

# Zurich mesoSPIM documentation

## V3.01

Software version: commit: 407344

3.10.18

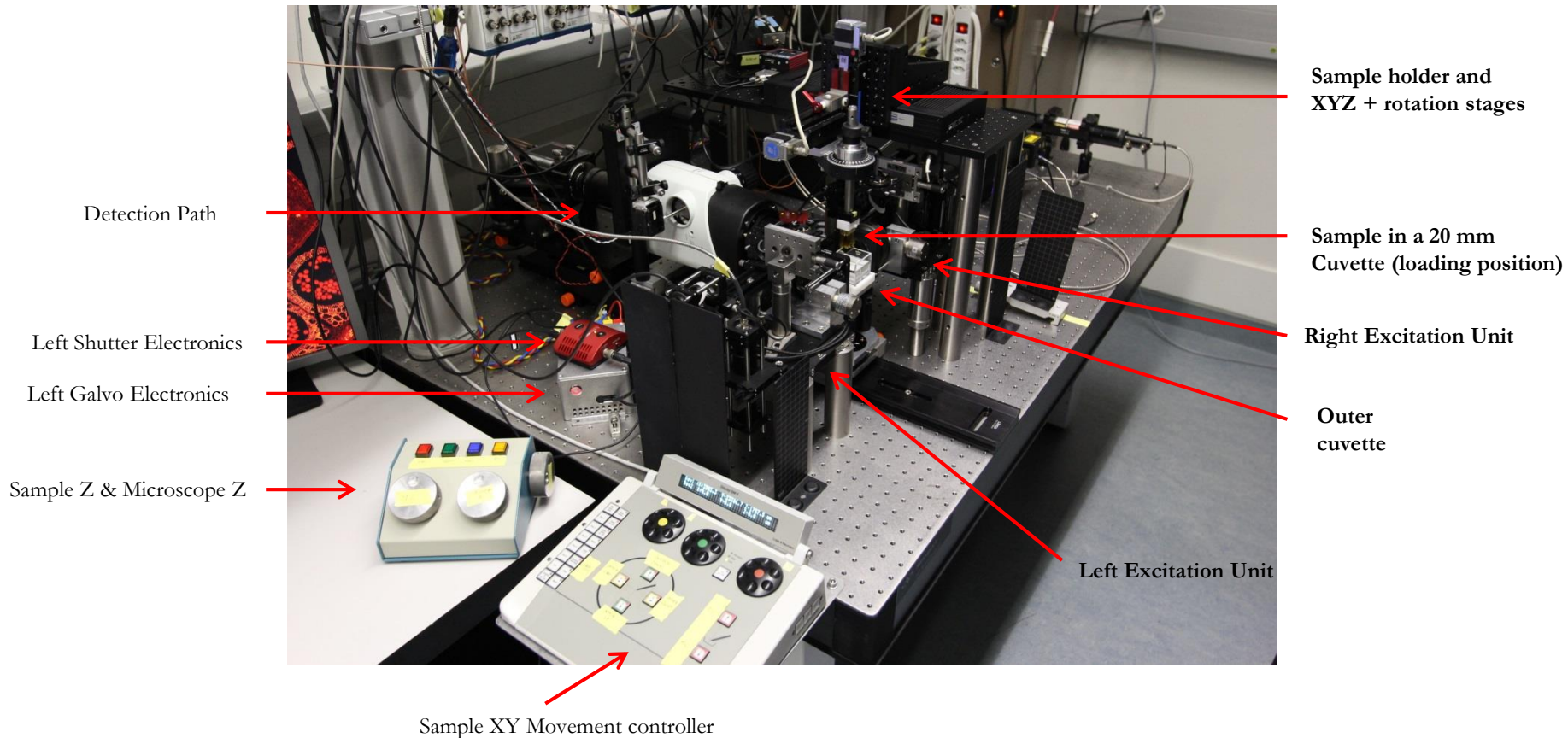
# Part I: Specifications

# FOV calibration

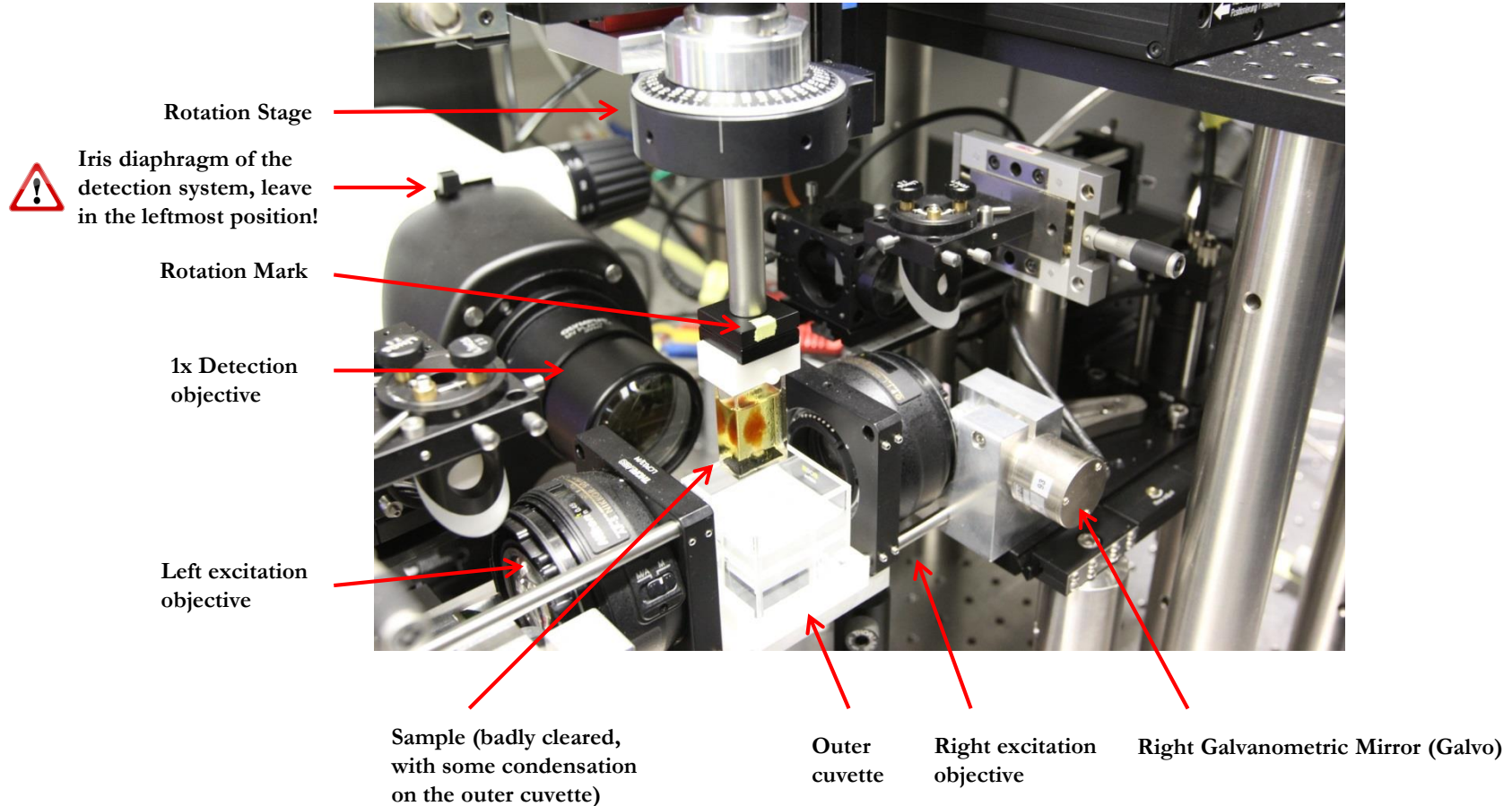
Objective	Zoom	FOV size (mm)	Pixel size (mm)
1x	0.63	21.56	0.0105263
1x	0.8	16.86	0.008234
<b>1x</b>	<b>1</b>	<b>13.42</b>	<b>0.006552</b>
<b>1x</b>	<b>1.25</b>	<b>10.79</b>	<b>0.0052666</b>
1x	1.6	8.36	0.0040844
1x	2	6.69	0.0032688
1x	2.5	5.34	0.0026059
1x	3.2	4.16	0.0020332
<b>1x</b>	<b>4</b>	<b>3.29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

