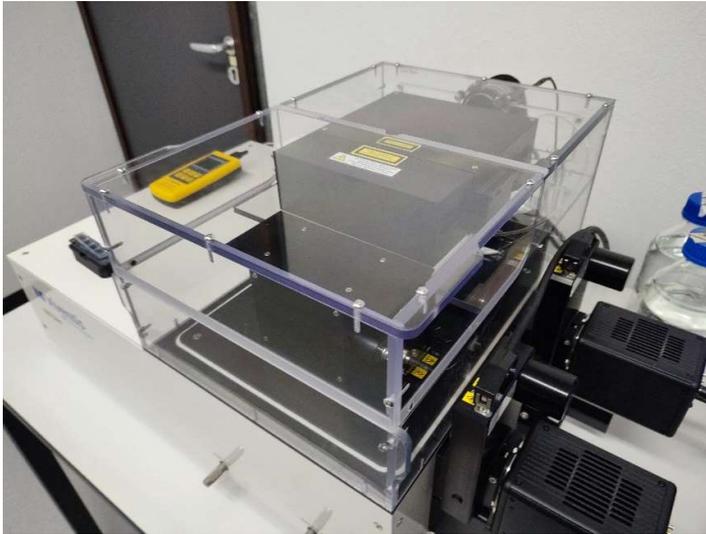


Leica Viventis LS2 Live (Deep)



CO2 : input pressure around 1.2-1.5 bar

Chamber water : Bidistilled + Autoclaved / Change 1X/Week

Computer : User account / Pass: LS2Live

Technical Information

Lens : 25X

Field of view : 600um

Pixel size : 260 nm

Lasers : 488nm / 561nm /638nm

Laser intensity from 20% to 100% (not linear below 20%)

Additional filter wheel with ND filters (OD1>10%, OD2>1%, OD3>0.1% and OD4>0.01%)

[Good starting setting 50%laser + OD2 > 5% Laser]

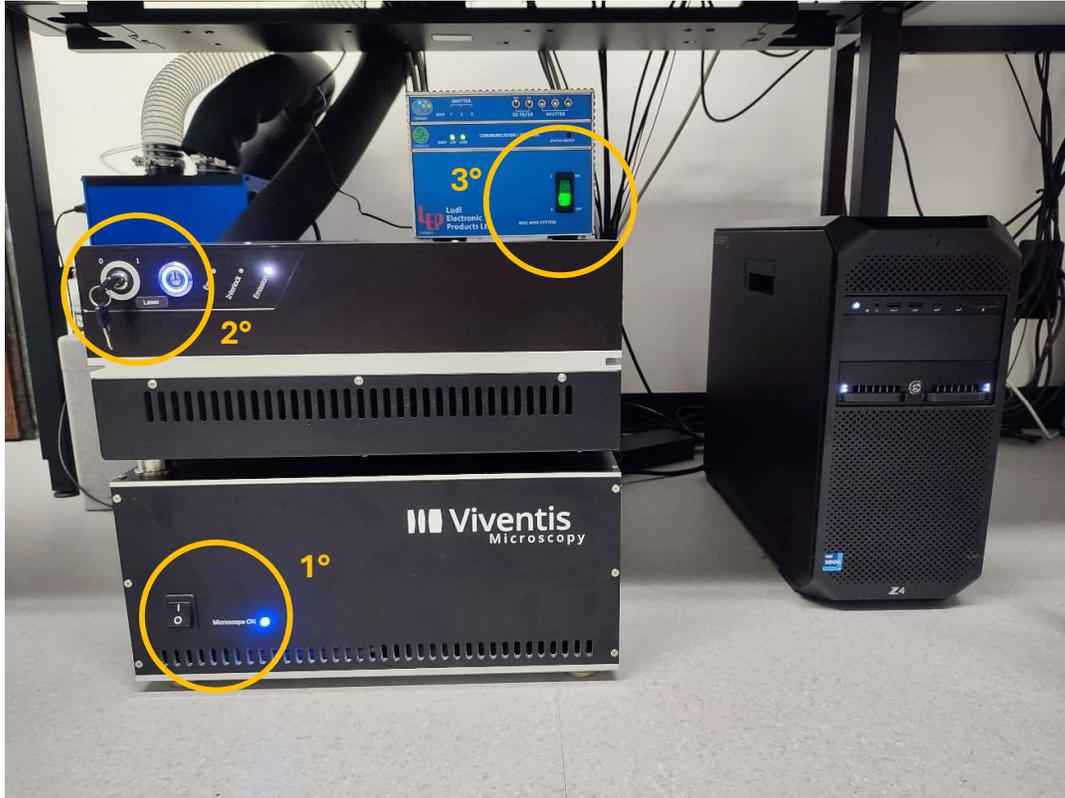
Heatin block (Cube) : Around 39.5°C to have chamber at 37°C / Turn ON the day before (or always ON)

