

# MesoSPIM Part 2: Image acquisition

This guide explains the steps required to acquire a z-stack on the MesoSPIM lightsheet microscope.

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mesoSPIM Main Window

File View

Movement

ETL Scripting

Log

Parameters

Alignment

Sample Translation

XY

↑

←

ZERO XY

→

↓

Increment

500 μm

LOAD

UNLOAD

Z

↑

ZERO Z

↓

Increment

500 μm

Focus

↑

ZERO F

↓

AUTO Focus

Increment

500 μm

Rotation

←

ZERO ROT

→

Increment

0.00°

STOP MOVEMENT

X

1182.9 μm

Y

5499.3 μm

Z

5593.7 μm

Angle

0.0°

Focus

3000.0 μm

Controls

405-488-561-640-Quadrupleblock

1x

Right

488 nm

10 %

Live

Snap

Run Selected Acquisition

Run Acquisition List

STOP

Acq. Progress

0%

Total Progress

0%

mesoSPIM-Control: Camera Window

Adjust levels

Overlay: none

Image dimensions: (24, 1024)

3000

2800

2600

2400

2200

2000

1800

1600

1400

1200

1000

800

600

400

200

ROT

Norm

mesoSPIM Acquisition Manager

	X_pos	Y_pos	Z_start	Z_end	Z_step	Planes	Rot	F_start	F_end	Laser	Intensity	Filter	Zoom	hutterconfi	Folder	Filename
Stack 0	0	0	0	100	10	10	0	0	0	488 nm	0	Empty-Al...	1x	Left	tmp	one.raw

Add Row

Duplicate Row

Delete Row

Move Row Up

Save Table

Tiling Wizard

Image Processing Wiz

Mark Current XY

Mark Rotation

Preview Selected Row

Delete all Rows

Move Row Down

Load Table

Filename Wiz

Focus Tracking Wiz

Mark Focus

Mark ETL values

Preview: Move to Z start

Predicted time (HH:MM:SS): 00:00:02

Set Folders

Mark State

Mark ALL

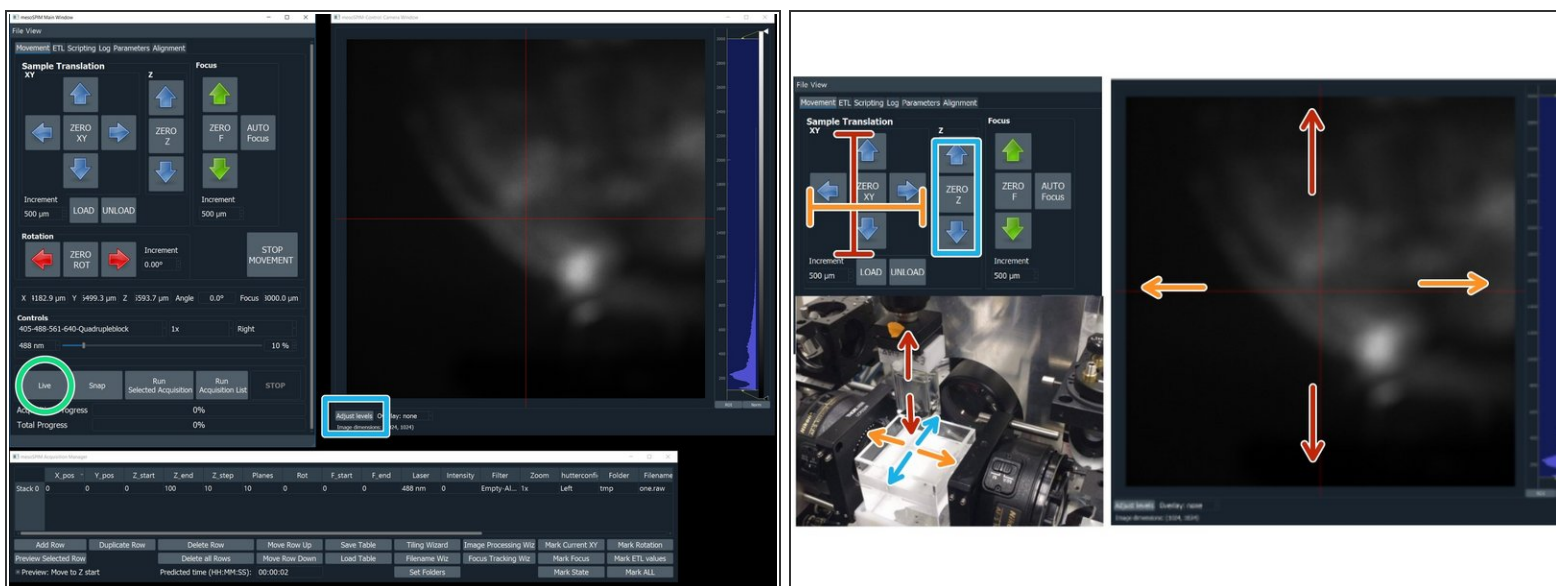
## INTRODUCTION

In this guide for the ZMB MesoSPIM lightsheet microscope you learn how to locate and focus a sample as well as setting up and acquiring a z-stack.