## Part II: Hardware overview

# Microscope overview

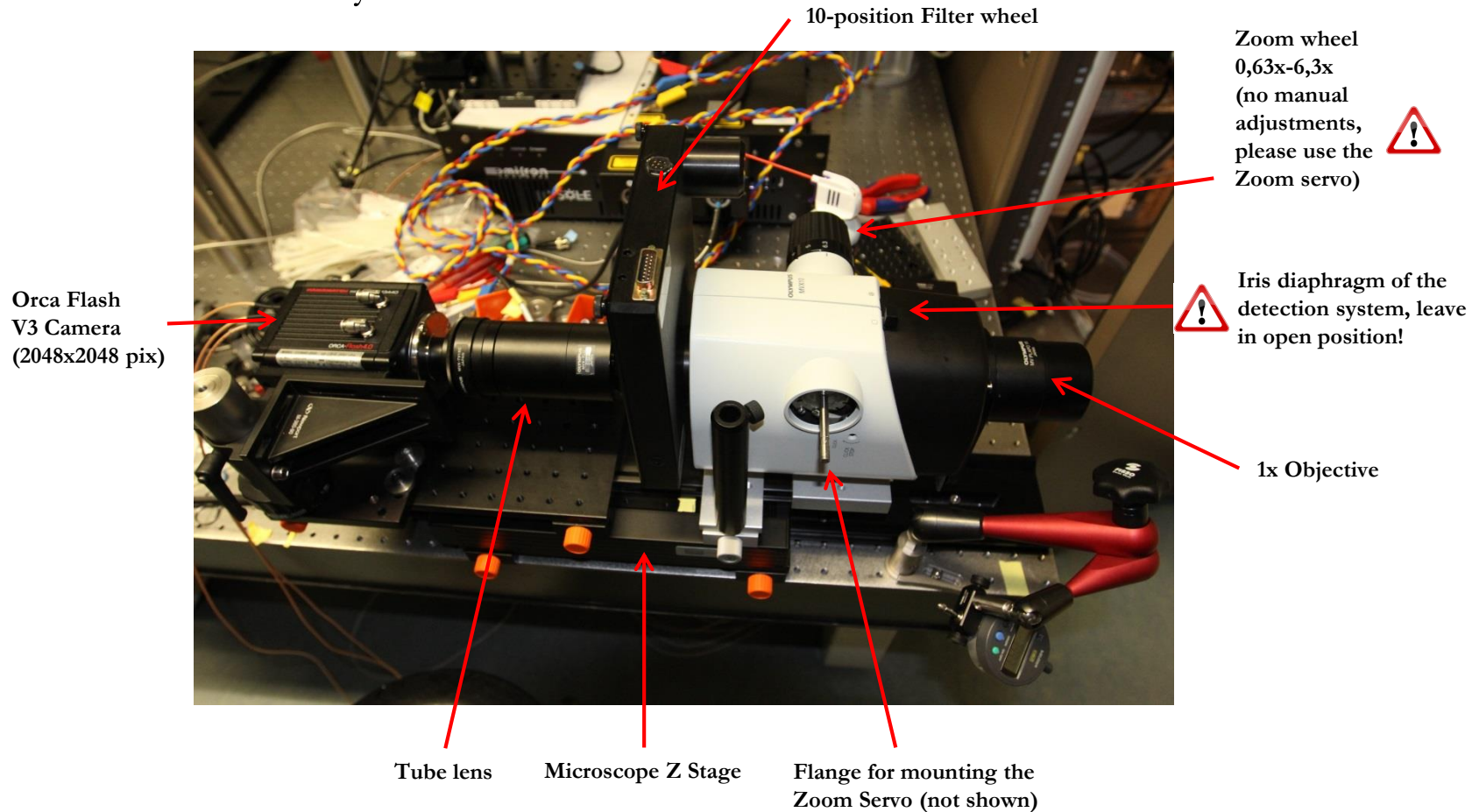


# Overview of the sample holder





# Overview of the detection system

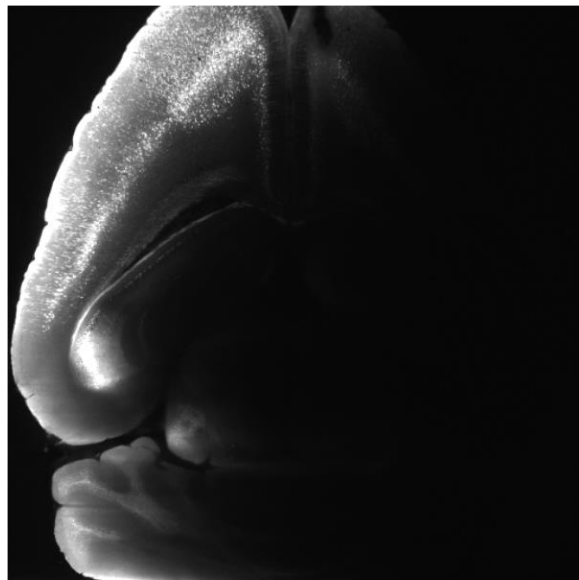
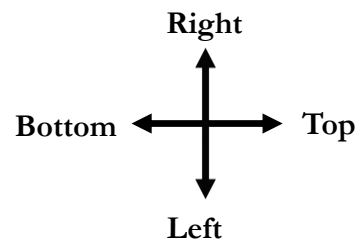


