

Zurich mesoSPIM documentation

V3.10

Software version: commit: 25ac01

20.11.18

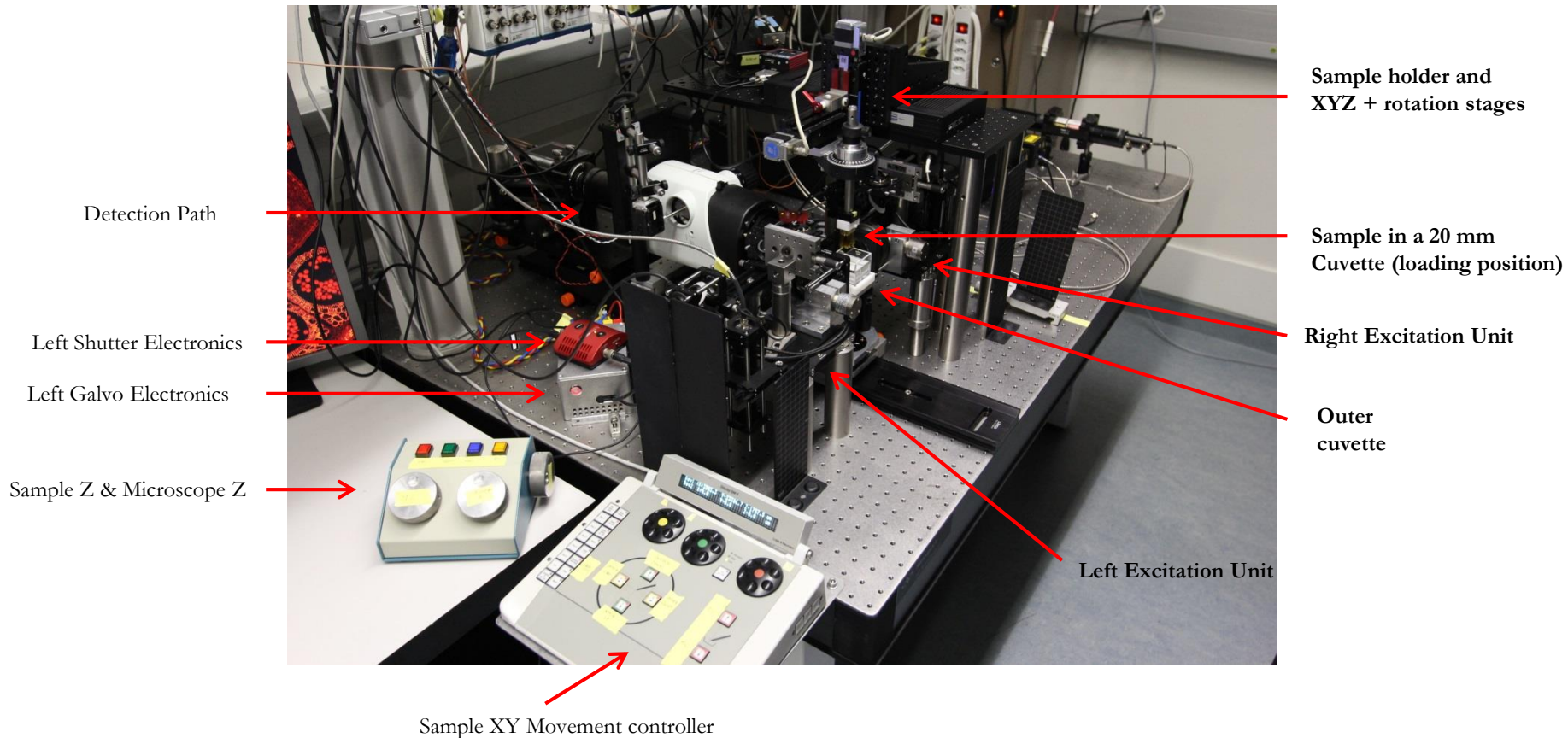
Part I: Specifications

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



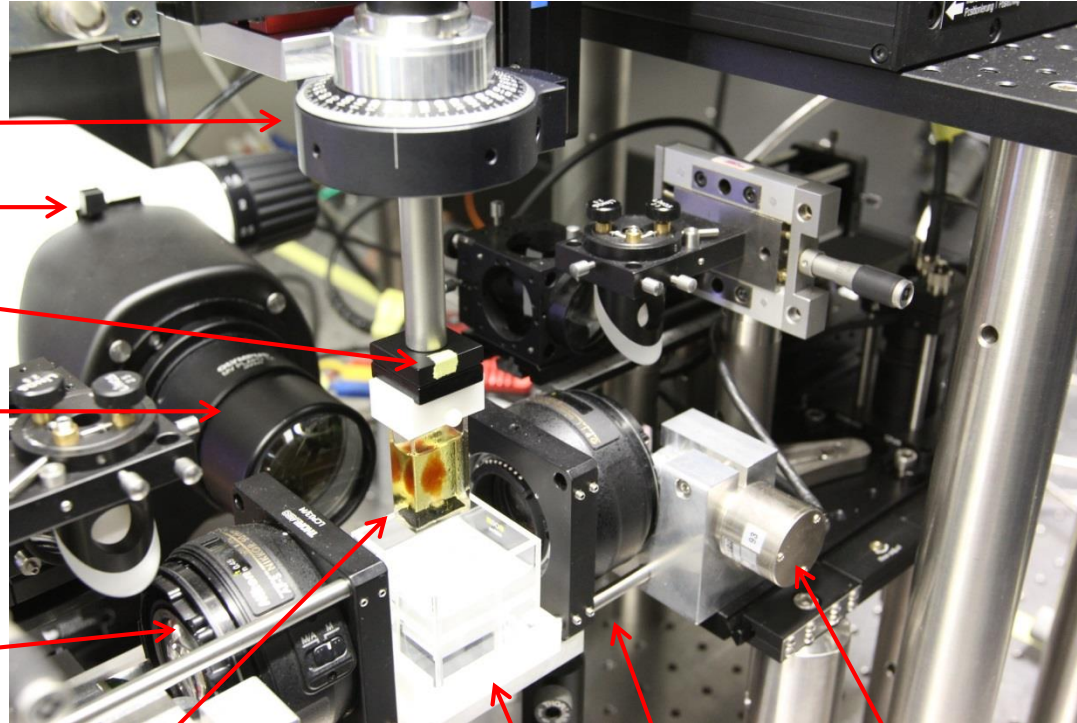
Iris diaphragm of the detection system, leave in the leftmost position!

