

Laserpower Measurement Tutorial

Instrument & measuring mode

- Use Powermeter PT 9610
- **Primary switch on** is at the top side of device (On/Off-slider)
→ After use you always have to switch off the primary switch in order to avoid a shift in instruments precision!
- **Secondary switch on** at the bottom of control panel (On/Off-pushbuttons)
- Measure in RMS L Mode X
- Set mode by using buttons: **RMS** and **HF/LF/WB**
- Record the upper value in the spreadsheet
- The measuring field on the detector head is the white spot, try to center the beam in it

General

Always use the same 10x objective (yellow ring, NA=0.3) for measurement. Lasers should be switched on at least 1 hour before measuring to warm up. Set the argon laser emission to approx. 30% for warming up. For measuring boost emission up to 100% (especially important for the argon laser).

Adjust the detector head with a weak laserline (like 458nm) with approx. 30-50% emission. The measuring field is the white spot on the head.

Don't forget to switch the different wavelengths on power meter, too (arrow keys). **Avoid outside light sources** because they will falsify the measurements. Record the values when they are stable.

Measurements are to be recorded in the spreadsheet of the particular microscope. URL:

docs.google.com

Login: lmf@mpi-cbg.de
PW: backfocalplane

Measurements at Leica TCS SP2 Confocal

1 hour before measurement

- To start system according to the start routine switch on the three red buttons "Laser", "Scanner" and "PC" on remote control at right hand side of table
- Turn controller for argon laser emission to MIN
- Turn the keys for the laser emission to the right:
 - ⇒ Turn argon key "488" to **Start**-mark until it flips back to **ON**-mark
 - ⇒ Turn keys for "543" and "633" to **ON**
 - ⇒ Control lights will turn on
- Turn controller for argon emission to the right approx. a quarter turn for warming up (Figure A)

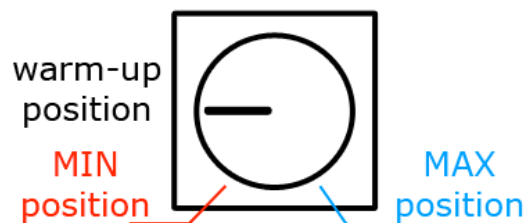


Figure A) Argon emission controller

Directly before measurement

- Turn controller for argon emission to the right "**MAX**" until stop for 100% emission
- After boot up start the **Leica Confocal Software** and choose personal mode then click **Start**



Microscope settings

- Chose bright field "**HF**" position at the wheel above objective revolver and also filter 4 with filter wheel above
- Pull out the slider of the scan-visual-camera-switch on left hand side of microscope

Detector head

- Place detector head with measuring field upwards on slide holder
- Use a piece of cardboard for fixation (e.g. from a cleaning tissue package)
- While maximizing first laser emission (later in procedure) center the beam in scan field
- For this use a low wavelength with approx 50% emission