Joystick :	X > long axis (50mm range)	Clockwise goes right
	Y > Vertical axis	Clockwise goes up
	Z > Back/Forth axis	Clockwise goes back

Switching ON

[Electronics should be turned ON 3-4H before usage (heat production > lens dilatation)]

Under the table :

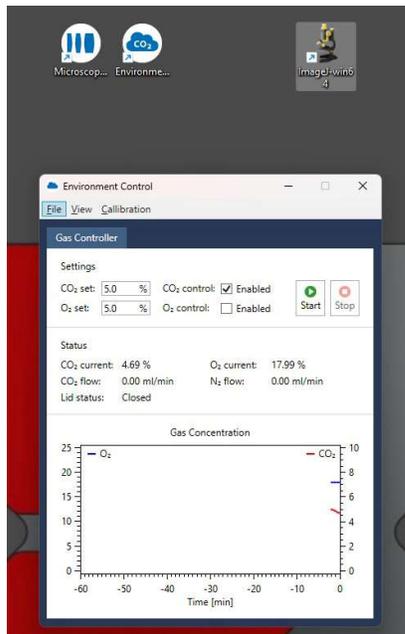


- 1° Black box
- 2° Laser combiner (button + key)
- 3° Controller of EMM filters (blue box) – Only the ON button (switches should be neutral/neutral/down/down/down)
- 4° Both cameras ON

Wait for initialization before opening Software.



Environment Control



You can control the temperature with the yellow thermometer



Only if **CO₂** is needed :

4° Open CO₂ Bottle (1.2-1.5 bar)



- 4''° Start Environment Control and **start** CO2
 - 4'''° **Minimize** window (don't close it or it will stop CO2)
- 5° Start **Microscope Control** software



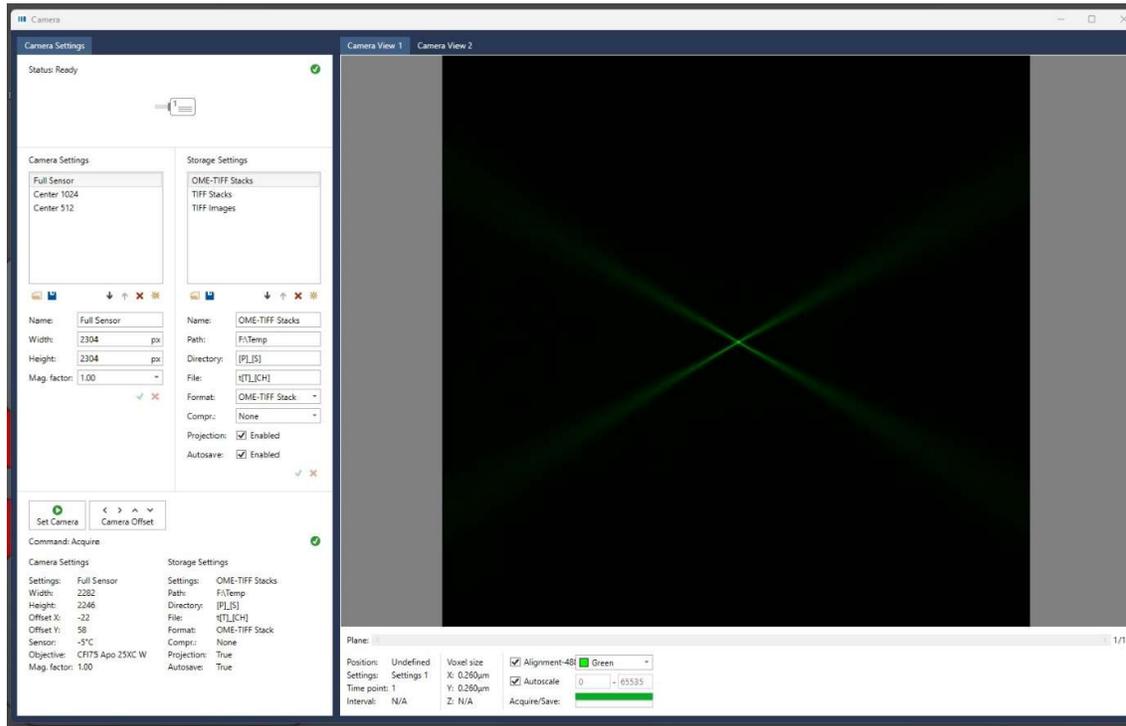
[Sample stage initialization : NO SAMPLE SHOULD BE INSIDE ! MUST BE CHECKED !]

