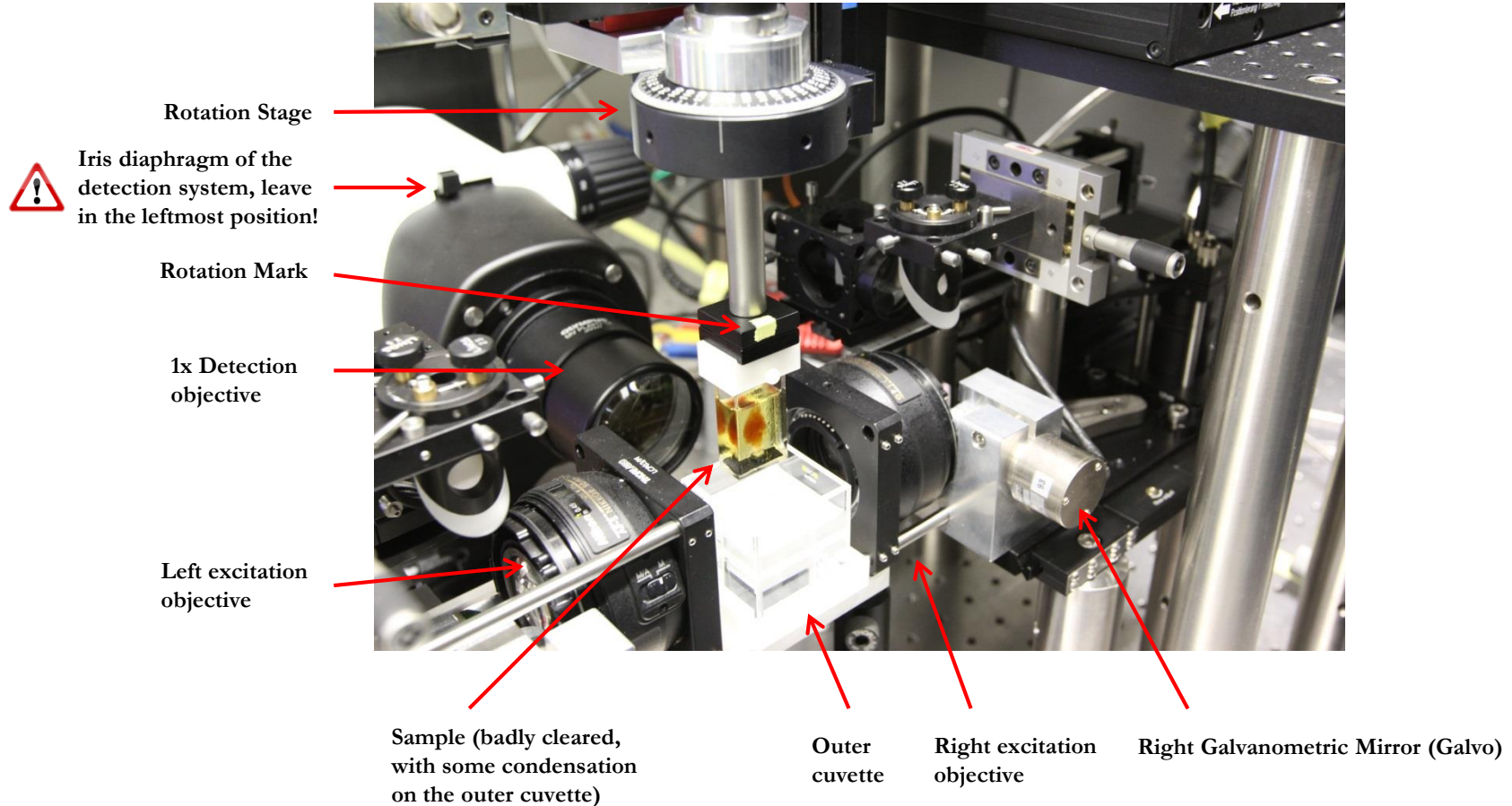
Objective	Zoom	FOV size (mm)	Pixel size (mm)
1x	0.63	21.56	0.0105263
1x	0.8	16.86	0.008234
1x	1	13.42	0.006552
1x	1.25	10.79	0.0052666
1x	1.6	8.36	0.0040844
1x	2	6.69	0.0032688
1x	2.5	5.34	0.0026059
1x	3.2	4.16	0.0020332
1x	4	3.29	0.001606
1x	5	2.62	0.0012788
1x	6.3	2.12	0.0010348

