

# mesoSPIM documentation V3.02



Software version: commit: 429b6f

December 6th, 2018

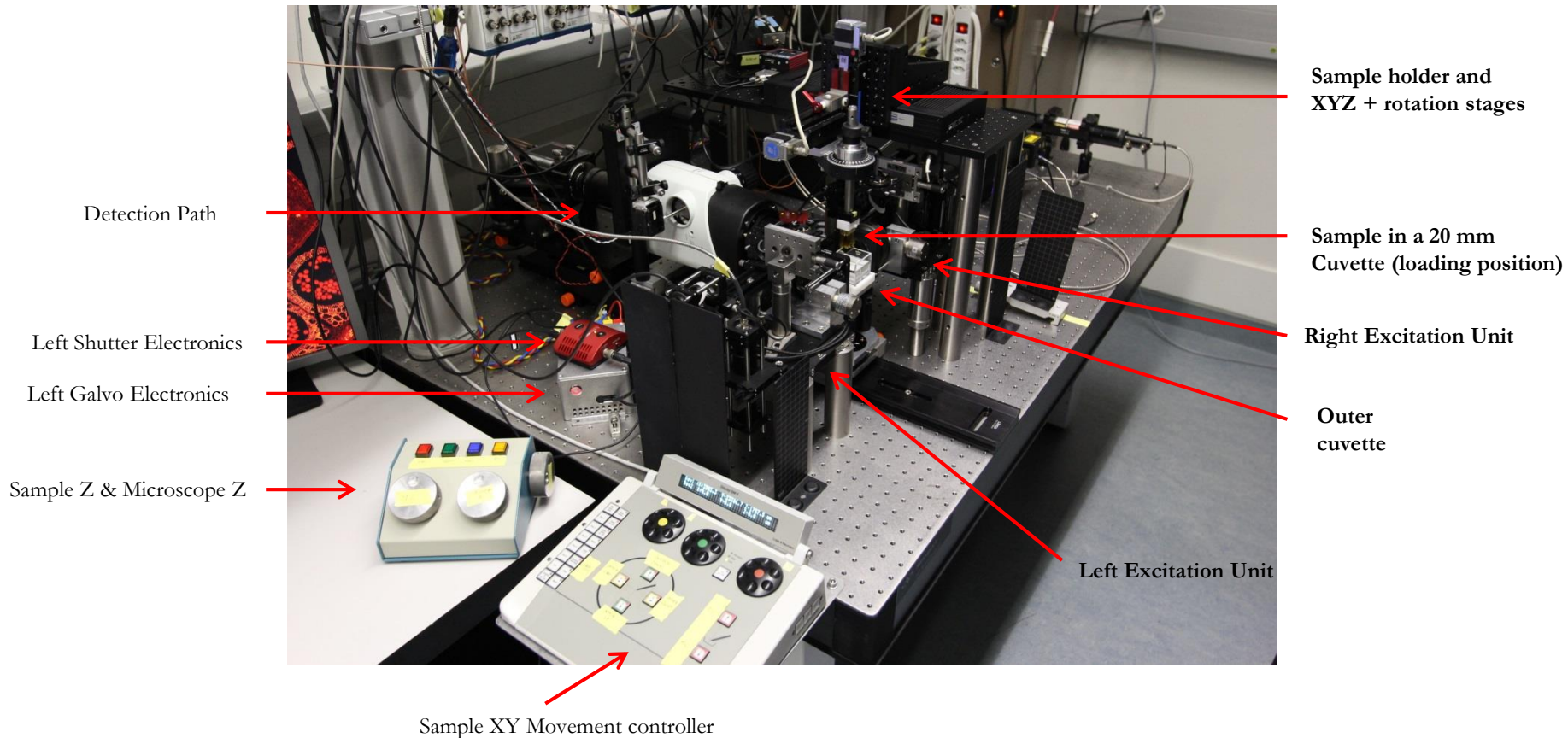
# Part I: Specifications

# FOV calibration

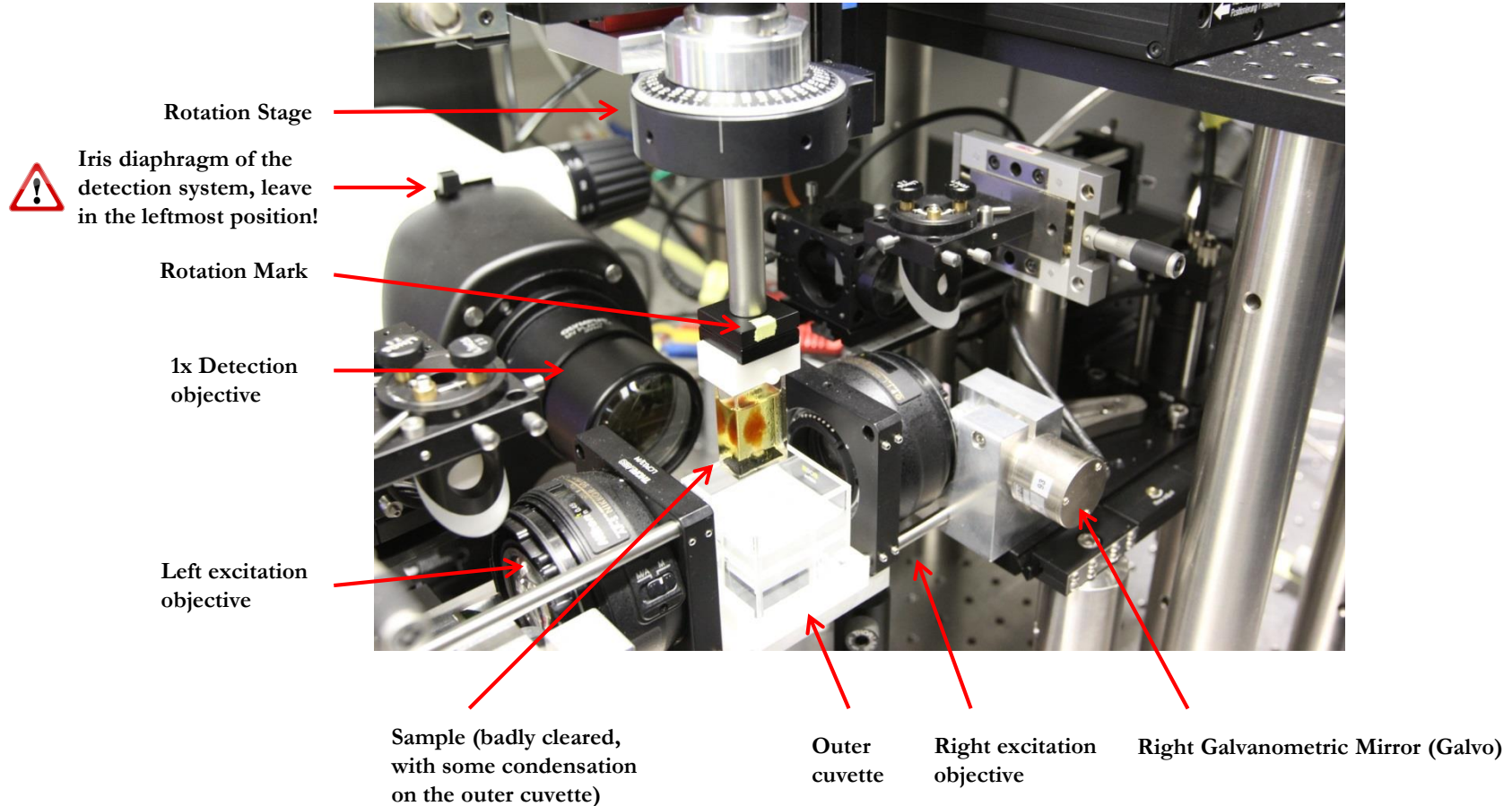
Objective	Zoom	FOV size (mm)	Pixel size (mm)
1x	0.63	21.56	0.0105263
1x	0.8	16.86	0.008234
<b>1x</b>	<b>1</b>	<b>13.42</b>	<b>0.006552</b>
<b>1x</b>	<b>1.25</b>	<b>10.79</b>	<b>0.0052666</b>
1x	1.6	8.36	0.0040844
1x	2	6.69	0.0032688
1x	2.5	5.34	0.0026059
1x	3.2	4.16	0.0020332
<b>1x</b>	<b>4</b>	<b>3.29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