Microscope Alignment

[Bring both lightsheets on **sample plane** AND in **focal plane**]

> Takes only a few minutes

1° **Snap** > 2 beams (static) [notice 2 cameras views]



If no beams > Problem

A. Laser not ON

B. No water in chamber

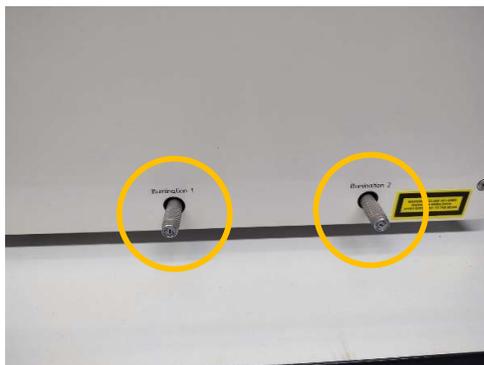
Now we will align both beams and both camera views (exactly identical). Camera 2 automatically and camera 1 with the micrometric screw.

2° Chose **ADVANCED MODULES** (Top left) > **MICROSCOPE ALIGNMENT**

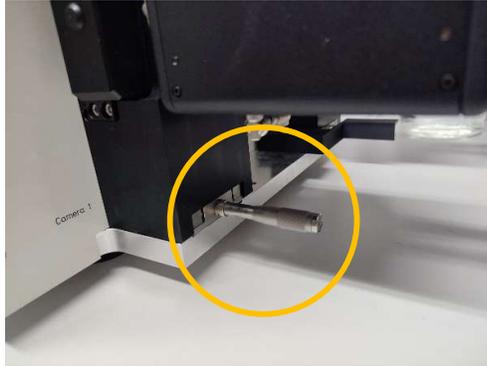
A. **Align and center beams** > Click **START** (*it automatically aligns beams*)

[If you right click image you can get overlays to see more easily the center]

- Center **Beam Waists** in the **Green Zone** using **Front Micrometer Screws 1 and 2** (follow instructions given in the black area) [*something around 1 turn should be enough*]
- Click **STOP**



- B. **Align and center views** (Focal plane of both cameras) > Click **START**
- Turn **Camera 1 micrometer screw** to go into the **Green Zone**
 - Click **STOP**



*[Wait for **XY centering (Digital)** before closing Microscope Alignment window > both images will be exactly identical]*

Now the microscope is ready to use. The sample can be loaded.

INSERTING SAMPLE

Click **LOAD**



The sample holder goes UP (out of the water)

When the sample is in position, click **IMAGING**

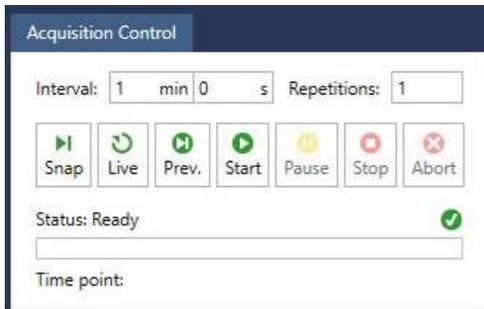
The sample holder goes DOWN (in the water)

[In Y, -1.5mm is the limit before the lens touches the holder, In X, +-25mm is the limit]

SOFTWARE

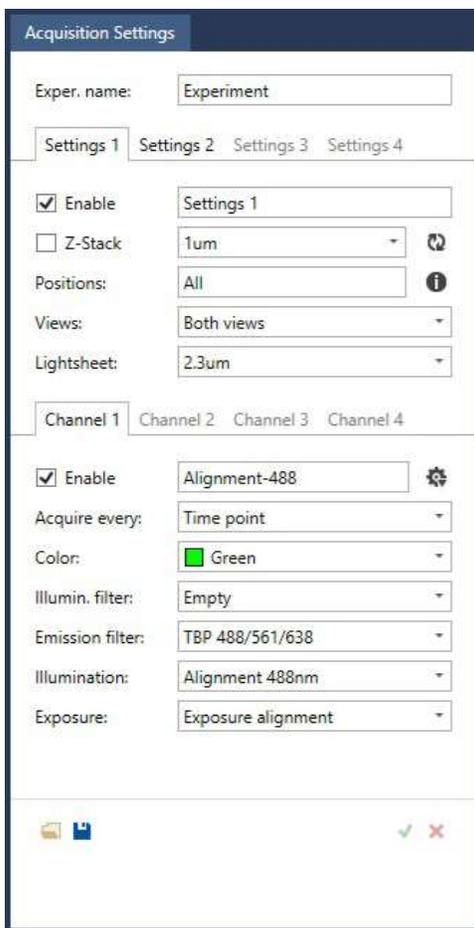
[Remember > Any change must be confirmed with ENTER or ✓]

Top : Acquisition Ctrl.