Part II: Hardware overview

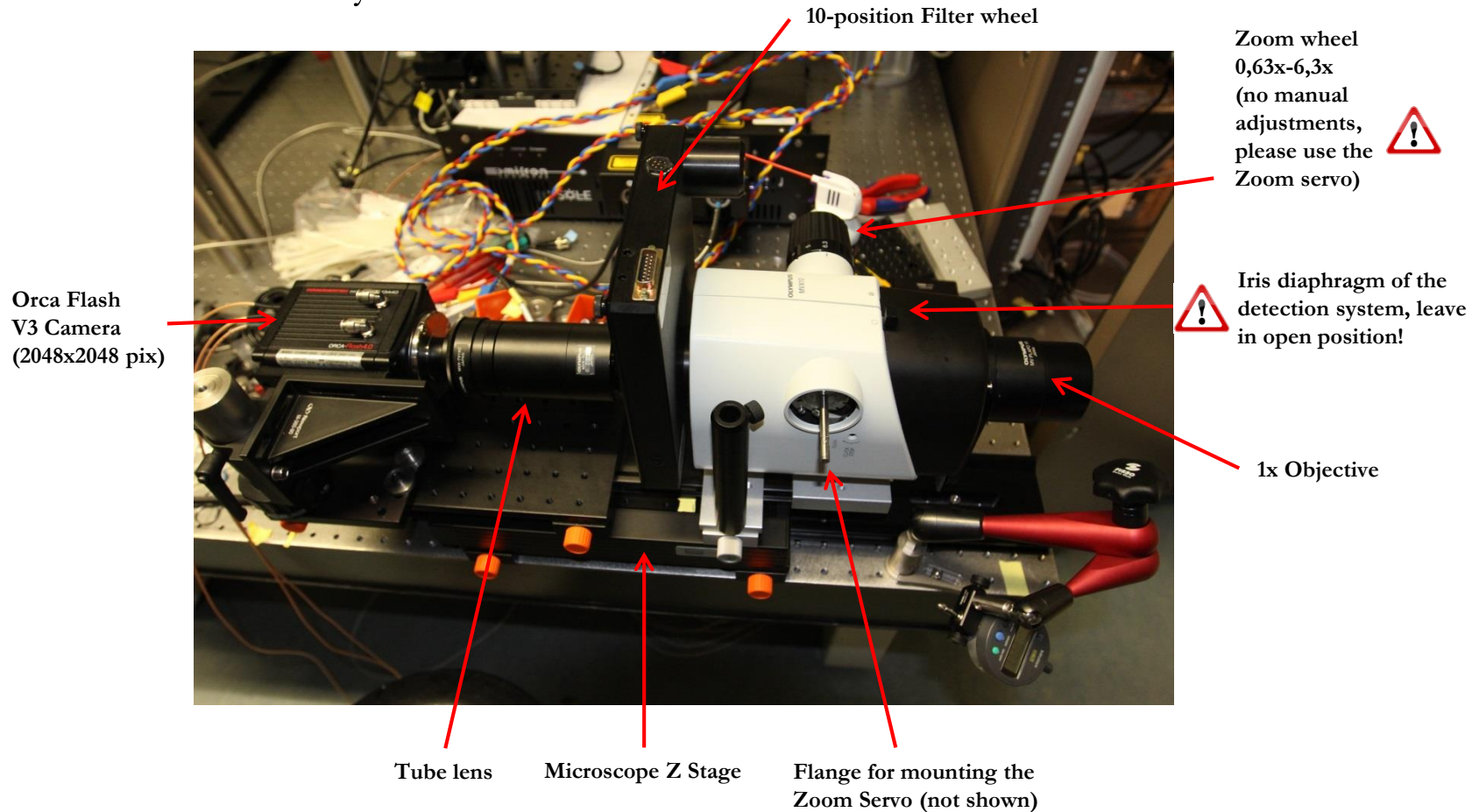
Microscope overview



Overview of the sample holder



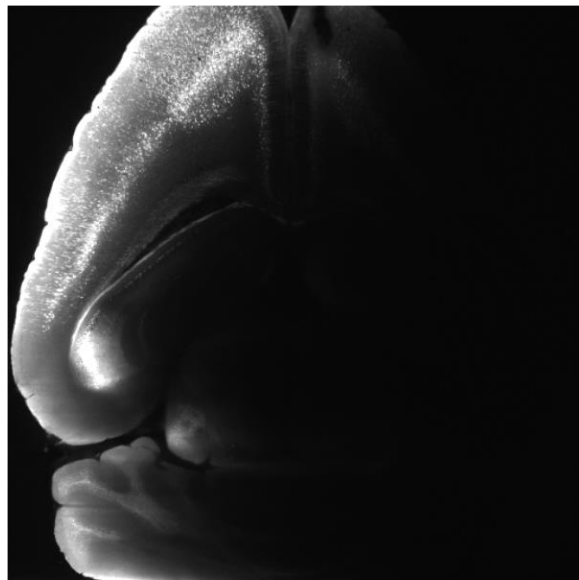
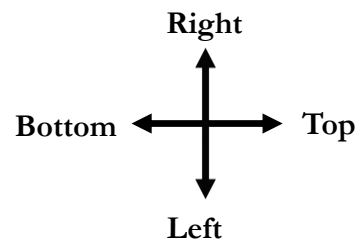
Overview of the detection system



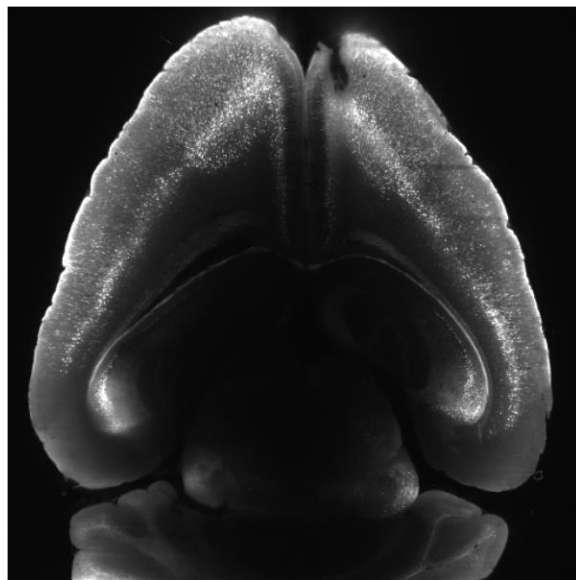
Part III: Learning to see with a light-sheet microscope

Light-sheet directions (@Zoom 1.25x)

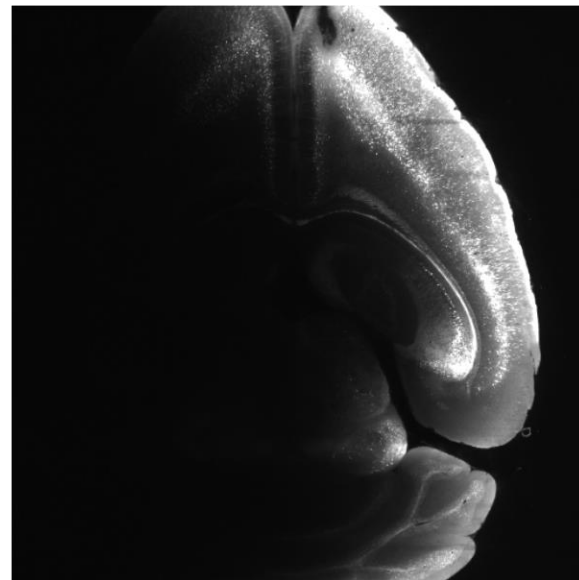
In order to allow the sweeping focus to be synchronized with the camera readout, the camera is rotated by 90°. In not-so-well-cleared samples, the penetration direction is clearly visible:



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 →

Z direction:
Acquisition movement
of a stack

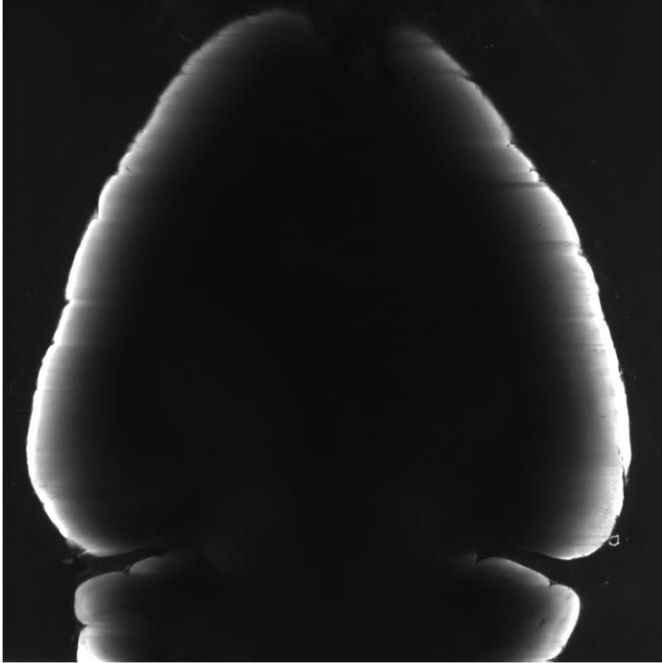
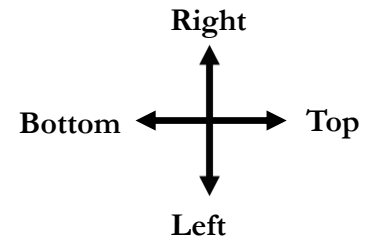
↓

← Lightsheet 2



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 strongly wavelength-dependent



405 nm + Quadrupleband



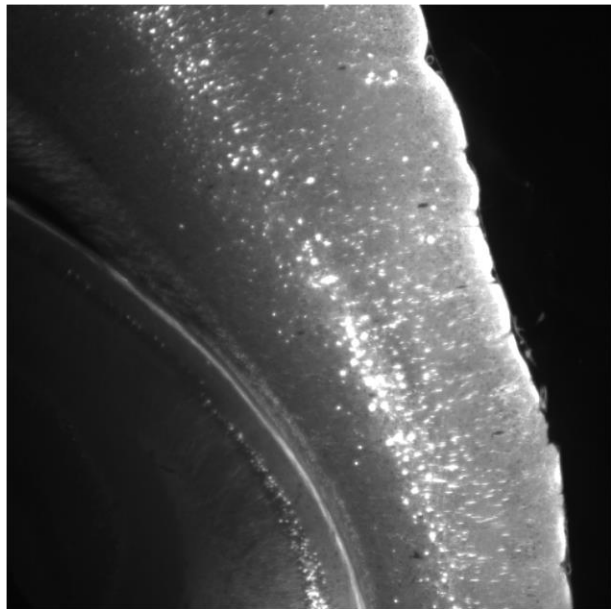
647 nm + Quadrupleband

What happens if the tunable lens parameters are incorrect?

