Protocols for using the BRUKER microscope at INMED

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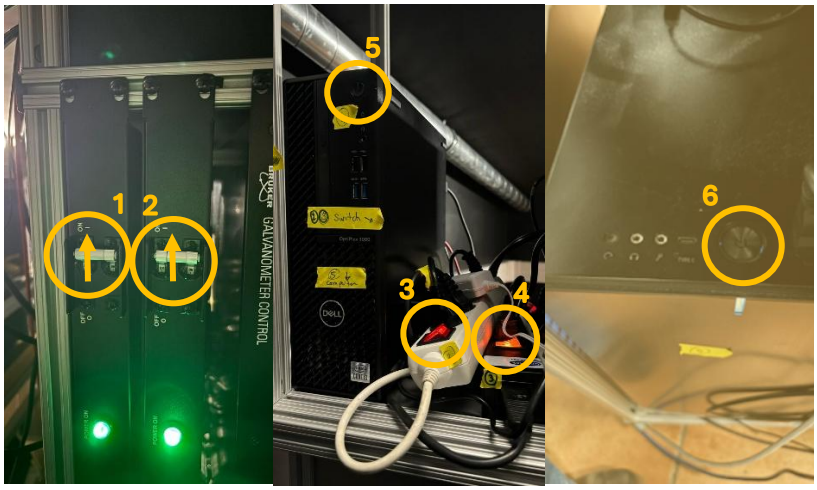
Startup Protocol:

1 – Power on:

Turn on the two side switches (1),(2) / Power strips (3),(4) / SLM computer (5) / Main tower computer (6):



⚠ After switching on power strips (3) and (4), wait for the SLM computer (5) to shut down before turning it back on, and verify it's properly turned on (indicator light), otherwise the software linked to the SLM will return two errors.

2 – Log in with admin session:

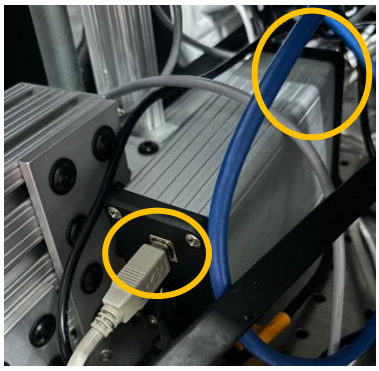
Select “Other User” – “How do I sign in to another domain” :
id = « USERBRU-XXXXXXX\user » replace “local” with “user” in the provided id.
Mdp = X (empty)

OR: Use your own session and password

3 – Open the Software: « Prairie View »

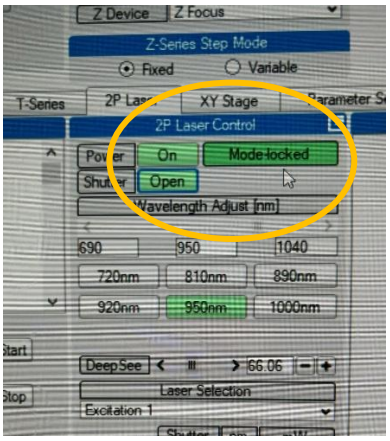
⚠ If a lot of errors occur: Check USB connections behind the computer (should light blue), close and reopen the interface.

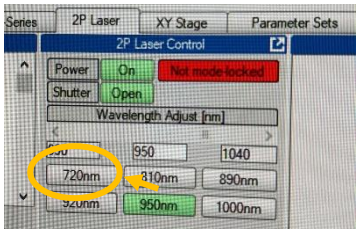
⚠ If « Orbital » error appears: Close the software and shut down the computer. Note that the SLM will shut down too. Check for bad contacts in the control box.
Then restart SLM first, followed by the computer.



4 – Laser imaging part:

In “*2P Laser Control*”, set “off” → “on”, “closed” → “open”, and wait for “*Mode-locked*” to turn green.

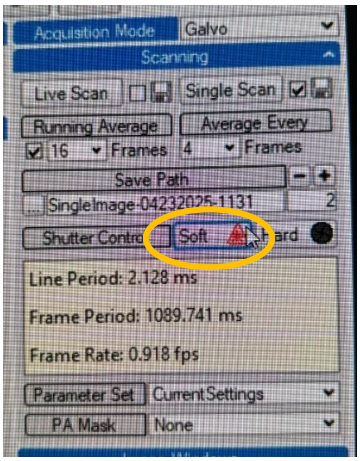




If « Not mode-locked » remains red: Lower the wavelength to reduce startup energy. And then, put it back to the one you intend to use.

5 – In the « Scanning » section:

Open the shutter “Soft” (top right) to warm up the Pockels cell



6 – In the « Acquisition Mode » section:
Select “Resonant Galvo”, and wait to hear a click that confirms the mirror position.

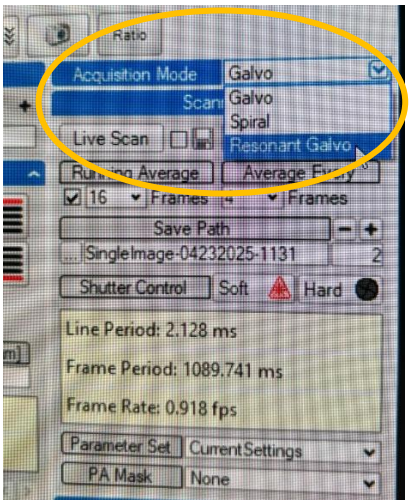
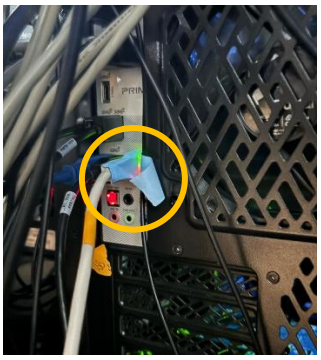
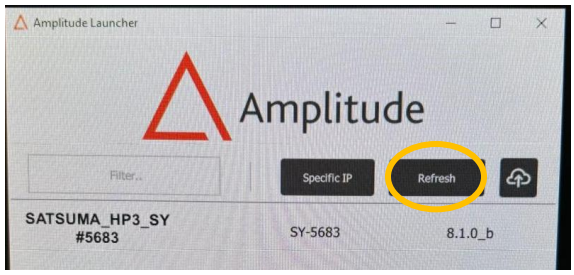