Rotation Stage

Rotation Mark

1x Detection objective

Left excitation objective



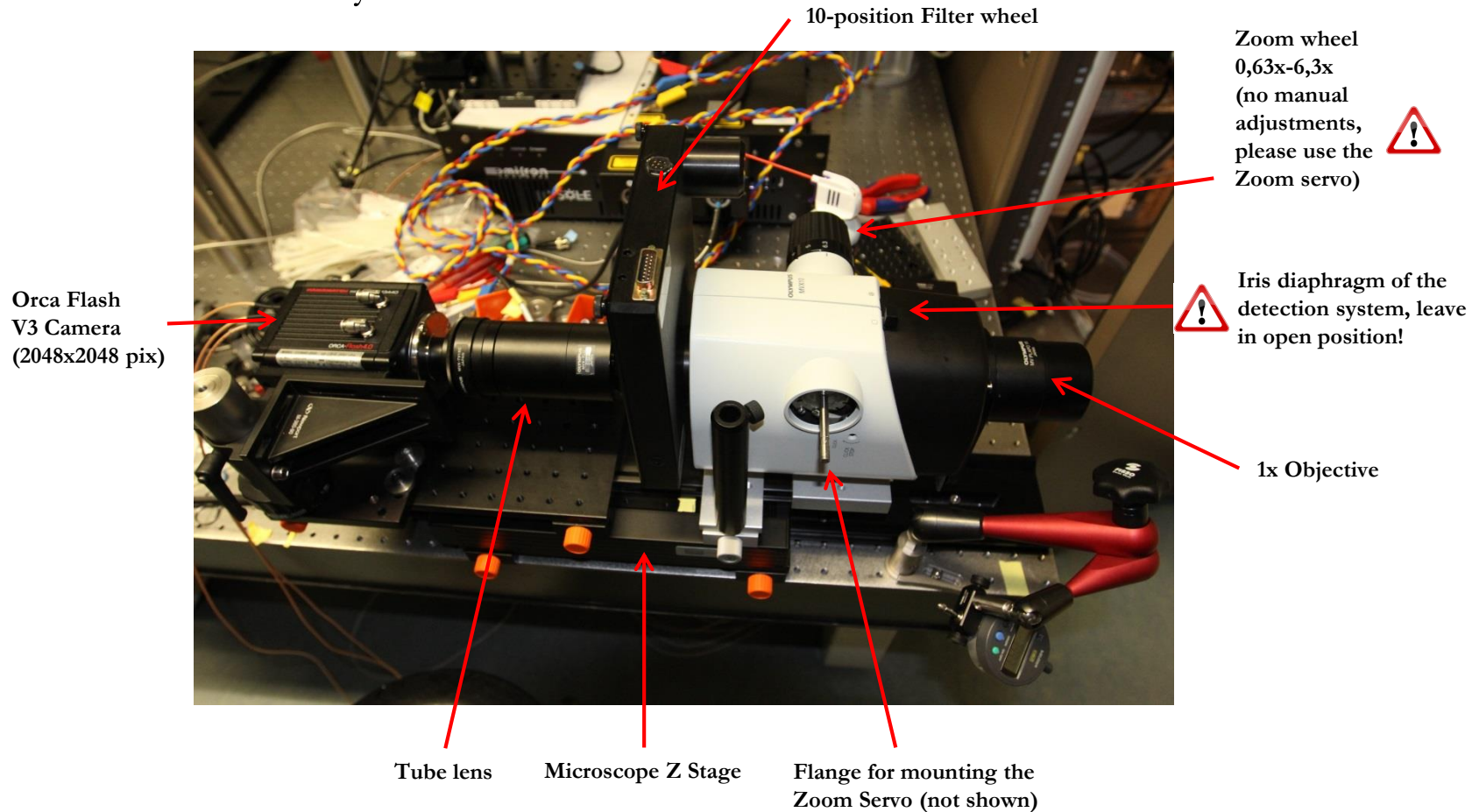
Sample (badly cleared, with some condensation on the outer cuvette)

Outer cuvette

Right excitation objective

Right Galvanometric Mirror (Galvo)

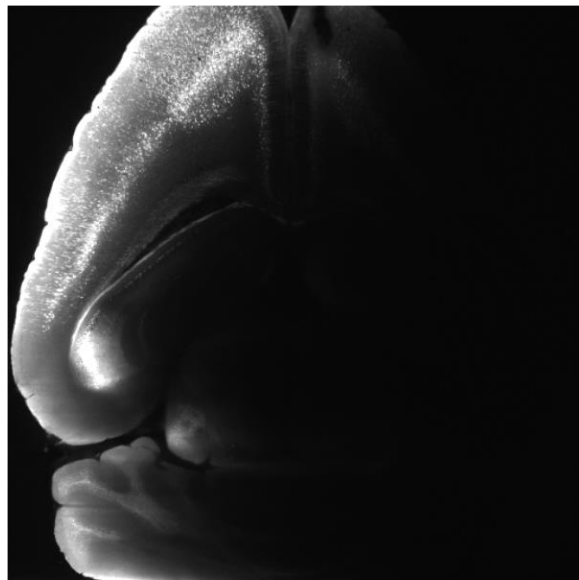
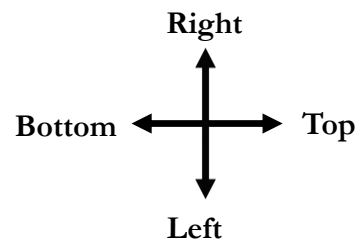
Overview of the detection system