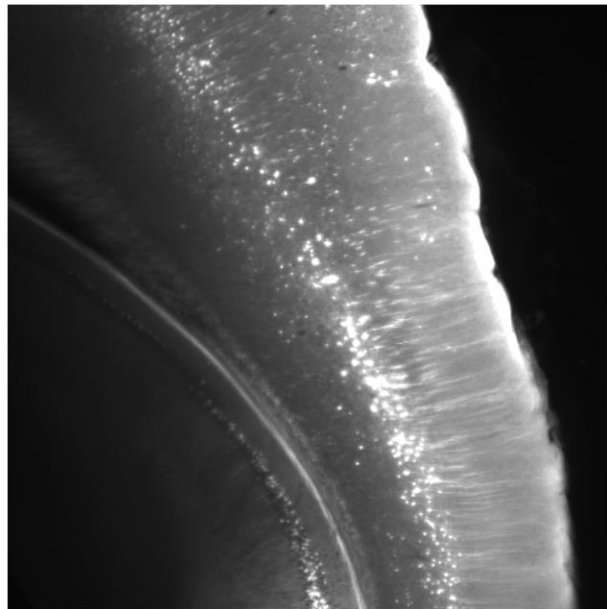


A thicker light-sheet leads to more features being visible at the expense of contrast

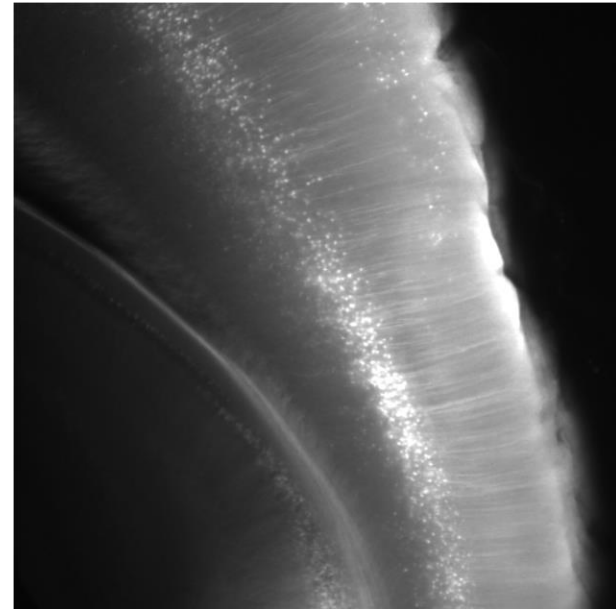
Thy1-YFP (H-line), 488 nm excitation, zoom 4



ETL offset = 2.47 V
ETL amplitude = 0.17 V



ETL offset = 2.47 V
ETL amplitude = 0.0 V



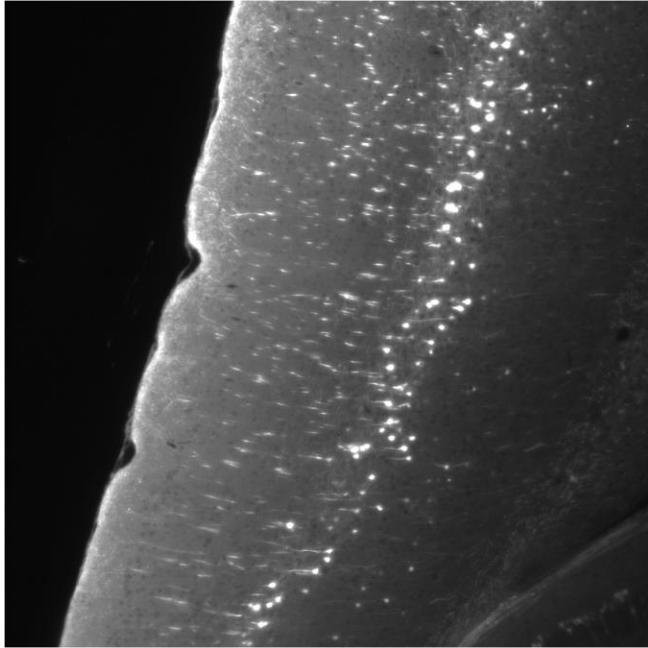
ETL offset = 2.2 V
ETL amplitude = 0.0 V



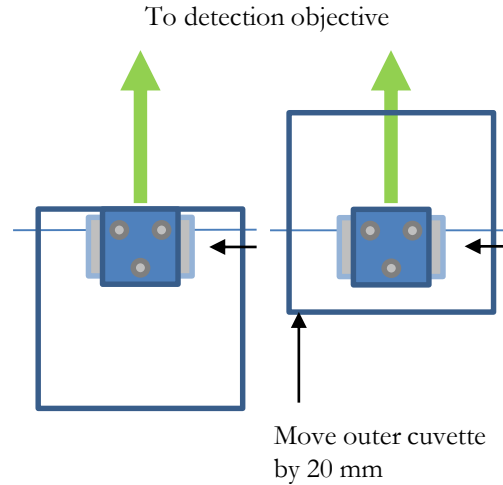
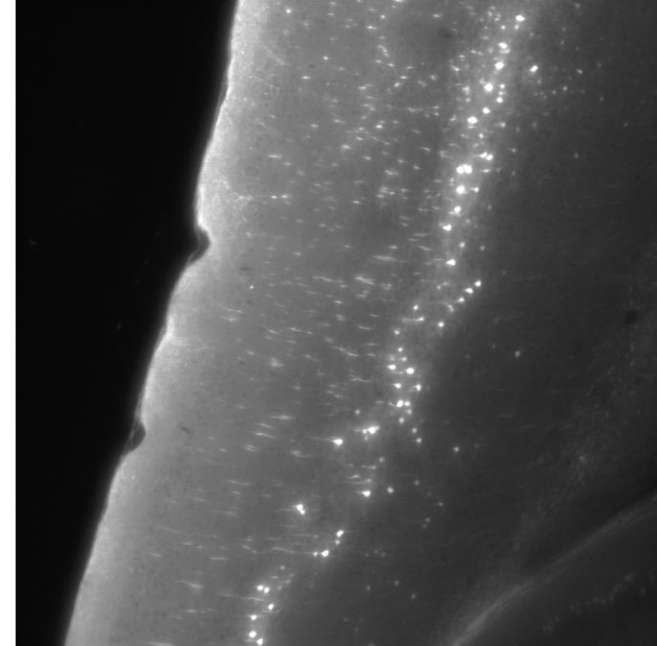
Counterstained cell-bodies and blood-vessel cross-sections are usually good structures to judge this

What happens if the path length is too 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