Photo-Stimulation Protocol:

1 – Open the interface: “Interface_v3…”

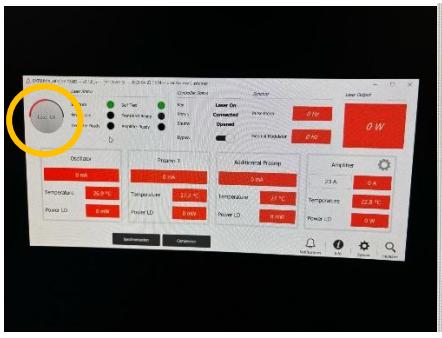
☹ If SATSUMA doesn't appear, check the back cable (green light) and hit “Refresh” in the interface:

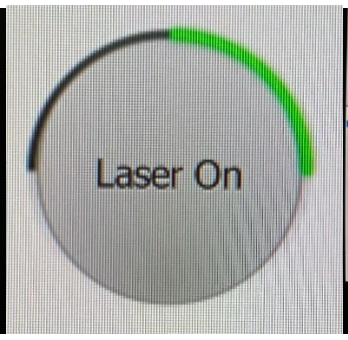


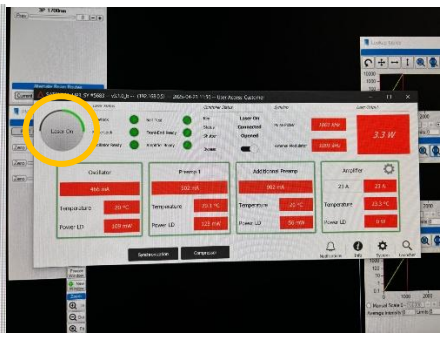


2 – Turn on the photo-stimulation laser:

Once detected, click on SATSUMA “open”, then on “Laser Off” → it should display “Laser On”.:







3 – Output power:

Switch the “Error” cursor from “External Modulator” to “Bypass”, then back to “External Modulator”. The error shouldn’t be there anymore.

⚠ You must hear the shutter sound – it confirms 40W output.

Note : Bypass is useful for 3-photon mode.

4 – To reduce output power :

In “Synchronization”: Select “Software” and type 3%, then switch to “Analog Input”:

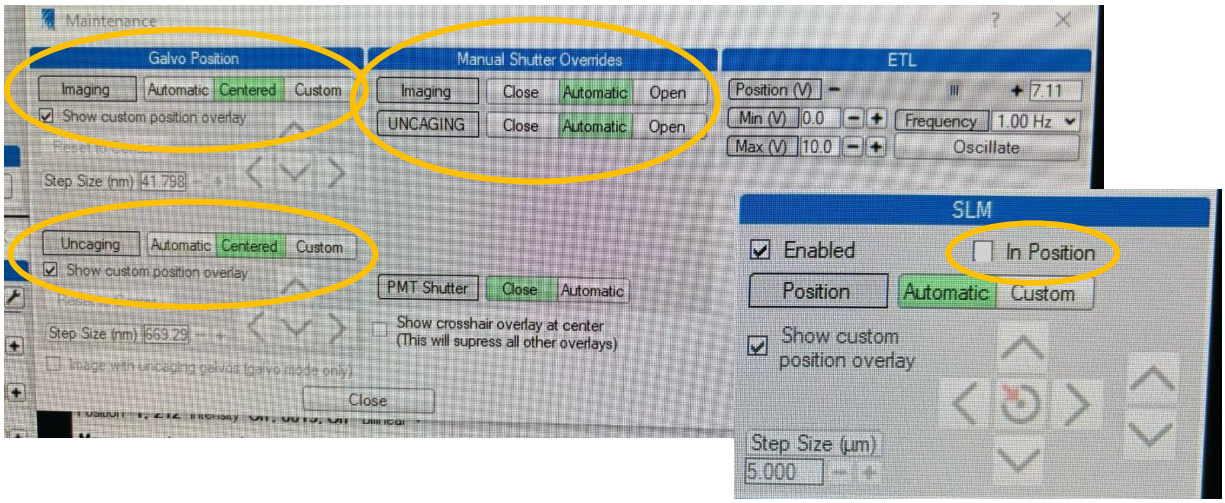
Info:

In the “Amplitude” box, it uses the 40W output from the fiber optic, to convert it into *OPA* wavelengths, with three outputs: 1030 nm (for 2-photon photo-stimulation, blue), 1300 nm, and 1700 nm.
After conversion, the output power from the “Amplitude” box, directed to the beam path and then to the microscope, is approximately 1W (due to losses in the conversion loop).
As a result, the total output is now only around 4W instead of 40W (for safety).

You can now:
Close the “Synchronization” window and minimize the SATSUMA window to keep it aside.

5 – Beam centering check:

Go to « Tools » -> « Maintenance »:



Under “Galvo Position”, select “Centered” for both *Imaging* and *Uncaging*.

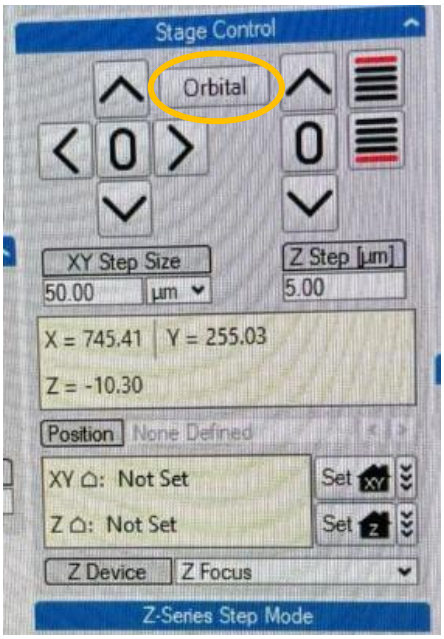
Make sure shutters are CLOSED: “Automatic” but not “Open”, and “Close” at the bottom.