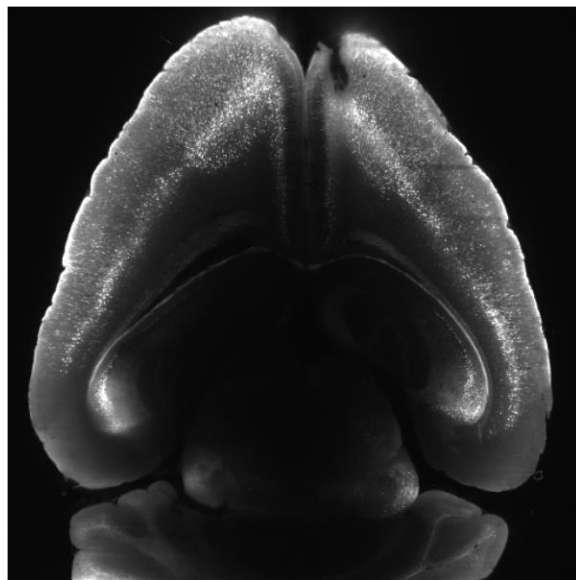
Part III: Learning to see with a light-sheet microscope

Light-sheet directions (@Zoom 1.25x)

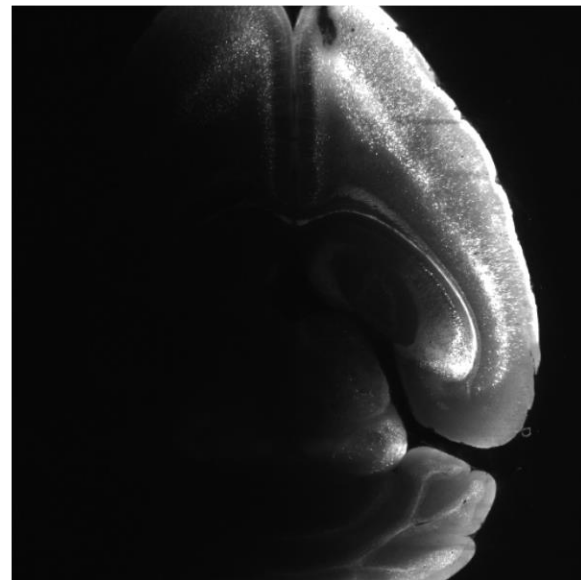
In order to allow the sweeping focus to be synchronized with the camera readout, the camera is rotated by 90°. In not-so-well-cleared samples, the penetration direction is clearly visible:



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

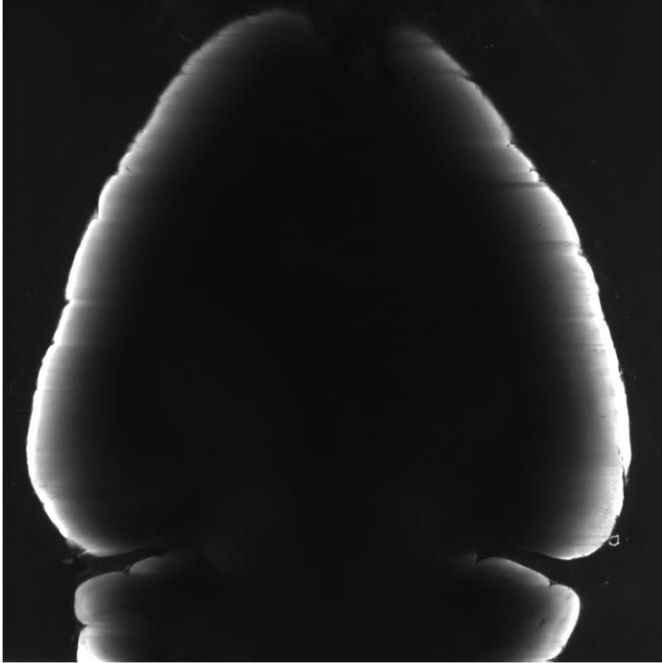
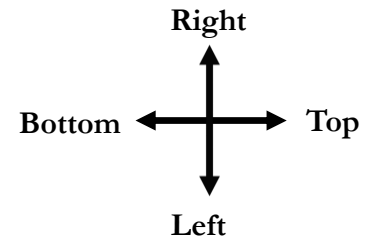
↓

← 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



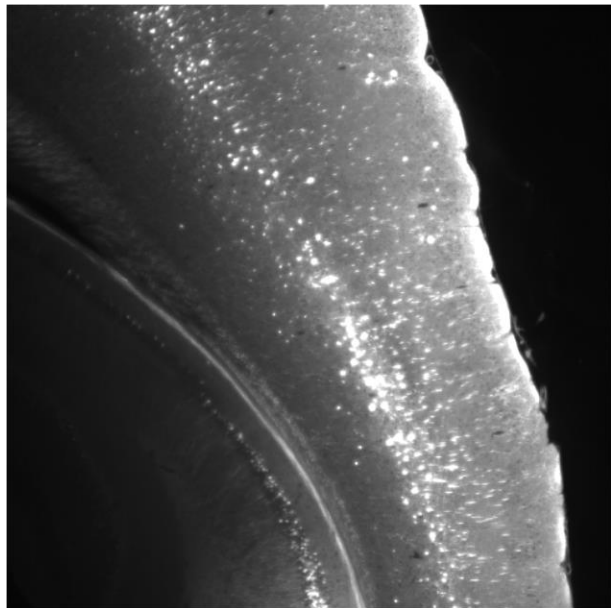
647 nm + Quadrupleband

What happens if the tunable lens parameters are incorrect?

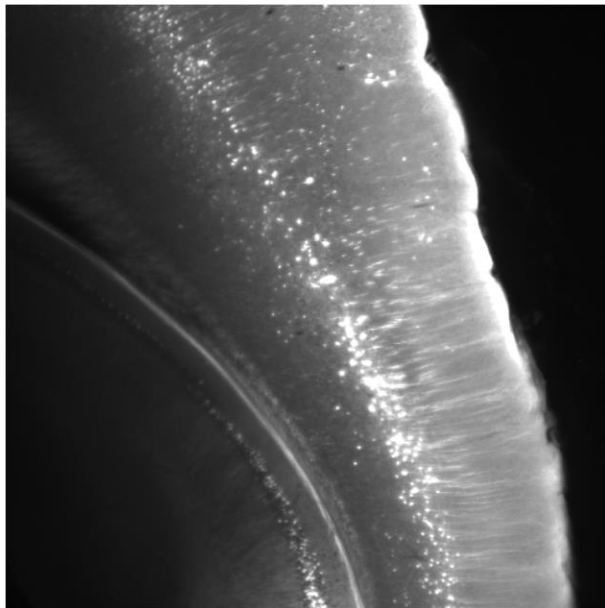


A thicker light-sheet leads to more features being visible at the expense of contrast