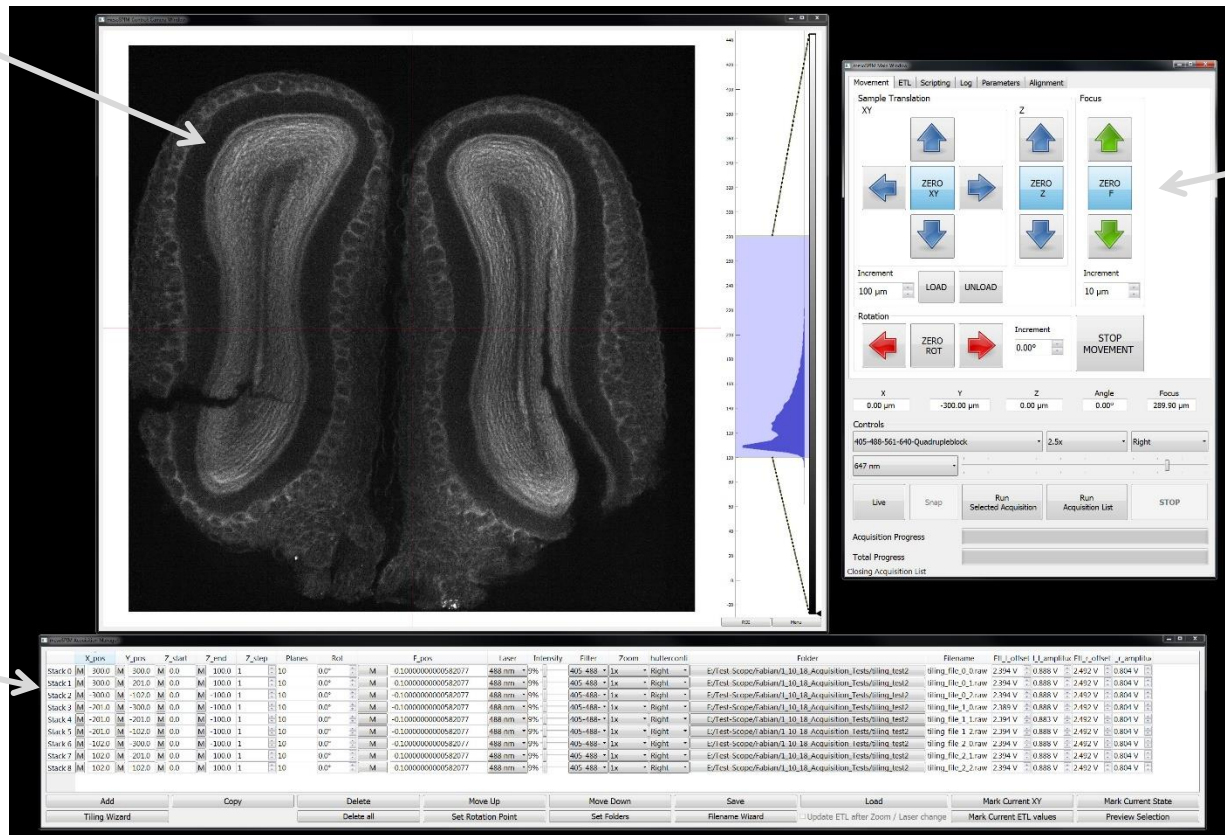
Part IV: User Interfaces

mesoSPIM-control: Overview

Camera Window

Main Window

Acquisition Manager



mesoSPIM-control: Main window: Movement tab

The screenshot shows the 'mesoSPIM Main Window' with the 'Movement' tab selected. The window is divided into several sections: 'Sample Translation' (XY and Z axes), 'Focus', 'Rotation', 'Coordinate readout', 'Controls', and 'Imaging mode buttons'. Annotations with arrows point to specific features:

- Movement arrows:** Points to the blue arrows in the XY and Z translation sections.
- Sample loading and unloading (Endpoints can be set In the config file):** Points to the 'LOAD' and 'UNLOAD' buttons.
- Increment selector for the movement:** Points to the 'Increment' field for the XY translation, set to '100 μm '.
- Coordinate readout:** Points to the 'X', 'Y', 'Z', 'Angle', and 'Focus' readout fields.
- Filter selector:** Points to the '405-488-561-640-Quadrupleblock' dropdown menu.
- Laser selector:** Points to the '647 nm' dropdown menu.
- Imaging mode buttons:** Points to the 'Live', 'Snap', 'Run Selected Acquisition', 'Run Acquisition List', and 'STOP' buttons.
- Zoom selector:** Points to the '4x' zoom level dropdown.
- Lightsheet direction:** Points to the 'Right' lightsheet direction dropdown.
- Progress bar for the current acquisition:** Points to the green progress bar showing '100% (Image 3100/3100)'.
- Progress bar for the list of acquisitions:** Points to the green progress bar showing '100% (Acquisition 3/3) (Image 9300/9300)'.

A warning icon (a red triangle with an exclamation mark) is located to the left of the 'Sample loading and unloading' annotation.

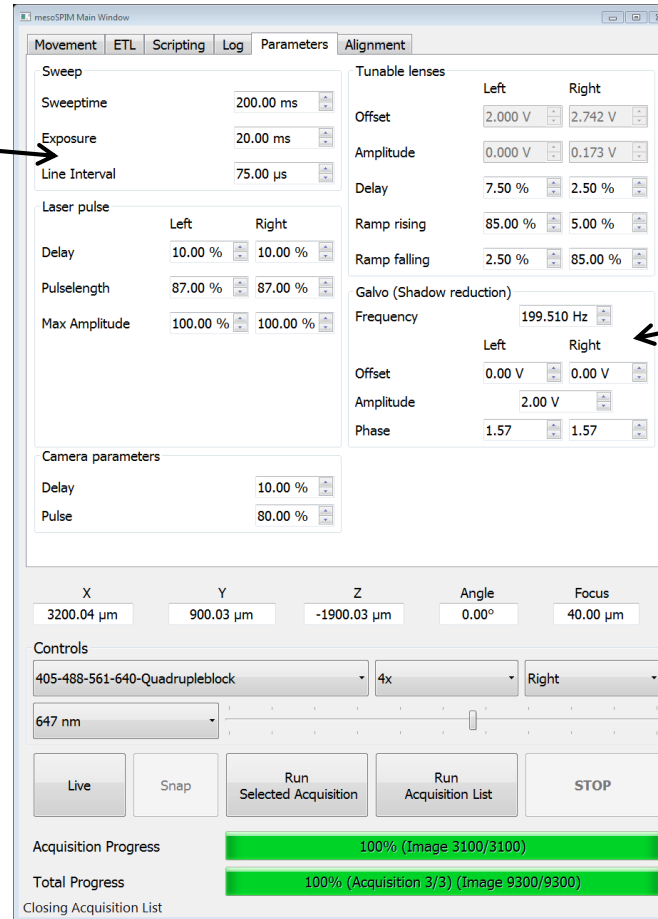
mesoSPIM-control: Main window: ETL tab

The screenshot shows the 'mesoSPIM Main Window' with the 'ETL' tab selected. The window is divided into several sections:

- Tunable lenses:** This section contains controls for 'Left' and 'Right' lenses. It includes 'Offset' (2.546 V and 2.742 V), 'Amplitude' (0.186 V and 0.173 V), and 'Increment' (0.001 V) fields. There are also buttons for 'Ampl=0' and 'AmplR=0'. Annotations point to these controls: 'ETL offset and amplitude' points to the offset fields; 'Buttons to set ETL Amp to 0 (saves last amplitude)' points to the 'Ampl=0' and 'AmplR=0' buttons; 'Increment selector for ETL parameters (especially for mouse wheel usage)' points to the 'Increment' field.
- Configuration:** Below the tunable lenses are buttons for 'Choose ETL config file' and 'Save current parameters to csv'. An annotation 'Buttons for choosing a ETL config file & saving parameters' points to these buttons. Below these is a 'Config File:' section showing the path 'E:/Code/mesoSPIM-control/mesoSPIM/config/etl_parameters/ETL-parameters - Fabian - DBE - H45.csv'. An annotation 'Config file indicator' points to this path.
- Positioning:** A row of five fields shows the current position: X (3200.04 μm), Y (900.03 μm), Z (-1900.03 μm), Angle (0.00°), and Focus (40.00 μm).
- Controls:** This section includes a dropdown menu for '405-488-561-640-Quadrupleblock', a dropdown for '4x', a dropdown for 'Right', and a dropdown for '647 nm'. Below these are buttons for 'Live', 'Snap', 'Run Selected Acquisition', 'Run Acquisition List', and 'STOP'.
- Progress:** At the bottom, there are two progress bars. The first is 'Acquisition Progress' at 100% (Image 3100/3100). The second is 'Total Progress' at 100% (Acquisition 3/3) (Image 9300/9300). Below these is a 'Closing Acquisition List' button.

mesoSPIM-control: Main window: Parameters tab

Camera exposure &
Line interval



The screenshot shows the 'Parameters' tab of the mesoSPIM Main Window. The interface is divided into several sections for configuring the microscope's operation.