## Part III: Learning to see with a light-sheet microscope

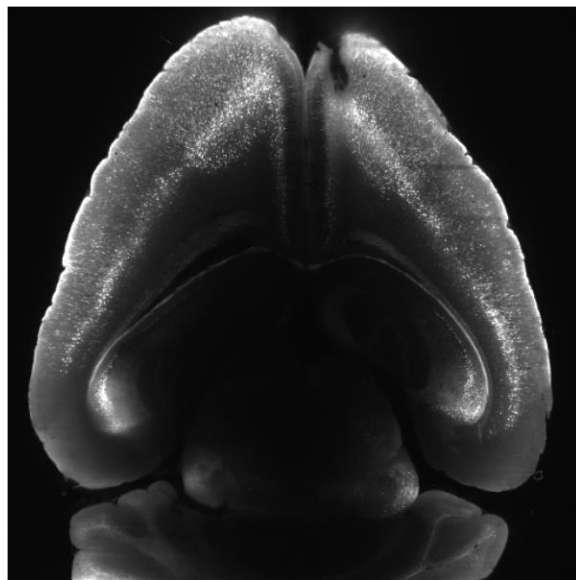


# Light-sheet directions (@Zoom 1.25x)

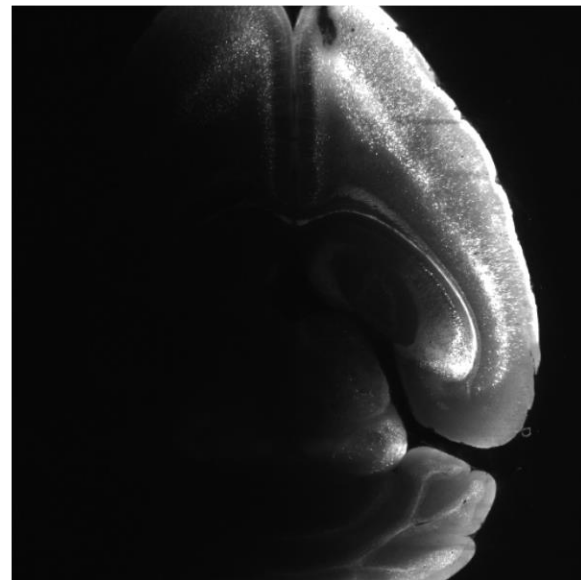
In order to allow the sweeping focus to be synchronized with the camera readout, the camera is rotated by 90°. In not-so-well-cleared samples, the penetration direction is clearly visible:



**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

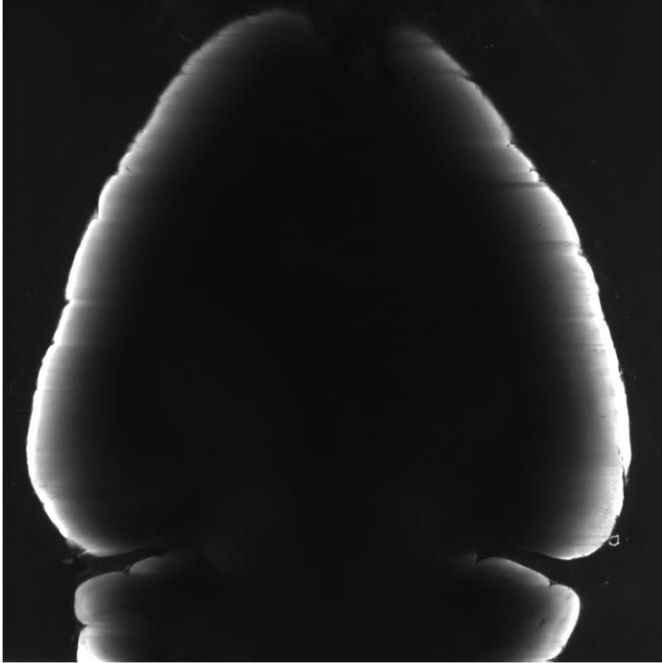
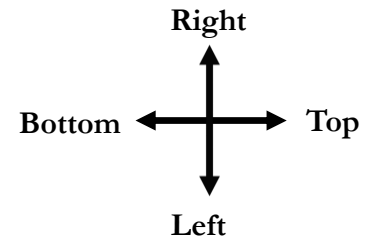
↓

← 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



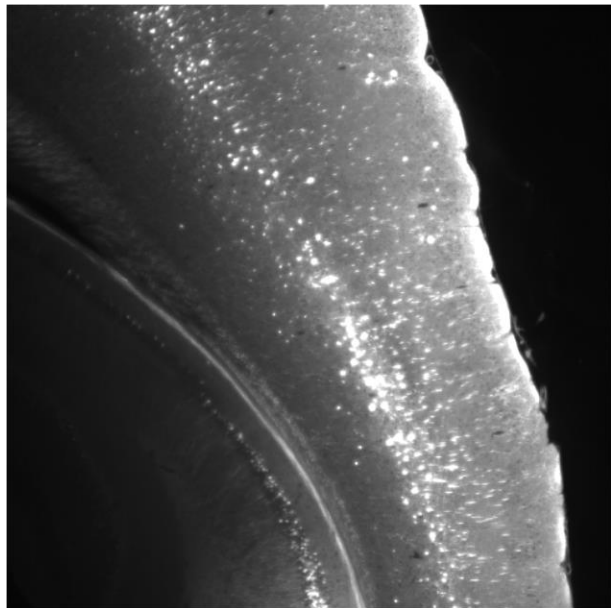
647 nm + Quadrupleband

# What happens if the tunable lens parameters are incorrect?

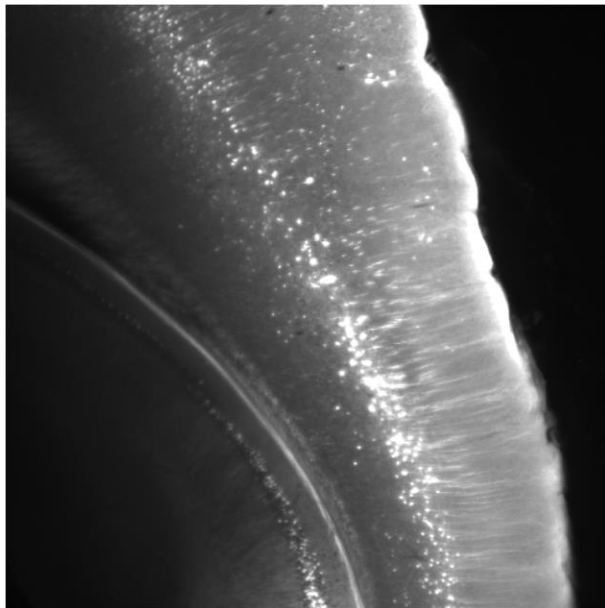


A thicker light-sheet leads to more features being visible at the expense of contrast