Uncheck “In Position” in SLM to redirect away from the SLM for proper *UNCAGING Galvo* alignment.

Info:
- Galvo laser is a technique that uses two mirrors to move the laser beam in different directions by adjusting the mirrors’ angles to steer the beam.
- Uncaging mode is used in two-photon photo-stimulation, where light activates an opsin (a protein) that can open neuronal channels.

6 – Set objective to neutral (0°):

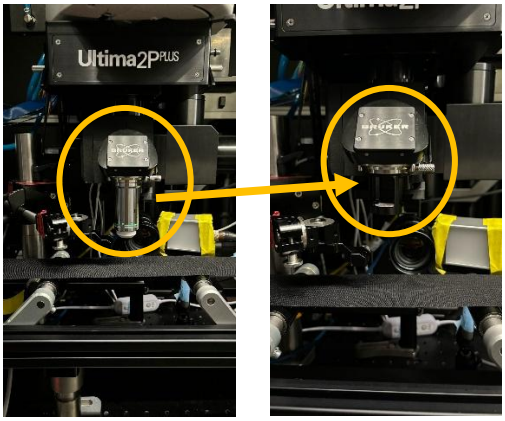
In « Stage Control », click on « Orbital »:



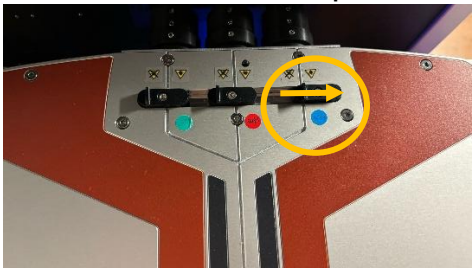
In « Pitch Angle », enter 0°and check the physical position.

7 – Check beam alignment:

Remove objective and place the target.

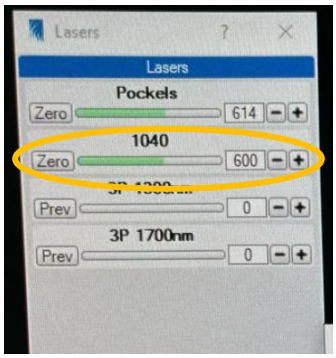


Open the blue 1030nm shutter on the “Amplitude” box (blue sticker).



⚠ Do NOT open the laser path box after this step!

To adjust power with the AOM (Amplitude box):
Use the “1040” slider – 550 for 2mW, 600 for 7mW (see conversion sheet on microscope table). Range: 0–1000:



Use the IR viewer to check beam position and intensity, and make sure that it is centered.

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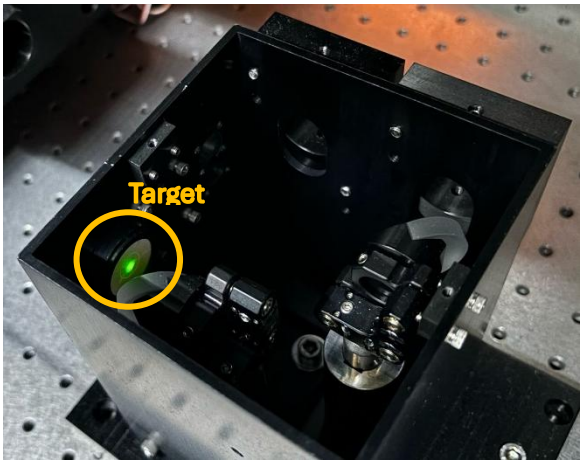
Note: If nothing is visible, check *Maintenance* > *Manual Shutter Overrides* > *UNCAGING* – must be set to “Open”, not “Automatic”.

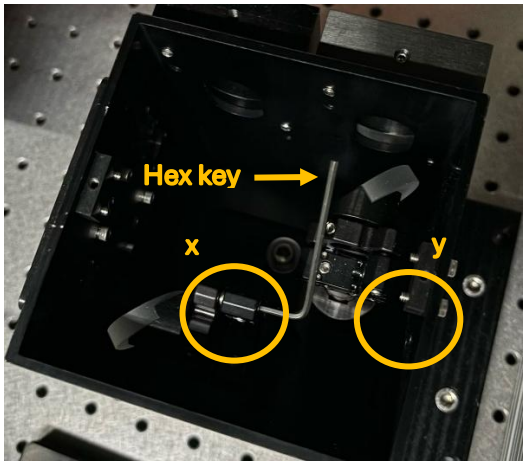
⚠ Close the maintenance window before starting live imaging, or the signal will be blocked.

Info: “Pockels” should ideally be at 0, not 614.

8 – Adjust beam if misaligned:

Use an intermediate target to verify beam alignment.
If off: adjust long-range beam path (outside microscope).
If centered on the intermediate target but not after the final target: fine-tune using X/Y hex key inside the box.





Using the wrench (hex key), rotate along the X and Y axes to center the visible beam on the final target using the infrared (IR) viewer.

9 – Power measurement:

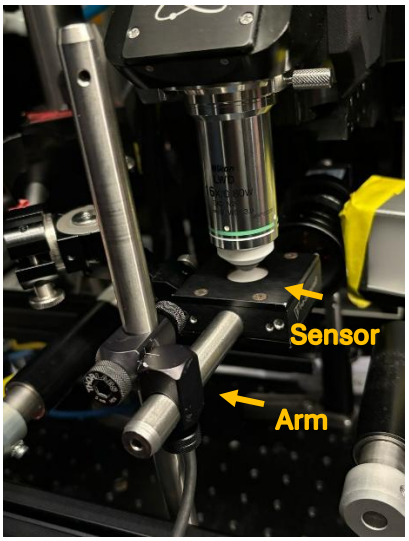
Positioning a « power meter »:

Remove the target and reinsert the objective.