- Sweep:** Contains fields for Sweeptime (200.00 ms), Exposure (20.00 ms), and Line Interval (75.00 μ s).
- Laser pulse:** A sub-section with two columns, Left and Right, for Delay, Pulselength, and Max Amplitude.
- Camera parameters:** Includes Delay (10.00 %) and Pulse (80.00 %).
- Tunable lenses:** A section for Left and Right lenses with parameters for Offset, Amplitude, Delay, Ramp rising, and Ramp falling.
- Galvo (Shadow reduction):** Includes Frequency (199.510 Hz) and parameters for Left and Right Offset, Amplitude, and Phase.

At the bottom of the window, there are position controls for X, Y, Z, Angle, and Focus, a Controls section with dropdown menus for acquisition type, magnification, and camera, and a series of buttons for Live, Snap, Run Selected Acquisition, Run Acquisition List, and STOP. Progress bars for Acquisition Progress and Total Progress are also displayed.

Galvo parameters

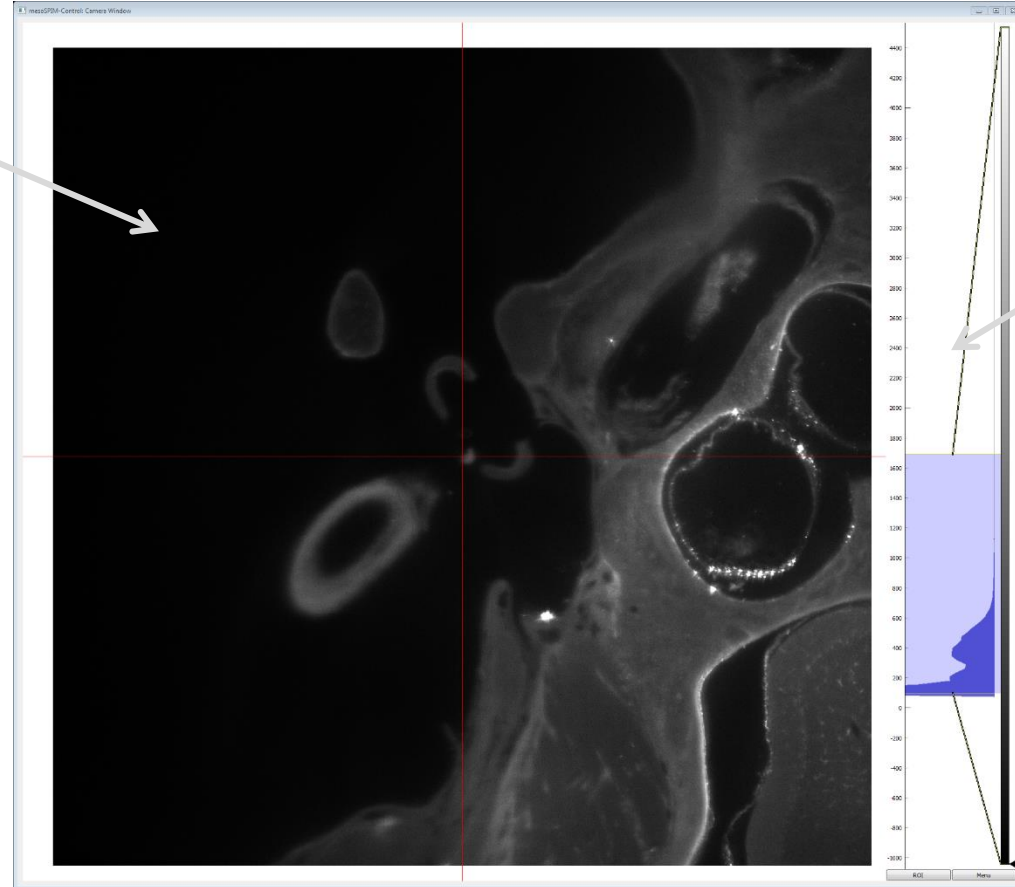


mesoSPIM-control: Camera window

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

mesoSPIM Acquisition Manager																										
	X_pos	Y_pos	Z_start	Z_end	Z_step	Planes	Rot	F_pos	Laser	Intensity	Filter	Zoom	Shutterconfig	Folder	Filename	Et1_offset	I_r_amplitud	Et1_r_offset	r_r_amplitud							
Stack 0	M	3200.04	M	900.03	M	1900.03	M	5000.07	1	3100	0.0°	M	40.0	488 nm	51%	508-520-35	4x	Right	E:/Test-Scope/Fabian/2_10_18_Paola_IDISCO_Sample_A/4x_Cochlea	488_nm_508-520-35_4x_Right_000000.raw	2.528 V	0.202 V	2.750 V	0.143 V		
Stack 1	M	3200.04	M	900.03	M	1900.03	M	5000.07	1	3100	0.0°	M	45.0	561 nm	54%	561LP	4x	Right	E:/Test-Scope/Fabian/2_10_18_Paola_IDISCO_Sample_A/4x_Cochlea	561_nm_561LP_4x_Right_000001.raw	2.517 V	0.157 V	2.737 V	0.120 V		
Stack 2	M	3200.04	M	900.03	M	1900.03	M	5000.07	1	3100	0.0°	M	45.0	647 nm	57%	405-488-561-6	4x	Right	E:/Test-Scope/Fabian/2_10_18_Paola_IDISCO_Sample_A/4x_Cochlea	647_nm_405-488-561-640-Quadrupleblock_4x_Right_000002.raw	2.546 V	0.186 V	2.742 V	0.173 V		
Add			Copy			Delete			Move Up			Move Down			Save			Load			Mark Current XY			Mark Current State		
Tiling Wizard						Delete all			Set Rotation Point			Set Folders			Filename Wizard			<input type="checkbox"/> Update ETL after Zoom / Laser change			Mark Current ETL values			Preview Selection		

mesoSPIM-control: Acquisition Manager

The screenshot shows the 'mesoSPIM Acquisition Manager' window. At the top is a table with columns: X_pos, Y_pos, Z_start, Z_end, Z_step, Planes, Rot, F_pos, Laser, Intensity, Filter, Zoom, Shutterconfig, Folder, Filename, and several ETL parameters. Below the table is a control bar with the following buttons and sections:

- Controls for table entries:** Add, Copy, Delete, Move Up, Move Down, Tiling Wizard, Delete all, Set Rotation Point, Set Folders.
- Save & load tables:** Save, Load, Filename Wizard.
- Update parts of a selected table entry & preview:** Mark Current XY, Mark Current ETL values, Mark Current State, Preview Selection.

Arrows point from descriptive text below to specific buttons: 'Tilting wizard' to 'Tiling Wizard', 'Set rotation point' to 'Set Rotation Point', 'Set folder for all rows' to 'Set Folders', 'Autogenerate filenames' to 'Filename Wizard', and 'Update parts of a selected table entry & preview' to the 'Mark Current XY' and 'Mark Current ETL values' buttons.