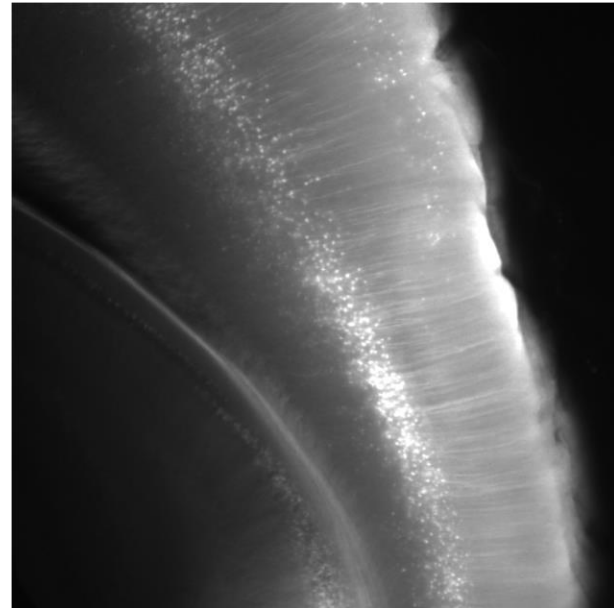
Thy1-YFP (H-line), 488 nm excitation, zoom 4



ETL offset = 2.47 V
ETL amplitude = 0.17 V



ETL offset = 2.47 V
ETL amplitude = 0.0 V



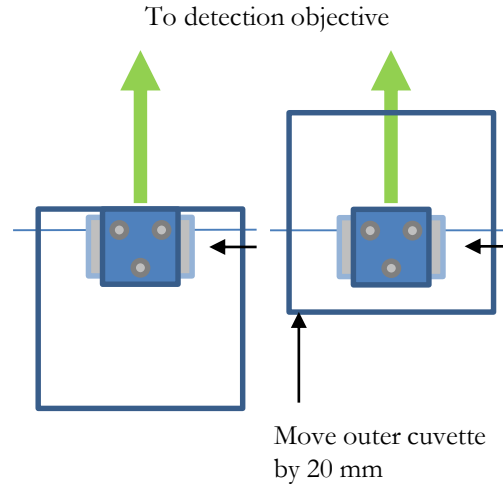
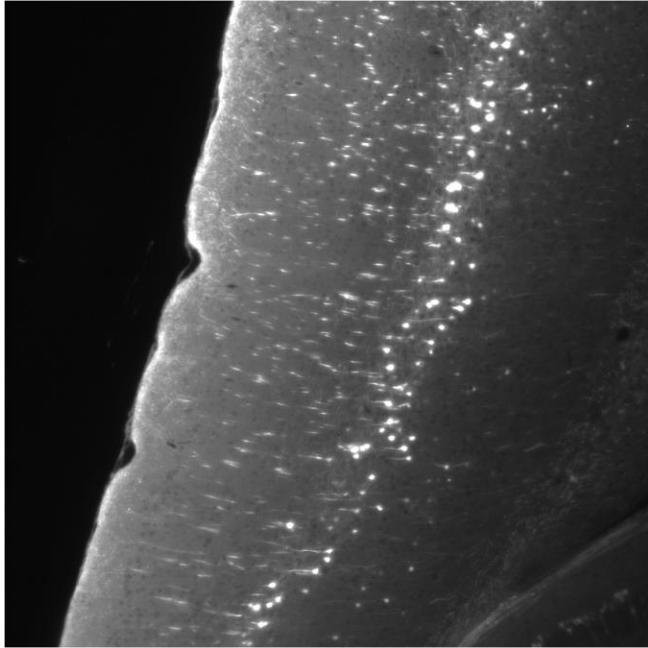
ETL offset = 2.2 V
ETL amplitude = 0.0 V



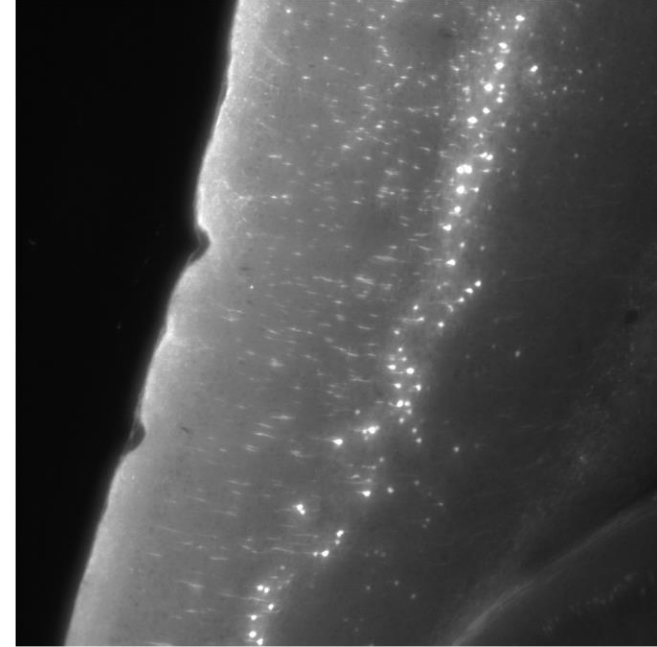
Counterstained cell-bodies and blood-vessel cross-sections are usually good structures to judge this