## Part II: Hardware overview

# Microscope overview

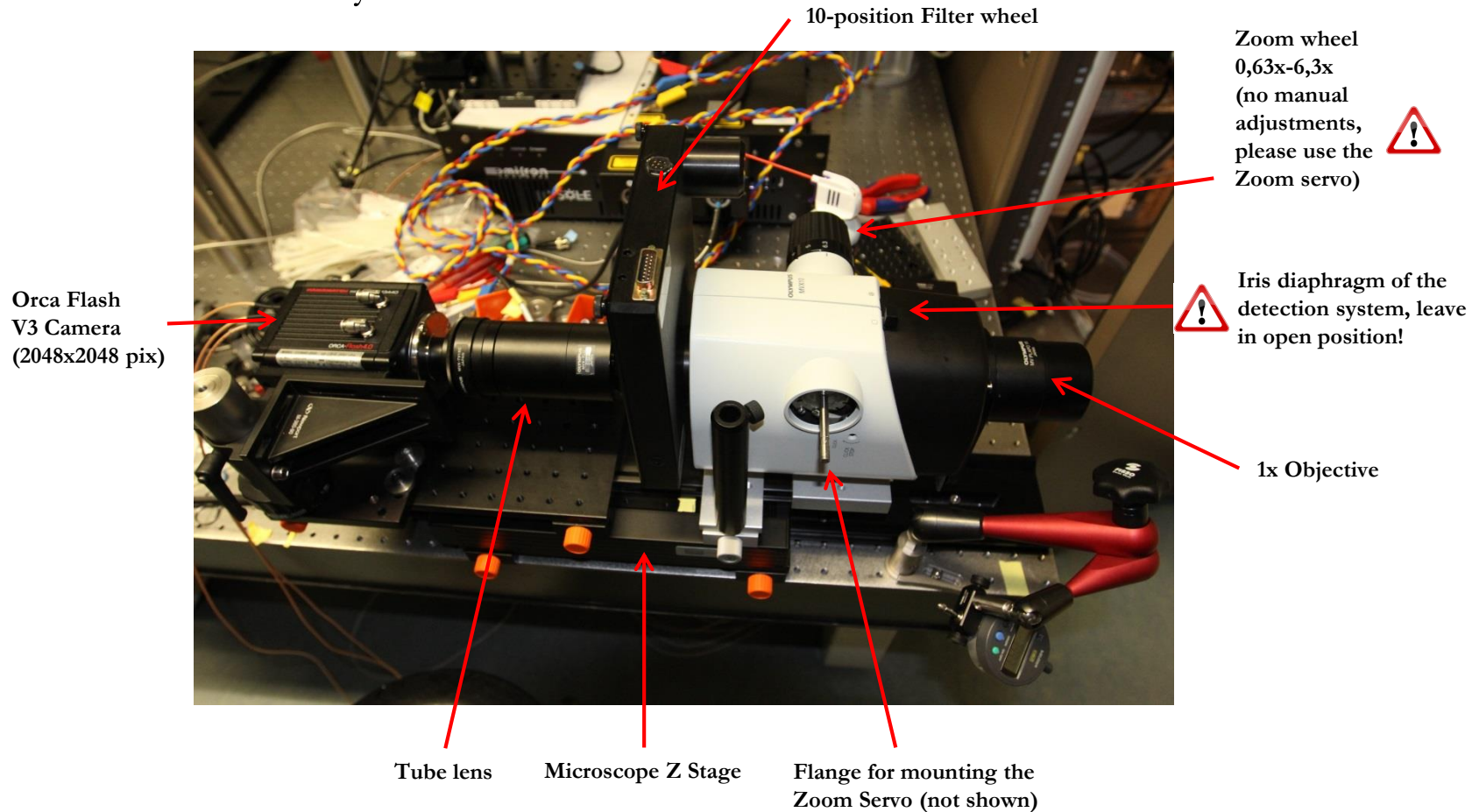


# Overview of the sample holder





# Overview of the detection system

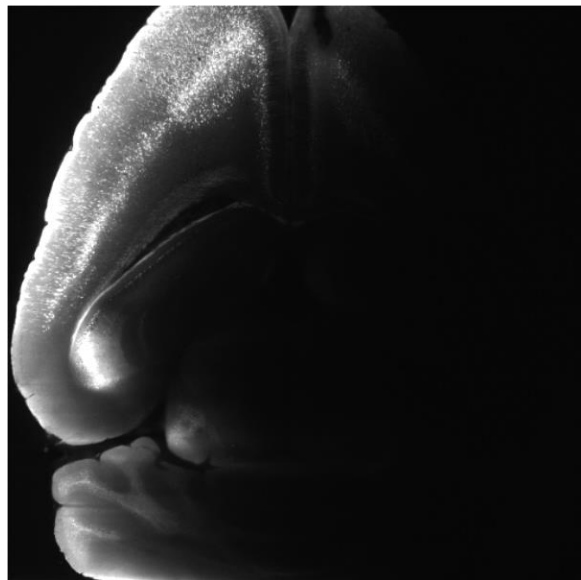
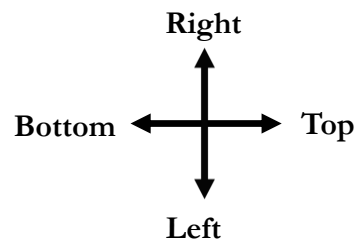


## Part III: Learning to see with a light-sheet microscope

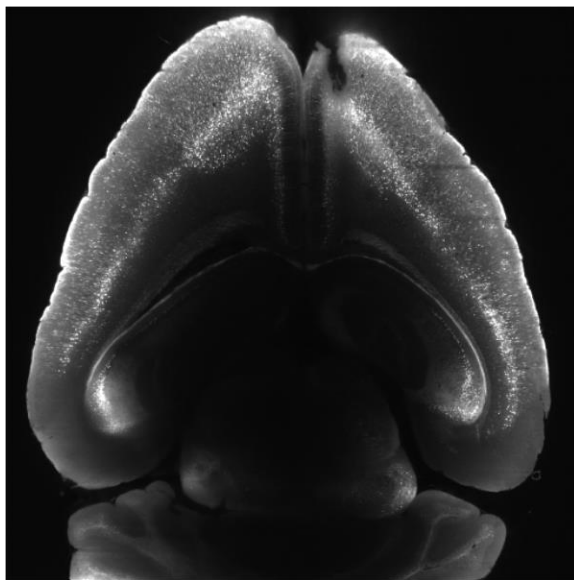


# Light-sheet directions (@Zoom 1.25x)

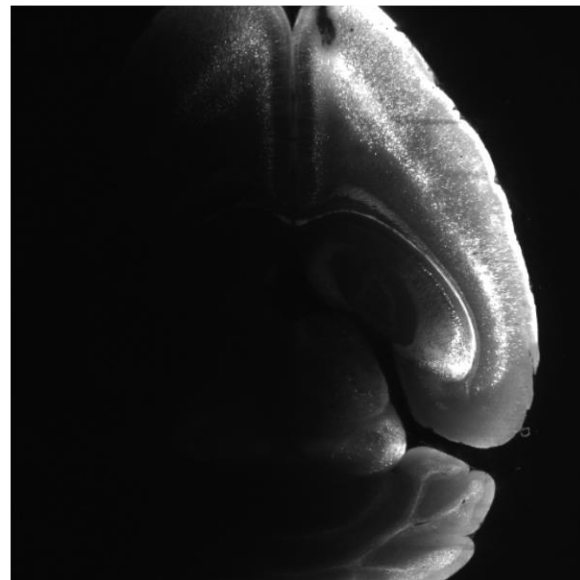
In order to allow the sweeping focus to be synchronized with the camera readout, the camera is rotated by 90°. In not-so-well-cleared samples, the penetration direction is clearly visible:



**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

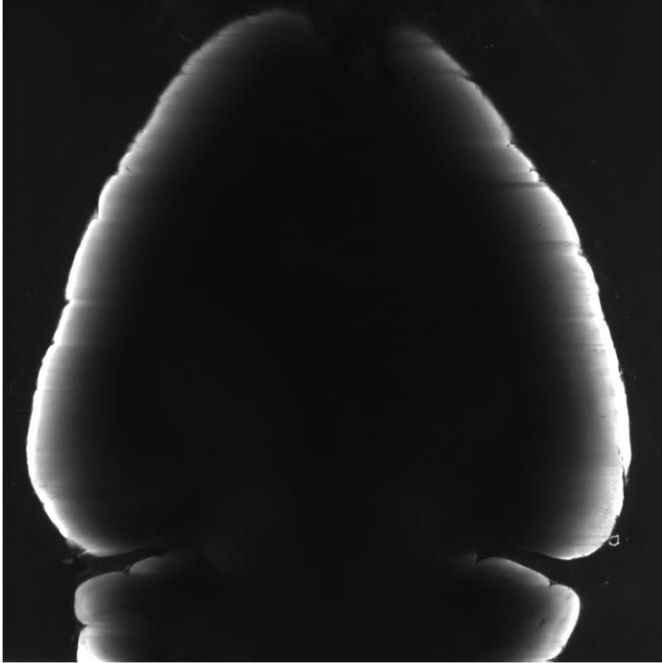
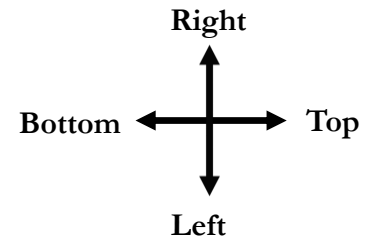
↓

← 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



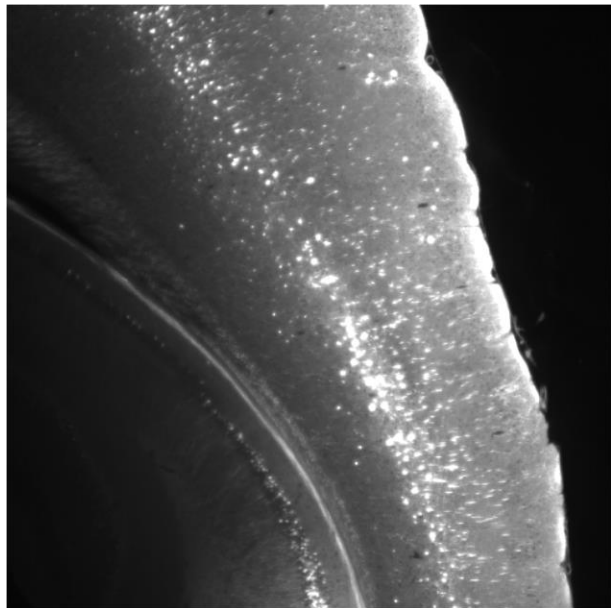
647 nm + Quadrupleband

# What happens if the tunable lens parameters are incorrect?

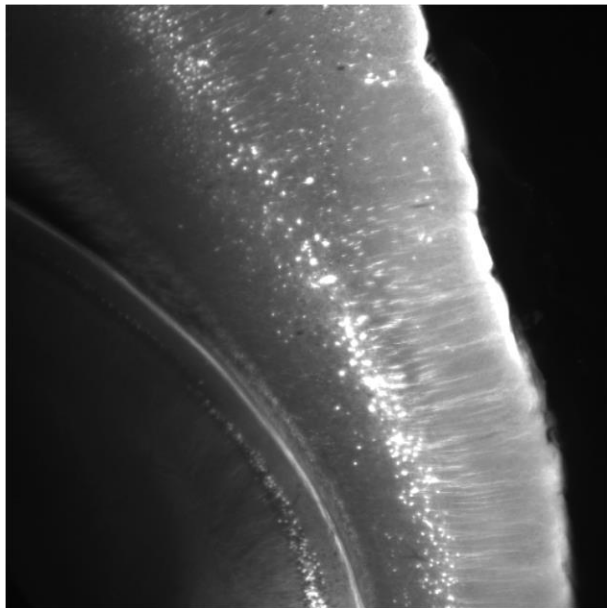


A thicker light-sheet leads to more features being visible at the expense of contrast

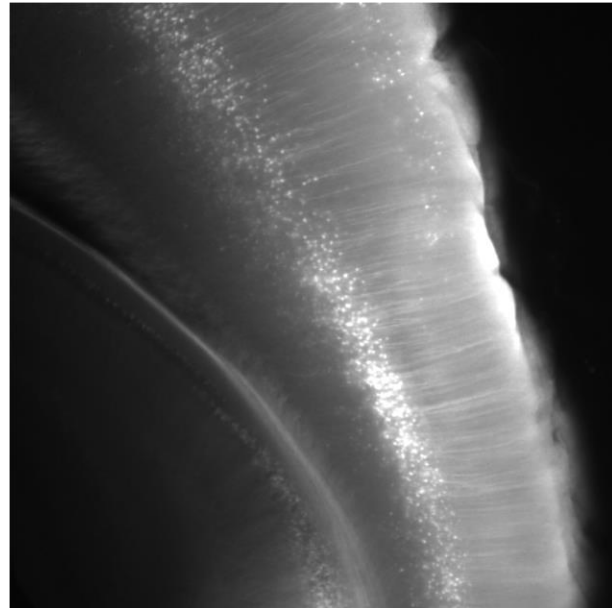
Thy1-YFP (H-line), 488 nm excitation, zoom 4



ETL offset = 2.47 V  
ETL amplitude = 0.17 V



ETL offset = 2.47 V  
ETL amplitude = 0.0 V



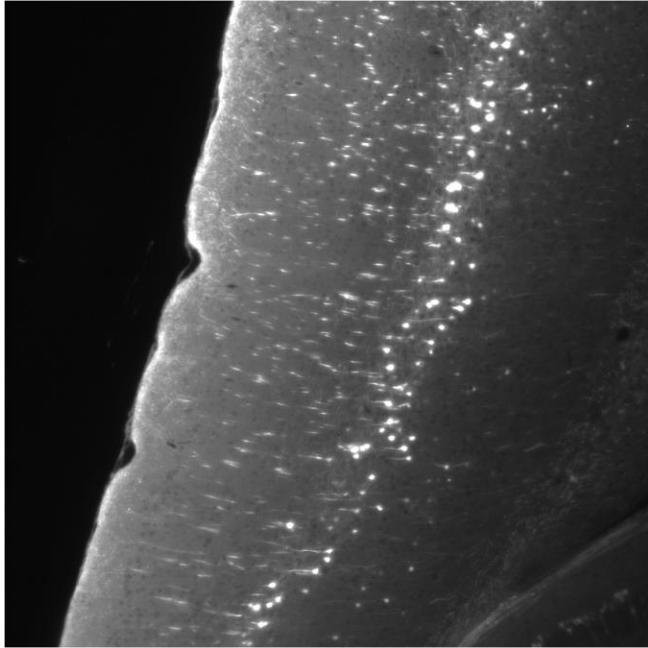
ETL offset = 2.2 V  
ETL amplitude = 0.0 V