Tilting wizard
(not fully supported yet)

Set rotation point
(For an acquisition list) – set
a location where the sample
can rotate without damage
(not fully supported yet)

Set folder for all rows

Autogenerate filenames

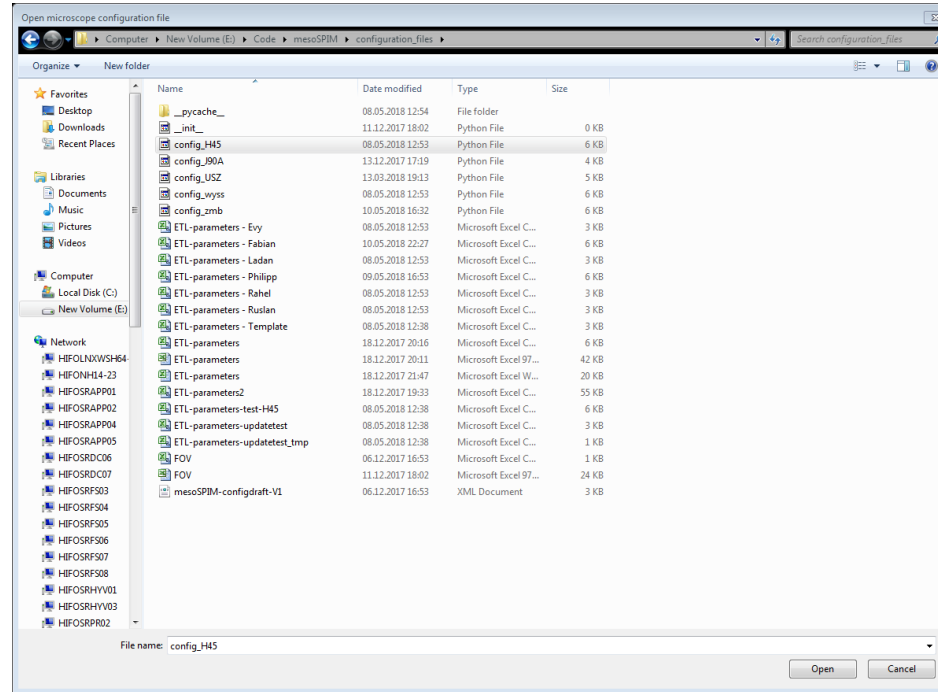
Update parts of a selected
table entry & preview

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»)

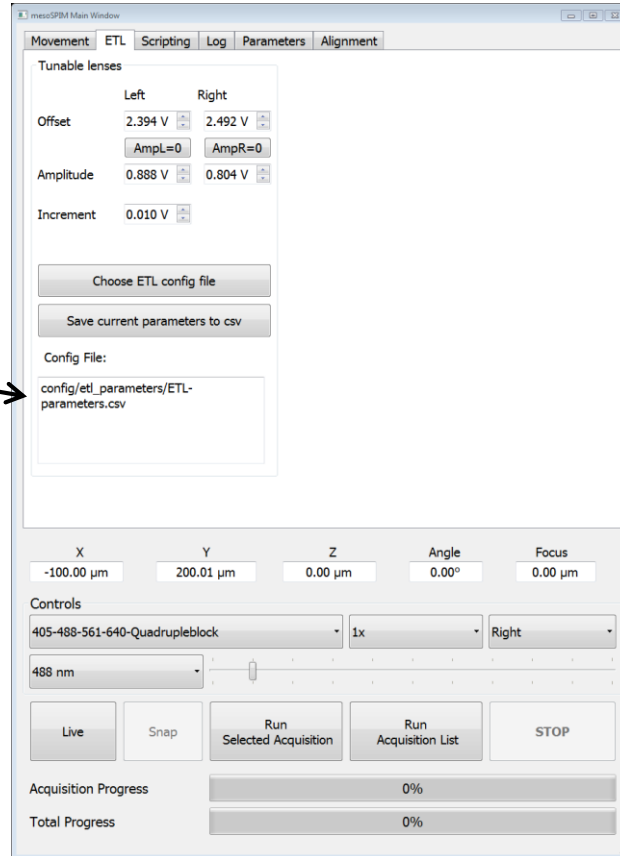
mesoSPIM-control: Startup I



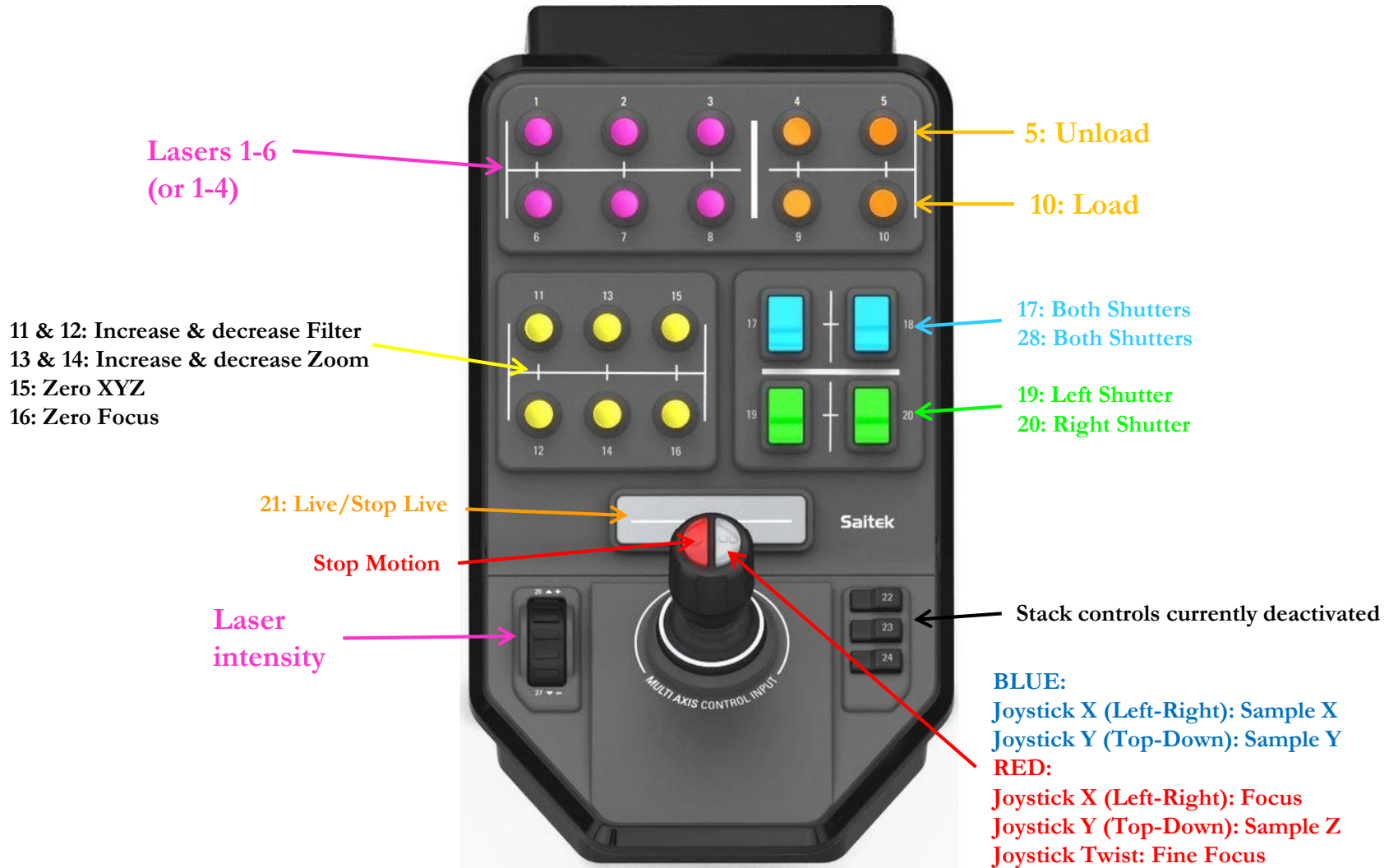
1. Choose config file according to your setup. This is a .py file containing the microscope configuration, NOT a ETL-parameter file.
2. The software takes 10-15s to start while performing a reference move with the focusing stage

mesoSPIM-control: Startup II

3. Select your ETL parameter file



Part VI: Joystick



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 HCIImage if the crosshair is switched on). If 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