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

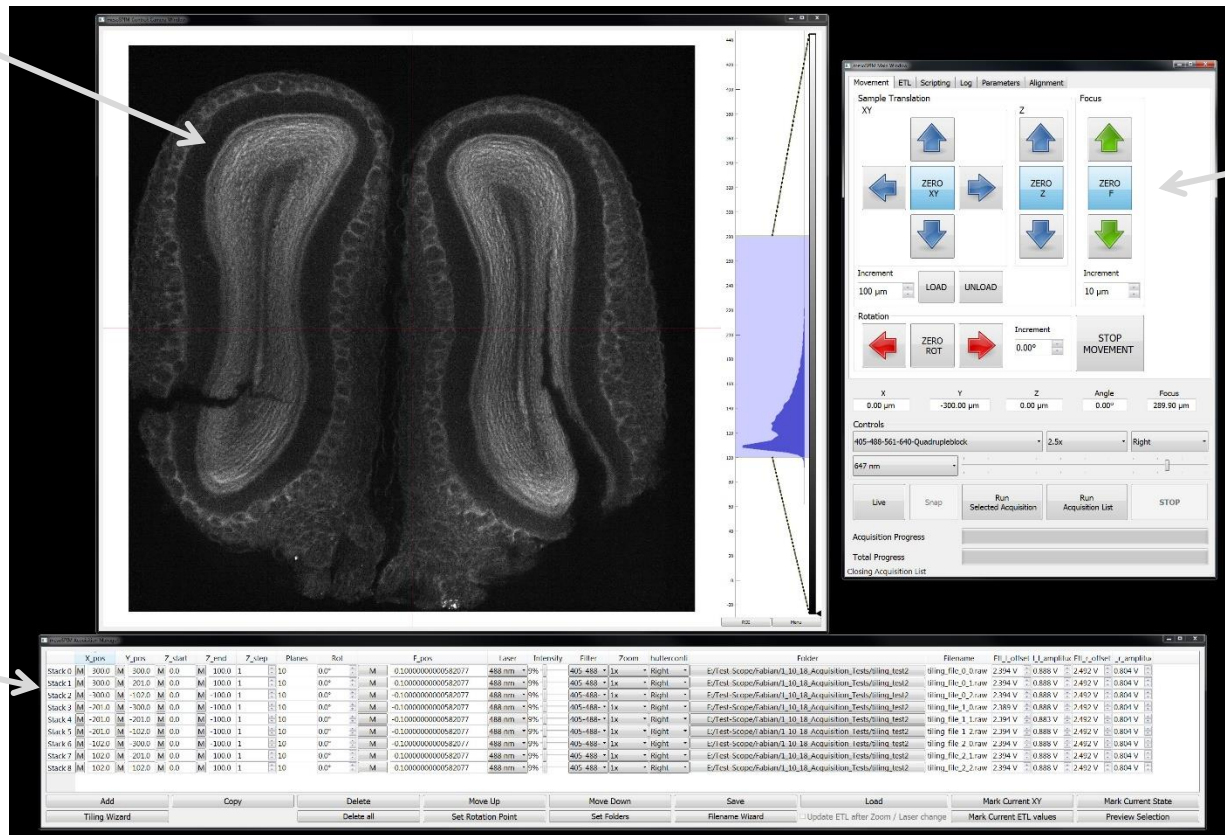
Part IV: User Interfaces

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 axis controls.
- 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 movement, 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 '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'. A 'Config File:' section shows the 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 config file path.
- Positioning:** A row of five fields shows '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 '4x' magnification dropdown, a 'Right' camera dropdown, and a '647 nm' wavelength dropdown. Below these are buttons for 'Live', 'Snap', 'Run Selected Acquisition', 'Run Acquisition List', and 'STOP'.
- Progress:** At the bottom, there are two green progress bars. The first shows 'Acquisition Progress' at '100% (Image 3100/3100)'. The second shows 'Total Progress' at '100% (Acquisition 3/3) (Image 9300/9300)'. A 'Closing Acquisition List' label is at the very bottom.

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 μ 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 μ m Y 900.03 μ m Z -1900.03 μ m Angle 0.00° Focus 40.00 μ 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

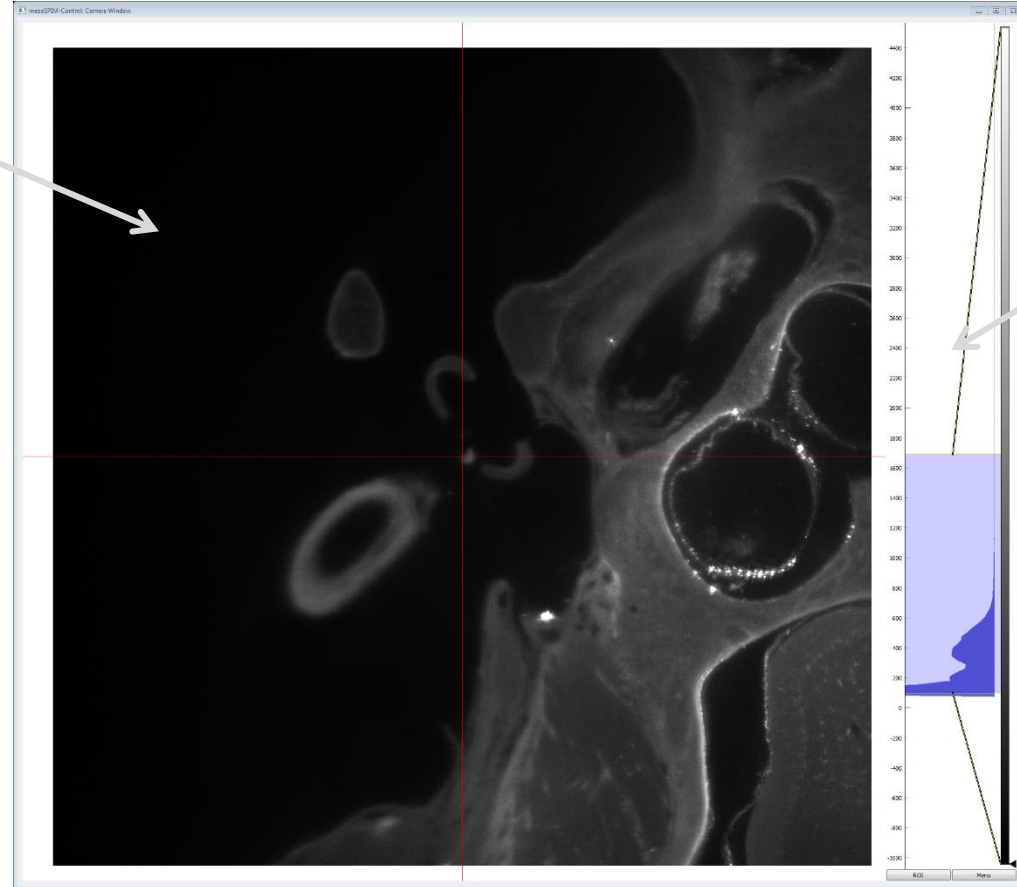


mesoSPIM-control: Camera window

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	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, ETL_offset, I_offset, I_amplitude, ETL_r_offset, and r_amplitude. Below the table is a control bar with several buttons and options. The buttons are: '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', and 'Preview Selection'. The 'Save' and 'Load' buttons are highlighted with a red box. The 'Mark Current XY', 'Mark Current State', 'Mark Current ETL values', and 'Preview Selection' buttons are highlighted with a green box. Arrows point from text labels below to specific buttons: 'Tiling wizard' points to 'Tiling Wizard', 'Set rotation point' points to 'Set Rotation Point', 'Set folder for all rows' points to 'Set Folders', 'Autogenerate filenames' points to 'Filename Wizard', and 'Update parts of a selected table entry & preview' points to the green box.

