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STEP-BY-STEP GUIDE FOR CONTINUOUS VOLUMETRIC MICROSCOPY

by

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August 2021

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1. Create and set Scanimage data folder.

2. Set wavelength to 935 nm.

3. Center XY motor stage: Position the objective lens at +11,000 um in X and Y.

4. Install 10x objective lens.

5. Set Z motor stage at the bottom of its travel range: This step was essential with older ScanImage versions, that occasionally sent the objective lens crashing downwards. I did not experience this bug this year.

6. Set correction collar to +

7. Align incoming beam: Turn on the alignment beam and use two irises to check if the beam is pointed towards the microscope. Switch back to 935nm beam within less than 30 minutes.

8. Maximize power below the objective lens: Place a power meter directly below the objective lens. Start scanning with high magnification and high power. Carefully turn the two distal knobs of the periscope mirror (not the middle one) until the power reading is maximal. Mark down that power level.

9. Verify PREAMP2 connection to TD2000 discriminator: Verify that the output port of the bottom DHPCA-100 preamplifier is connected to the input port of the TD2000 discriminator.

10. Verify SYNC connection to Flex RIO input port 2: Verify that the BNC cable labelled as 'SYNC' is connected to an unshaped output port of the TD2000, as well as to second input port of the Flex RIO crate.

11. Verify bandwidth and gain of PREAMP2: Verify that the bandwidth of the bottom DHPCA-100 preamplifier is set to 200 MHz, and its gain is set to 10^2 (low noise).

12. Show and save Scanimage acquisition from TD2000 sync channel: In Scanimage 'Channels' window, make sure that both imaging channels are displayed and saved.

13. Tune Japanese flag: Dilute a drop of fluorescein solution in a DDW dish. Homogenize the solution using a disposable Pasteur pipette. Turn on both PMTs. Set the frame rolling average factor to 100. Switch to 1x magnification, and 128 lines per frame. Switch to Red High color map. Right click the green imaging channel window and click 'Show Crosshair'. Carefully turn the two distal knobs of the periscope mirror (not the middle one) until the intensity distribution is centered. Check if the intensity distribution at the red imaging channel is reasonably centered as well. Remove the fluorescein dish and carefully clean the objective lens.

14. Pick TAG lens assembly: The TAG lens is installed within an aluminum cup, with a black lens tube on top and an aluminum adapter plate at the bottom. Stretch your hand into the far right end of the Faraday cage and carefully pick the TAG lens assembly. Remove the SM1EC2B snap-on dust cap and check if the relay lens is clean. Recall where you keep the dust cap.

15. Set input aperture: The effective clear aperture of the TAG lens at its 189kHz resonance is 4mm. Accordingly, the input beam aperture should be slightly smaller than 4mm, otherwise a slight lens misalignment may lead to a considerable degradation in lens performance. Check that the aperture of SM1D12C graduated iris diaphragm is set to about 3.5mm.
    ! Critical
    The SM1D12C iris cannot fully close! Handle it with care or else its reading will no longer be accurate.

16. Install TAG lens: Place the TAG lens adapter plate on top of its translation stage. Check that its coaxial cable is not twisted nor interfering with the optical path. Fasten the TAG lens adapter plate to its translation stage using four M6 screws with washers. Use the available short screws (probably 12mm or 16mm), as longer screws might disrupt the translation stage.

17. Verify 18mm distance between the lens tube and scanner head entrance: This should already be the case, more or less. This distance corresponds to a L2-MOM entrance distance of 27.5mm, with 6mm lens thickness from front to focal plane and 3.4mm from lens tube to retaining ring.

18. Maximize power below the objective lens: Place a power meter directly below the objective lens. Start scanning with high magnification and high power. Do not touch

the periscope mirror! Carefully turn the two knobs of the TAG lens translation stages until the power reading is maximal. Mark down that power level.

19. Mount pollen sample: Fetch the orange Pelican case of the TAG lens from the bottom left white drawer. Among else, it should contain two petri dishes with loctite-sealed rectangle cover glasses. Preferably pick the dish with two rectangle cover glasses glued one on top of the other. Rinse with DDW. Center the sample below the objective lens. Secure it using two table clamps - not too tight so it won't crack and not too loose so it won't move. With the 10x objective lens fixed at the bottom of its travel range, carefully raise the lab jack until the distance between the sample and front surface of the objective lens is well below 8 mm. Fill with DDW. Start scanning with 1x magnification, 512 or 1024 pixels per line, relatively high power and some reasonable frame rolling average factor. Slowly raise the objective lens until you come across some clear features. Zero XYZ on those features and reduce power as needed. If the observed features resemble a regular pattern of defocused circles or streaks, you're probably focused at the bottom of the petri dish. Keep raising the objective lens until you come across some irregular pattern of small bright blots. Note that individual pollen are sometimes hardly detectable with 1x magnification and 512x512 imagery.

