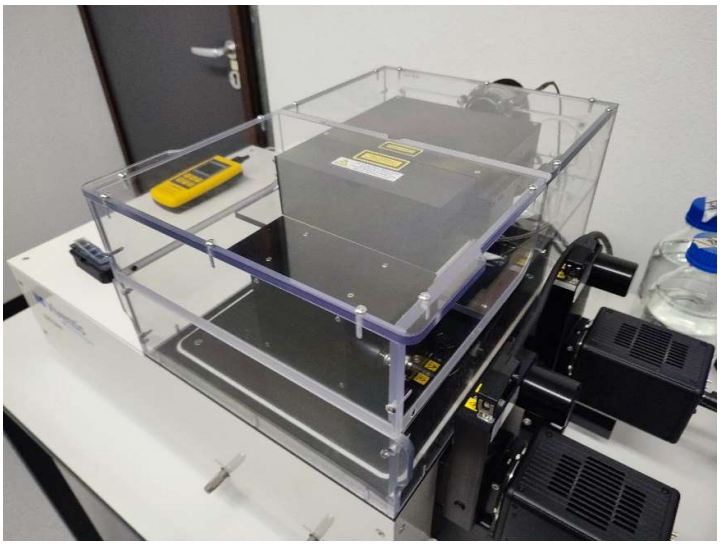
Leica Viventis LS2 Live (Deep)



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]

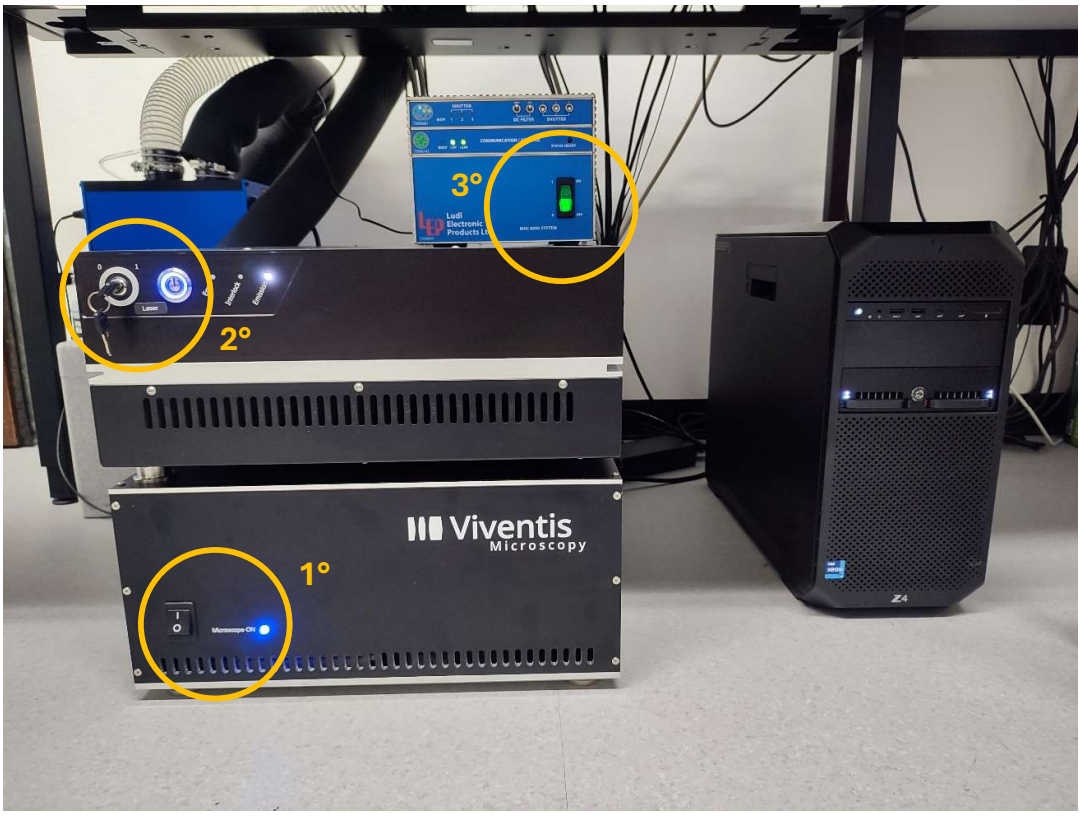
Heatin block (Cube) : Around 39.5°C to have chamber at 37°C / Turn ON the day before (or always ON)

