

Multiphoton Laser

Booking: Select "**MP laser yes**" on the microscope booking page.

Warning: The Multiphoton-laser emits in the infrared range, so it is an **invisible laser!!!**

- *FLIM-PMT (PMT 3): has an AUTOSHUTTER, turns off when the light is too bright (screen stripy / black)*
- *Laser power is weaker close to extremes (690 nm and 1020 nm)*
- *12.5ns between pulses (80,00Hz), a few hundreds of fs pulse*

Check MP chiller:

- **DO NOT turn off at any time**
- Temperature should be at 21°C
- The 2 switches should be in the on "I" position
- Red warning light must be OFF, otherwise refill with *distilled water* (system can stay on)
- Air filter needs to be checked and hoovered / replaced occasionally

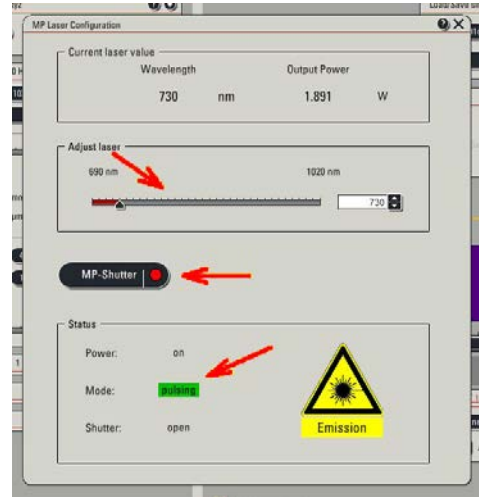
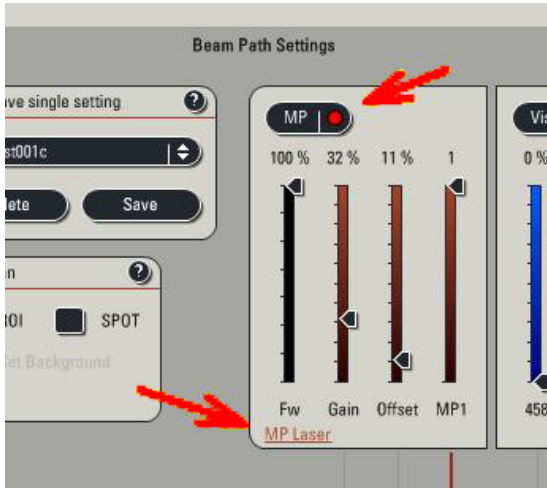
Startup:

- Run Leica software ('LAS AF Confocal')
- Set configuration to "**SP5 MP with Deepsee control**"
- Select Microscope Stand

MP-laser software control:

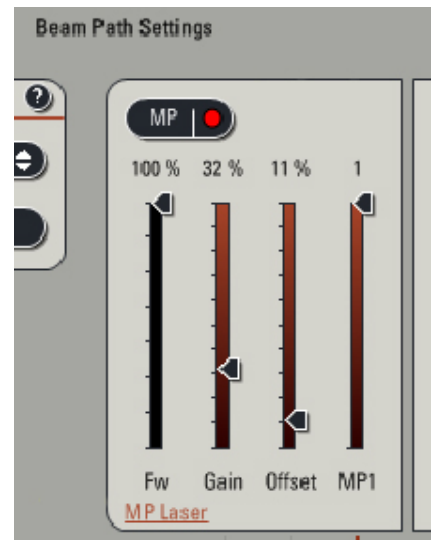
- In configuration –lasers tab: select MP
- In the Acquire-tab, click on MP-Laser.
 - Set the desired wavelength (subsequent hardware adjustment always needs some time: please be patient)
 - Open the shutter by holding the MP-Shutter button for several seconds
 - Wait until laser switches from "cw" to "**pulsing**"
(NB if it fails to start pulsing after a couple of minutes try temporarily changing to a different wavelength and trying again)
 - In the GDD window (below laser status)_select the objective you are using from the drop-down list: this will use the appropriate compensation curve for the objective.

- Close window and press MP in the beam path settings



Optimize/ calibrate MP-laser output with the sliders in the MP-section of the beam path settings:

- **Open pinhole fully** (stays open for MP)
- Increase laser power with "Fw" (filter wheel) until an image appears on the screen (6 - 25%)
- Switch "MP1" to 0
- Change OFFSET until image is black
→ this is not a real offset, but a polarised crystal, so "0" and "100%" are not at bottom / top
→ re-calibrating this makes sure the laser is off for fly-back (no bleaching)
→ position varies for different wavelengths
- Switch "MP1" to 1
- Adjust the GAIN to get the maximum brightness
- Repeat



GDD calibration (pre-compensation)

- Motor position of the DeepSee module can be adjusted in small increments: it will vary the pre-compensation of the pulse & needs to be calibrated for each wavelength and objective used (can use SHG to do that)
- Adjust the motor position with +/- to get the maximum brightness
- Ask Staff if unsure.

Shutting down:

- Check booking for a last minute change.
- Switch off the laser in the Leica software

Never switch off the cooling or the power!!!