	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

Tiling wizard

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

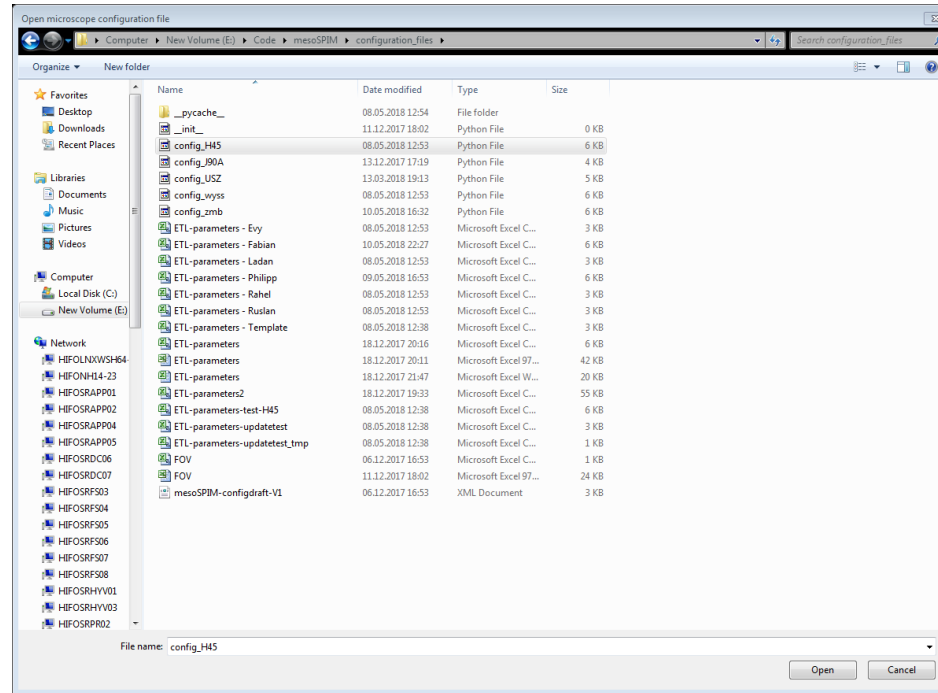
Autogenerate filenames

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»)