20. Identify suitable pollen FOV for alignment: Select some FOV with clear features, reduce laser beam power and increase magnification to 6x. Right click the green imaging channel window and click 'Show Crosshair'. Use the XY knobs to center the crosshair on one of the observed features, while keeping other features visible within the same FOV. Slowly move the objective lens above and below the observed features. If the features change their planar position as a function of z then their tilted orientation is not suitable for alignment. Pick another FOV. If the features retain their planar position while getting in and out of sight, they are suitable for TAG lens alignment. Zero XYZ.

21. Turn on the power source for TAG lens driver: Look for an ugly off-white box labeled 'Heavy quiet TAG lens power source' on top of the Sutter PMT power source, to the left of the function generator. Turn it on. Worry not if you forget to turn it off.

22. Set TAG lens frequency: Click the icon of the TAG lens driver. A GUI window will open. Click 'Connect' to establish connection to the TAG lens driver through COM port 4. A round red light should indicate that the lens is turned off.

! Critical

The default frequency of the TAG lens driver is wrong and must be changed before turning on the lens! First select a resonance frequency of 189kHz from the drop down menu, and only then click 'Power On'. A round green light should now indicate that the lens is turned on.

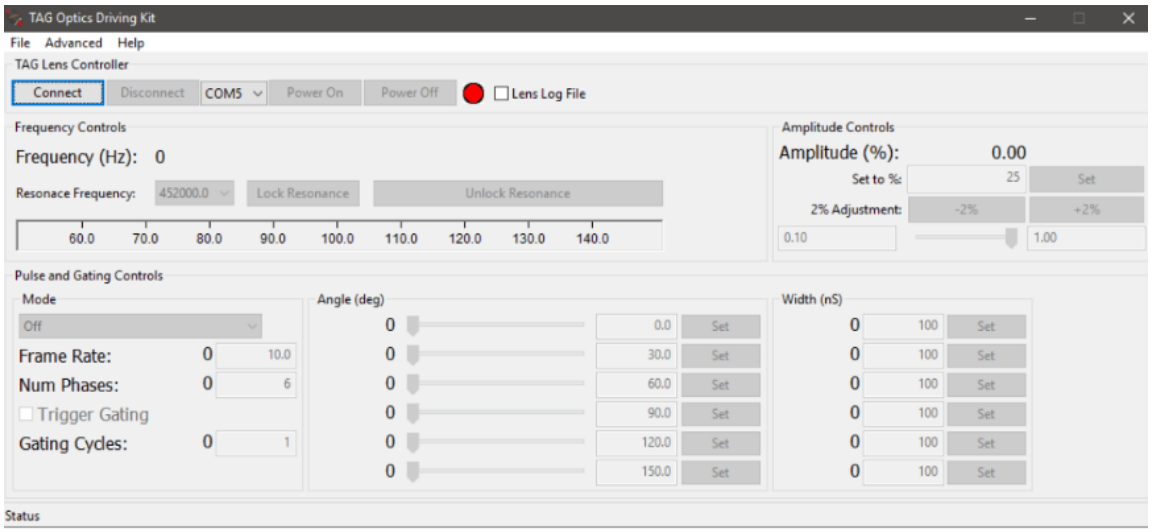
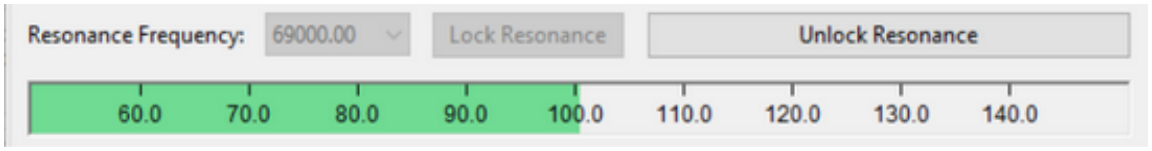


Figure 1: GUI of TAG lens driver.

23. Set TAG lens parameters: Allow the lens to remain powered on and unlocked for several minutes, at 189kHz and 62% amplitude. In the mean time, in the bottom left pulse menu, change the Mode from 'Off' to 'RGB'. The top three sliders control the phase of the TTL synchronization pulses sent through the three TTL ports of the TAG lens driver. The others are meaningless. Set the phase of the top slider to 0 degrees. 0 degrees correspond to the maximal diverging phase of the lens, i.e. maximal depth. 180 degrees correspond to the maximal converging phase of the lens, i.e. minimal depth. 90 and 270 degrees correspond to null optical power of the lens, i.e. nominal imaging depth.



! Critical

The lens can be physically damaged by locking it wrong! Please read the following instructions carefully.

