

The Mai Tai Two Photon Laser System, Confocal Four.

Overview and Laser Safety.

NB: All users **MUST** be trained in the use of the Mai Tai and must read and sign the Mai Tai specific risk assessment prior to use.

The Mai Tai two photon laser is a powerful infra-red, pulsed, Class 4 device. Unshielded, it would pose a high risk of operator eye damage.

Beam path enclosures, interlock switches, and shutters built into this system, and the material of the microscope's incubation chamber together facilitate safe operation. Users **MUST NOT** attempt to circumvent these safety provisions, or deviate from the operational procedures detailed in the Mai Tai tutorial. Doing so may result in laser exposure, with attendant eye damage and loss of vision.

NB: Three lower doors on the confocal's incubation chamber do not have safety interlocks. These doors are marked "Ensure System is Laser Safe Before Opening This Door". You can ensure the system is laser safe by checking the ZEN software to ensure a scan is not in progress.

Mercury Arc Lamp Safety.

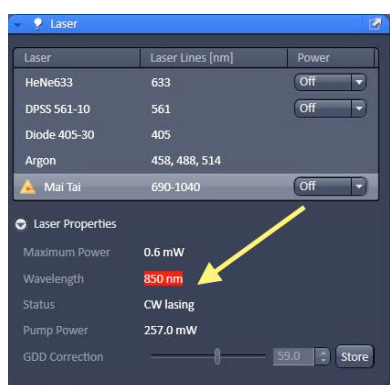
Do Not change filter cubes whilst looking through the eyepieces, as you may be exposed to intense white light.

Startup.

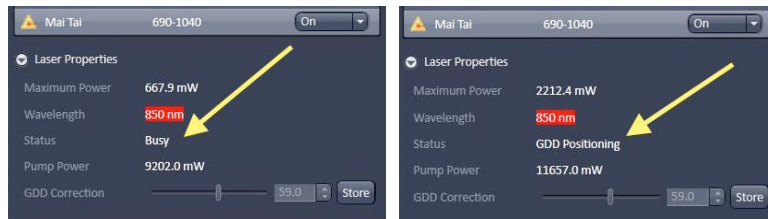
- Obtain the blue tagged Mai Tai key from Microscopy Manager's office.
- Examine the laser system and microscope. The system **must not be used** if any component appears damaged, incomplete, or partially disassembled. Contact IMB microscopy staff if in doubt.
- Ensure all the doors on the microscope's incubation chamber are closed, and that the Zeiss ZEN capture software is NOT running.
- Insert the key into Mai Tai control panel *. Turn the key to on.
- If necessary, start the microscope system and computer. Log in to Windows. Observe the start order and necessary delays for trouble free start up.
- Start ZEN. Ensure ZEN starts without errors.
- On the ZEN "Acquisition" screen, click "Show all tools".
- Open the Laser panel, select Mai Tai laser, and open the Mai Tai's information drop down. You will see an initial display as below.



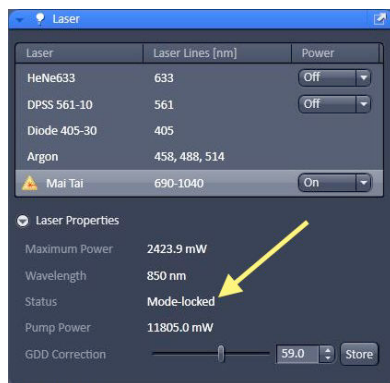
- Click on drop down box to the right of the Mai Tai name and toggle the selection to "On".
- The Mai Tai information drop down will show the laser's presently selected wavelength highlighted in red and a status of "CW Lasing", as the laser begins its warm up sequence.



- The Mai Tai takes about 3 minutes to warm and mode-lock. During this time it will gradually build up power and transition through “Busy” and “GDD Positioning” states.



- When ready for use ZEN will show the Mai Tai’s status as “Mode-locked”.



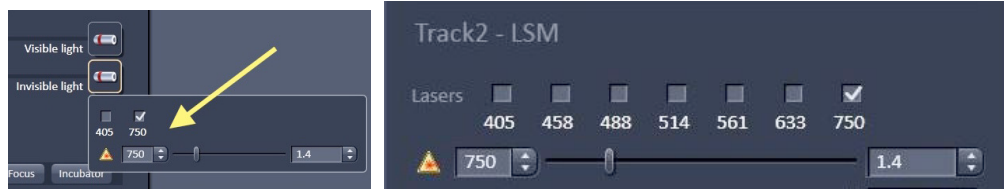
Users note: *

You must turn the Mai Tai’s key to on before starting ZEN. Though the laser may still operate when called in ZEN, starting the Mai Tai after ZEN will produce a GDD positioning warning.