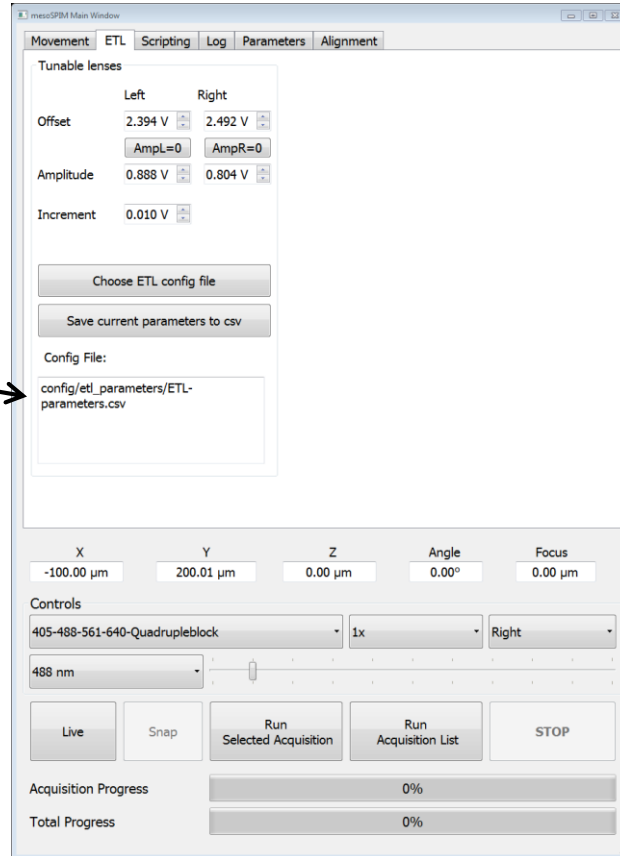
mesoSPIM-control: Startup I



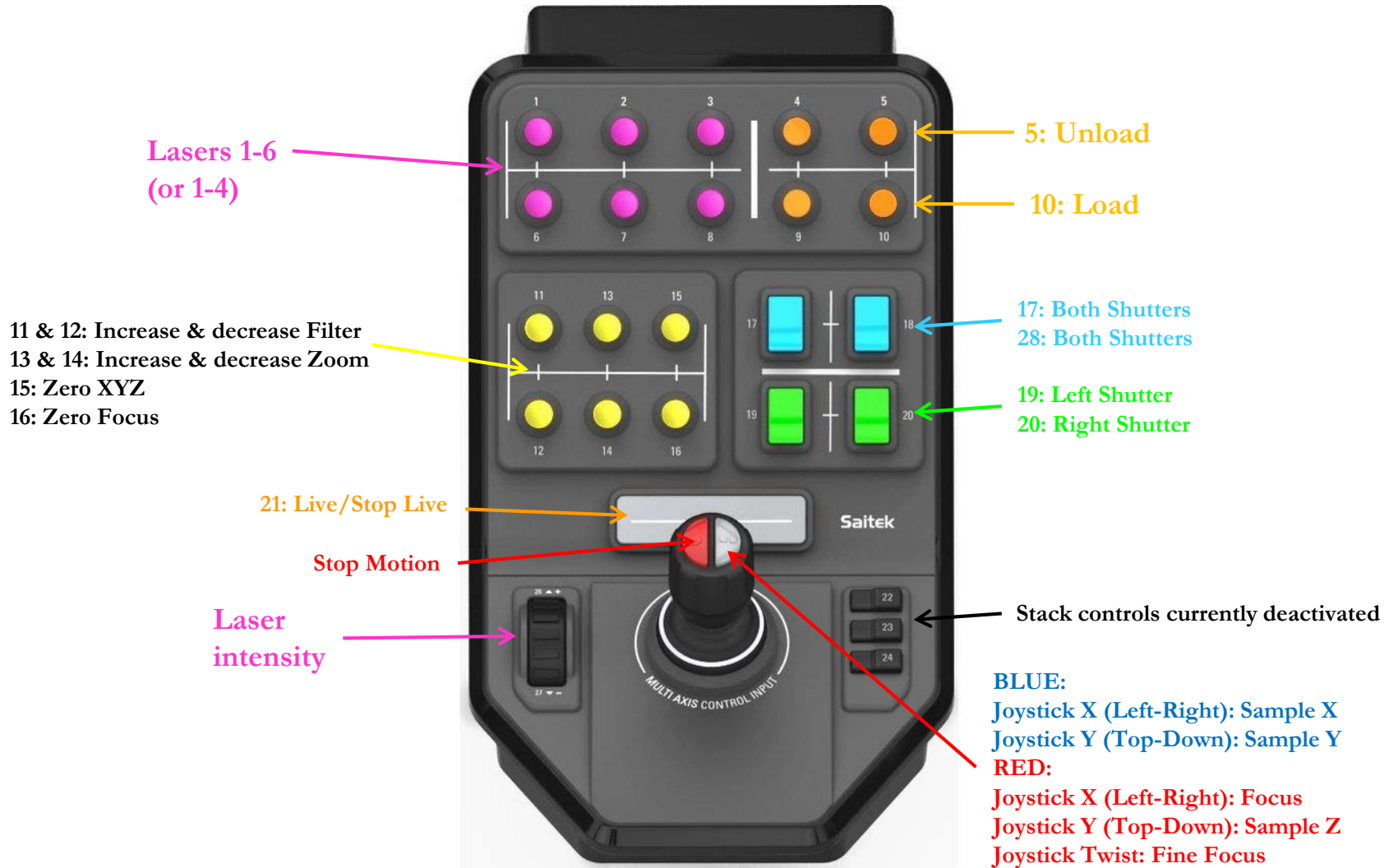
1. Choose config file according to your setup. This is a .py file containing the microscope configuration, NOT a ETL-parameter file.
2. The software takes 10-15s to start while performing a reference move with the focusing stage

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 HCLImage 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»)

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

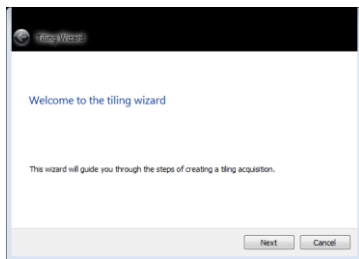
Data is saved as raw image streams, the «.raw» file extension should be used

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

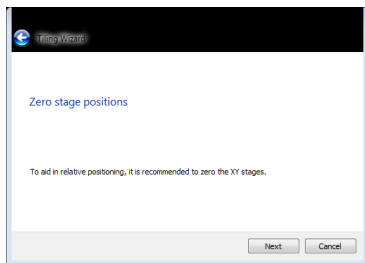
Mark X + Y position
For a selected row

Tiling Wizard

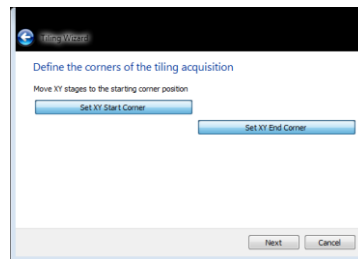
1. Start the Tiling Wizard



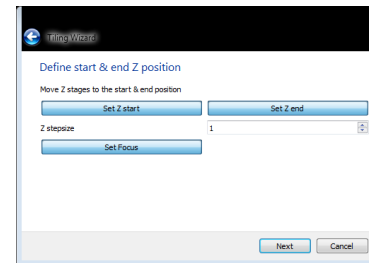
2. If desired, zero the stages using the buttons in the main user interface



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



4. Define Start & End Position & Focus

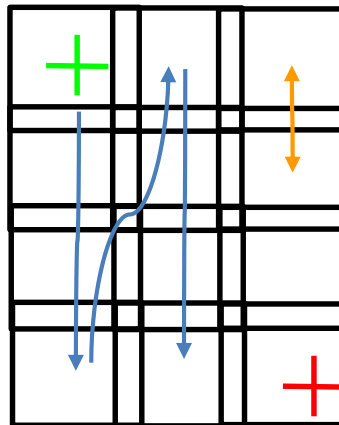


For some reason, screenshots have a «black bar» on top (not visible in the window during usage)



The first FOV is centered on the start position

Start



Offset



«Offset» is the mechanical FOV-to-FOV (ΔX or ΔY) 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	l_l_amplitud	Etl_r_offset	l_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

mesoSPM Acquisition Manager

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

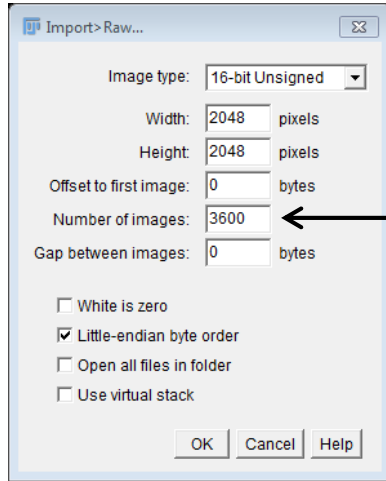
[ETL PARAMETERS]
[ETL CFG File] E:/Code/mesoSPIM-control/mesoSPIM/config/etl_parameters/ETL-parameters - Fabian - DBE - H45.csv
[etl_l_offset] 2.5479999999999983
[etl_l_amplitude] 0.5819999999999999
[etl_r_offset] 2.732
[etl_r_amplitude] 0.4099999999999999

[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

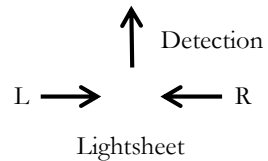


Number of images can
be larger than the number of acquired images,
the import will work normally