24. Lock TAG lens: Verify that the resonance frequency is 189kHz and the amplitude is 62%. Tick the 'Lens Log File' box next to the round green light. Start scanning the sample at 1x magnification. Now click 'Lock Resonance' and watch a horizontal green bar growing from 0 to 100. If the the bar disappears and a 'Lens locked' status appears at the bottom left corner of the GUI window, then the lens is locked and everything is fine. However, **if the bar fluctuates in size and occasionally turns reddish, the lens is unstable and should be stopped immediately!** Simply click 'Unlock Resonance' and then click back 'Lock Resonance'. **Do not allow the lens to remain unstable under any circumstances!**

25. Align TAG lens using pollen: Now that you selected a suitable FOV for alignment and the lens is locked, it is time for fine alignment of the TAG lens. Switch to 2x magnification. Increase the frame rolling average factor and the beam power until you obtain decent pollen imagery. Right click the green imaging channel window and click 'Show Crosshair'. Use the XY knobs of the microscope to center the crosshair on one of the observed features. Slowly turn the Z knob upwards and downwards while watching out for lateral drift of the observed features. If the center feature remains stationary while the distal features mildly radiate out evenly, like the petals of an opening flower, the TAG lens is aligned properly. If the center feature moves and the distal features radiate more towards that direction, then the TAG lens is misaligned with the laser beam and should be carefully moved using its translation stages. Simply turn one translation stage at a time while turning the Z knob back and forth, and see if the lateral drift decreases (good) or increases (bad, move the TAG lens translation stage back in the other direction). After a few corrections with the TAG lens translation stages, you may have to slightly shift the position of the objective lens using its XY knobs, so as to recenter the cross hair on your chosen feature. Once the FOV is sufficiently aligned in 2x magnification, switch to 4x magnification and check if finer alignment may be needed using the same procedure. Finally, switch to 8x magnification and optimize the TAG lens alignment.

26. Verify TAG lens alignment: The goal here is to make sure that our TAG lens alignment is not biased by some tip/tilt of the alignment target. Click 'Power off' in the GUI of the TAG lens driver. Move away from the FOV you selected earlier by at

least 2mm in X and Y and preferably by more than a hundred microns in Z. As before, use the XY knobs to center the crosshair on one of the observed features, while keeping other features visible within the same FOV. Slowly move the objective lens above and below the observed features. If the features change their planar position as a function of z then their tilted orientation is not suitable for alignment. Pick another FOV. If the features retain their planar position while getting in and out of sight, they are suitable for TAG lens alignment. Turn on the TAG lens and lock its 189kHz resonance at 62% amplitude, while taking the same cautionary steps as detailed above to avoid damaging it. Switch to 8x magnification and turn the Z knob of the microscope upwards and downwards while watching out for lateral drift of the observed features. If the center feature remains stationary while the distal features mildly radiate out evenly, like the petals of an opening flower, the TAG lens is aligned properly. Congratulations!

! Warning
The intravital imaging session will surely fail if the system is misaligned. If the pollen remain misaligned at 3x magnification in unrelated FOVs, regardless of the position of the TAG lens translation stages, then the incoming beam is probably off. In this case, the only solution is to dismount the TAG lens, realign the beam as per the steps above, remount the TAG lens and realign it using pollen. Don't forget to put on some good music while doing so.

27. Measure FOV size: Click 'Power off' in the GUI of the TAG lens driver. Switch to 3.5x magnification. Pick some small feature and walk it from edge of the FOV to the other, horizontally and vertically, using the XY knobs of the microscope. Document that FOV size.

0.2 Axial range measurement

1. Acquire pollen z-stack: Switch to 1x magnification and identify a field of view that has clear, distinct features across at least $600\mu m$, and preferably $1000\mu m$. If you can't find such a FOV, you can easily generate one using the laser beam, as described in the following optional step. In Scanimage main controls window, verify that stack is enabled and 'Save' is ticked. Set its acquisition parameters to 100 frames per slice, an averaging of 100 acquired frames per saved frame, zoom of 1x, scan angle multipliers of 1. In Scanimage channels window, verify that both imaging channels are saved. In Scanimage configuration window, set the number of lines to 1024. In

the image controls window, set the frame rolling average factor to 1. In the stack menu, set the start and end depths, and choose a slice spacing of 10 microns. This z-stack is only used to estimate the axial range of our aligned TAG lens and verify its linearity, so a finer spacing won't be necessary. Imaging power can remain fixed, or almost fixed, since the imaged sample is nearly transparent. Choose an informative file name and start z-stack acquisition.

