

Bruker Luxendo InVi SPIM Lattice Pro

Quick start guide

29 October 2022

This is a quick reference for users who have been trained on the system. See the user manual for detailed operating instructions.

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POWER ON



Twist the two overhead sockets to the on position. Check that the microscope power supply and the camera switches are on.



Start up the workstation. Wait a couple of minutes, then turn on the display using the remote.

Login and launch LuxControl



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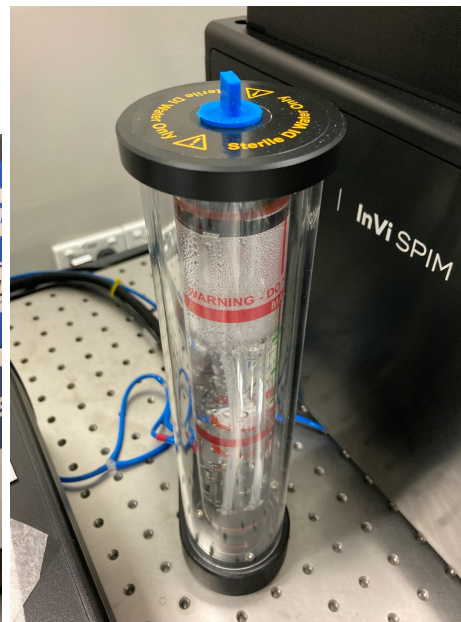
INCUBATION (skip this section if not needed)

Note that incubation set-up should be done about an hour in advance.

First check that the water levels for the temperature and gas control systems are between the high and low markings:



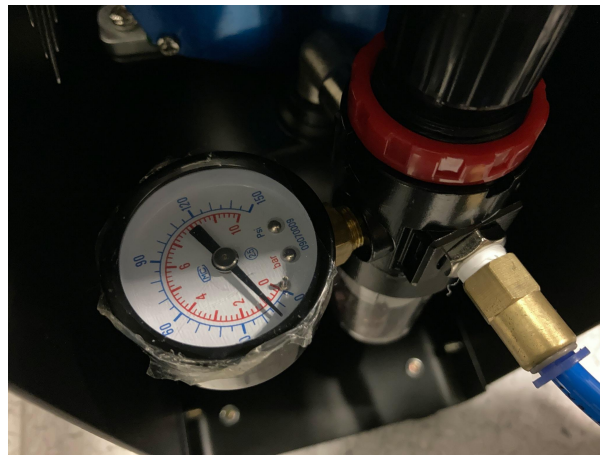
Refill this with tap water if necessary.
Do not use purified water.



Refill this with purified water if necessary.



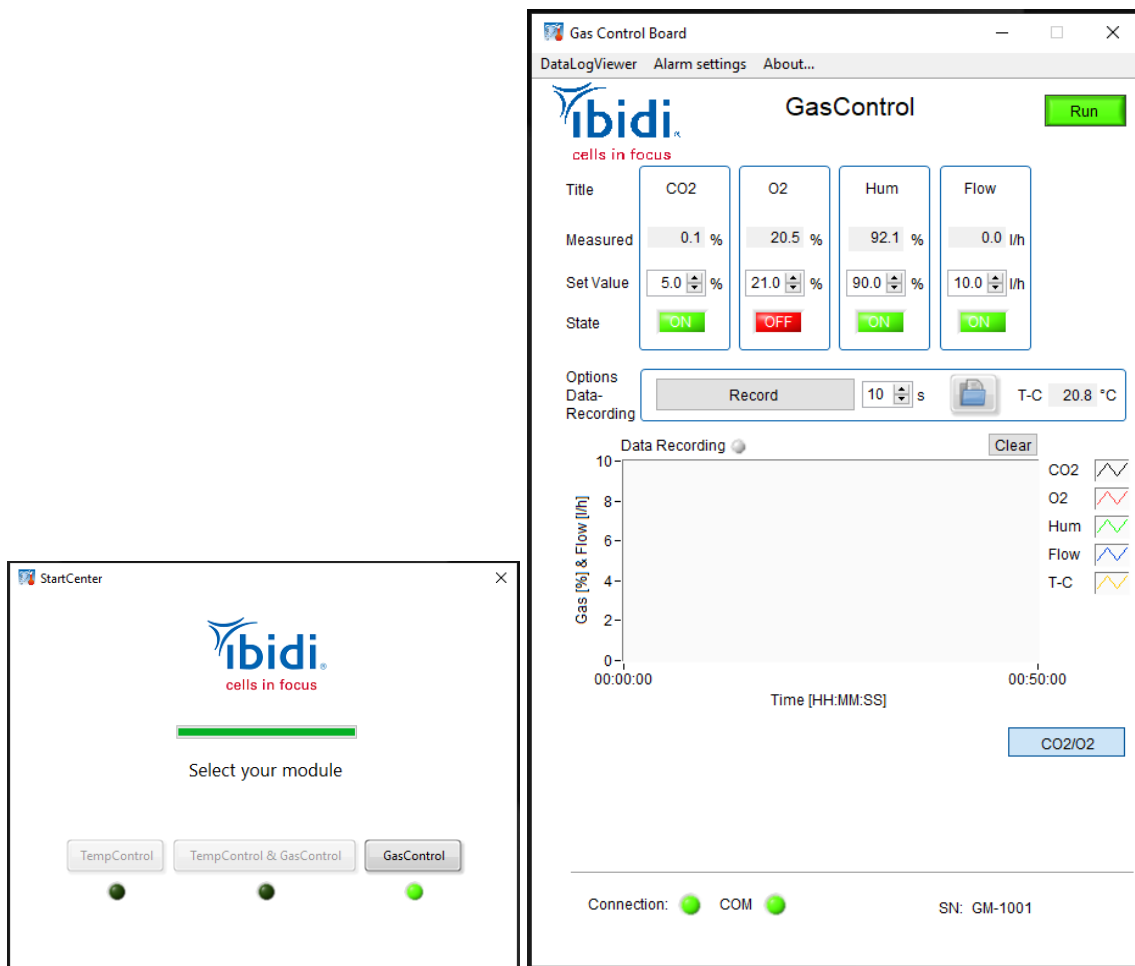
Turn on the temperature (left) and gas (right) controllers. The switch for the gas controller is on the back.