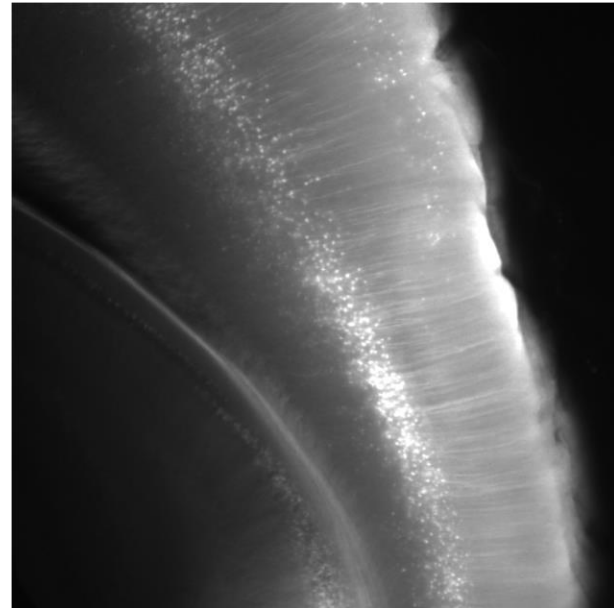
Thy1-YFP (H-line), 488 nm excitation, zoom 4



ETL offset = 2.47 V  
ETL amplitude = 0.17 V



ETL offset = 2.47 V  
ETL amplitude = 0.0 V



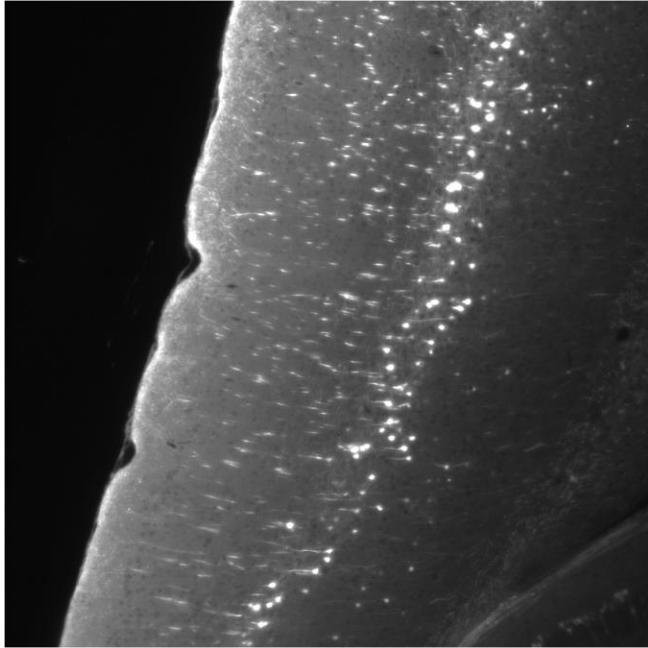
ETL offset = 2.2 V  
ETL amplitude = 0.0 V



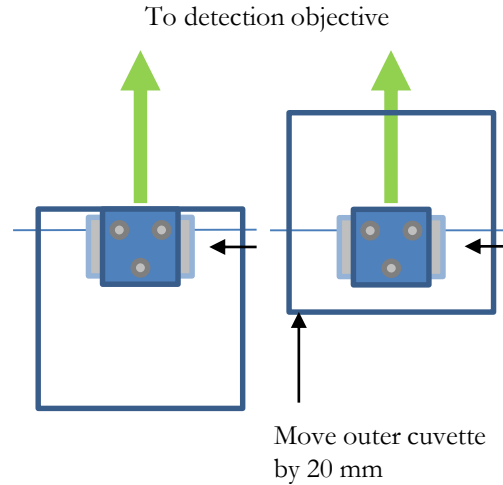
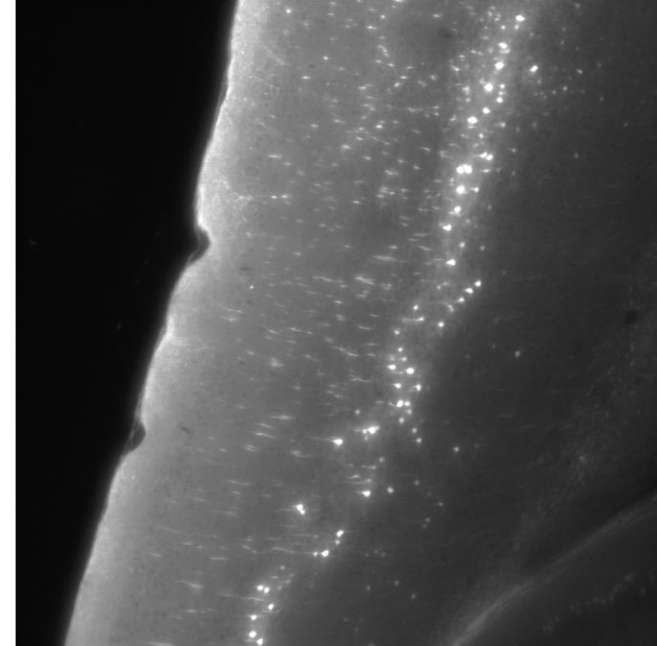
Counterstained cell-bodies and blood-vessel cross-sections are usually good structures to judge this