⚠ Close shutters before doing so!

Info: A « power metre » converts photon energy to heat, measured in mW.

a) Connect and position the sensor ~3mm below objective using an articulated arm:



b) Adjust placement using X/Y/Z knobs near computer. Reopen shutter to get a visible spot, not a point (avoid burning).

c) Turn on the power meter:
- Adjust the wavelength by selecting 1040 nm to match the laser.
- Set the “Range” to 300 by clicking on (+) at first.
- Close the shutter and turn off the lights to perform the zeroing – select “On” to do a “Tare”.

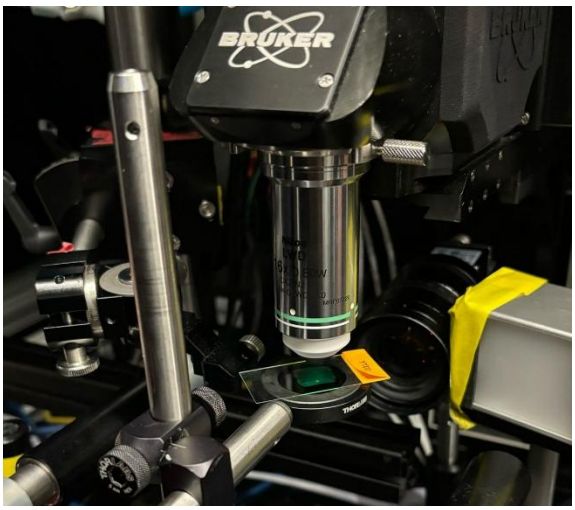
d) Plot a power measurement curve. A reference list is available on the desktop. From 0 to 500, the output is 0 mW, at 550 → 2.5 mW […] and at maximum, 1002 mW for a setting of 1000.

Bleacher Protocol:

Note: Bleaching = photodestruction of fluorescent molecules.

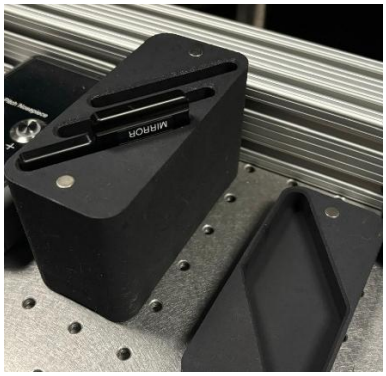
1 – Place the coverslip: Use a coverslip prepared with fluorescent dye for testing.

2 – Add water: Between the coverslip and the objective.Be careful to NOT put water on the ink side.



3 – Initial camera adjustment:

a) Remove the dichroic mirror “T685spxxr” and replace it with the “MIRROR” (from the small storage box in front, magnetic opening):

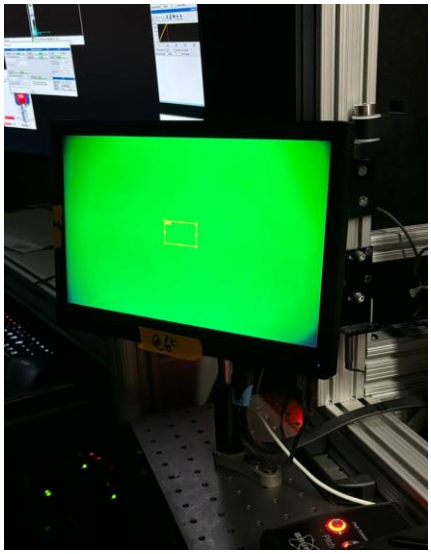


Info:
Dichroic mirror = Transmits infrared and reflects visible light. It separates wavelengths. For example, 685 reflects $\lambda \leq 685\,nm$ and transmits $\lambda > 685\,nm$.

Mirror = Reflects all wavelengths toward the camera, nothing goes to the detector.

b) Turn on the camera light (LED-Driver): twist once to activate, again to adjust intensity:

c) Use the X, Y, and Z knobs to find the imaging plane. You can change precision with the red button:



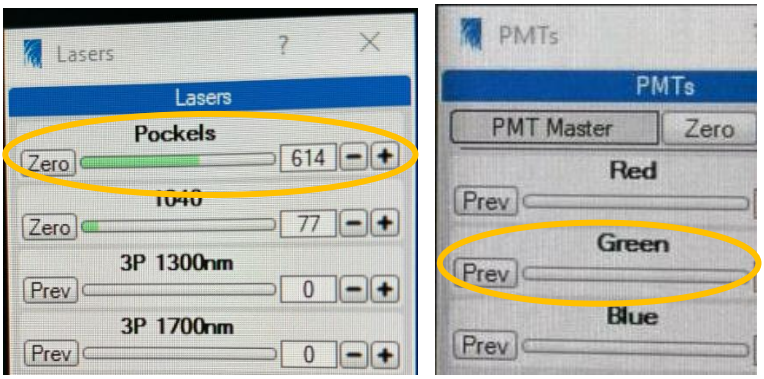
4 – Switch to imaging mode:

a) Turn off the LED, remove the mirror, and reinsert the dichroic. Don’t forget to check the water.

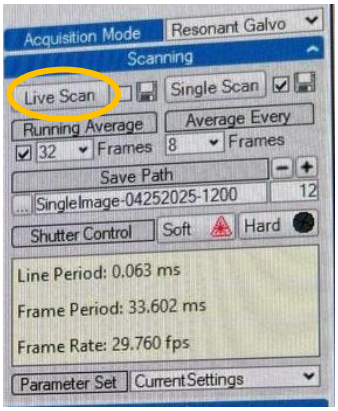
b) Turn off the laser box light and room light, and close the enclosure.