Check that the gas pressure gauge under the table reads 1 bar.



To set temperature, press “SEL” button to choose “SV” (left). Press up/down triangle buttons to set temperature. Press “RET” button and check that the bottom of the display shows “MODE Learn” (right).

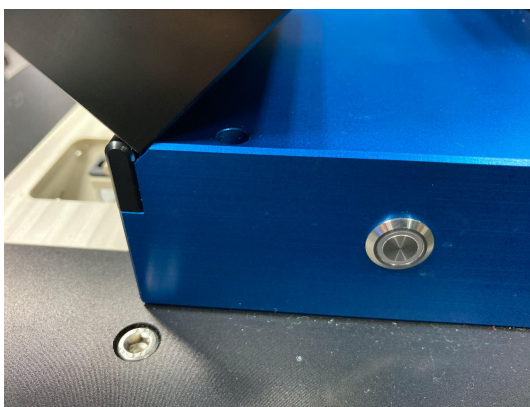


To set carbon dioxide and humidity, launch IncuControl software  and click "GasControl" (left). Set the desired conditions (right) and click the top right button to run or stop the gas control. When the button shows "Run" in green, it is running. When it shows "Stop" in red, it is stopped.

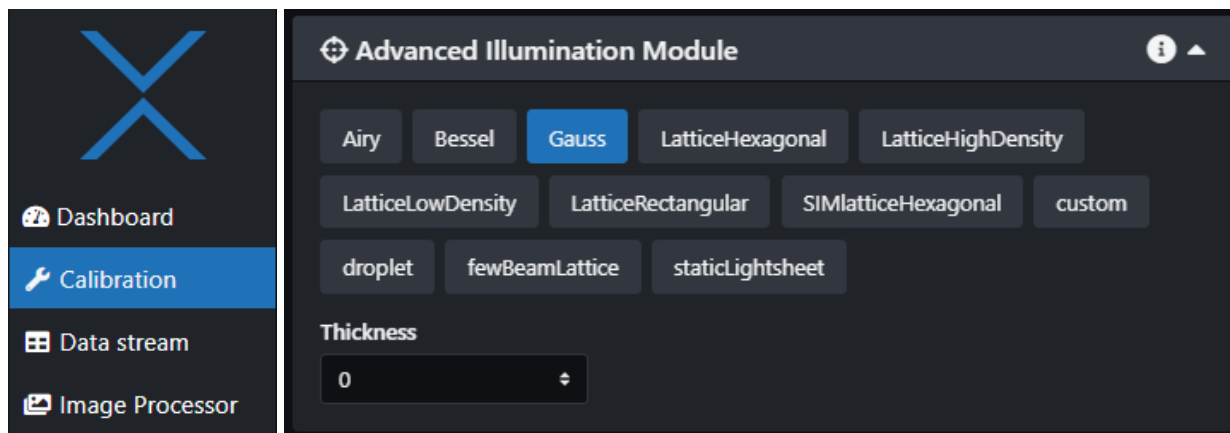
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CALIBRATION