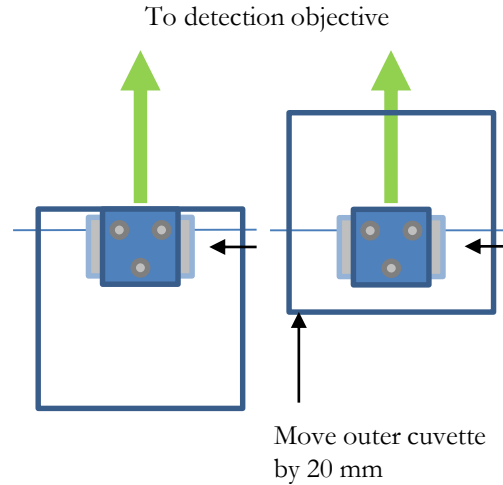
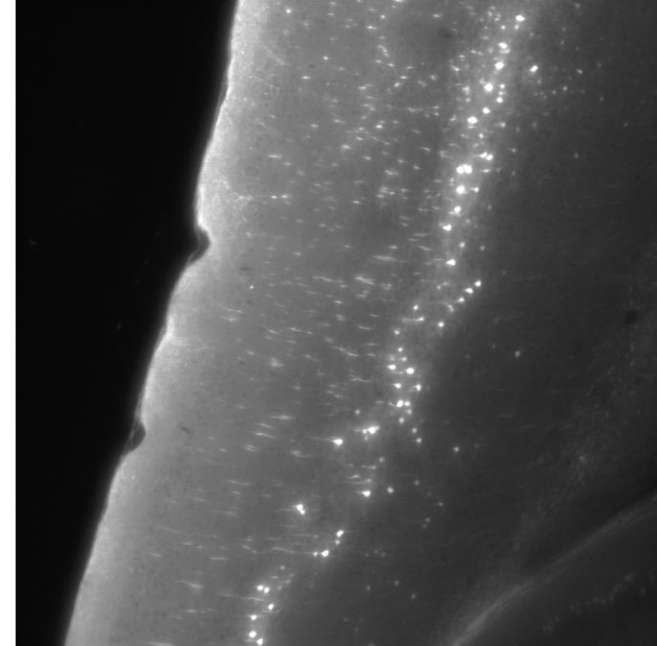
Counterstained cell-bodies and blood-vessel cross-sections are usually good structures to judge this

# 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



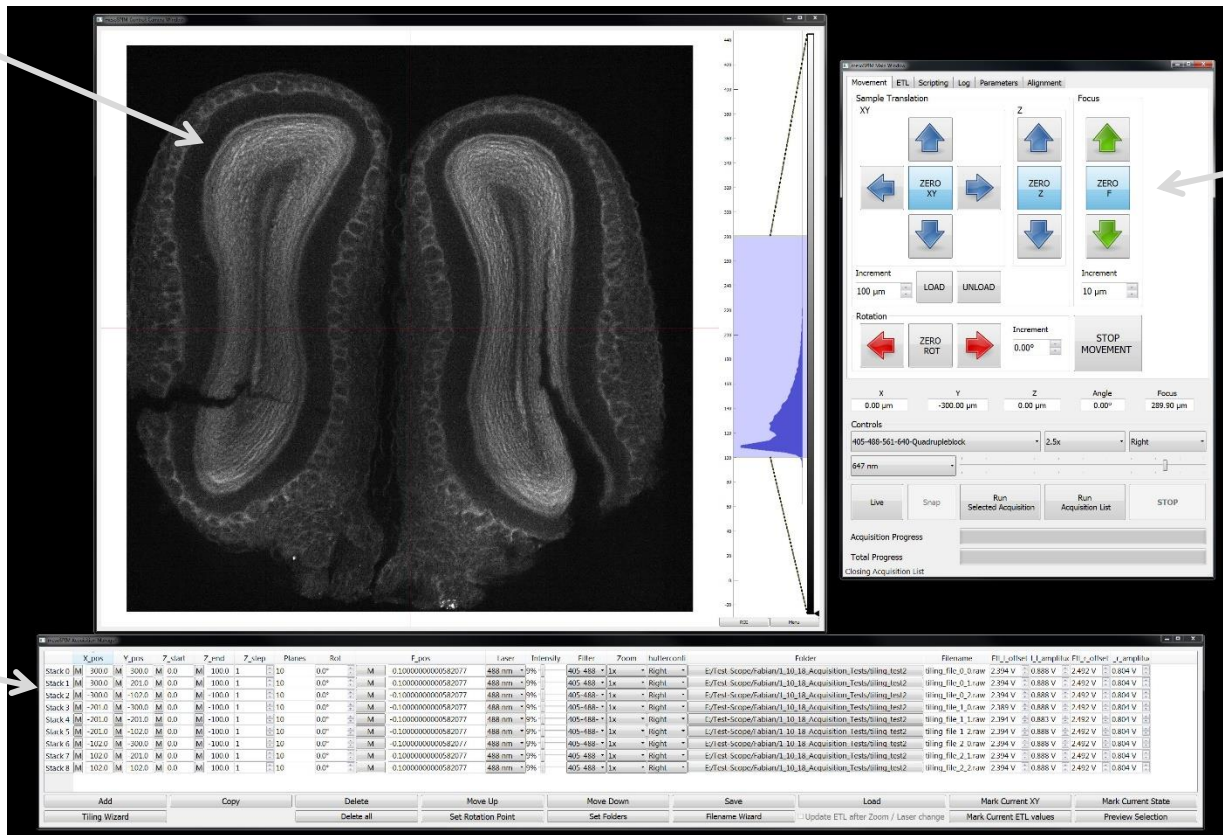
Select a position of the outer cuvette that minimizes spherical aberration while offering enough space for sample movement during stacks & rotations

## Part IV: User Interfaces

## Camera Window

## Main Window

## Acquisition Manager





# mesoSPIM-control: Main window: Movement tab



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

Increment selector  
for the movement

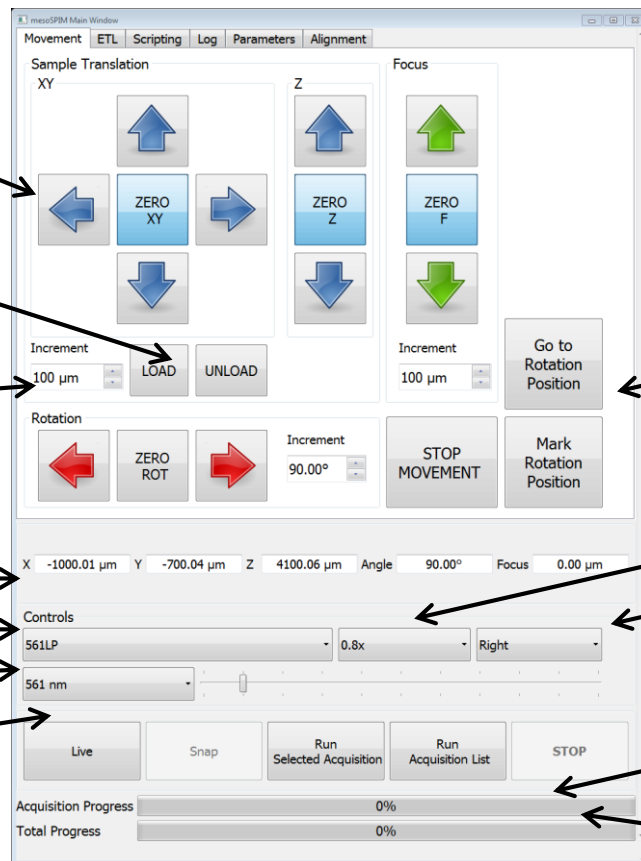
Movement  
arrows

Coordinate readout

Filter selector

Laser selector

Imaging mode buttons



Buttons to check & mark  
rotation position  
(user-selected safe position where  
the sample can be rotated  
without collisions)

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 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



mesoSPIM Main Window

Movement ETL Scripting Log Parameters Alignment

Sweep

Sweeptime 200.00 ms

Exposure 20.00 ms

Line Interval 75.00  $\mu$ s

Laser pulse

	Left	Right
Delay	10.00 %	10.00 %
Pulselength	87.00 %	87.00 %
Max Amplitude	100.00 %	100.00 %

Camera parameters

Delay 10.00 %

Pulse 80.00 %

Tunable lenses

	Left	Right
Offset	2.000 V	2.742 V
Amplitude	0.000 V	0.173 V
Delay	7.50 %	2.50 %
Ramp rising	85.00 %	5.00 %
Ramp falling	2.50 %	85.00 %