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

Mark buttons («M»)

The screenshot shows the 'mesoSPIM Acquisition Manager' window. It contains a table with columns: X_pos, Y_pos, Z_start, Z_end, Z_step, Planes, Rot, F_pos, Laser, Intensity, Filter, Zoom, shutterconfi, Folder, Filename, Etl_l_offset, _l_amplitu, Etl_r_offset, _r_amplitu. Two rows are visible: 'Stack 0' and 'Stack 1'. Arrows point to the 'M' (Mark) buttons in the X_pos and Y_pos columns for 'Stack 0'. Another arrow points to the 'Rot' column, which shows '0.0°' for 'Stack 0' and '90.0°' for 'Stack 1'. A third arrow points to the 'Filename' column, which shows 'one.raw' for both stacks. Below the table, there are several buttons: 'Add', 'Copy', 'Delete', 'Move Up', 'Move Down', 'Save', 'Load', 'Mark Current XY', 'Mark Current State', 'Tiling Wizard', 'Delete all', 'Set Rotation Point', 'Set Folders', 'Filename Wizard', and 'Update ETL after Zoom / Laser change'. A warning icon and text are overlaid on the table area, stating 'Acquisition lists with varying rotations («multiview») are currently disabled.' Another text box states 'Data is saved as raw image streams, the «.raw» file extension should be used'.

	X_pos	Y_pos	Z_start	Z_end	Z_step	Planes	Rot	F_pos	Laser	Intensity	Filter	Zoom	shutterconfi	Folder	Filename	Etl_l_offset	_l_amplitu	Etl_r_offset	_r_amplitu
Stack 0	M 0	M 0	M 0	M 100	10	10	0.0°	M 0	488 nm	0%	515LP	1x	Left	E:/tmp	one.raw	0.000 V	0.000 V	0.000 V	0.000 V
Stack 1	M 0	M 0	M 0	M 100	10	10	90.0°	M 0	488 nm	0%	515LP	1x	Left	E:/tmp	one.raw	0.000 V	0.000 V	0.000 V	0.000 V

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

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

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 Acquisition List» allows to run the entire table

Mark X + Y position
For a selected row

Acquisition checklist

mesoSPM Acquisition Manager																										
		X_pos	Y_pos	Z_start	Z_end	Z_step	Planes	Rot	F_pos	Laser	Intensity	Filter	Zoom	Shutterconfig	Folder	Filename	ETL_l_offset	l_l_amplitud	ETL_r_offset	r_r_amplitud						
Stack 0	M	3200.04	M	900.03	M	1900.03	M	5000.07	1	3100	0.0°	M	40.0	488 nm	51%	508-520-35	4x	Right	E:/Test-Scope/Fabian/2_10_18_Paola_IDISCO_Sample_A/4x_Cochlea	488_nm_508-520-35_4x_Right_000000.raw	2.528 V	0.202 V	2.750 V	0.143 V		
Stack 1	M	3200.04	M	900.03	M	1900.03	M	5000.07	1	3100	0.0°	M	45.0	561 nm	54%	561LP	4x	Right	E:/Test-Scope/Fabian/2_10_18_Paola_IDISCO_Sample_A/4x_Cochlea	561_nm_561LP_4x_Right_000001.raw	2.517 V	0.157 V	2.737 V	0.120 V		
Stack 2	M	3200.04	M	900.03	M	1900.03	M	5000.07	1	3100	0.0°	M	45.0	647 nm	57%	405-488-561-6	4x	Right	E:/Test-Scope/Fabian/2_10_18_Paola_IDISCO_Sample_A/4x_Cochlea	647_nm_405-488-561-640-Quadrupleblock_4x_Right_000002.raw	2.546 V	0.186 V	2.742 V	0.173 V		
Add			Copy			Delete			Move Up			Move Down			Save			Load			Mark Current XY			Mark Current State		
Tiling Wizard						Delete all			Set Rotation Point			Set Folders			Filename Wizard			<input type="checkbox"/> Update ETL after Zoom / Laser change			Mark Current ETL values			Preview Selection		

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

```
488_nm_508_520-35_1_25x_Left_000000.raw_meta - Notepad
File Edit Format View Help
[Metadata for file] E:/Test-Scope/Fabian/2_10_18_Pao1a_IDISCO_Sample_A/1_25x_overview_scan/488_nm_508_520-35_1_25x_Left_000000.raw
[z_stepsize] 2
[z_planes] 3600

[CFG]
[Laser] 488 nm
[Intensity (%)] 81
[Zoom] 1.25x
[Pixelsize in um] 5.26
[Filter] 508 520-35
[Shutter] Left

[POSITION]
[x_pos] 0.0
[y_pos] 0.0
[f_pos] 0.0
[z_start] 0.0
[z_end] -7200.1
[z_stepsize] 2
[z_planes] 3600

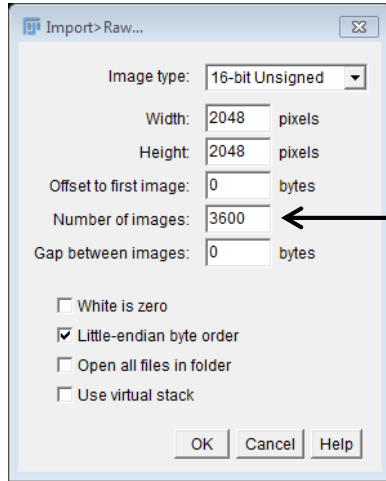
[ETL PARAMETERS]
[ETL CFG File] E:/Code/mesoSPIM-control/mesoSPIM/config/etl_parameters/ETL-parameters - Fabian - DBE - H45.csv
[etl_l_offset] 2.5479999999999983
[etl_l_amplitude] 0.5819999999999999
[etl_r_offset] 2.732
[etl_r_amplitude] 0.4099999999999999

[GALVO PARAMETERS]
[galvo_l_frequency] 99.9
[galvo_l_amplitude] 6.0
[galvo_l_offset] 0.0
[galvo_r_amplitude] 6
[galvo_r_offset] 0.0

[CAMERA PARAMETERS]
[camera_exposure] 0.02
[camera_line_interval] 7.5e-05
```

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

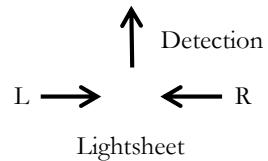


Number of images can
be larger than the number of acquired images,
the import will work normally

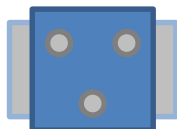
3. The stack will open in the center

Controlling sample rotation

Rotations of a 20 mm cuvette:

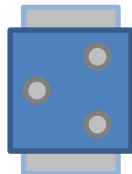


0/360°

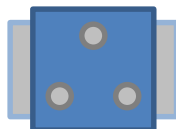


Position mark

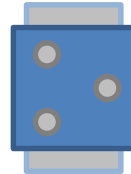
90°



180°



270°



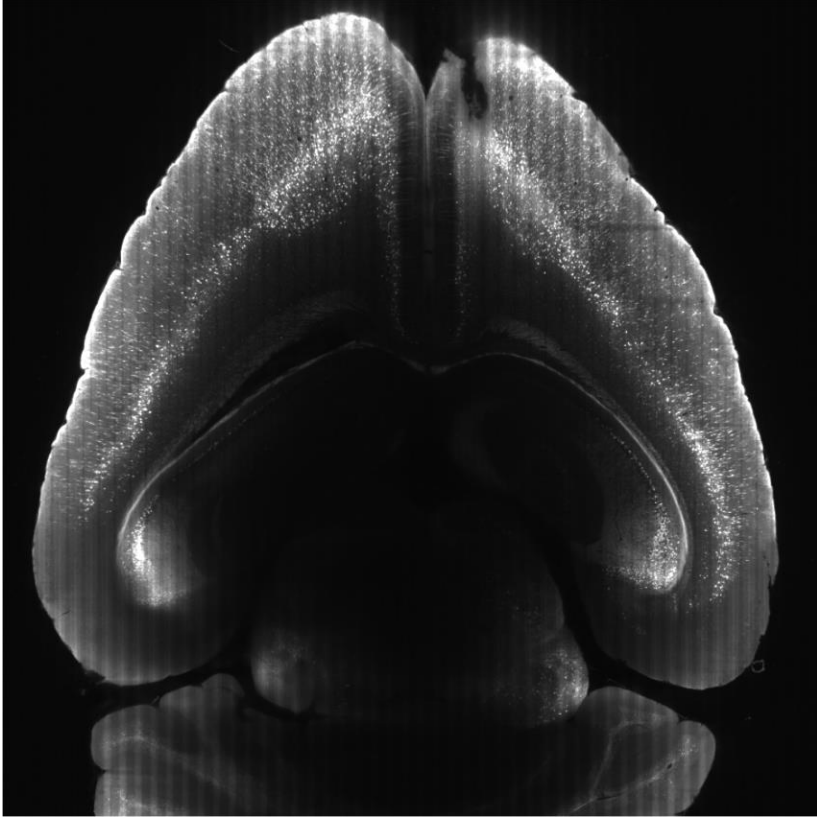
Viewed from the top,
positive rotations are oriented
clock-wise



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

Part VI: Troubleshooting

Horizontal Stripes in the image

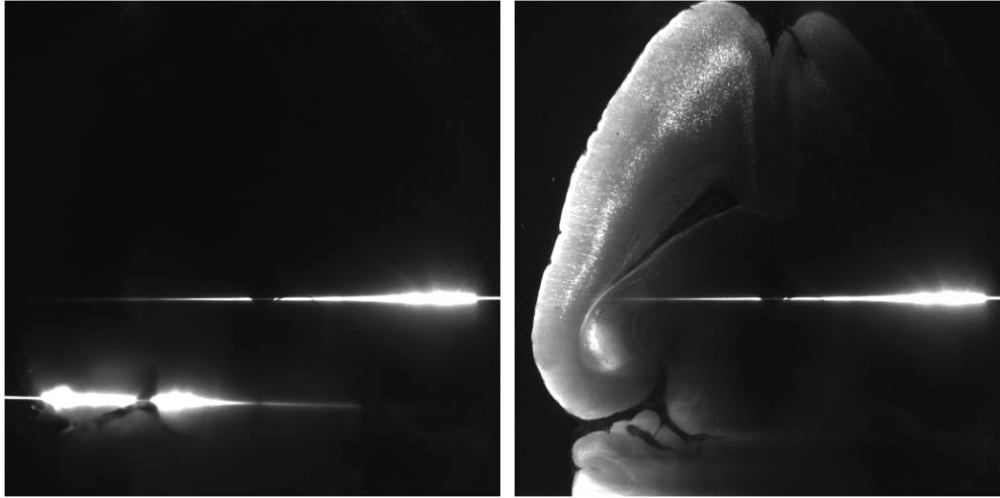


Stripes indicate that the Galvo Frequency is not averaged out by the exposure.

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:

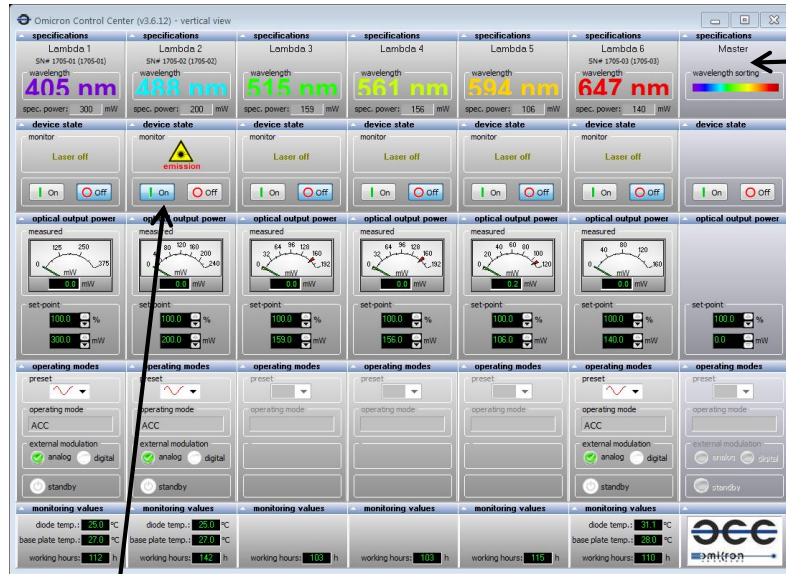
- Galvos are switched off
- Galvos switched off due to overheating (usually at amplitudes >3.7 V)



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

messSPM-Control
File Configuration

Sweep

Sweeptime (ms)

Exposure (ms)

Line Interval (μs)

Laser pulse

	Left	Right
Delay (%)	<input type="text" value="10.00"/>	<input type="text" value="10.00"/>
Pulselength (%)	<input type="text" value="50.00"/>	<input type="text" value="50.00"/>
Max Amplitude (%)	<input type="text" value="80.00"/>	<input type="text" value="80.00"/>

☐ Laser interleaving

Tunable lenses

	Left	Right
Offset	<input type="text" value="2.52"/>	<input type="text" value="2.56"/>
Amplitude	<input type="text" value="0.18"/>	<input type="text" value="0.17"/>
Delay (%)	<input type="text" value="10.00"/>	<input type="text" value="10.00"/>
Ramp rising (%)	<input type="text" value="10.00"/>	<input type="text" value="10.00"/>
Ramp falling (%)	<input type="text" value="10.00"/>	<input type="text" value="10.00"/>

Galvo (Shadow reduction)

Frequency

	Left	Right
Offset	<input type="text" value="0.00"/>	<input type="text" value="0.00"/>
Amplitude	<input type="text" value="3.90"/>	
Phase	<input type="text" value="90.00"/>	<input type="text" value="90.00"/>

Choose ETL config file

Save current parameters to csv

Camera parameters

Delay (%)

Pulse (%)

X [μm] Y [μm] Z [μm] Angle [°] Foc [μm]

405-780-561-640-Quadrupleblock 1x Left

488 nm

Live Snap Run Stack Run Acquisition STOP

0%