2. **Optional** - increasing the useful thickness of the pollen sample through laser ablation: Pick a FOV with many small pollen. Shut off the PMTs. Insert the metal shutter that physically blocks light from hitting the PMTs. Switch to maximal magnification and maximal laser power. Scan the sample for half a minute. Reduce laser power, switch to 1x magnification, remove the metal shutter, turn on the PMTs, resume imaging. A dark spherical bubble surrounded by bright edges should now appear in the center of your FOV. If the resulting FOV is not thick enough, ablate another bubble at a different spot in your FOV, $300\mu m$ above it or so. It's ok to create a large bubble, hundreds of microns across, as long as its edges contain distinct fluorescent features.

3. Set TimeTagger data folder.

4. Select depth for continuous volumetric imagery: Turn on the TAG lens and lock its 189kHz resonance at 62% amplitude, while taking the same cautionary steps as detailed above to avoid damaging it. Bring the objective lens to the middle of the axial range previously covered by the conventional z-stack. For instance, if the z-stack was taken from $z = 0\mu m$ to $z = 1000\mu m$, bring the objective lens to $z = 500\mu m$. Have a look at the resulting imagery at nearby depths (e.g. between $z = \pm 100\mu m$ in the said example) and park the objective lens at a depth that offers the most detailed imagery at 1x magnification.

5. Connect Fast preamplifier: Disconnect the PMT cable leading to DHPCA-100 PREAMP1 (the top unit, whose output goes directly to Flex RIO input port 1) and connect it instead to Fast Comtec's TA-1000 input port. Now you can only monitor the experiment online through the other PMT channel.

6. Configure the Time Tagger: Verify that the TimeTagger is powered on (its power source is labeled and supposed to be plugged to power outlet splitter #2). Open a new file explorer window. Choose '1-Quickstart' from the right quick access

bar. Copy the folder path to clipboard. Open a new anaconda prompt window and change directory to the pasted path to '1-Quickstart' folder. Open the file '_dump_it_right_lior.py' using Visual Studio Code or some other Python editor. Set the values of the header variables (data_directory, recording_length, num_lines, magnification, acceptable_laser_event_rate, sample_type, tag_flag, depth, fov_num, mouse_num) as needed. Specifically, the value of 'recording_length' determines the recording duration, in seconds. Preferably set a recording length of 600-1200 seconds, to obtain a detailed continuous volumetric image.

7. Acquire continuous volumetric imagery: In the prompt window, type 'python _dump_it_right_lior.py'. Wait a couple of seconds for the code to print a header message titled 'STEP 1: Write events from selected channels into a file'. The TimeTagger is now acquiring detection events. Now start scanning the sample with Scanimage, with the same magnification and number of lines as acquired during the conventional z-stack and documented in the python script (1x magnification and 1024 lines, respectively). The TimeTagger will record a small (3-4 kB) file 'filename.ttbin' followed by a series of 1GB long files 'filename.1.ttbin', 'filename.2.ttbin', and so forth. While data taking, check file validity as explained in the following step.

8. Check pickle file validity: While acquiring volumetric imagery, copy the first few ttbin files to a separate folder. Open '.../1-Quickstart/tt2pickle.py' with Visual Studio Code or some other Python editor. Set the values of its header variables ('data_directory' and 'file_wile_card') and then run it (either through Visual Studio or by typing 'python tt2pickle.py' in a separate prompt window). The script should create a series of pickle file (files ending with '.p' extension) and print out some statistics for the first pickle file it creates. Check that the TAG lens periodicity is sensible (roughly 5290000 ps), that the line periodicity is fine (roughly 63,100,000 ps), that the frequency-divided laser clock is stable (almost 250,000 ps apart for a 4MHz clock), and that the number of events per PMT channel is fine - typically between 100kcps and 9Mcps. Usually, once the PMT threshold is set sufficiently far from its noise floor, its count rate doesn't change much with its threshold.

9. Parse a short ttbin file to check volumetric imagery: Copy 1-2 pickle files to QNAP and parse them with PySight. Choose a small number of slices (5) and a small number of columns (64) to reduce processing time. You may check the resulting zarr datasets with napari or seaborn, or use '/data/lior/code/sum_stacks.py' to normalize

their brightness along the z axis, exported to both zarr and hdf5 format. FiJI could then import these hdf5 datasets.

10. Reacquire continuous volumetric imagery: Park the objective lens $200\mu m$ higher (or deeper) than in the previous volumetric imaging session, while holding all the other imaging parameters fixed, and acquire another volumetric dataset just like before. That would provide me with more data points for axial range estimation.

11. Copy imagery to QNAP: Copy the acquired z-stack and ttbin files to QNAP for offline analysis. Confirm successful file copying and then erase them from the MOM computer.

12. Axial range estimation: I'll conduct this offline step myself.

0.3 Beam power modulation

Phototoxicity considerations limit the tolerable beam power to 80mW at the surface, and about 250mW while imaging deep layers. In practice, the optimal beam power should be lower, for two reasons:

1. High beam power at the surface may saturate the probability of photon emission per laser pulse at the center of the focal volume, thereby broadening the point spread function ([3, 2]).