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 :



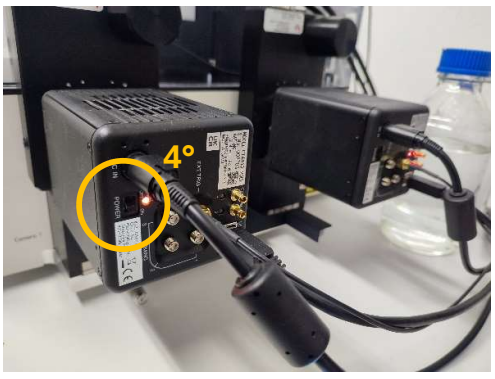
1° Black box

2° Laser combiner (button + key)

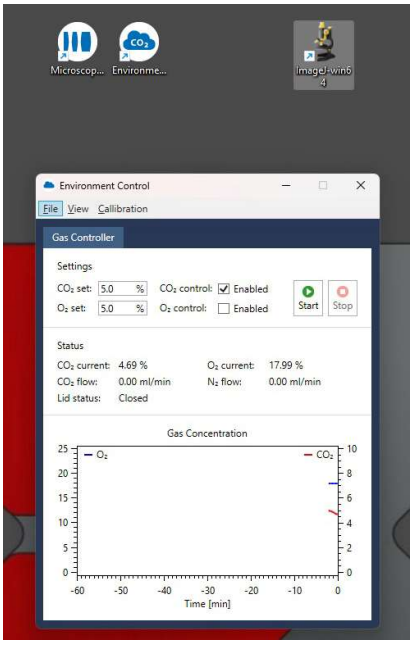
3° Controller of EMM filters (blue box) – Only the ON button (switches should be neutral/neutral/down/down/down)

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

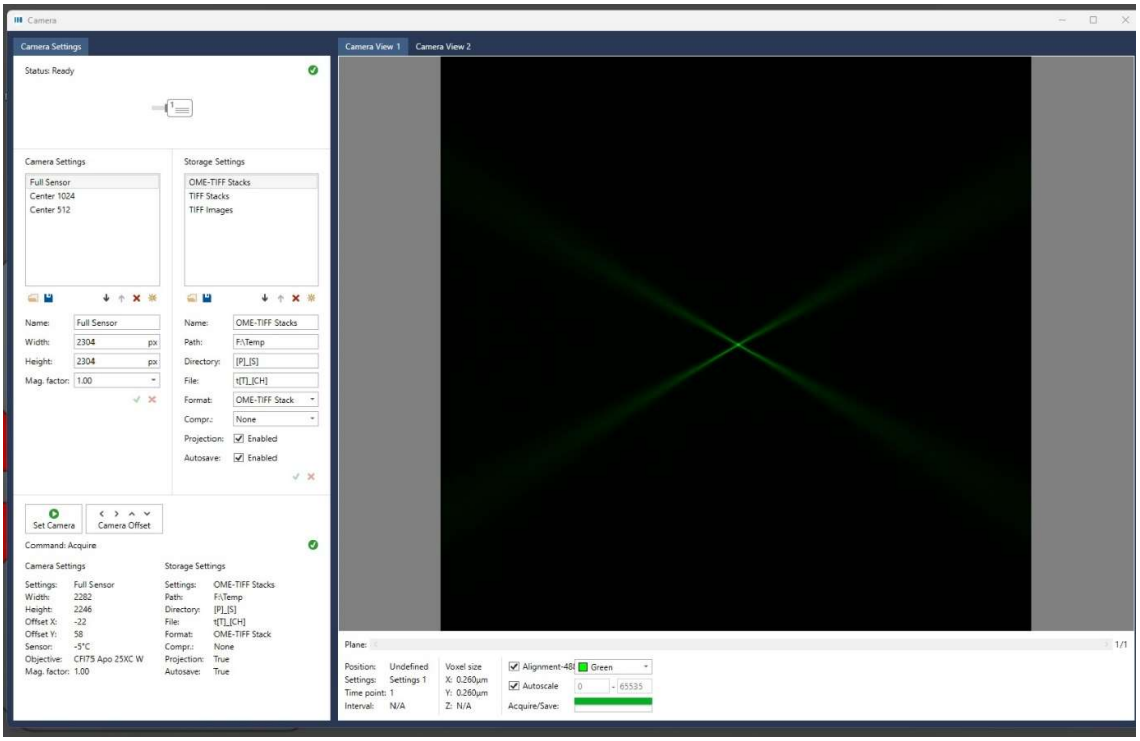
[Sample stage initialization : NO SAMPLE SHOULD BE INSIDE ! MUST BE CHECKED !]