Galvo (Shadow reduction)

Frequency 199.510 Hz

	Left	Right
Offset	0.00 V	0.00 V
Amplitude	2.00 V	
Phase	1.57	1.57

X 3200.04  $\mu$ m Y 900.03  $\mu$ m Z -1900.03  $\mu$ m Angle 0.00° Focus 40.00  $\mu$ m

Controls

405-488-561-640-Quadrupleblock 4x Right

647 nm

Live Snap Run Selected Acquisition Run Acquisition List STOP

Acquisition Progress 100% (Image 3100/3100)

Total Progress 100% (Acquisition 3/3) (Image 9300/9300)

Closing Acquisition List

Galvo parameters

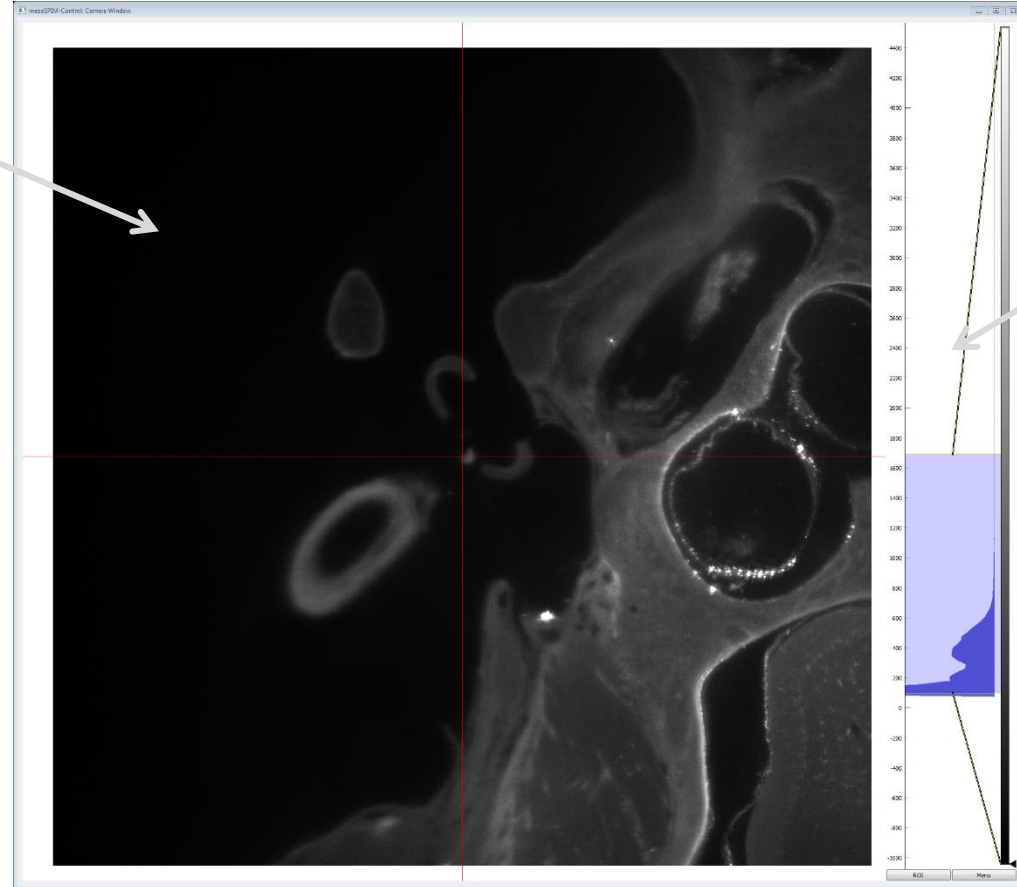


# mesoSPIM-control: Camera window

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	hutterconfi	Folder	Filename	EtI_l_offset	I_l_amplitu	EtI_r_offset	r_amplitu								
Stack 0	M	-600.01	M	-400.02	M	1000001	M	1000001	2	50	90.0°	M	0.0	561 nm	10%	561LP	0.8x	Right	E:/Test-Scope/Fabian/6.12.18_RotationTests	tiling_file_0_0.raw	2.642 V	0.600 V	2.661 V	0.750 V			
Stack 1	M	-600.01	M	-500.02	M	1000001	M	1000001	2	50	90.0°	M	0.0	561 nm	10%	561LP	0.8x	Right	E:/Test-Scope/Fabian/6.12.18_RotationTests	tiling_file_0_1.raw	2.642 V	0.600 V	2.661 V	0.750 V			
Stack 2	M	-600.01	M	-600.02	M	1000001	M	1000001	2	50	90.0°	M	0.0	561 nm	10%	561LP	0.8x	Right	E:/Test-Scope/Fabian/6.12.18_RotationTests	tiling_file_0_2.raw	2.642 V	0.600 V	2.661 V	0.750 V			
Stack 3	M	-700.01	M	-400.02	M	1000001	M	1000001	2	50	90.0°	M	0.0	561 nm	10%	561LP	0.8x	Right	E:/Test-Scope/Fabian/6.12.18_RotationTests	tiling_file_1_0.raw	2.642 V	0.600 V	2.661 V	0.750 V			
Stack 4	M	-700.01	M	-500.02	M	1000001	M	1000001	2	50	90.0°	M	0.0	561 nm	10%	561LP	0.8x	Right	E:/Test-Scope/Fabian/6.12.18_RotationTests	tiling_file_1_1.raw	2.642 V	0.600 V	2.661 V	0.750 V			
																Mark Current XY				Mark Current Focus				Mark Current Rotation			
																Mark Current State				Mark Current ETL values							
																<input type="checkbox"/> Update ETL after Zoom / Laser change											
																Add				Copy				Delete			
																Move Up				Save				Load			
																Preview Selection				Tiling Wizard				Delete all			
																Move Down				Set Folders				Filename Wizard			

# mesoSPIM-control: Acquisition Manager

Save & load tables

The screenshot shows the 'mesoSPIM Acquisition Manager' window. It features a large table with columns for 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\_l\_amplitud, Etl\_r\_offset, and r\_amplitud. Below the table is a row of buttons: 'Add', 'Copy', 'Delete', 'Move Up', 'Save', 'Load', 'Delete all', 'Move Down', 'Set Folders', and 'Filename Wizard'. To the right of these buttons are four buttons: 'Mark Current XY', 'Mark Current Focus', 'Mark Current Rotation', and 'Mark Current State'. A red box highlights the 'Save' and 'Load' buttons, and a green box highlights the 'Mark Current' buttons. Arrows point from the text labels below to specific buttons in the interface.

	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_l_amplitud	Etl_r_offset	r_amplitud					
Stack 0	M	-600.01	M	-400.02	M	1000001	M	1000001	2	50	90.0°	M	0.0	561 nm	10x	561LP	0.8x	Right	E:/Test-Scope/Fabian/6.12.18_RotationTests	tiling_file_0_0.raw	2.642 V	0.600 V	2.661 V	0.750 V
Stack 1	M	-600.01	M	-500.02	M	1000001	M	1000001	2	50	90.0°	M	0.0	561 nm	10x	561LP	0.8x	Right	E:/Test-Scope/Fabian/6.12.18_RotationTests	tiling_file_0_1.raw	2.642 V	0.600 V	2.661 V	0.750 V
Stack 2	M	-600.01	M	-600.02	M	1000001	M	1000001	2	50	90.0°	M	0.0	561 nm	10x	561LP	0.8x	Right	E:/Test-Scope/Fabian/6.12.18_RotationTests	tiling_file_0_2.raw	2.642 V	0.600 V	2.661 V	0.750 V
Stack 3	M	-700.01	M	-400.02	M	1000001	M	1000001	2	50	90.0°	M	0.0	561 nm	10x	561LP	0.8x	Right	E:/Test-Scope/Fabian/6.12.18_RotationTests	tiling_file_1_0.raw	2.642 V	0.600 V	2.661 V	0.750 V
																				tiling_file_1_1.raw	2.642 V	0.600 V	2.661 V	0.750 V

Buttons: Add, Copy, Delete, Move Up, Save, Load, Delete all, Move Down, Set Folders, Filename Wizard, Mark Current XY, Mark Current Focus, Mark Current Rotation, Mark Current State.