The Acquisition Control window features a title bar with the text "Acquisition Control". Below the title bar, there are two input fields: "Interval: 1 min 0 s" and "Repetitions: 1". A row of seven control buttons follows: "Snap" (green play icon), "Live" (green refresh icon), "Prev." (green double left arrow), "Start" (green play icon), "Pause" (yellow double vertical bar), "Stop" (red square), and "Abort" (red X). Below the buttons, the status is shown as "Status: Ready" with a green checkmark icon. At the bottom, there is a "Time point:" label and an empty input field.

Bottom : Acquisition Settings



The Acquisition Settings window has a title bar "Acquisition Settings". It starts with "Exper. name: Experiment" in a text box. Below are four tabs: "Settings 1", "Settings 2", "Settings 3", and "Settings 4". Under "Settings 1", there is a checked "Enable" checkbox, a "Z-Stack" checkbox (unchecked), "Positions: All", "Views: Both views", and "Lightsheet: 2.3um". Below these are four channel tabs: "Channel 1", "Channel 2", "Channel 3", and "Channel 4". Under "Channel 1", there is a checked "Enable" checkbox, "Acquire every: Time point", "Color: Green", "Illumin. filter: Empty", "Emission filter: TBP 488/561/638", "Illumination: Alignment 488nm", and "Exposure: Exposure alignment". At the bottom, there are icons for a folder, a document, and a green checkmark/red X.

[Any change must be confirmed with ENTER or ✓]

✓ Z-stack

Positions : All > Same parameter for all ex.)

Selection (1-2 if settings for positions 1 and 2 for

Position Settings

Center

Center

Position X: 0 μm

Position Y: 0 μm

Position Z: 0 μm

Skip:

Update ✓ ✗

To define positions:

- Type a **name** : (**Position A** for ex.)
- Move to the desired position with the control
- Click **Update**

Views : Both views = both cameras (if sample thick)

Lightsheet : thickness (thicker is more homogenous on the FOV)

Z-Stack Settings

1um

2um

5um

1um

Step: 1 μm

Planes: 201

Range: 200 μm

✓ ✗

Illumination Filter (ND) to control intensity of excitation (OD1>10%, OD2>1%, OD3>0.1% and OD4>0.01%)

Illumination Settings

Alignment 488nm
Laser 488nm
Laser 561nm
Laser 638nm
Transmitted

↓ ↑ ✕ ✨

Name:	Alignment 488nm
488nm:	100 %
561nm:	0 %
638nm:	0 %
Transm.:	0 %

✓ ✕

[Good starting setting 50%laser + OD2 > 5% Laser, not less than 20% laser (not linear anymore)]

[Good Exposure : 50->100ms]

Range : Size of the plane of the Lightsheet

Z-Stack Settings

1um
2um
5um

↓ ↑ ✕ ✨

Name:	1um
Step:	1 μm
Planes:	201
Range:	200 μm

✓ ✕

[use 800um to cover the whole field]

HOLDER

To put sample holder (or take it out), bring it out of the water : Y UP (clockwise)

[In Y, -1.5mm is the limit before the lens touches the holder, In X, +-25mm is the limit]

CAMERA SETTINGS

Change Crop to use part of the chip (smaller data)

*[Don't forget to click **SET CAMERA** to confirm any change]*

SAVING DATA

In *F:Temp*

FUSING DATA

Chose **ADVANCED MODULES** (Top left) > **FUSE VIEWS**

- **Reference channel** : Name (name of laser normally (488,...))
[Chose most homogenous one]
- Select **positions** (eliminate bad ones)
- Click **START**

TRACKING

[To keep samples in the field of view, in Z also]

Chose **ADVANCED MODULES** (Top left) > **OBJECT TRACKING**

[The experiment should have already started before you use tracking (at least 1 acquisition done)]

1° Use **Offline Mode** to optimize

Enter folder path of the already acquired sample

Choose the type of **projection** : Max or Average (smoother, better for Center of Mass)

2° Now chose **Online mode** to **start** the tracking