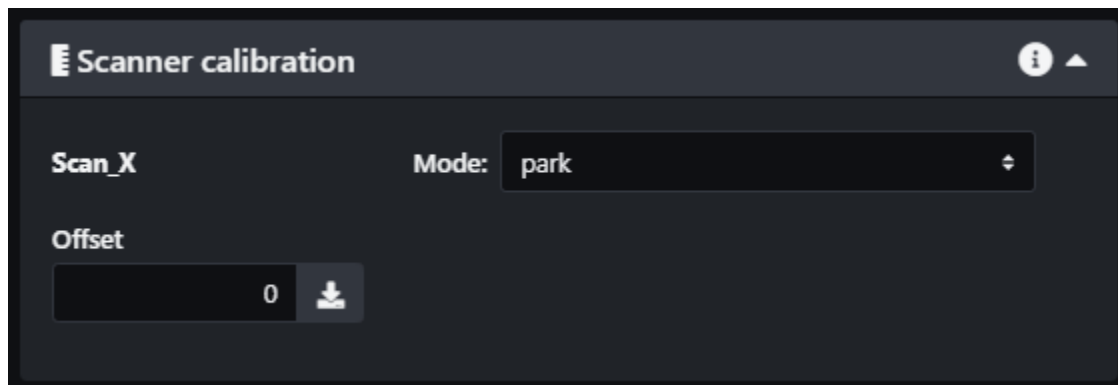
Fill sample chamber with purified water or the imaging medium you will be using.



Remember to press the laser interlock button to reactivate laser.



Select the calibration tab in LuxControl. Set beam to Gauss of thickness 0.



In the Scanner calibration box, select Mode: park.



Turn on the laser you will be using for the experiment. Use high laser power to get a better signal for calibration. If you are using lasers from 488 nm to 642 nm, you only have to calibrate one of them. If using 405 nm, calibrate it separately and save the adjustments in a separate channel.

Click "Start live" at the top of the LuxControl window.

Detection

Filter wheel short

BP 418-462

Filter wheel long

LP 498

Magnification changer

62.5x

Dichroic switch

LP 499

Select the filters that give the clearest signal on the camera feeds.

Cameras

Exposure time

1
10
100
1000
5000

200 ms

Delay

11 ms

Camera

Mode
☒ link

Lines
☒ link

Short

area

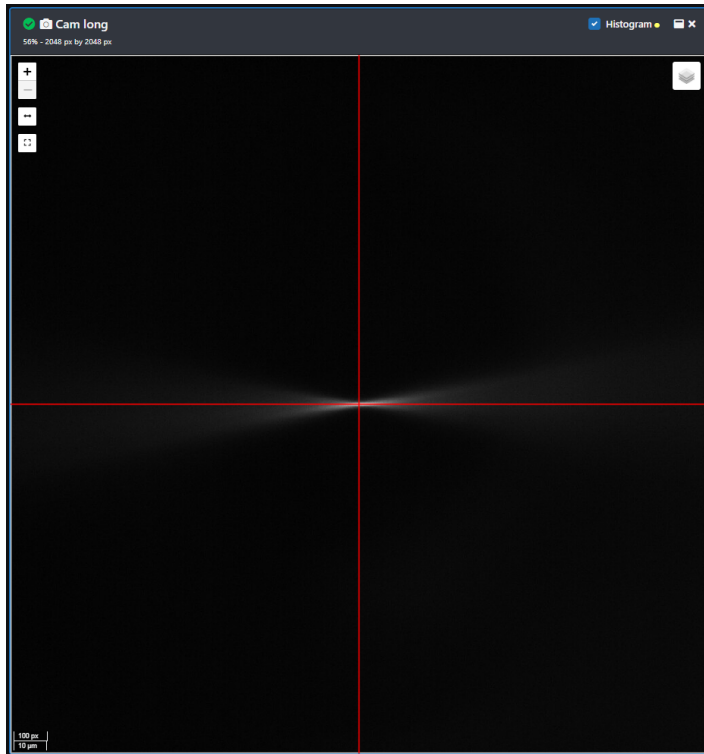
20 px

Long

area

20 px

Set camera exposure to get a good signal without saturation. 200 ms is a good starting point.