# 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



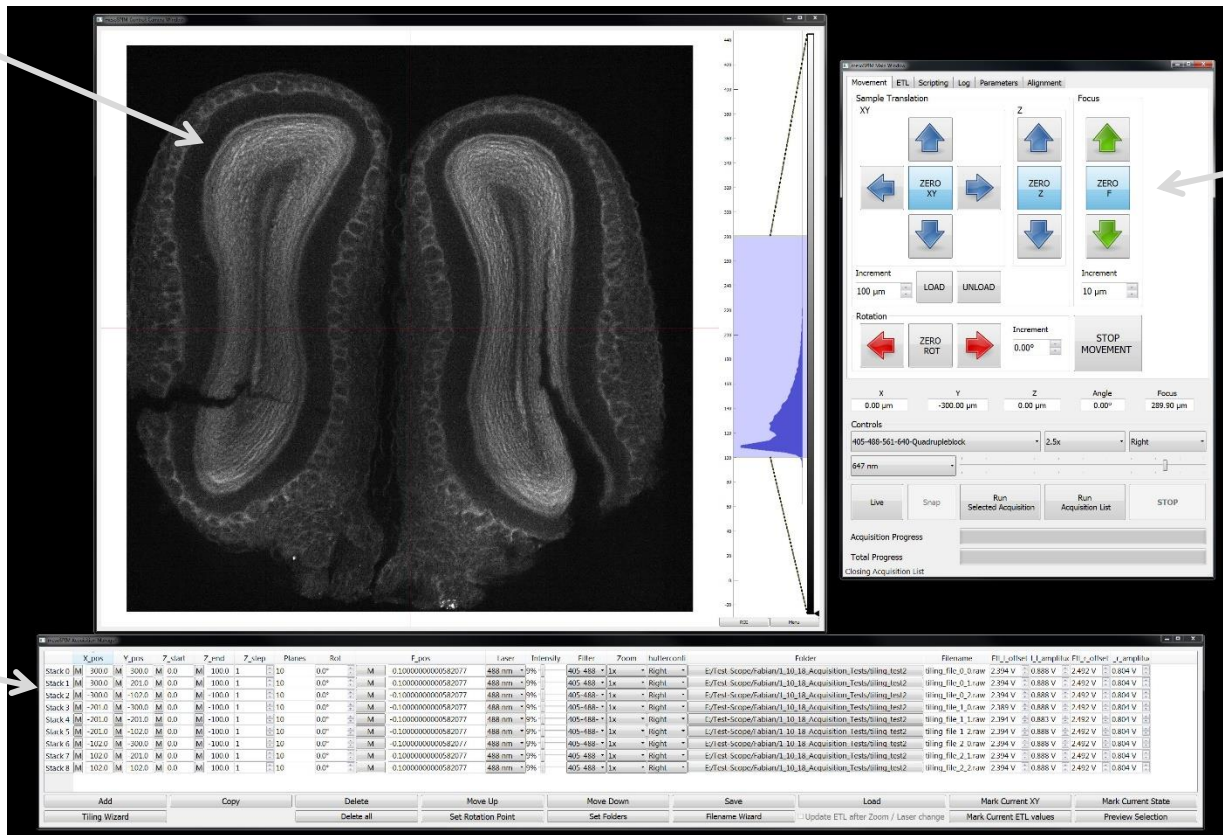
Select a position of the outer cuvette that minimizes spherical aberration while offering enough space for sample movement during stacks & rotations

## Part IV: User Interfaces

## Camera Window

## Main Window

## Acquisition Manager





# mesoSPIM-control: Main window: Movement tab

mesoSPIM Main Window

Movement ETL Scripting Log Parameters Alignment

Sample Translation

XY Z Focus

Movement arrows

Sample loading and unloading (Endpoints can be set In the config file)

Increment selector for the movement

Coordinate readout

Filter selector

Laser selector

Imaging mode buttons

Zoom selector

Lightsheet direction

Progress bar for the current acquisition

Progress bar for the list of acquisitions

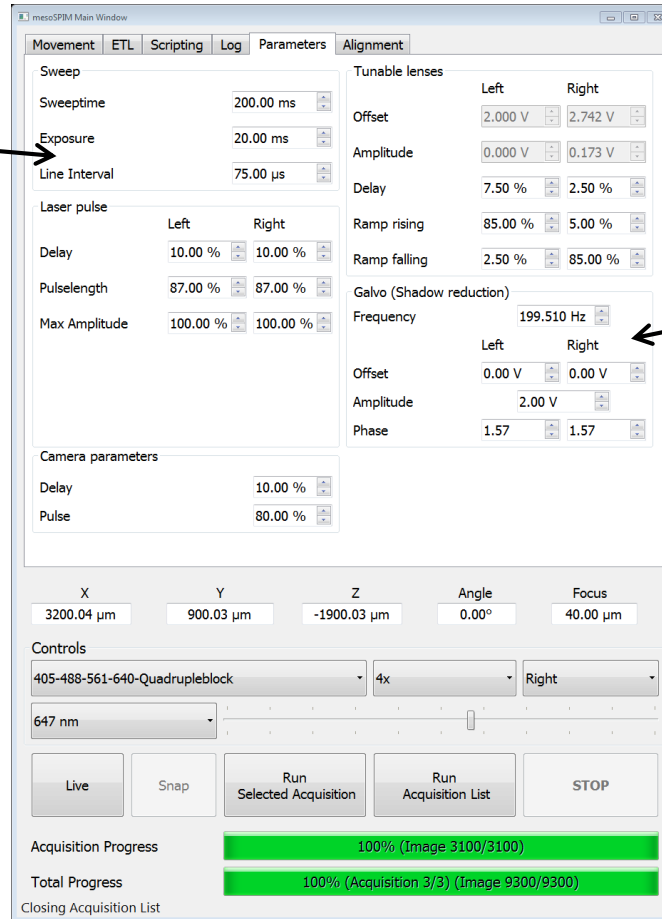
# mesoSPIM-control: Main window: ETL tab

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 'AmplL=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 'AmplL=0' and 'AmplR=0' buttons, and 'Increment selector for ETL parameters (especially for mouse wheel usage)' points to the 'Increment' field.
- Configuration:** Below the tunable lenses, there are buttons for 'Choose ETL config file' and 'Save current parameters to csv'. A 'Config File:' section shows the current file path: 'E:/Code/mesoSPIM-control/mesoSPIM/config/etl\_parameters/ETL-parameters - Fabian - DBE - H45.csv'. Annotations point to these: 'Buttons for choosing a ETL config file & saving parameters' points to the two buttons, and 'Config file indicator' points to the file path text.
- 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 the acquisition block (405-488-561-640-Quadrupleblock), a dropdown for magnification (4x), and a dropdown for the camera (Right). There is also a slider for the wavelength (647 nm).
- Buttons:** At the bottom, there are buttons for 'Live', 'Snap', 'Run Selected Acquisition', 'Run Acquisition List', and 'STOP'.
- Progress:** Two green progress bars are shown: 'Acquisition Progress' at 100% (Image 3100/3100) and 'Total Progress' at 100% (Acquisition 3/3) (Image 9300/9300).

# 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  $\mu$ s).
- Laser pulse:** A sub-section with two columns, Left and Right, for Delay (10.00 %), Pulselength (87.00 %), and Max Amplitude (100.00 %).
- Camera parameters:** Includes Delay (10.00 %) and Pulse (80.00 %).
- Tunable lenses:** A section for Left and Right lenses with fields for Offset, Amplitude, Delay, Ramp rising, and Ramp falling.
- Galvo (Shadow reduction):** Includes a Frequency field (199.510 Hz) and sub-fields for Left and Right Offset, Amplitude, and Phase.