2. Increasing beam power while imaging deep layers eventually leads to increasing two-photon excitation at the surface, as well as to two-photon excitation at the vicinity of the focal volume [1]. Both background mechanisms essentially limit the attainable signal-to-background ratio, unless dedicated background rejection measures are taken [1]. Raising the beam power beyond the surface excitation threshold does not improve the signal to background ratio.

The following guide specifies how to setup an intensity modulation waveform synchronized with the TAG lens oscillation, so as to sufficiently reduce the excitation light intensity when imaging the superficial layers of the brain. An exact waveform that precisely compensates for optical scattering pends measuring the attenuation length of the mouse brain at the selected excitation wavelength (935nm), and to a lesser degree across the emission wavelengths. In contrast, our approximated waveform is only meant to spare

our imaging conditions from surface excitation and the brain from photo-toxic irradiation, as exemplified in Supplementary Figure 2.

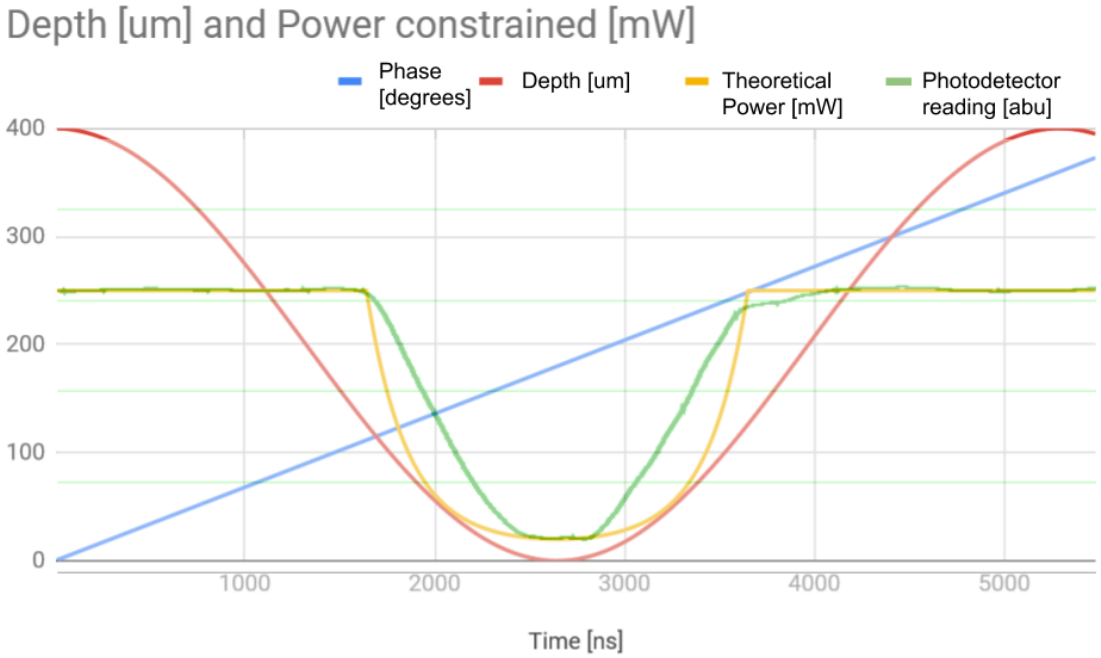


Figure 2: Optimal (yellow) and practical (green) beam power modulation waveforms, with respect to lens phase (blue) and nominal depth (red) oscillation over time. Note differing units of measure for each trace.

1. Measure the beam power below the objective lens: Click 'Power off' in the GUI of the TAG lens driver. Remove the pollen sample and store it in the orange Pelican case. Place a power meter directly below the objective lens. Start scanning with high magnification and full power. Mark down the resulting power level. Compare it to the power level you've marked during the 7th alignment step, i.e. before installing the TAG lens. It should be slightly lower due to beam clipping by the iris aperture.

2. Connect A-303 HV amplifier: Grab a stable bench. Firmly place it below our A-303 HV amplifier. Grab the top platform. Climb up. Turn off the illuminated red switch at the rear panel of the A-304 HV amplifier. Disconnect its red and black banana cables and connect them to the A-303 HV amplifier. Turn on the red switch at the

rear panel of the A-303 HV amplifier. Grab the top platform. Climb down.