For centering adjustments, turn on the crosshairs by clicking the white button at the upper right corner of the camera view.

Lightsheet alignment

i

▲

Y1

◀◀

◀

0

▶

▶▶

Step10

↺

Z1

◀◀

◀

0

▶

▶▶

Step10

↺

Y2

◀◀

◀

0

▶

▶▶

Step0.1

↔

Z2

◀◀

◀

0

▶

▶▶

Step0.1

↻

⬇️

Store

Focus the beam using Z2, then center it left-right using Y2. Y1 and Z1 should not need adjustment. Once done, click Store.

Scanner calibration

i

▲

Scan_X

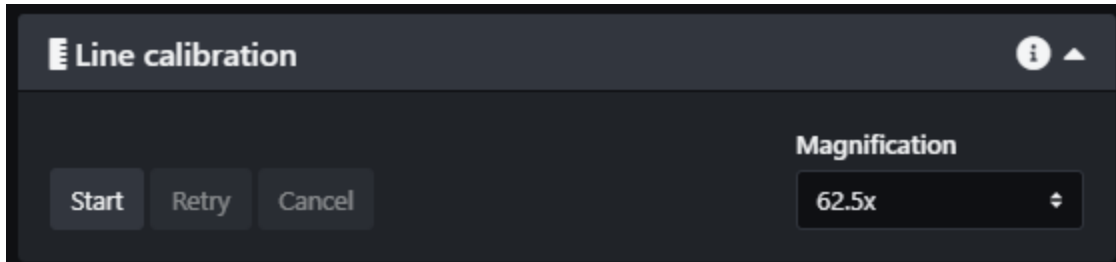
Mode:park

Offset

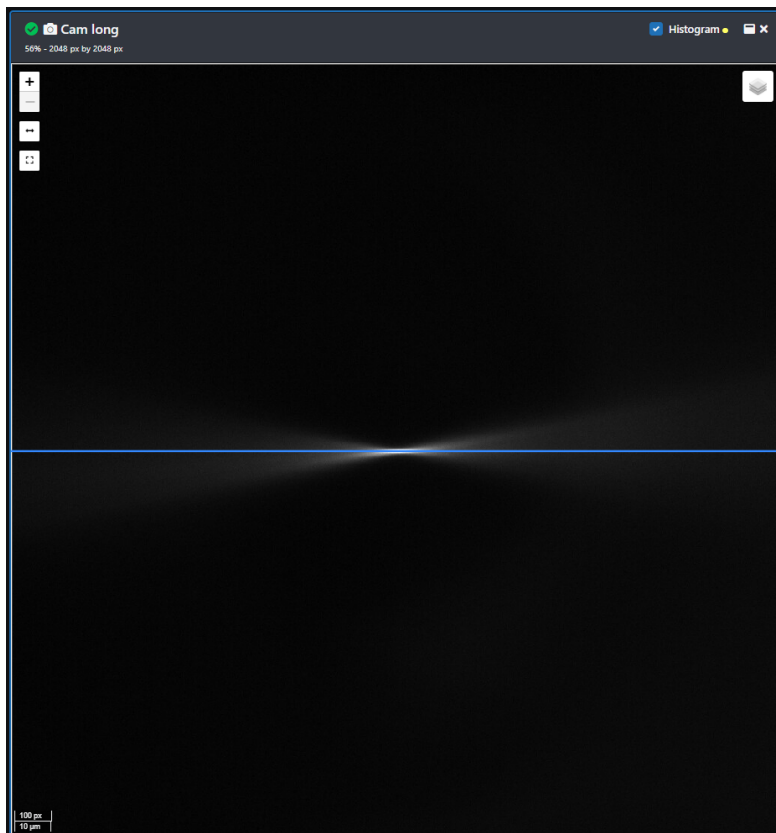
0

⬇️

Center the beam in the vertical direction by adjusting the Offset value. Once satisfied, click the store button.



Use the Line calibration box to sync the rolling shutters to the scanning beam. Click Start and check the auto alignment results.