At the bottom of the window, there are position controls for X (3200.04  $\mu$ m), Y (900.03  $\mu$ m), Z (-1900.03  $\mu$ m), Angle (0.00°), and Focus (40.00  $\mu$ m). Below these are controls for the acquisition, including a dropdown for the filter (405-488-561-640-Quadrupleblock), a dropdown for the objective (4x), and a dropdown for the camera (Right). A wavelength selector is set to 647 nm. At the bottom are buttons for Live, Snap, Run Selected Acquisition, Run Acquisition List, and STOP. Progress bars show 100% completion for both the acquisition (3100/3100 images) and the total run (9300/9300 images).

Galvo parameters

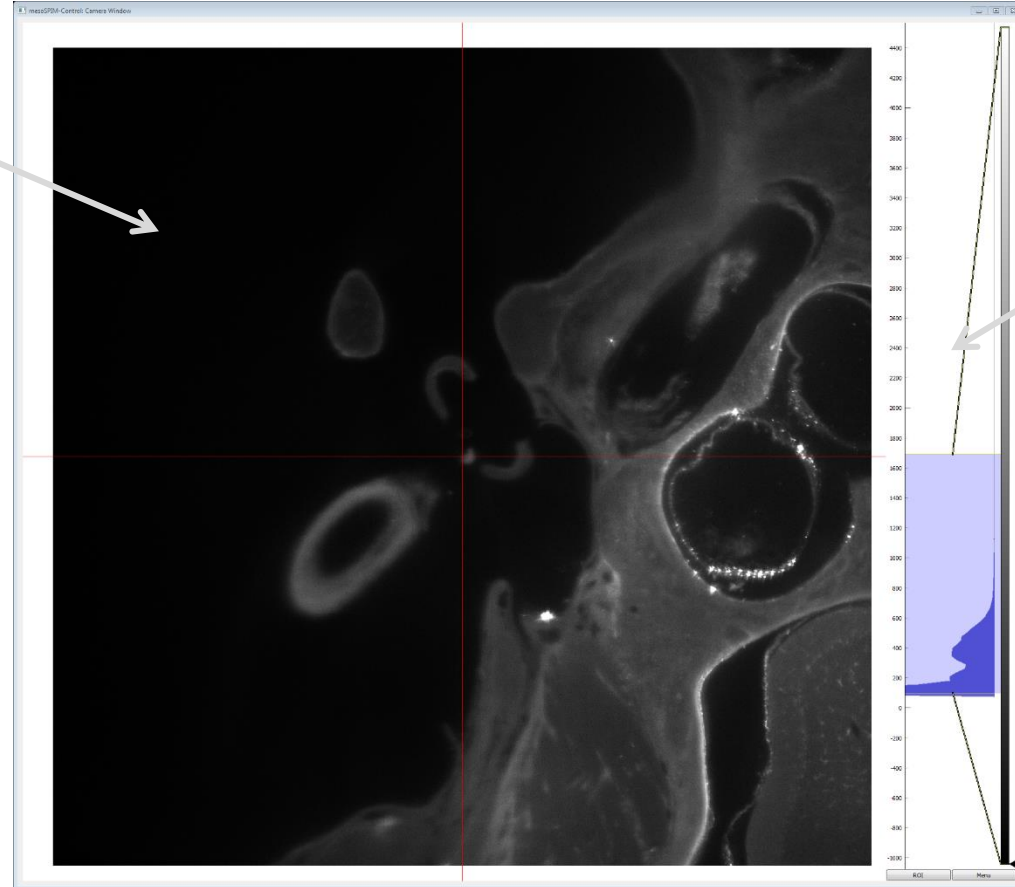


# mesoSPIM-control: Camera window

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 ETL\_offset. Below the table is a control bar with several buttons and options. The 'Save' and 'Load' buttons are highlighted with a red box and labeled 'Save & load tables'. The 'Mark Current XY', 'Mark Current ETL values', 'Mark Current State', and 'Preview Selection' buttons are highlighted with a green box and labeled 'Update parts of a selected table entry & preview'. Other buttons include 'Add', 'Copy', 'Delete', 'Delete all', 'Move Up', 'Move Down', 'Set Rotation Point', 'Set Folders', 'Filename Wizard', and 'Update ETL after Zoom / Laser change'.

	X_pos	Y_pos	Z_start	Z_end	Z_step	Planes	Rot	F_pos	Laser	Intensity	Filter	Zoom	Shutterconfig	Folder	Filename	ETL_offset	I_offset	I_amplitude	ETL_r_offset	r_amplitude				
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