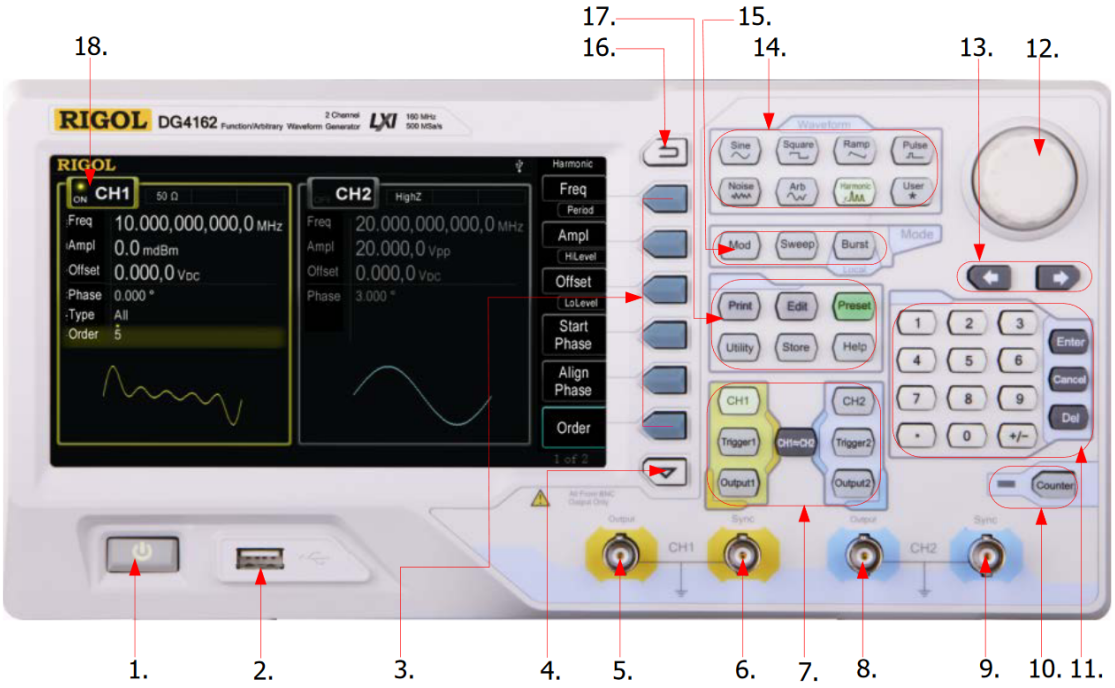


Figure 3: Front panel of DG4162 Function Generator

3. Maximize optical transmission for low voltages: Verify that the BNC cable labeled 'Fast HV amplifier' is connected to the signal output port of the second function generator channel (button 8 in Figure 3). Click 'Arb' and scroll down the menu display (button 4 in Figure 3). Select the 'DC' waveform option that shows up. Set the output DC level to -9 Volts. Press the 'Output2' button. Now slowly rotate the half wave plate until the optical power is **maximal** for that low voltage.

4. Map voltage to beam power: Start scanning with 3.5x magnification. Raise the DC voltage in 1 Volt steps and mark down the resulting beam power for each voltage. Identify the voltages for which the beam powers are 220mW, 80mW and 20mW. Also identify the beam power for 0V - a high beam power at 0V implies that the mouse could not be imaged while the function generator output is turned off. In this case, planar imaging of the mouse will be conducted with a sufficiently high DC voltage that corresponds to a sufficiently low constant beam power.

Figure 4: Rear panel of DG4162 Function Generator

! Critical

Always turn off the function generator output by pressing the 'Output2' button, before changing the parameters of its waveform. The function generator can and will generate excessively sharp voltage slopes when changing other parameters! Our HV amplifier only has limited protection against the resulting loads and will underperform and eventually fail. **THE VOLTAGE SLOPE SHOULD NEVER BE SHARPER THAN 1V per 20ns!** Check the leading/falling edge times and increase them as needed before turning the function generator output back on.

5. Trigger function generator by TAG lens driver: Verify that TAG driver CH3 SYNC channel is connected to CH2 trigger port at the rear panel of the function generator (button 7 in Figure 4). Press 'Pulse' and define a pulse with a frequency of 200kHz, a width of 1000ns and leading/falling edge times of 670ns each. The low and high voltage levels should those for which the beam powers are 220mW and 20mW, respectively. Press the 'Burst' button and select the 'Gated' burst type. Verify that the leading/falling edge times are sufficiently broad, as specified above. Only then, press the 'Output2' button.