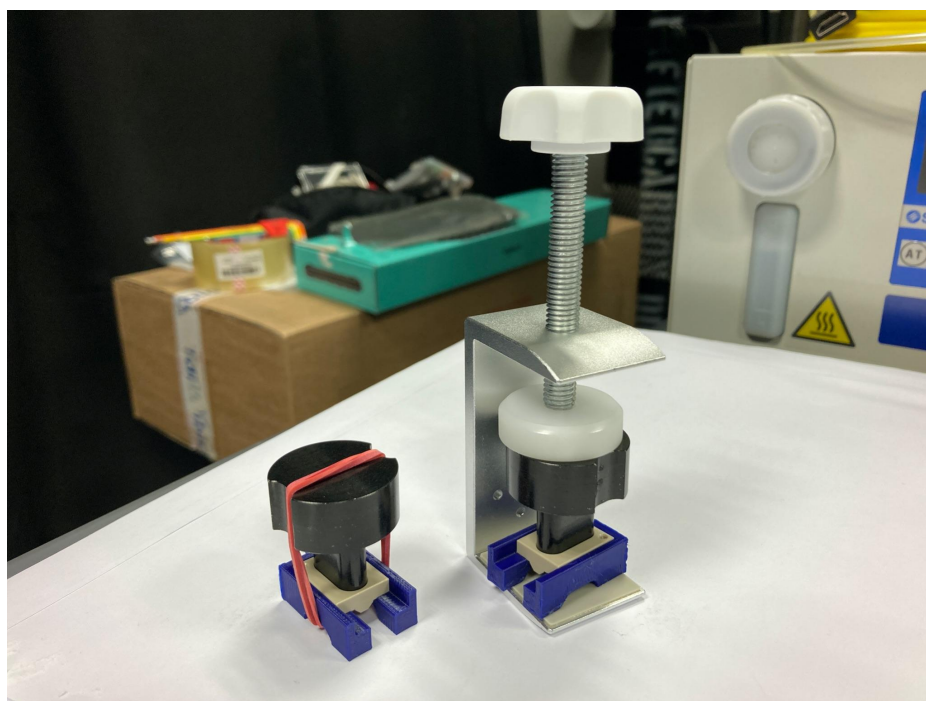


Click Retry if the blue line doesn't coincide with the beam. Once aligned, click to the next step.

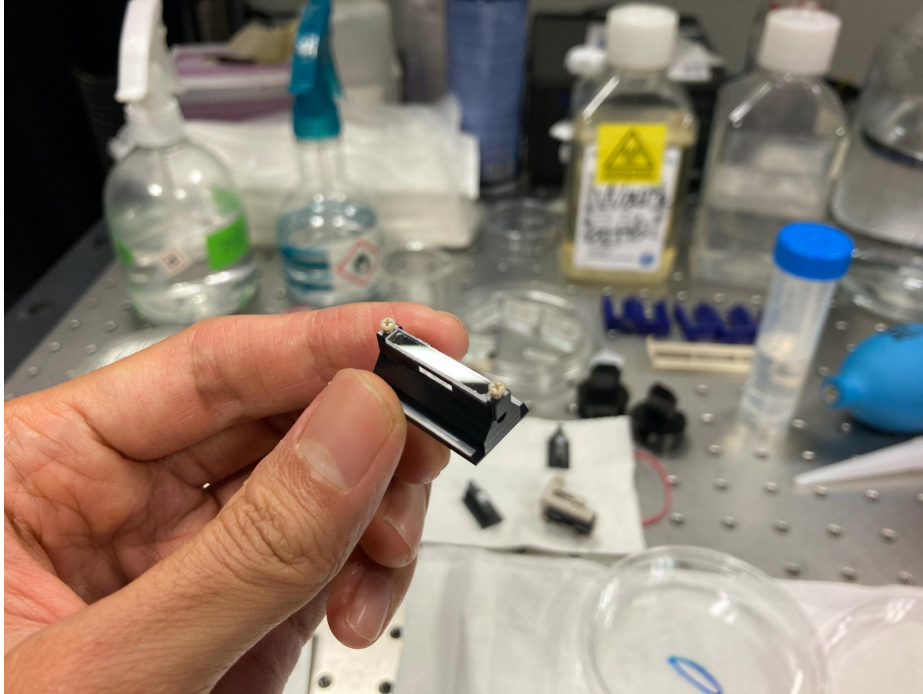
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SAMPLE MOUNTING