5 – Prepare calibration in the software (on the computer):

a) In the « Laser » window: Set a power. « Pockets », ex: 50.
In the « PMTs » window: Increase the gain of the appropriate PMT, ex: 700

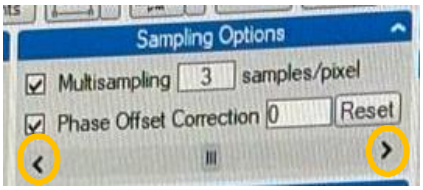


b) In « Scanning »: Start “Live Scan” to view the signal. Adjust the averaging (ex: 32) to influence real-time feedback.



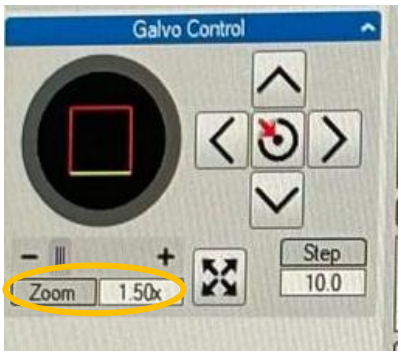
c) Use the knobs to find a region of interest.

d) If line doubling appears: use « Offset » in « Sampling Options » to correct a visible object.



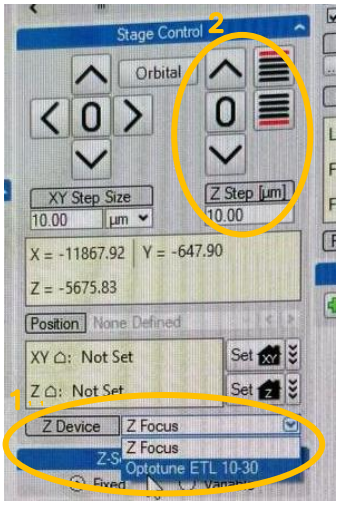
⚠ *Offset is zoom-dependent. Recalibrate if zoom changes in “Galvo Control”.*

⚠ Uncheck “Phase Offset Correction” before starting the experiment, as it may interfere.



e) Calibrate alignment between photo-stimulation laser and imaging laser (focal point overlap):

- In “Stage Control”, select “Optotune ETL 10-30”.
- Define the focus for a reference point by activating “Live Scan” and identifying the signal maximum.

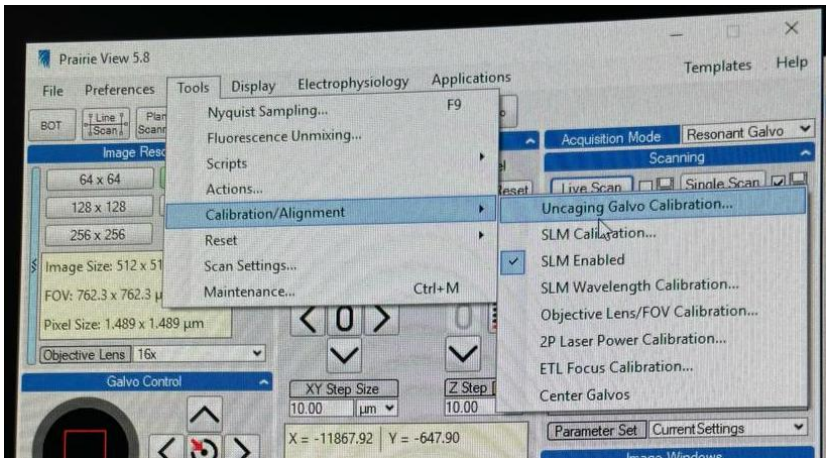


Note: ETL = Electrically Tunable Lens, a flexible lens with a liquid membrane that adjusts focus without moving the objective. It is useful for reaching deeper even when objective limits are reached. Plus, since there are no moving parts, the switch between imaging planes in z is faster

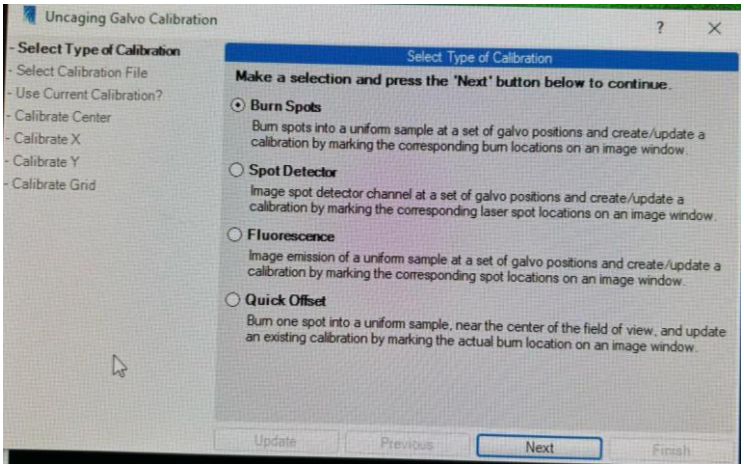
6 – Launch the calibration:

Calibration depends on the chosen zoom – select it before starting.