**Controls for table entries**

**Save & load tables**

**Update parts of a selected table entry & preview**

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

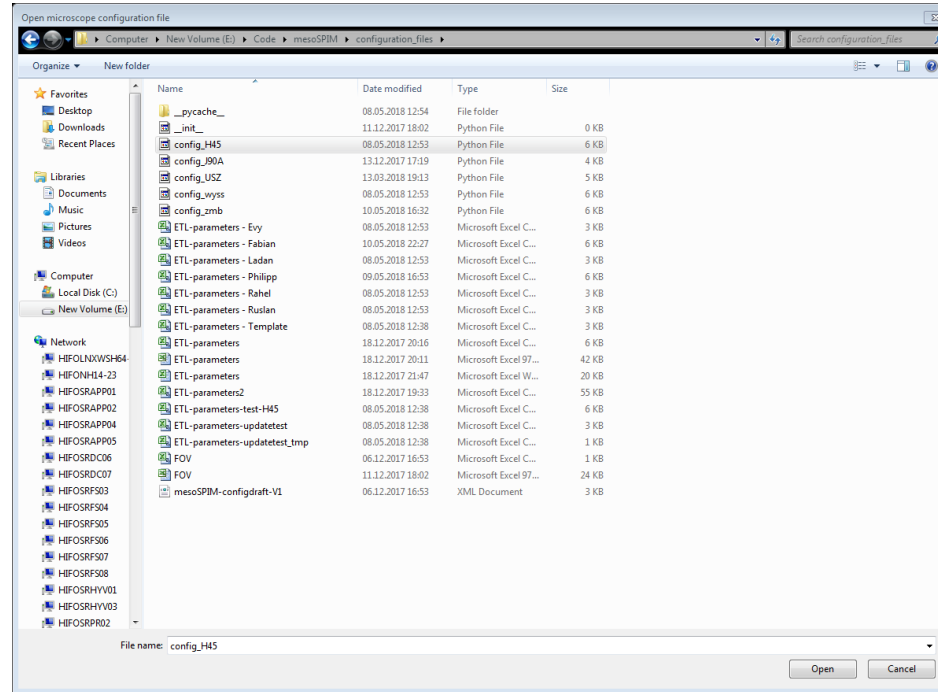
## Part V: Startup



# 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»)

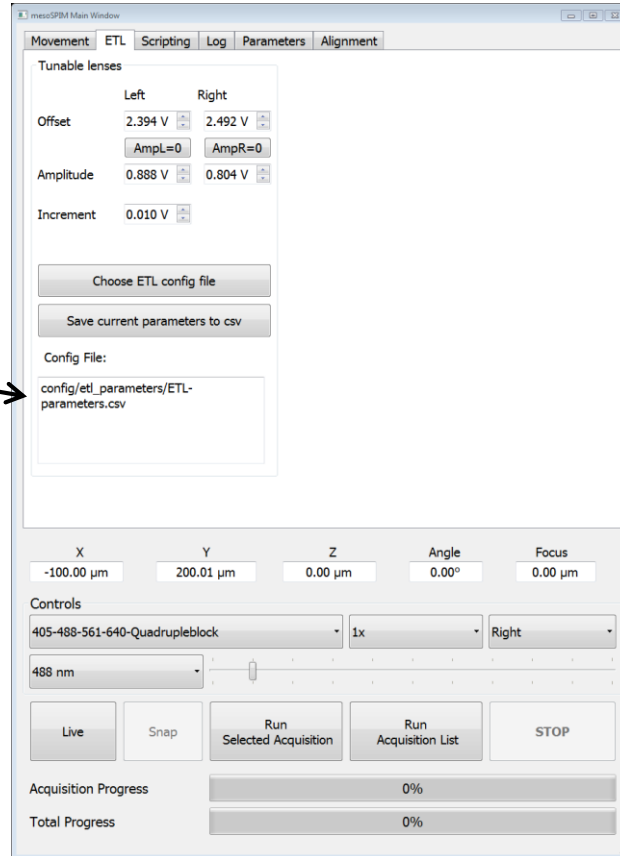
# mesoSPIM-control: Startup I



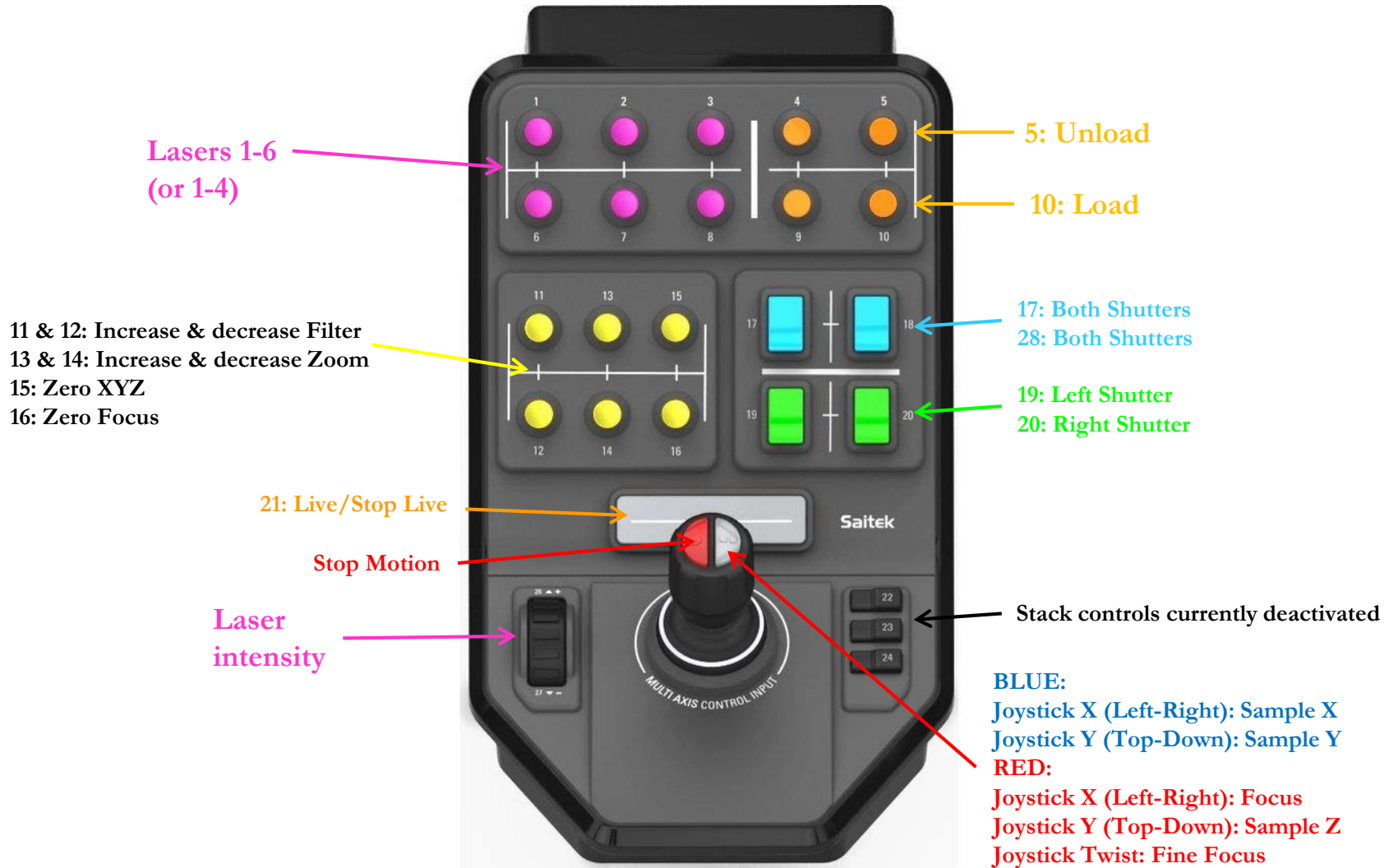
1. Choose config file according to your setup. This is a .py file containing the microscope configuration, NOT a ETL-parameter file.
2. The software takes 10-15s to start while performing a reference move with the focusing stage

# 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 HImage 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 for 'Stack 1'. 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 a checkbox 'Update ETL after Zoom / Laser change'. At the bottom right, there are buttons 'Mark Current ETL values' and 'Preview Selection'. A warning icon and text are overlaid on the table area, stating 'Acquisition lists with varying rotations («multiview») are currently disabled.' Another text overlay 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

# 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

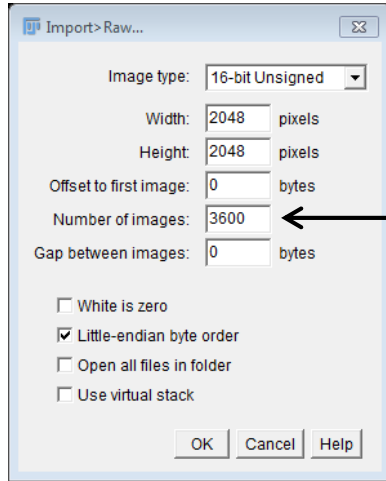
[GALVO PARAMETERS]
[galvo_l_frequency] 99.9
[galvo_l_amplitude] 6.0
[galvo_l_offset] 0.0
[galvo_r_amplitude] 6
[galvo_r_offset] 0.0