Software settings

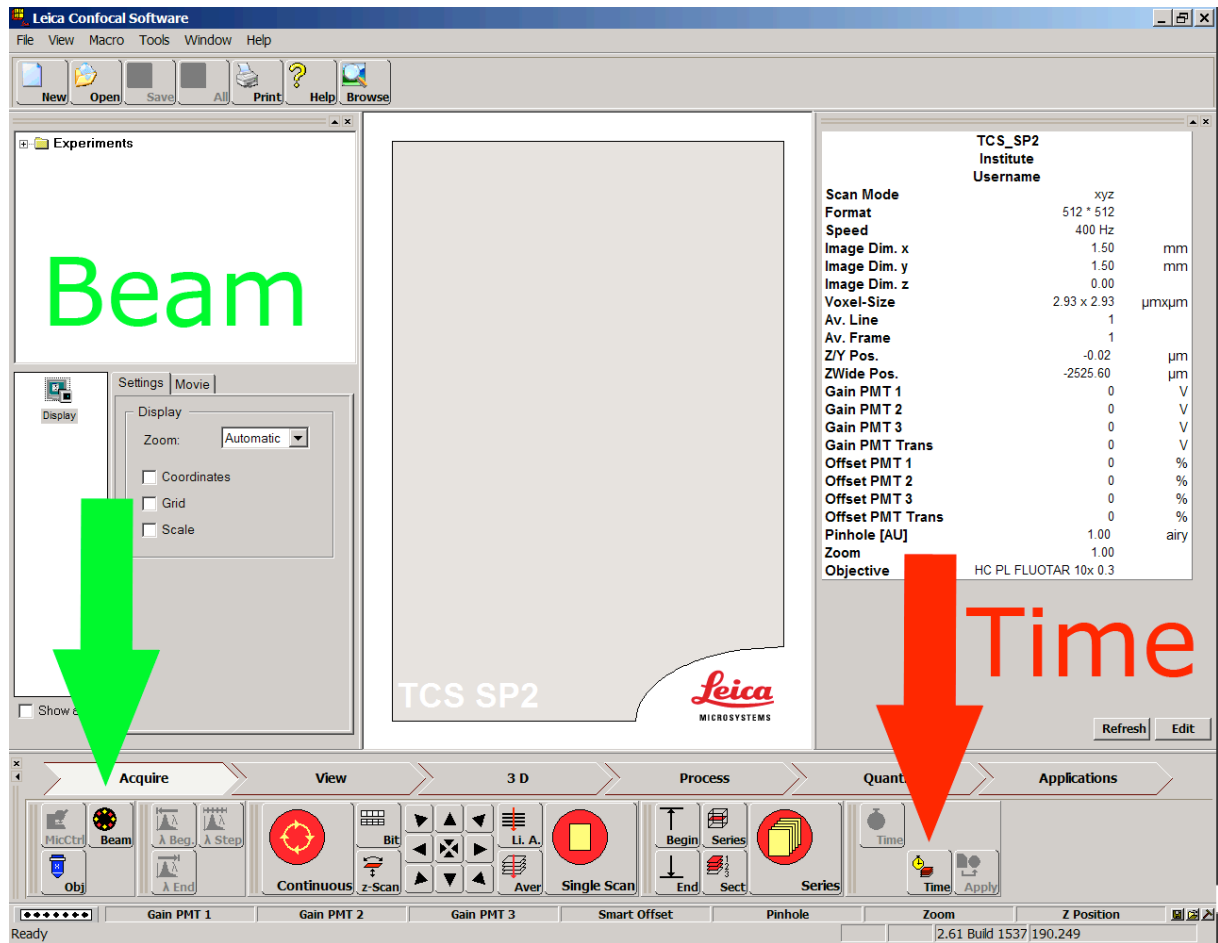


Figure B) Leica Confocal Software main window

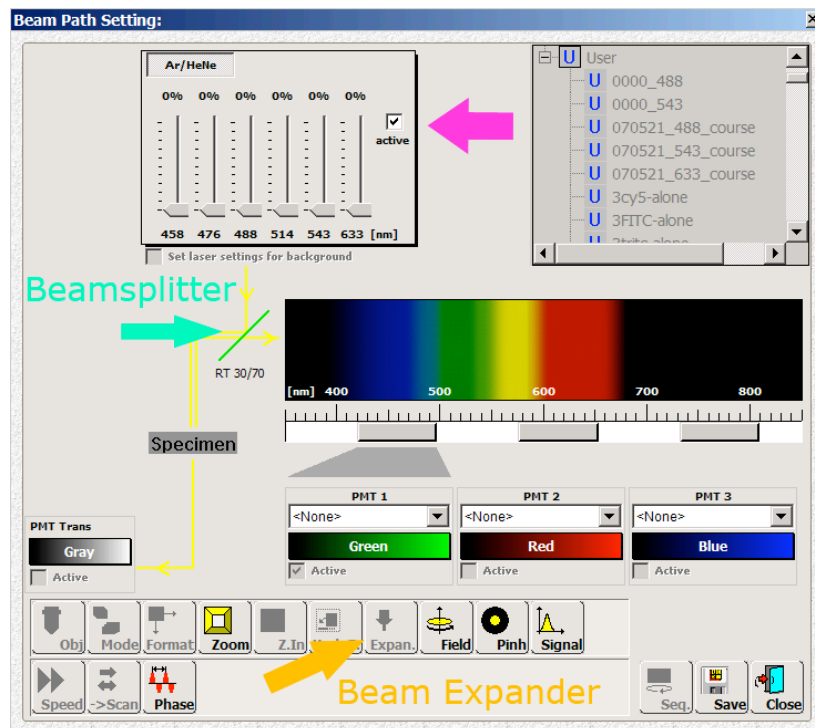


Figure C) Beam Path Setting window

- After software start click **Beam** in main window to choose **Beam Path Setting** window (Figure B)
- Activate laser emission by checking the **Active** field near by the emission controller in **Beam Path Setting** window (Figure C)
- Click on icon "**Expan.**" to set the **Beam Expander** to "---"
- Then click **Time** in main window to choose **Advanced Time-Lapse** window (Figure D)

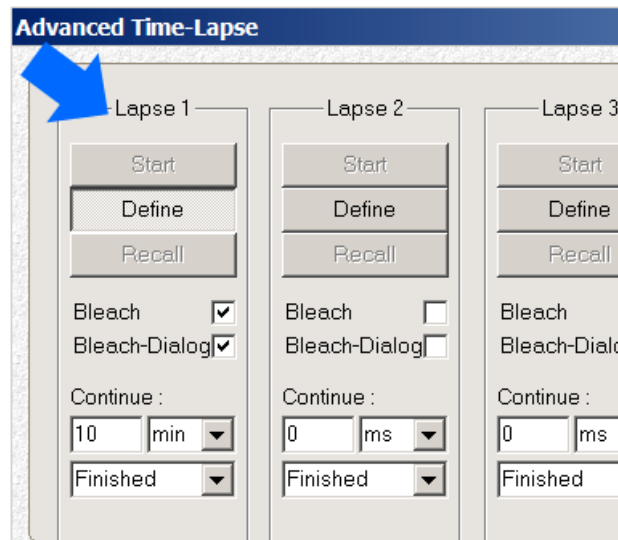


Figure D) Advanced Time-Lapse window left hand side

- Check the **Bleach** and **Bleach-Dialog** fields in Lapse 1 column left hand side
- Enter 10min in **Continue field** until Finished is preset
- Click **Define** then **Start**
- Click **Continue** in pop up window and wait until a short scan is done
- Click **Define** again then set ROI-point (*region of interest*) in right hand side black window via mouse click
- Then enter 10min in **Duration field** for the ROI scan and then click **Continue** now the beam is fixed to a ROI
- Afterwards return to emission controller in **Beam Path Setting** window in order to start measurement
- Therefore maximize emission of each laser one after another to 100% and also set the correct wavelength on power meter
- While measuring laserlines choose RT 30/70 **Beamsplitter** in **Beam Path Setting** window (Figure C)

After measurement – shutting down routine

- Make sure that emissions of all laserlines in emission controller field are 0%
- Deactivate laser emission by unchecking the **Active** field near by the emission controller in **Beam Path Setting** window
- Click **STOP** button in **Advanced Time-Lapse** window right hand side (Figure E)
- Close program



Figure E) Advanced Time-Lapse window right hand side

Remote control

- If another user is coming, log off windows account and turn controller for argon emission to the left for approx. a half turn to **warm-up position** (Figure A)
- Turn controller for argon emission back to the left until "**MIN**" for 0% emission if no user is following
- Turn the keys for the laser emission to the left (control lights turn off)
- Shut down the computer
- Switch off the red buttons "**Scanner**" & "**PC**" **but switch off the "Laser" button at the earliest 5min after turning off the keys** to allow the fan to cool down the argon laser