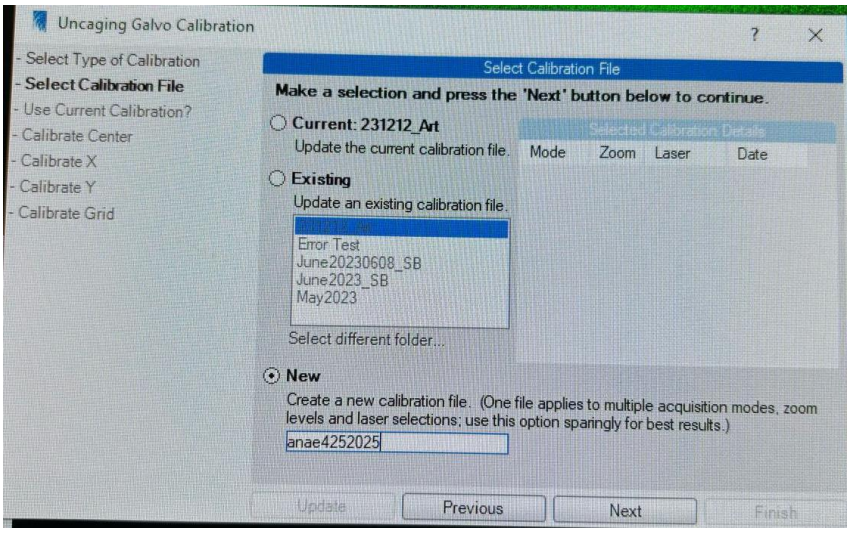
Go to: “Tools”, “Calibration/Alignment”, “Uncaging Galvo Calibration”:



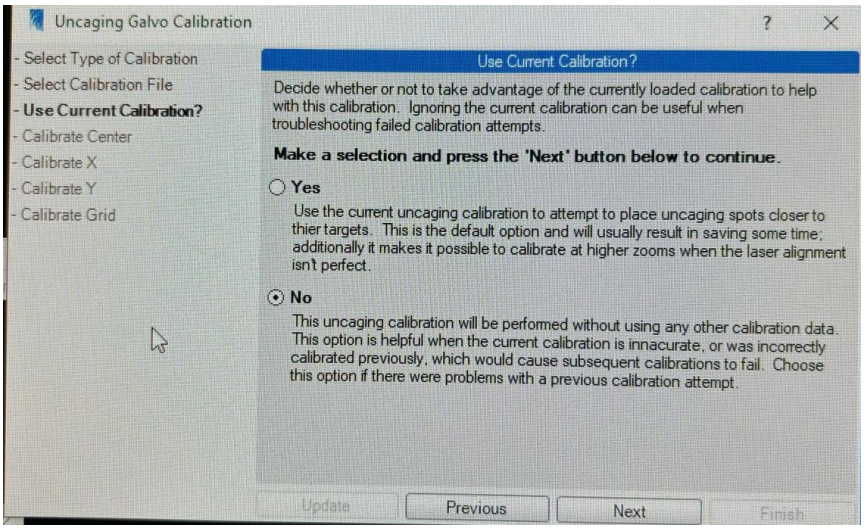
a) Select « Burn Spots », then click on « Next »:



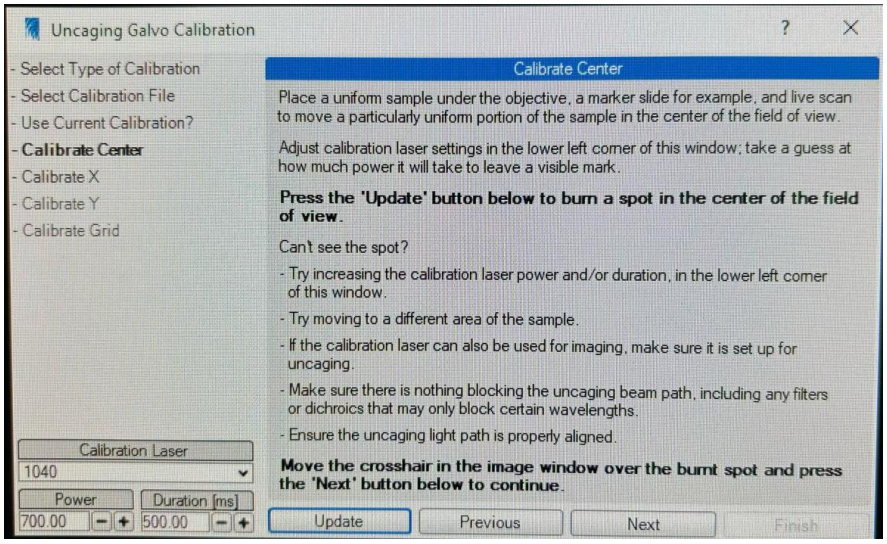
b) Create a new file with your first name and the date, then click “Next”:



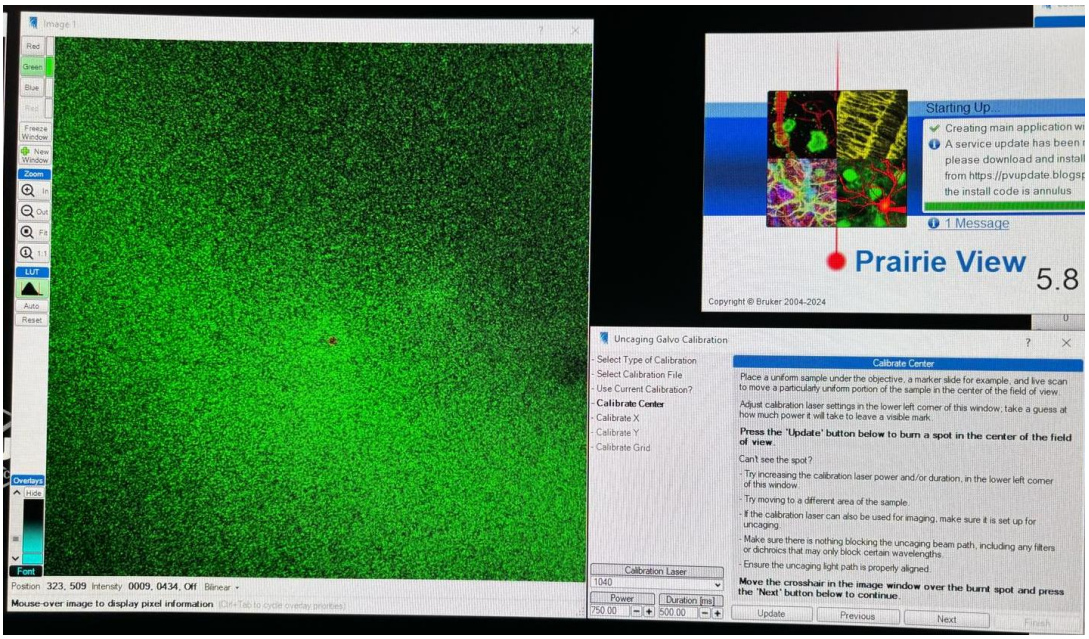
c) Do not use the current calibration → select “No”, then “Next”:

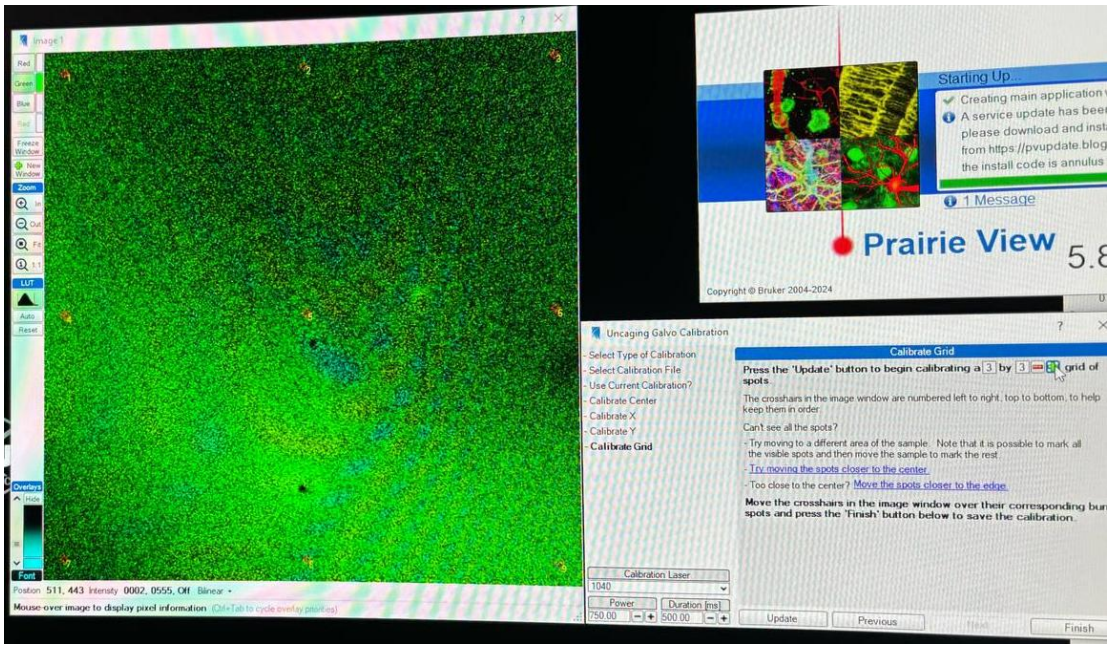


d) Set the photo-stimulation laser power and the duration (burn time). Use the power scale from the photo-stimulation protocol (see the sheet on the computer table).
For testing:
→ Power = 700 (≈ 60 mW) and Duration = 500 ms
Then click “Update”.

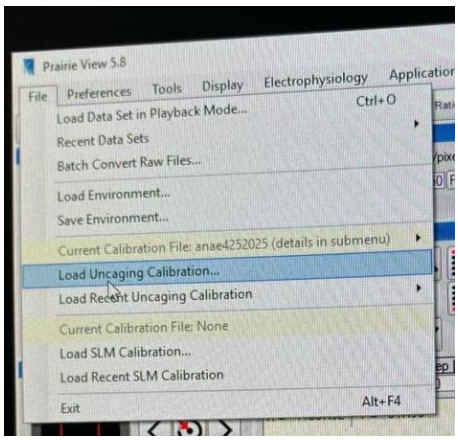


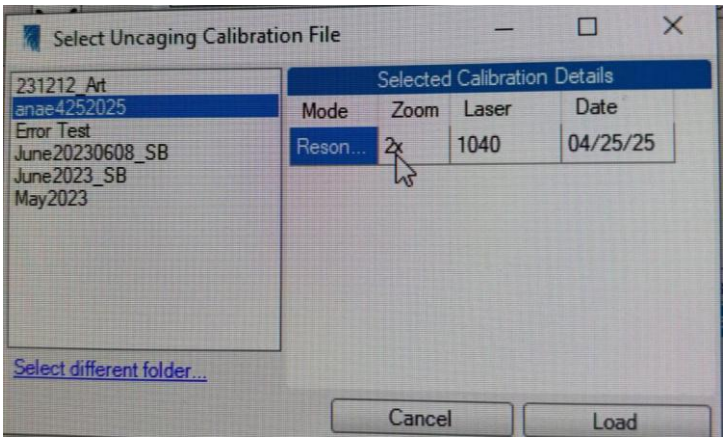
e) Follow the calibration steps: Use “Live Scan” to locate the burn point, align the red cursor on it, click “Next”, click “Update” again, realign, “Next”... The last step uses multiple points (you can adjust this), then click “Finish”.





f) Go to “File”, “Load Uncaging Calibration”, then select the calibration folder you just created.