Controls for table entries

Tiling wizard

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

Set folder for saving  
data for all rows

Autogenerate filenames

Update parts of a selected  
table entry & preview

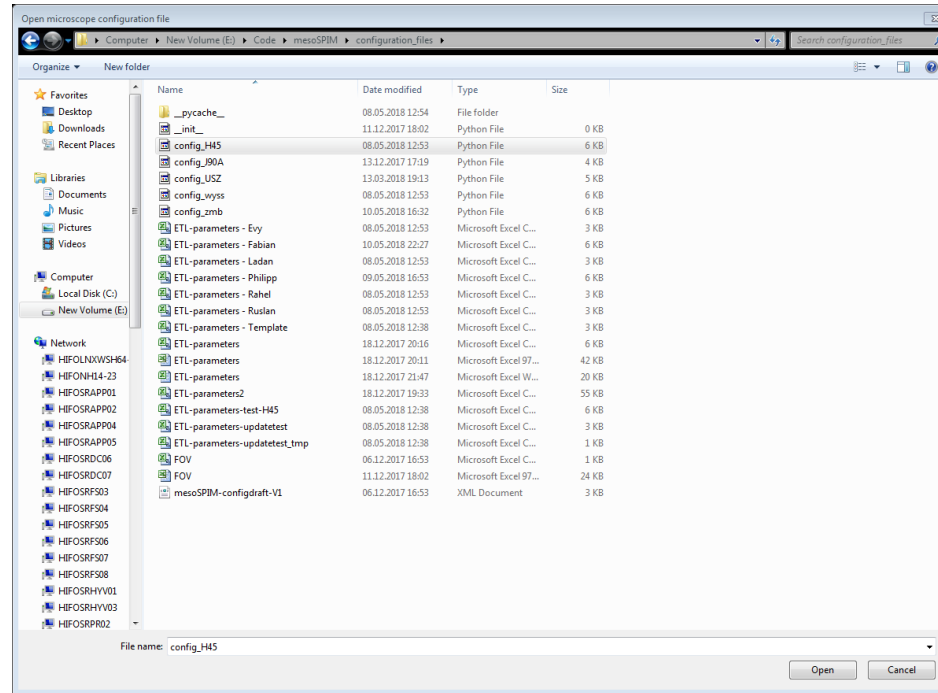
## Part V: Startup



# 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»)

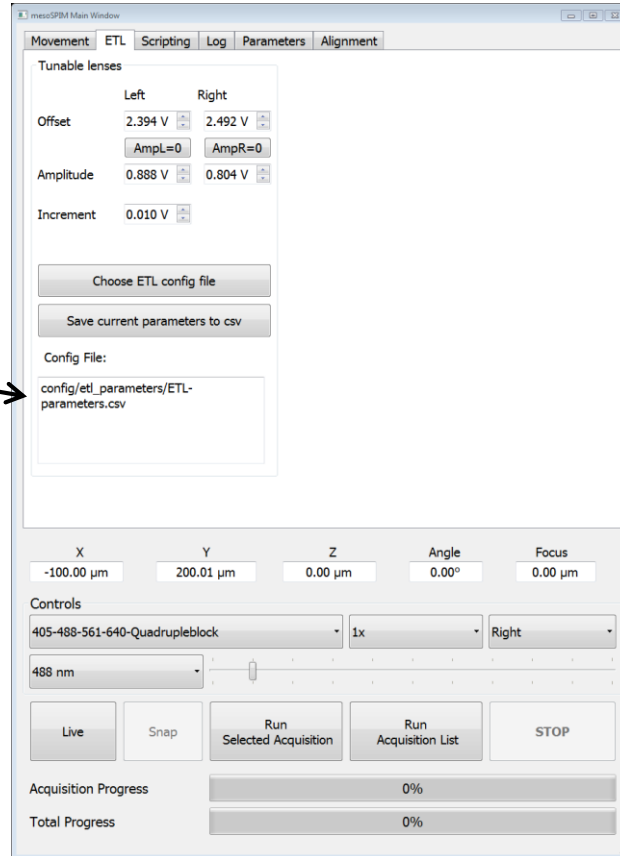
# mesoSPIM-control: Startup I



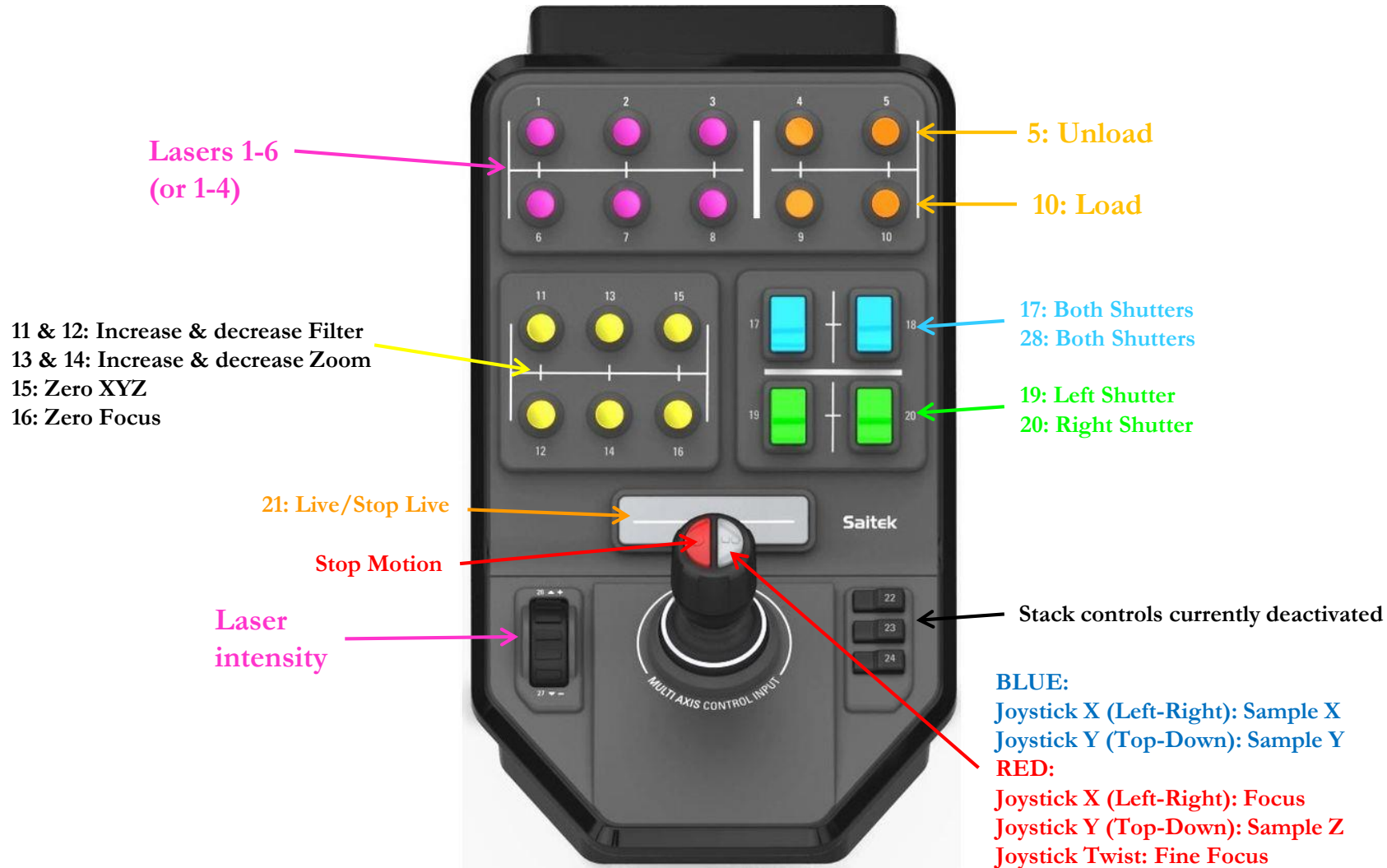
1. Choose config file according to your setup. This is a .py file containing the microscope configuration, NOT a ETL-parameter file.
2. The software takes 10-15s to start while performing a reference move with the focusing stage