Chameleon laser users note: whereas you must wait for the Chameleon’s front panel to display “Mode-locked” before starting ZEN, in this case ZEN is started after the laser key is turned to on. Note that the Mai Tai will not start to warm up until it is called from ZEN.

Changing the Mai Tai’s power output and emission wavelength.

- Output power can be set in Zen’s Imaging Setup panel or in the Channels panel, in the same manner as the other lasers.
- Start with a low power value (eg. 0.2%) and increase gradually. Note that two photon excitation is non linear; there is a fine line between poor excitation and sample destruction.
- The Mai Tai emission tuning range is 690 to 1040 nm. Type the desired value in the dialog box next to the power slide bar and press “enter”. The newly entered value will be highlighted in red whilst the Mai Tai adjusts, and become plain text once the laser achieves mode-lock at the new wavelength.



Setting up the light path and pinhole.

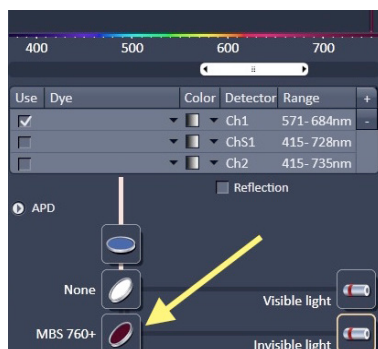
Fluorophore emission in response to two photon excitation may be captured by either the standard detectors, or the non-descanned detectors (NDDs).

The non-descanned detectors are considerably more sensitive than the standard detectors, but are not adjustable for capture wavelength. Those used here are equipped with bandpass filters (BP500-550 and BP575-610nm, detector R1 and R2, resp.) suitable for green and orange-red emission fluorophores only.

Note that ZEN's "Smart Setup" is not two-photon aware; both the excitation and emission light paths must be configured manually.

To capture via the standard detectors:

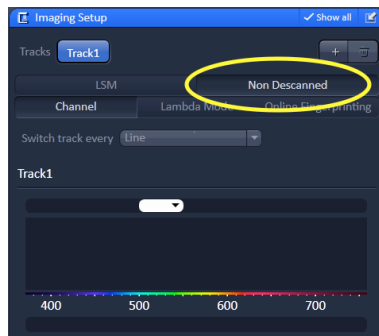
- Open the ZEN "Imaging Setup" panel. Set the excitation light path to direct the two photon excitation to the sample, selecting either the 650+ or 760+ Main Beam Splitter (MBS), as appropriate to your intended excitation and fluorophore emission wavelengths.



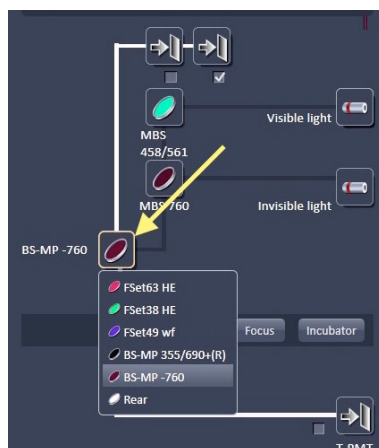
- Set the emission (or capture portion of the) light path as normal.
- Open the ZEN "Channels" panel. Gain and offset are controlled as normal. Note, however, that two photon excitation is confined to the focal point and thus, is inherently confocal. Therefore, the detector pinhole should be set wide open.

To capture via the non-descanned detectors:

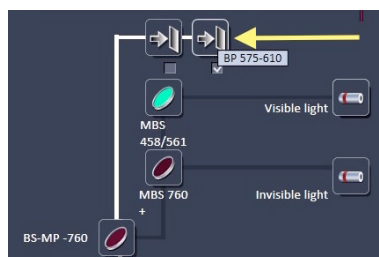
- Open the ZEN “Imaging Setup” panel. Ensure you select “Show All” in the bar’s header to display all available options.
- Select the “Non Descanned” button. The non-descanned light path will be displayed.



- Select the appropriate Main Beam Splitter (MBS) for your two photon wavelength and fluorophore (as above).
- Select the appropriate Secondary Beam Splitter (BS-MP 355/690+® or BS-MP-760) to direct your fluorescence emission to the non descanned detectors.

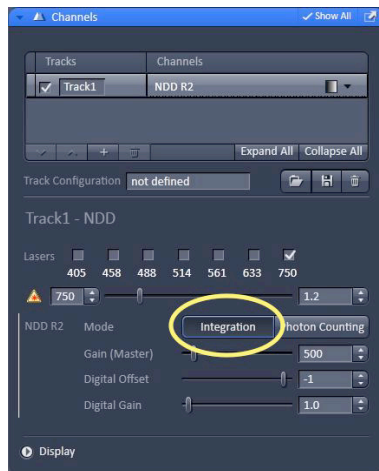


- Select one or both of the NDD channels. These have fixed bandpass filters covering the emission ranges 500-550 and 575-610nm, respectively.