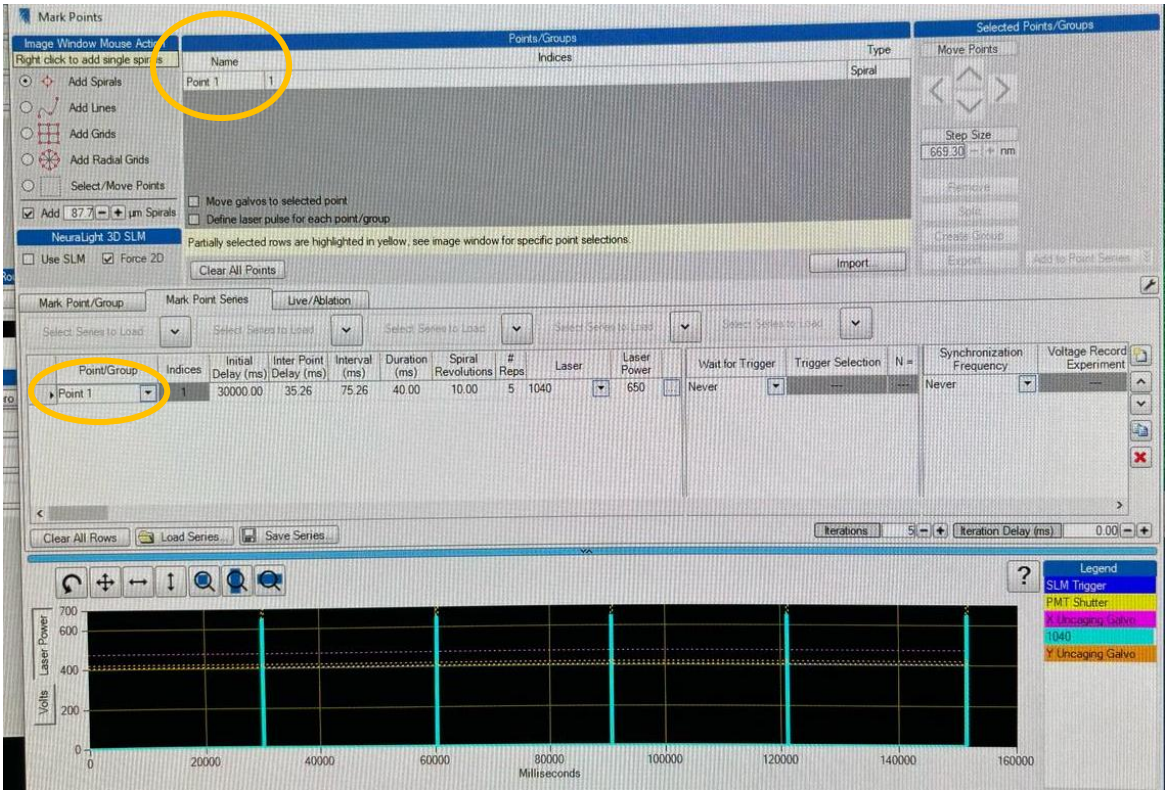
Note: There is a bug with the “Zoom” in “Selected Calibration Details”: it always shows “2x” even if it is not, but it is okay.

7 – Use « Mark points » :

Go to “Mark Point” : 

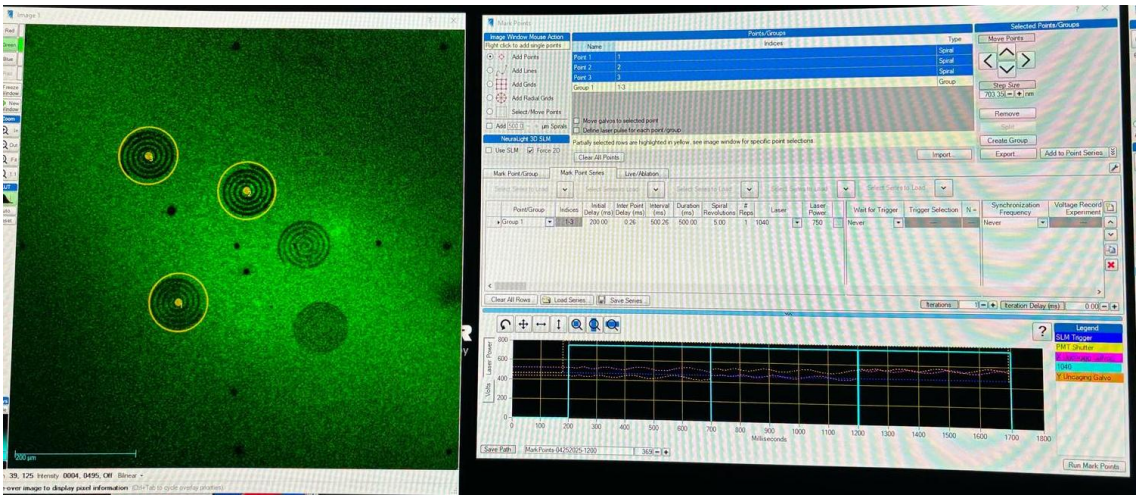
Select the points/spirals listed in “Points/Groups”.
Then launch the marking using the desired variables.

Be sure to select the correct items to run in “Mark Point Series” → “Point/Group”.



Info: The values shown in this example are for experiments with live animals.

Explanation of key variables for test phase:



- A selected point is a “point”: to use a spiral, check “Spiral/Size” (top right) and choose the scale. Then place it where you like.

- Uncheck “Add” to avoid confusion with spiral size.

- « Spiral Revolutions » = Number of turns in the spiral.

- « #Reps » = Number of repetitions = how many times the laser action is repeated.

- Use the same “Power” and “Duration” as in the calibration.

- « Initial Delay » = baseline = recording time before laser activation to compare pre/post effects.

- Before clicking “Run Mark Points”, use “Live Scan” to adjust the Z-position and optimize the signal.

Shutdown Protocol:

1 – In Amplitude: Click « Laser Off » and close the window.

2 – On the photo-stimulation laser box: Physically turn off the right-hand shutter with the blue sticker (move it to the left), then tape it with the “Do not touch”.

3 – In the Software:
Set both “Pockels” and “1040” sliders back to 0.
In “2P Laser”: Set “Open” → “Closed” and “On” → “Off”.
Then close the window and the software.

4 – Turn off in this order: Computer (6) with SLM computer (5), power strips (4) and (3), then the two main switches (2) and (1) on the side.

5 – Raise or rotate the objective to its maximum height, and carefully remove the water without scratching.

6 – Tidy up, clean, and take all personal belongings with you. 😀