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]

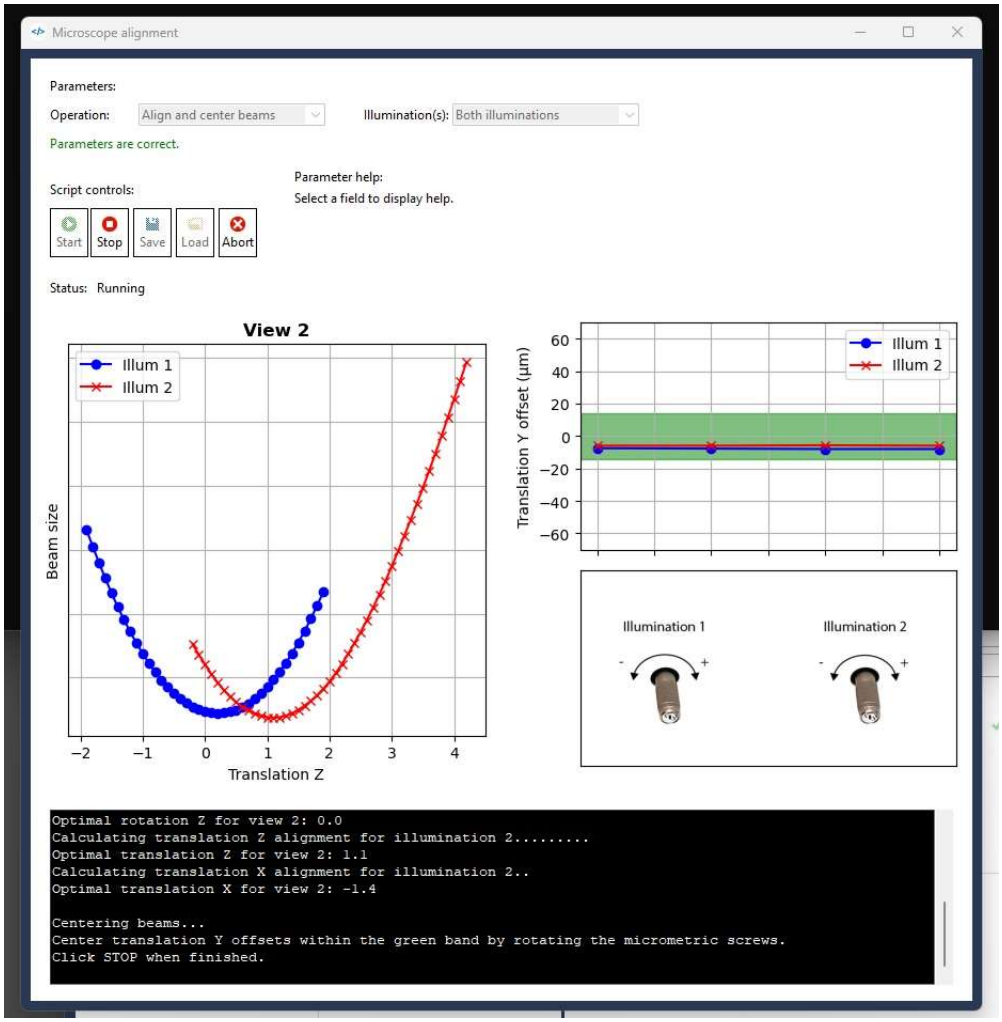


If no beams > Problem A. Laser not ON B. No water in chamber

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

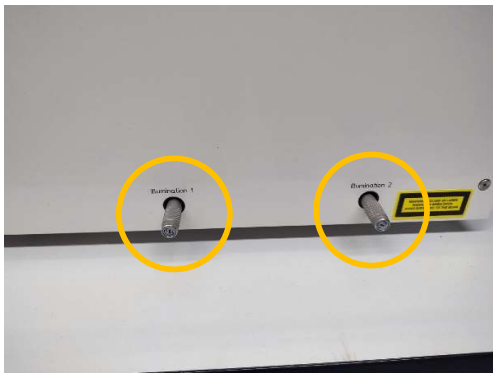
2° Chose **ADVANCED MODULES** (Top left) > **MICROSCOPE ALIGNMENT**