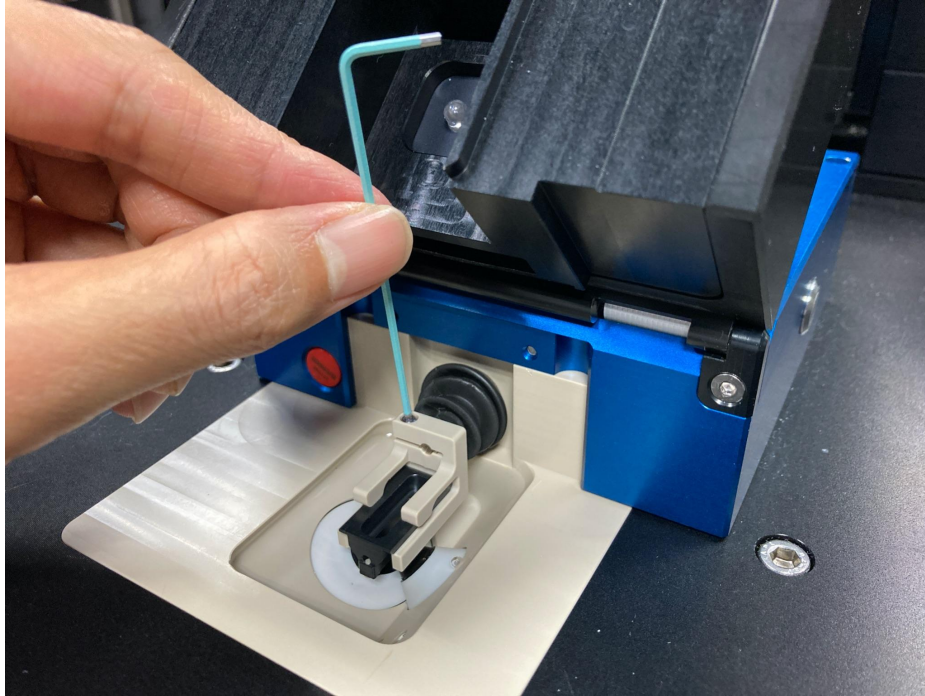
If using FEP foil, wear gloves and cut a piece measuring about 2 cm by 3 cm. Spread a small amount of Silpuran along the edge of the dish with a toothpick and use the mould to press the foil in.



Secure the mould with rubber band or clamp and leave for a few minutes.



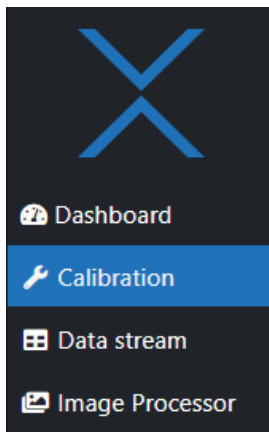
If using glass slide, push the slide into the slot on its mounting dish and carefully tighten the screws.



Gently slot the sample holder into the stage pin. Do not force it as the stage pin may be pushed out of position. Tighten with the light blue hex key.