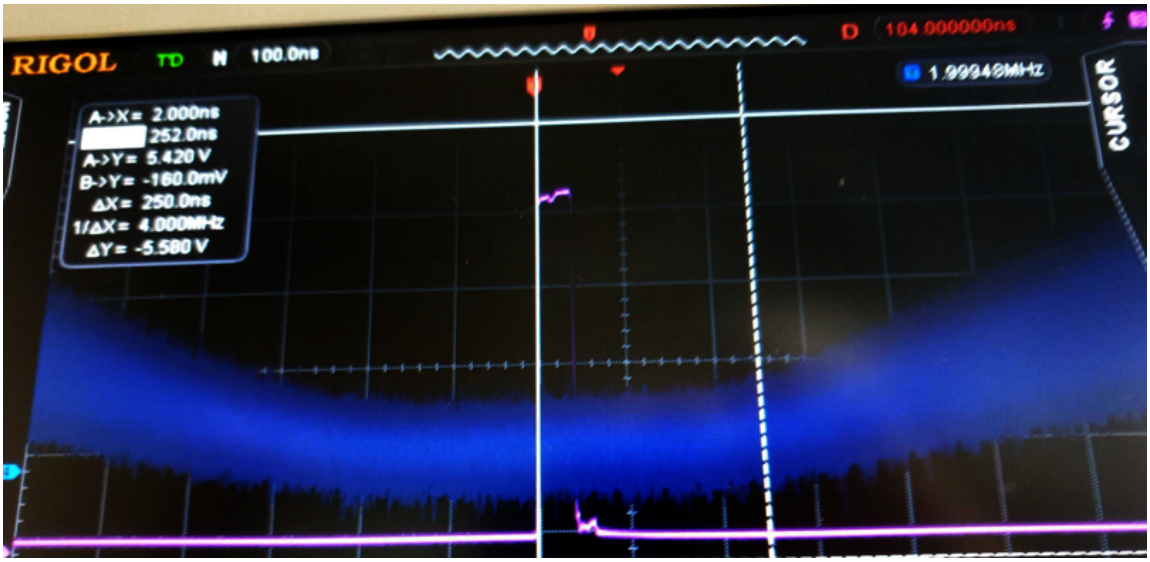


Figure 5: Modify the phase of the CH3 SYNC channel of the TAG lens driver, which triggers the function generator, until the un-filtered trough of the beam power waveform (blue trace) is centered at 180 degrees, as marked by the rising edge of the CH2 SYNC channel of the TAG lens driver (pink trace).

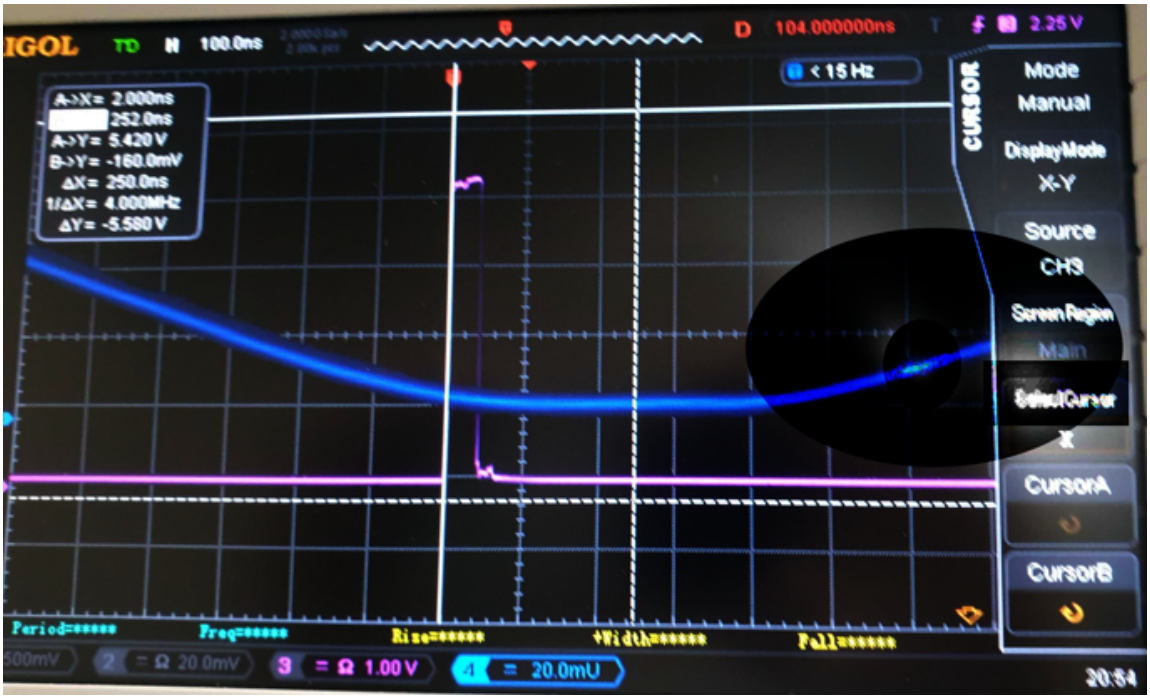


Figure 6: The low-pass filtered beam power waveform lags by 250ns with respect to the unfiltered waveform, so the rising edge of the CH2 SYNC channel of the TAG lens driver (pink trace) should be registered 250ns ahead of it.