- Open the ZEN “Channels” panel. Ensure “Integration” is selected. The alternative, “Photon Counting” does not form an image.

- Control of NDD gain and black level is as normal. **Note:** there is no pinhole in the NDD light path.

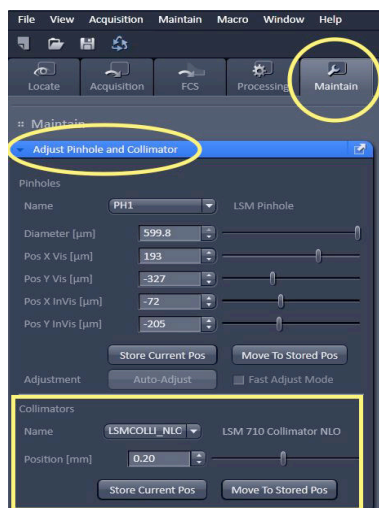


Warning: The non-descanned detectors are extremely sensitive and easily damaged by excess light. Start with **LOW** laser powers and detector gain settings.

Collimation.

The infra-red emission of a two-photon laser focuses to a slightly different z plane than the light from visible lasers. The magnitude of this difference is wavelength dependent. Laser focus can be adjusted to coincidence via the collimator.

- Select a well defined object or point in your sample towards the centre of the image field. Capture a reference image using the appropriate visible laser.
- Open ZEN's "Maintain" screen and select the "Adjust Pinhole and Collimator" panel.
- Under Collimators, select the "LSMCOLLI_NLO" option from the drop down box; the description LSM 710 Collimator NLO will be displayed to the right of the selection. You must ensure that you select this collimator and NOT the 405/UV device.



- Switch back to the “Acquisition” screen and click “New” to create a new image container. Set this up for two photon excitation. Initiate a “Live” or “Continuous” scan. Then, whilst scanning, switch back to the “Maintain” screen.
- The collimator is adjusted to bring the live, two photon produced image of your sample object or point to the same focus obtained in the visible laser image. Collimator position is best adjusted with slow, smooth motion of the scroll bar.
- Click “Store Current Pos”.
- Switch back to the “Acquisition” screen to stop the live \ continuous scan.
- Compare the visible reference image to the two photon image to verify your setting.

Note: this procedure may well bleach your sample. Collimation is best performed on an expendable sample.

Changing the “GDD” Setting.

Image sharpness, confocality and excitation efficiency are influenced by the group velocity dispersion of the laser pulse. The system stores a set of default “GDD” compensation positions for each objective and emission wavelength. These can, however, be altered to attempt optimisation for your particular sample and wavelength needs.

- GDD positions are shown in the Mai Tai information drop down on the ZEN Laser panel.



- The default value can be altered by moving the sliding bar, using the arrows, or typing a value into the value box. The GDD control will freeze and the value will be highlighted in red as the Mai Tai adjusts. Be patient.
- **DO NOT** Click the “Store” button. Doing so alters the default value in the database and thus affects all other users. **Be aware** that, if you choose to alter the GDD position, **you are expected to first note the original value, and reset to that position** prior to shutting down the Mai Tai and ZEN. We suggest also that you take note of your sample’s GDD value, so that you may easily re-enter it in future sessions.

Note: 1) Adjust in small increments and test by imaging. 2) Remember, the GDD setting is global; your alteration will affect all other users. Ensure it is reset to the original, default value at the close of your session. **Note that if you have accidentally clicked “store” for your altered, sample GDD value, you MUST re-enter the original value and click “store” again before turning off the Mai Tai and closing ZEN.**

Shutdown.

- Open the ZEN Laser panel, select Mai Tai laser, and open the Mai Tai's information drop down.
- If you have altered the GDD setting, reset it to its initial value.
- Click on drop down box to the right of the Mai Tai name and toggle the selection to “Off”.
- Wipe up any oil or media spilt on the microscope, the microscope stage, and tables, clean the microscope objectives of oil, and set the incubation chamber back to the default temperature, as appropriate.
- Shut down ZEN and log out of or shut down the the computer, as appropriate.
- Turn the Mai Tai control key to Off, extract the key and return it to the Microscopy Facility Manager's office.

Note: You must turn the Mai Tai off in ZEN before turning the Mai Tai's key to off. Turning the key off while ZEN shows the laser on will result in loss of communication with the laser module. Zen must be restarted, without error, before communication can re-establish.