For a full documentation about the microscope please, visit the project webpage:

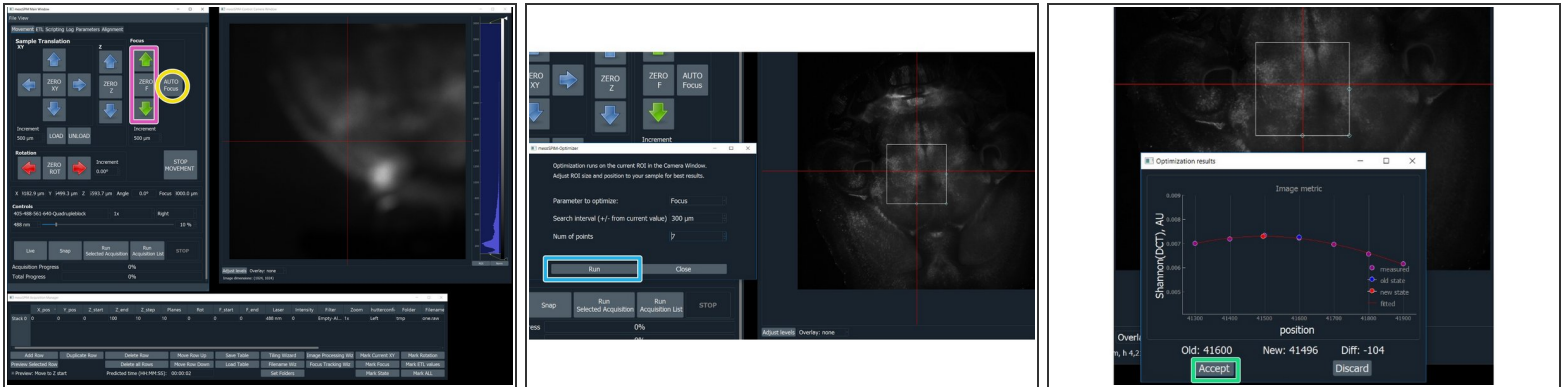
<http://MesoSPIM.org>

## Step 1 — Find and center the sample



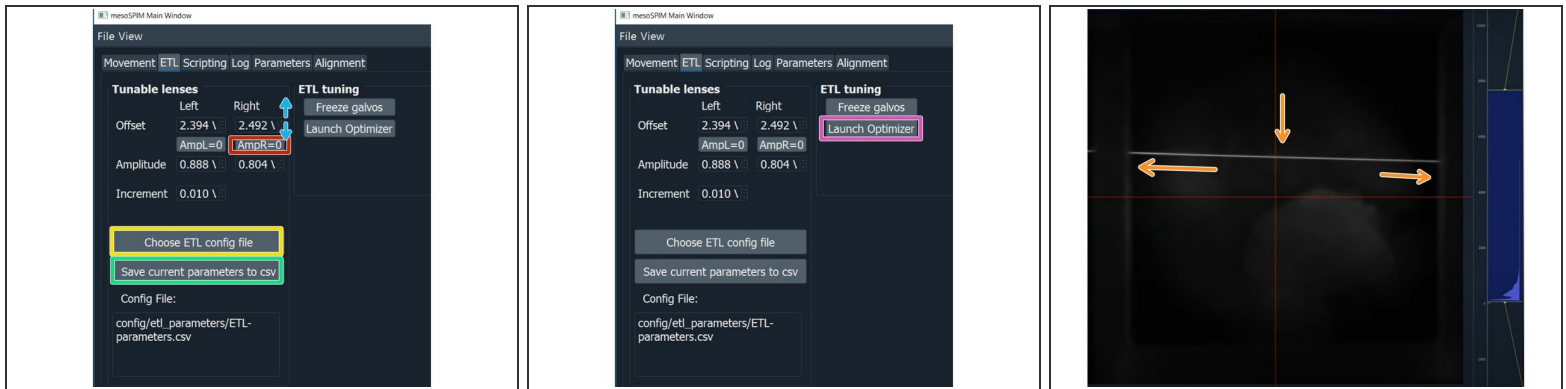
- Press "live" to get the first image and stop.
  - ❗ Default settings: Right laser illumination; Zoom: 1; 488 nm; Filter 405-488-561-640-quadrupleblock filter. Change this in the corresponding boxes if you need other settings.
  - Click "Adjust Levels" for adjusting the histogram.
  - Use the Y arrows to move the sample up or down (Y).
  - Use the X arrows to move the sample left or right (X).
  - Use the Z arrows to bring the sample into the plane of the lightsheet.
- ⚠ It is recommended, after some movements, to stop the scan and check the position of the cuvettes to avoid collisions between the inner and outer cuvettes.