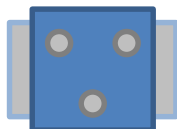
3. The stack will open in the center

Controlling sample rotation

Rotations of a 20 mm cuvette:

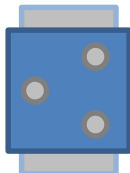


0/360°

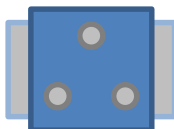


Position mark
↑

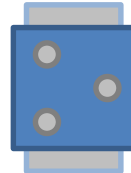
90°



180°



270°



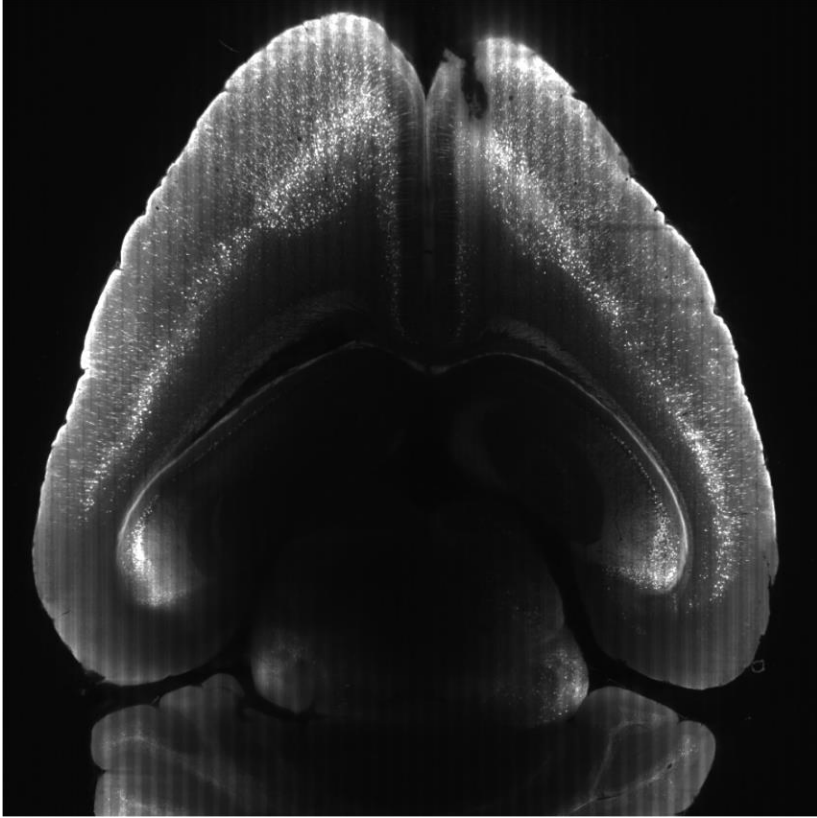
Viewed from the top,
positive rotations are oriented
clock-wise



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

Part VI: Troubleshooting

Horizontal Stripes in the image

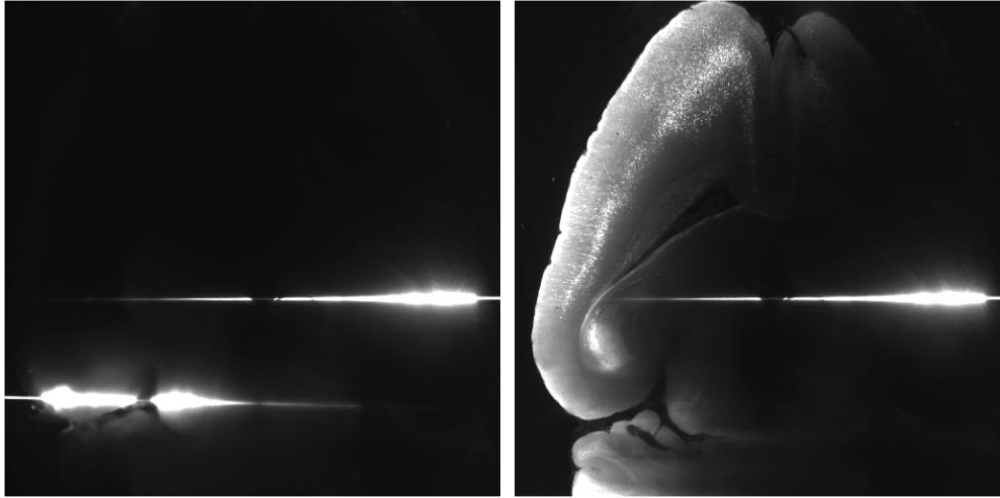


Stripes indicate that the Galvo Frequency is not averaged out by the exposure.

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:

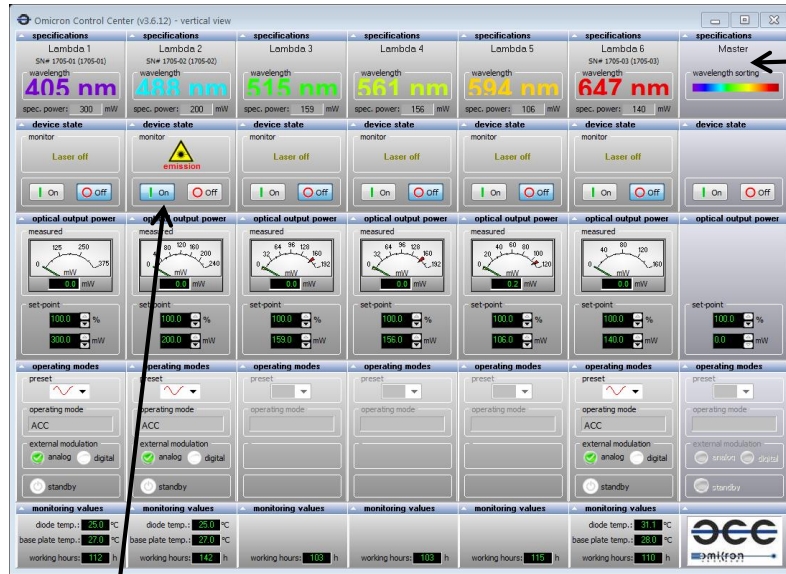
- Galvos are switched off
- Galvos switched off due to overheating (usually at amplitudes >3.7 V)



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