6. Determine correct pulse phase: Photograph the exact BNC port to which the BNC output cable of the PDA8A/M detector is connected through a 4MHz Crystek CLPFL-0004-BNC low-pass filter. Disconnect that cable and connect it to the oscilloscope, through the 4MHz low-pass filter. Use a #0 rectangle cover glass to reflect part of the beam into the PDA8A/M detector. Connect the CH2 SYNC channel of the TAG lens driver to an oscilloscope input channel, and set its phase to 180 degrees through the GUI of the TAG lens driver. Set the impedance of that oscilloscope input channel to 50 Ohm impedance. The rising edge of this synchronization pulse corresponds to the maximally convergent phase of the TAG lens, where beam power should be minimal. Through the GUI of the TAG lens driver, modify the phase of the CH3 SYNC channel of the TAG lens driver, which triggers the function generator, until the trough of the un-filtered beam power waveform is centered at 180 degrees, as marked by CH2 SYNC channel of the TAG lens driver. See example in figure 5. The 4MHz low-pass filter introduces a delay of 250ns, so the trough of filtered beam power waveform should lag by 250ns after the said synchronization pulse. See example in figure 6 Write down the identified phase of the CH3 SYNC channel. Finally, remove the rectangle glass that points at the PDA8A/M detector, to minimize beam deflection upstream of the microscope.
   ! Warning
   The typical latency between the trigger port of the function generator and the triggered burst is in the order of 300ns. Do not try to pre-guess the correct pulse phase without looking at the actual waveform reported by the photo-detector.

7. Measure power below the objective lens using a thermopile: Place a power meter directly below the objective lens. Press the 'Burst' button and select the 'Gated' burst type. Verify that the leading/falling edge times are sufficiently broad, as specified above. Only then, press the 'Output2' button. Start scanning with 3.5x magnification. Mark down the resulting power level. This is the average power which will be deposited in the mouse brain tissue during imaging.

Congratulations, the microscope is now ready for intravital imaging. Let's setup the behavioural camera before bringing in a mouse.

0.4 Behavioural monitoring

1. Center XY motor stage: Position the objective lens at +11,000 um in X and Y using the XY knobs.

2. Place treadmill: Place a mouse treadmill beneath the objective lens.

3. Position the behavioural camera: Fetch the behavioural camera, its triggering box, its IR illuminator and the power source of the IR illuminator from escargot room. Mount the behavioural camera on the top lab jack, such that it observes the treadmill from the left, with clear view of the future location of the mouse face and front paws. Mount the IR illuminator right next to it, such that it illuminates the mouse face. There is no need to worry about the IR illuminator contaminating the PMT readings: The PMT count rate was not affected by turning the illuminator on and off.

4. Set cable connections: Connect the triggering box of the behavioural camera with a frame synchronization signal carried by a BNC cable from PFI6 port of the NI DAQ board. This cable should already be connected, secured to the top platform and labeled. Power the IR illuminator. Connect the camera to a USB3 port.

5. Setup the behavioural camera: Set 'AcquisitionMode' to 'Continuous'. Set 'TriggerMode' to 'On'. Set 'TriggerSelector' to 'Frame Start'. Set 'TriggerSource' to the line input to which the frame synchronization cable is connected. Set 'TriggerActivation' to 'Rising Edge'. Set 'TriggerOverlap' to 'ReadOut'. Set 'TriggerDelay' to 0. Set a destination folder for the saved imagery. Notably, while the camera can be set to write to disk 1 in n frames, in practice it will write to disk 1 in n+1 frames. Therefore, asking the camera GUI to write to disk 1 in 1 frames will result in writing to disk every second frame.

6. Test behavioural imagery: Place some dummy object below the objective lens to test its illumination. Select a ROI that covers the expected location of the mouse face and its front legs. Start triggered acquisition with the behavioural camera. Scan 1000 frames with scanimage. Check that the behavioural camera wrote 500 frames to disk. Remove dummy object. Take out the treadmill. The imaging setup is ready.

0.5 Intravital imaging

1. Inject Texas Red / FITC / Alexa 680
2. clamp mouse stage to lab jack
3. (Here be science)
4. Acquire planar dynamics for the same FOV
5. Anaesthetize the mouse
6. Acquire detailed z-stack of the imaged FOV
7. Reacquire z-stack of the imaged FOV with a 25x objective lens
8. Acquire TAG volumes in fluorescein dish with same zoom and pixelization as TAG volumes in imaged samples
9. Realign beam Maximize power below objective lens without the TAG lens
10. Acquire exact beam modulation power using a fast photodetector
11. Follow end of day steps
12. Double backup data

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