## Step 2 — Focusing the sample



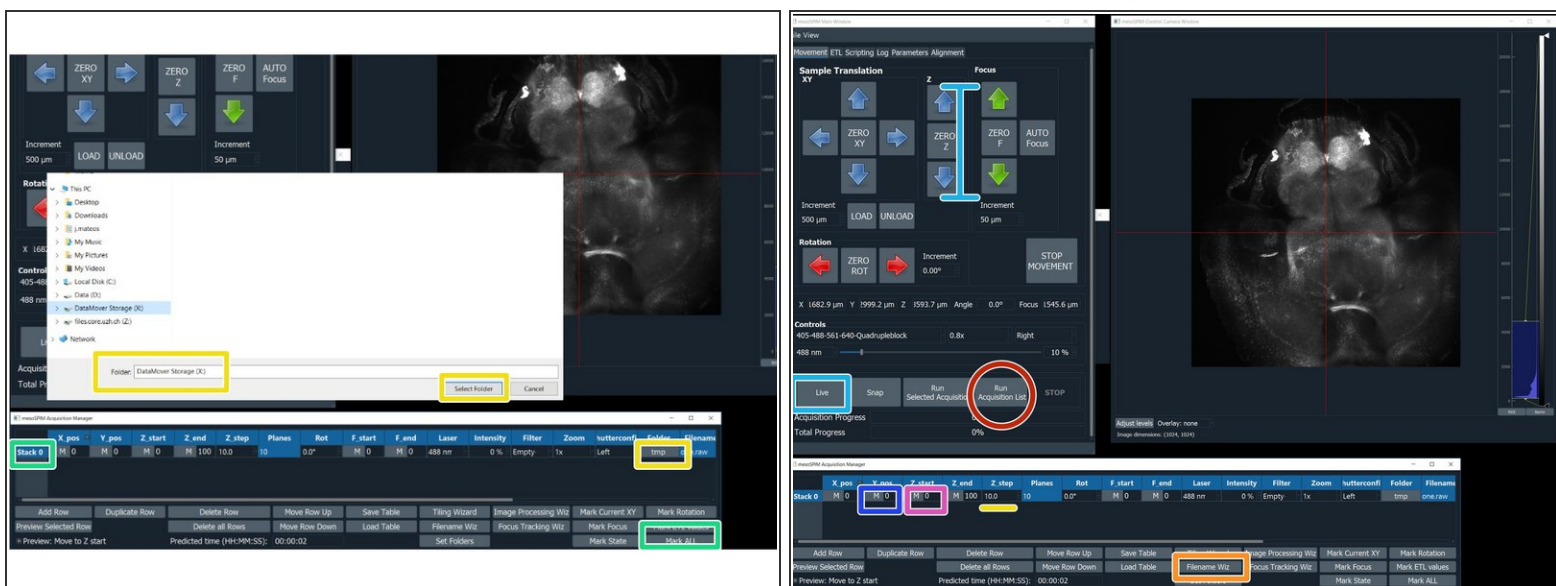
- Manual Focus: use the Focus arrows to focus the lightsheet. For a fine focus, change the focus "Increment" to 20  $\mu\text{m}$ .
- AUTO Focus: click on the "AUTO Focus" to launch an optimizer that lets you automatically search for the best focus within a given range from the current position.
- Click on "RUN"
- Once the autofocus has run a new window will appear showing the optimization results. If you are satisfied with the new focus click "Accept".

## Step 3 — Adjustment of ETL parameters



- Click "Choose ETL config file", select your ETL parameters file. The file is also specific for the clearing method you are using.
- **Manual ETL adjustment with sample**: For the ETL optimization press on "AmpL = 0" to set the ETL amplitude to 0.
- Hover with the mouse arrow over the corresponding offset value and use the mouse wheel to adjust it. Find the waist in the camera image and bring it to the center.
- Press again on "AmpL = 0" to set the value back to the stored value.
- Press "save current parameters to csv".
- **ETL parameter optimization**: It best performs using a highly transparent sample with sparse but evenly distributed labeling, such as vasculature.
- **Manual method without sample** using a parked galvo and scattered laser beam:  
["https://www.youtube.com/watch?v=dcJ9a7VA..."](https://www.youtube.com/watch?v=dcJ9a7VA...)

## Step 4 — Z-stack acquisition



- In the "mesoSPIM Acquisition Manager", click on "Stack 0", followed by "Mark ALL"
- **Saving data:** Click on "Folders" and select the Network drive X:\
- Press live and use the Z arrows to select the beginning of the stack.
- Click on the "M" button to the left of the "Z\_start" to record this position.
- Use the Z arrows to localize the end of your stack. Then press the "M" button to the left of "Z\_end".
- Insert the step size in micrometers. The number of planes will be automatically calculated.
- Click "Filename Wizard". In the appearing menu select "save as" BigDataViewerfile: h5; give a name to the file and press "finish".
- Click "Run Acquisition List"