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/Offpushbuttons)
- 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

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 ONmark
 - \Rightarrow Turn keys for "543" and "633" to **ON**
 - \Rightarrow 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)

| Adv | anced Time-Lapse | 2 | |
|-----|--------------------------------------|------------------------------------|--------------------------------|
| | Lapse 1 | Lapse 2 | Lapse 3 |
| | Start | Start | Start |
| | Define | Define | Define |
| | Recall | Recall | Recall |
| | Bleach 🔽 Bleach-Dialog | Bleach 📃 Bleach-Dialog | Bleach Bleach-Dialc |
| | Continue : 10 min v Finished v | Continue : 0 ms v Finished v | Continue : 0 ms Finished |

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