# 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 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») = copy current coordinate

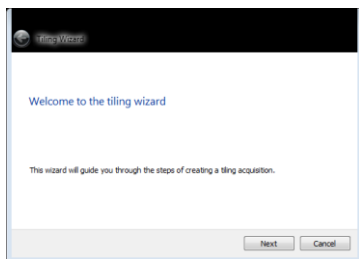
	X_pos	Y_pos	Z_start	Z_end	Z_step	Planes	Rot	F_pos	Laser	Intensity	Filter	Zoom	shutterconfi	Folder	Filename	EtL_offset	I_L_amplitud	EtL_r_offset	I_r_amplitud
Stack 0	M -600.01	M -400.02	M 1000001	M 1000001	2	50	90.0°	M 0.0	561 nm	10%	561LP	0.8x	Right	E:/Test-Scope/Fabian/6_12_18_RotationTests	tiling_file_0.raw	2.642 V	0.600 V	2.661 V	0.750 V
Stack 1	M -600.01	M -500.02	M 1000001	M 1000001	2	50	90.0°	M 0.0	561 nm	10%	561LP	0.8x	Right	E:/Test-Scope/Fabian/6_12_18_RotationTests	tiling_file_0.1.raw	2.642 V	0.600 V	2.661 V	0.750 V

Buttons: Add, Copy, Delete, Move Up, Save, Load, Mark Current XY, Mark Current Focus, Mark Current Rotation, Preview Selection, Tiling Wizard, Delete all, Move Down, Set Folders, Filename Wizard, Update ETL after Zoom / Laser change, Mark Current State, Mark Current ETL values

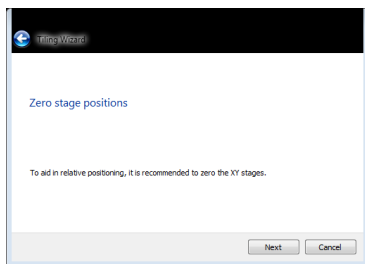
3. Using the mark buttons, select the X,Y,Focus, Z\_start and Z\_end positions
4. Select, laser, intensity, filter, shutter for a row
5. Select a folder for the stack to be saved in by clicking on the button in the folder column. The current folder will be displayed. Using the «Set Folders» button, the 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

# Tiling Wizard

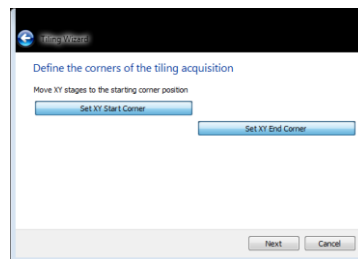
1. Start the Tiling Wizard



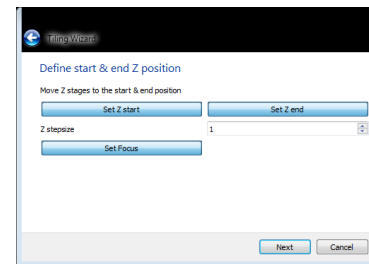
2. If desired, zero the stages using the buttons in the main user interface



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



4. Define Start & End Position & Focus

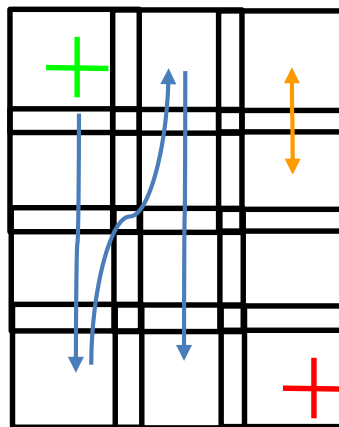


For some reason, screenshots have a «black bar» on top (not visible in the window during usage)



The first FOV is centered on the start position

Start



Offset



«Offset» is the mechanical FOV-to-FOV ( $\Delta X$  or  $\Delta Y$ ) movement distance  
- You have to calculate offset depending on your FOV



The last FOV contains the End position (not centered)

End

# Tiling Wizard

## 5. Define other imaging parameters

Define other parameters

Zoom: 1x

Laser: 488 nm

Intensity: [slider]

Filter: 405-488-561-640-QuadrupleBlock

Shutter: Right

XY Offset: 500 μm

ETL: ☒ Copy current ETL parameters

Next Cancel

## 6. Define Folder

Select folder

Please select the folder in which the data should be saved.

Select Folder

E:/Test-Scope/Fabian/20\_11\_18\_Tiling\_Wizard\_Tests

Next Cancel

## 7. Check FOV counts:

Check Tiling Page

Here are your parameters

X FOVs: 11

Y FOVs: 8

Values are ok?

Next Cancel

## 8. Finished!

Finished!

Attention: This will overwrite the Acquisition Table. Click 'Finished' to continue. To rename the files, use the filename wizard.

Finish Cancel

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

	X_pos	Y_pos	Z_start	Z_end	Z_step	Planes	Rot	F_pos	Laser	Intensity	Filter	Zoom	shutterconfi	Folder	Filename	Etl_L_offset	I_L_amplitud	Etl_r_offset	I_r_amplitud
Stack 0	M 5889.25	M 9948.79	M 12096.2	M 11996.2	1	100	0.0°	M 95000.0	488 nm	40%	405-488-	1x	Right	y/20_11_18	tiling_file...	2.394 V	0.888 V	2.492 V	0.804 V
Stack 1	M 5889.25	M 9898.79	M 12096.2	M 11996.2	1	100	0.0°	M 95000.0	488 nm	40%	405-488-	1x	Right	y/20_11_18	tiling_file...	2.394 V	0.888 V	2.492 V	0.804 V
Stack 2	M 5889.25	M 9848.79	M 12096.2	M 11996.2	1	100	0.0°	M 95000.0	488 nm	40%	405-488-	1x	Right	y/20_11_18	tiling_file...	2.394 V	0.888 V	2.492 V	0.804 V
Stack 3	M 5889.25	M 9798.79	M 12096.2	M 11996.2	1	100	0.0°	M 95000.0	488 nm	40%	405-488-	1x	Right	y/20_11_18	tiling_file...	2.394 V	0.888 V	2.492 V	0.804 V
Stack 4	M 5889.25	M 9748.79	M 12096.2	M 11996.2	1	100	0.0°	M 95000.0	488 nm	40%	405-488-	1x	Right	y/20_11_18	tiling_file...	2.394 V	0.888 V	2.492 V	0.804 V
Stack 5	M 5889.25	M 9698.79	M 12096.2	M 11996.2	1	100	0.0°	M 95000.0	488 nm	40%	405-488-	1x	Right	y/20_11_18	tiling_file...	2.394 V	0.888 V	2.492 V	0.804 V
Stack 6	M 5889.25	M 9648.79	M 12096.2	M 11996.2	1	100	0.0°	M 95000.0	488 nm	40%	405-488-	1x	Right	y/20_11_18	tiling_file...	2.394 V	0.888 V	2.492 V	0.804 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 Update ETL after Zoom / Laser change Mark Current ETL values Preview Selection

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

# Acquisition checklist

mesoSPIM Acquisition Manager

	X_pos	Y_pos	Z_start	Z_end	Z_step	Planes	Rot	F_pos	Laser	Intensity	Filter	Zoom	hutterconfi	Folder	Filename	EtI_offset	I_Lamplitud	EtI_r_offset	I_r_amplitud
Stack 0	M -600.01	M -400.02	M 1000001	M 1000001	2 50		90.0°	M 0.0	561 nm	10%	561LP	0.8x	Right	E:/Test-Scope/Fabian/6_12_18_RotationTests	tiling_file_0_0.raw	2.642 V	0.600 V	2.661 V	0.750 V
Stack 1	M -600.01	M -500.02	M 1000001	M 1000001	2 50		90.0°	M 0.0	561 nm	10%	561LP	0.8x	Right	E:/Test-Scope/Fabian/6_12_18_RotationTests	tiling_file_0_1.raw	2.642 V	0.600 V	2.661 V	0.750 V