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IMAGING



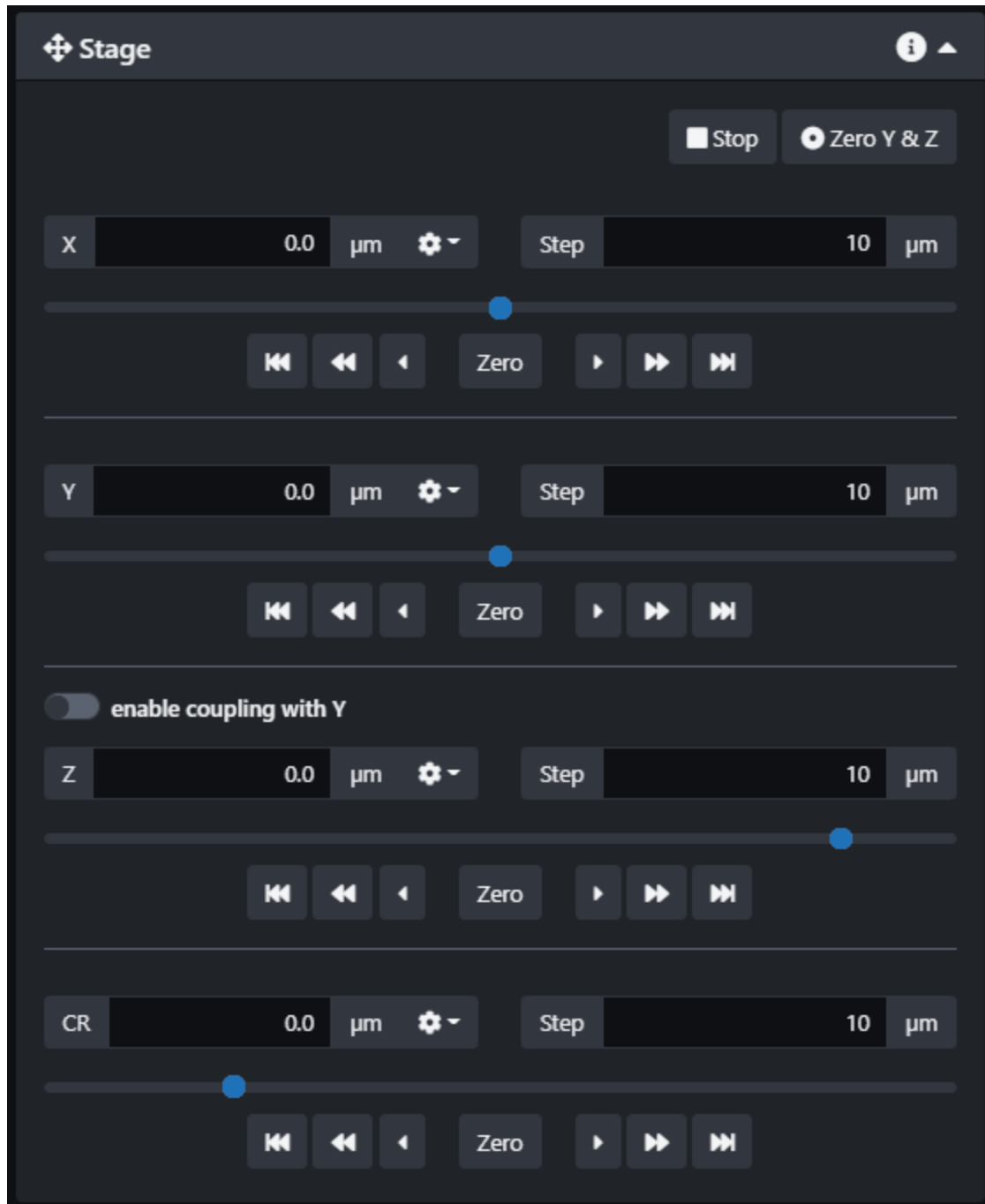
Click the Dashboard tab.



Select the desired magnification in the Detection box.



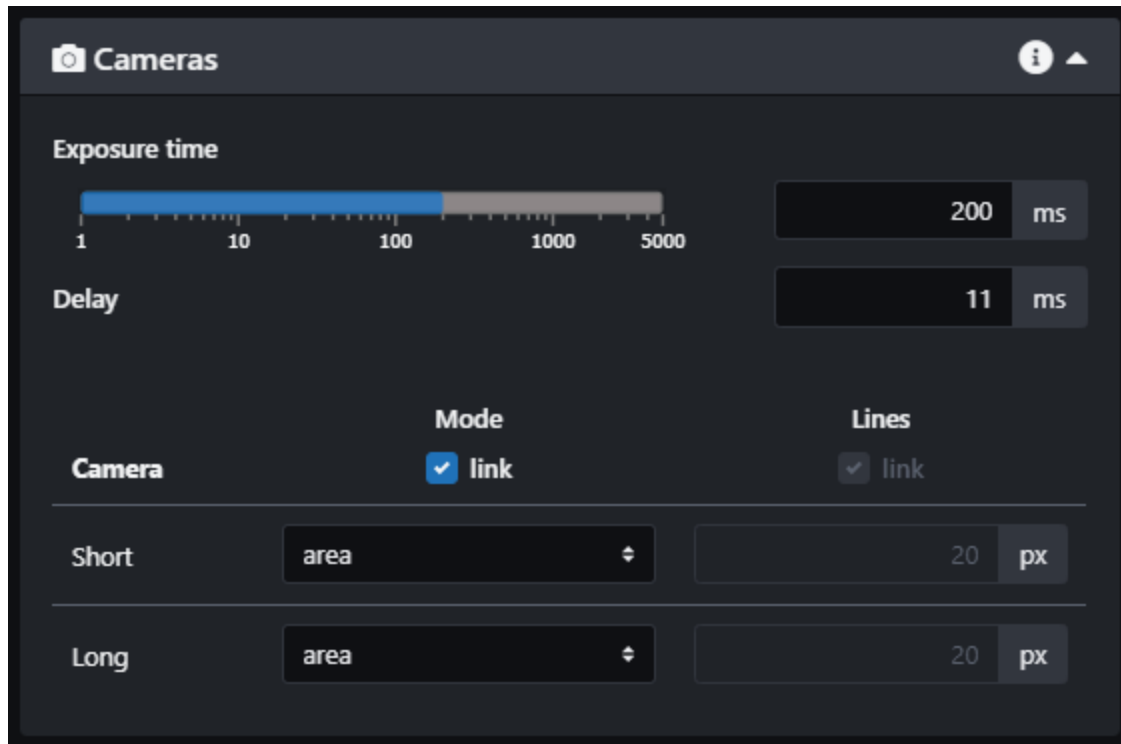
Turn on the sample chamber LED using the switch at upper right of Illumination box.