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



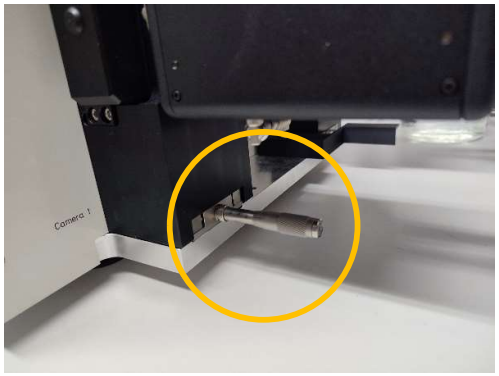
[If you right click image you can get overlays to see more easily the center]

- 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**

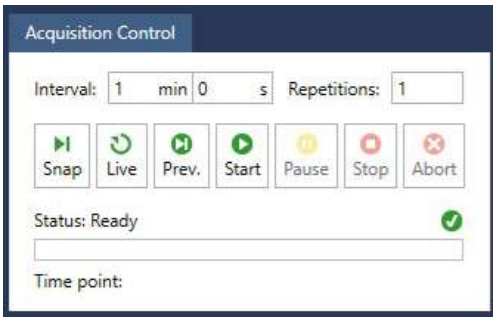
The sample holder goes DOWN (in the water)

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

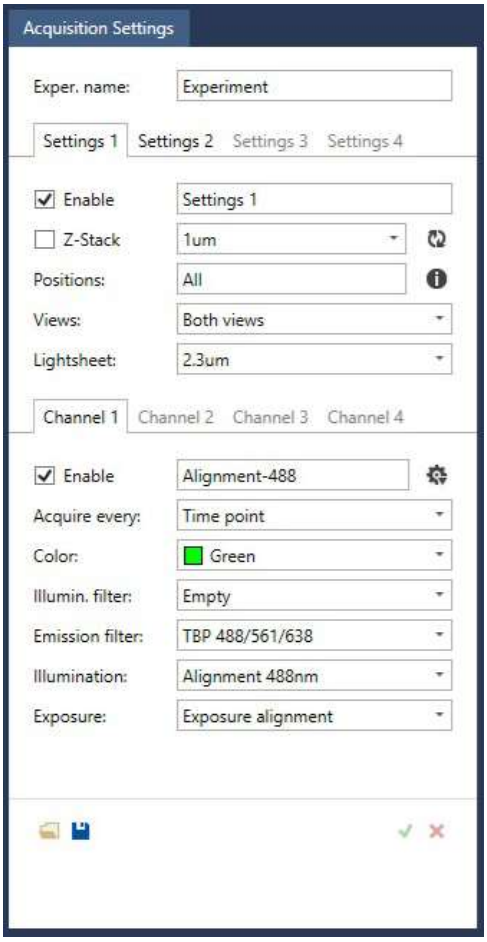
SOFTWARE

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

Top : Acquisition Ctrl.



Bottom : Acquisition Settings

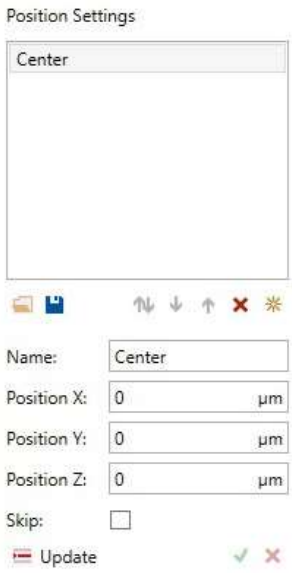


[Any change must be confirmed with ENTER or ✓]

✓ Z-stack

Positions : **All** > Same parameter for all ex.)

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



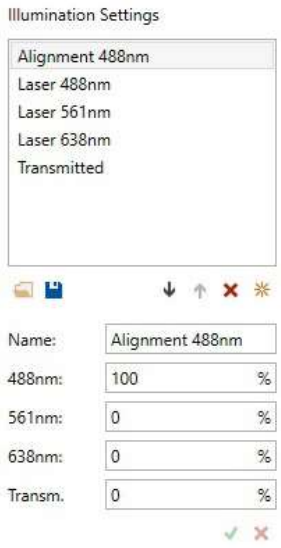
To define positions:

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

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

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

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



[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

[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