[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	

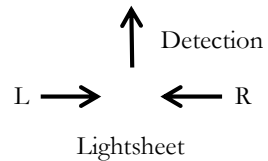


Number of images can  
be larger than the number of acquired images,  
the import will work normally

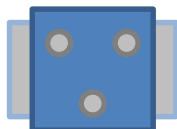
3. The stack will open in the center

# Controlling sample rotation

Rotations of a 20 mm cuvette:

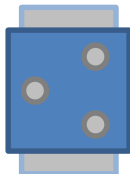


0/360°

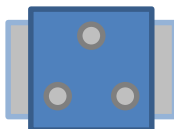


Position mark  
↑

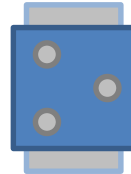
90°



180°



270°



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

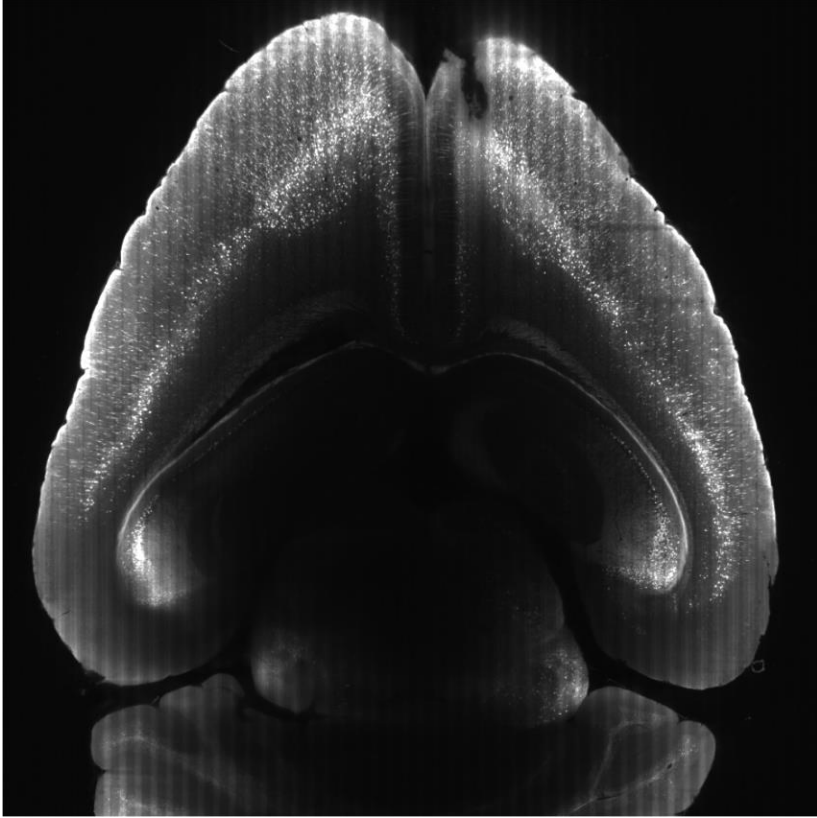


**Especially when using 20 mm  
cuvettes, watch out for collisions with  
the outer cuvette!**

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## Part VI: Troubleshooting

## Horizontal Stripes in the image



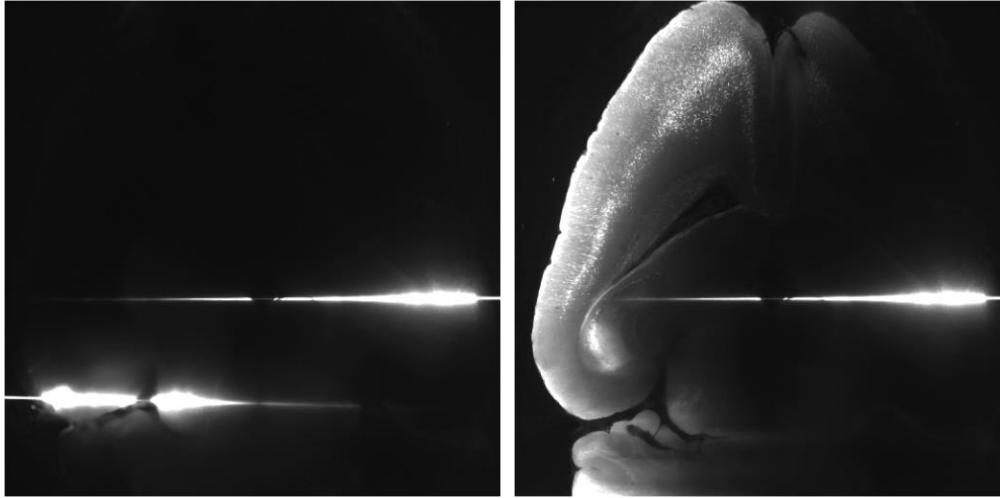
Stripes indicate that the Galvo Frequency is not averaged out by the exposure.

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:

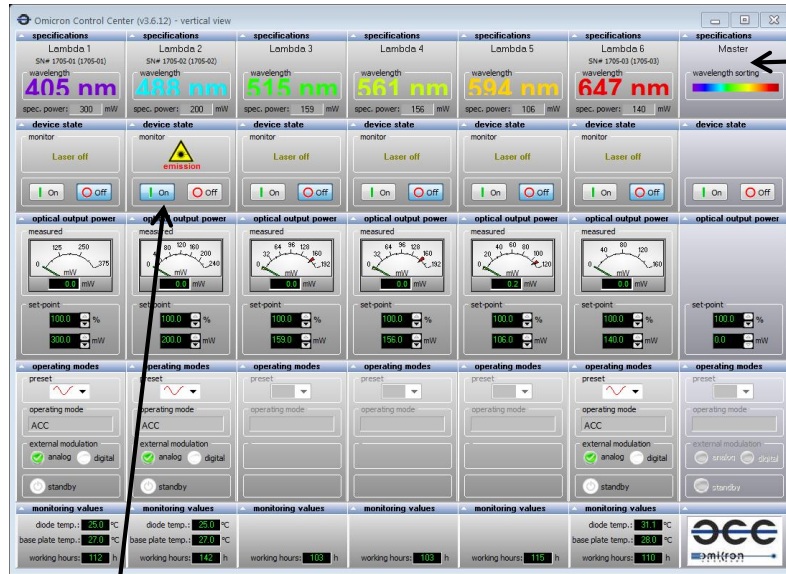
- Galvos are switched off
- Galvos switched off due to overheating (usually at amplitudes  $>3.7$  V)



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

Amplitude 1.4 V

**Galvo Parameters:**

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