Find the sample using the stage controls. CR (refractive index compensation) should be left at 0 for most aqueous imaging media.

Turn off the LED and turn on the laser(s). Adjust exposure settings.

[Channels box coming soon]



"Delay" should be at least 11 ms in most circumstances.

Stacks2

stack_1

Add

Remove

Current	Start	Center	End	Delta
<div>X</div> <div>0.0 μm</div>	→ 0		→ 0	400
<div>Y</div> <div>0.0 μm</div>	→ 0		→ 0	400
<div><input checked="" type="radio"/> Z</div> <div>0.0 μm</div>	→ 0	→ 0	→ 0	1
<div><input type="radio"/> CR</div> <div>0.0 μm</div>	→ 0		→ 0	

Planes 1
Repetitions 1

↶ Move to start

⦿ Move to center

↷ Move to end

Delete all

If applicable, define z-stack and xy tiles. Move the sample and assign the travel limits by clicking the appropriate arrows.

Events1

Event_0

Add

Remove

Number of tasks	1
Number of triggers	1

Delete all

Add an Event, which is an experiment with a set of tasks and triggers.

Tasks - Event_0 1

Add Combine...

Stack Channel

stack_0 channel_0

Delete all

Define the tasks (combinations of stacks and channels to acquire) in your imaging experiment/event.

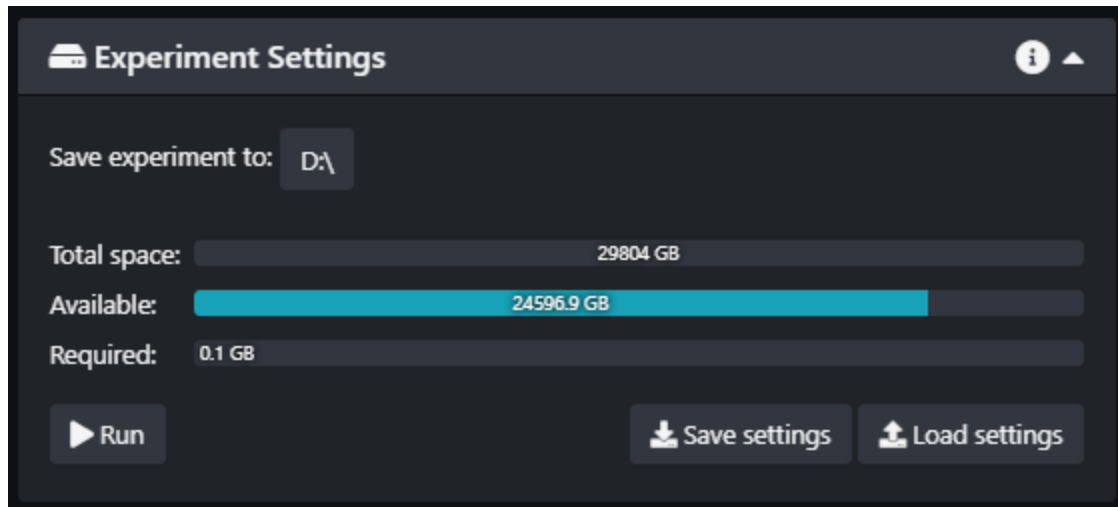
Triggers - Event_0 1

Add

	Hours	Minutes	Seconds
Start After	0	0	0
Interval	0	0	0
Repetitions	1		

Delete all

Define the triggers. This is where you can set time lapse. Set all timings to 0 and Repetitions to 1 if there is only one time point. If timings are set to 0 with multiple Repetitions, the system will repeat acquisitions as fast as possible.



Set the directory to save your data in.

Click Run.

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IMAGE PROCESSING

[coming soon]

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END OF EXPERIMENT

Zero the stage axes. Remove the sample. Empty and clean the sample chamber.

Transfer your data. Remember that portable drives are not allowed.

Power off the system if there is no more user for the day. Otherwise just logout, but if applicable also turn off the incubation controllers if the next user hasn't requested for them to be left on.