☐ Update ETL after Zoom / Laser change

## 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

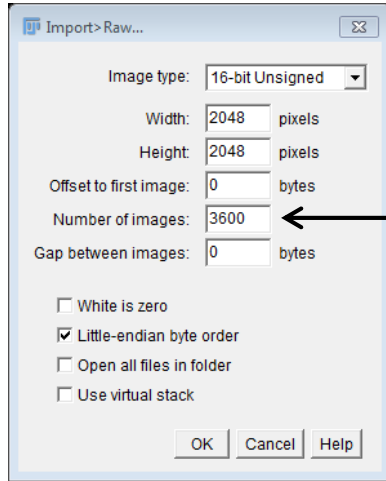
[ETL PARAMETERS]
[ETL CFG File] E:/Code/mesoSPIM-control/mesoSPIM/config/etl_parameters/ETL-parameters - Fabian - DBE - H45.csv
[etl_l_offset] 2.5479999999999983
[etl_l_amplitude] 0.5819999999999999
[etl_r_offset] 2.732
[etl_r_amplitude] 0.4099999999999999

[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



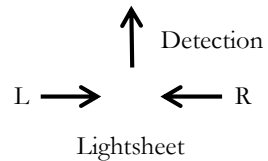
Number of images can  
be larger than the number of acquired images,  
the import will work normally

3. The stack will open in the center

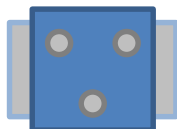


# Controlling sample rotation

Rotations of a 20 mm cuvette:

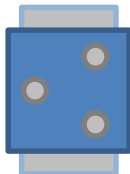


0/360°

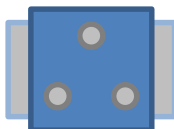


Position mark  
↑

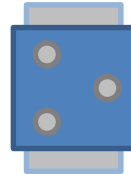
90°



180°



270°



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

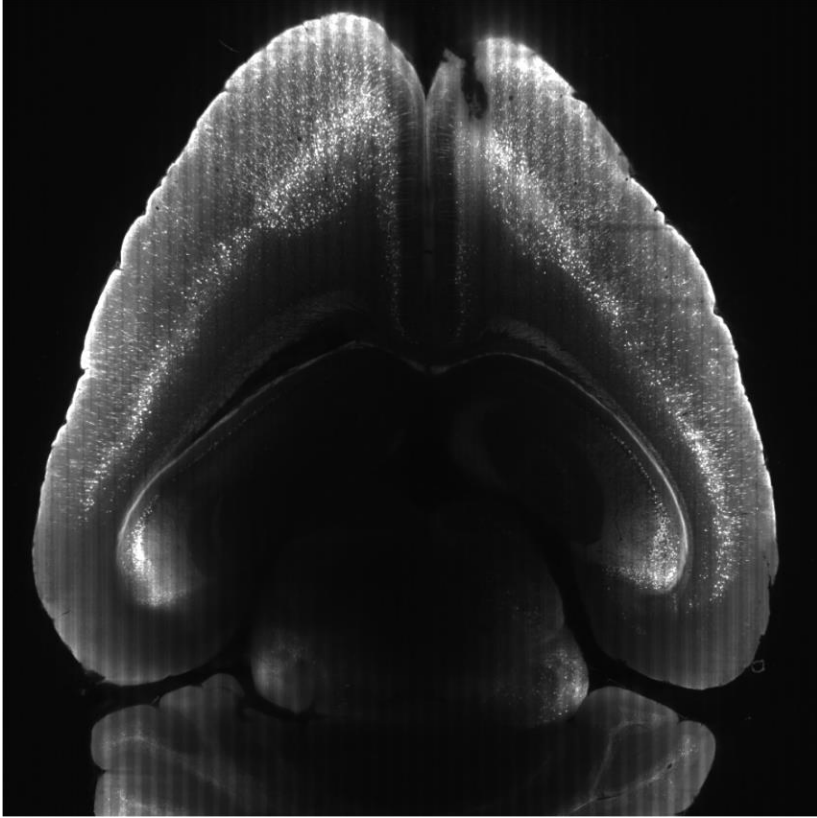


**Especially when using 20 mm  
cuvettes, watch out for collisions with  
the outer cuvette!**

---

## Part VI: Troubleshooting

## Horizontal Stripes in the image

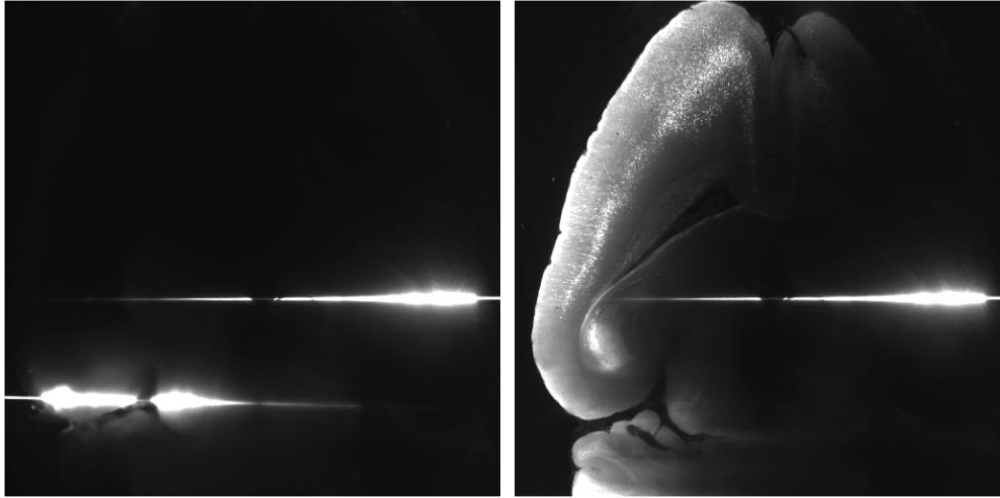


Stripes indicate that the Galvo Frequency is not averaged out by the exposure.

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:

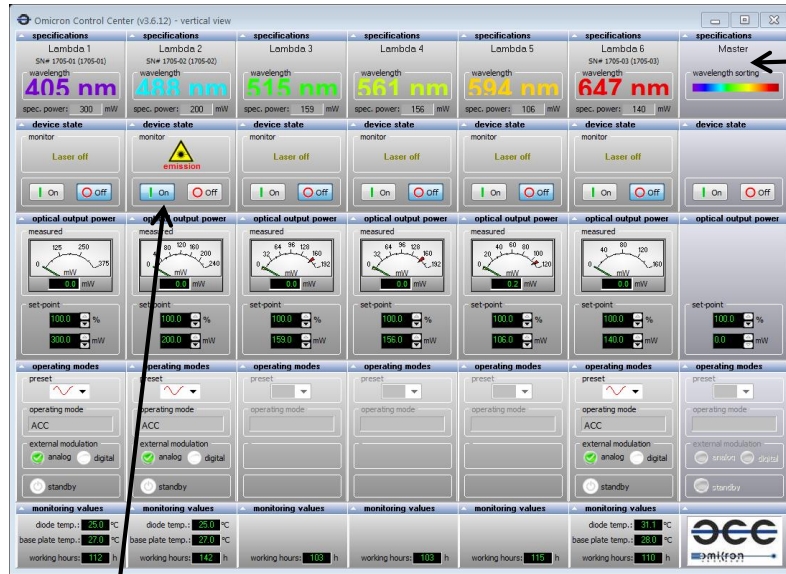
- Galvos are switched off
- Galvos switched off due to overheating (usually at amplitudes  $>3.7$  V)



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

Amplitude 1.4 V

**Galvo Parameters:**

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

