

# AMBIO Laserpower Measurement Protocol

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## For measurements out of objective at room temperature

- Switch on system according to its switch-on procedure
- Switch on lasers and let them equilibrate for 10 min at RT
- Put objectives in ESCAPE position and choose objective with highest magnification for measurements/always measure with the same objectives:
  - Widefield Ti2: 40x
  - A1Rsi+: 60x oil
  - SD CSU-X: 60x oil
  - STORM/TIRF: 100x oil
  - USD S2: 25x
  - (Multiphoton: 40x water)
- Start NIS software
- Center laserbeam on detector field, therefore, use the center laserbeam-slide and a low nominal laserpower (1-0.5%)
- Mount powermeter detector onto the slide holder
- Switch off room/incubator light
- Make sure that eventual FRAP filters are OUT
- Pay attention to the system-specific preparations/settings below
- Measure at 10% and 100% nominal laserpower in all laser lines one after another
- Therefore, select respective wavelengths on powermeter - “ $\lambda$ ” button
- Eventually change between 638/640/647 nm on the powermeter:
  - Hold “OK” button within  $\lambda$  menu
  - Change wavelength with arrow buttons and click 2x “OK”
- Reduce nominal laserpowers to 1% after each measurement
- Document measurements in [Laserpower measurements.xlsx](#) (Teams/Performance checks or S:\AG\AG-AMBIO\#Public\AMBIO Info\AMBIO Systems\Laserpower)

## System-specific preparations/settings

### Widefield Ti2

- Change lasers and settings via OCs
- Check if correct quad/triple filter cube is selected within the “Turret-up”

### A1Rsi+

- Turret 1: Empty
- Pinhole 1.2 AU
- Scan area: 512x512 pixels
- Speed: 1/32 frames/sec
- Within light path setup:

- 1<sup>st</sup> Dichroic Mirror: 405/488/561/640
- Ch1 Dichroic Mirror: 450/50
- Ch2 Dichroic Mirror: 525/50
- Ch3 & 4 Dichroic Mirrors: 595/50 & 700/75
- Measure in live mode
- For measuring each channel: check/activate only the actual channel/detector, uncheck the others!

#### SD CSU-X

- EPI Dichroics: Out
- (Quadband emission filter: In)
- Scan area: 538x538 pixels
- Measure in live mode

#### USD S2

- EPI Dichroics (Turret 1): Out
- (Quadband emission filter (CSD DM): In)
- Change lasers via OCs
- (CSU Pad 2500 rpm)

#### STORM/TIRF

- Put laser condenser out
- Do laser focus procedure (laser angle: WF)
- Choose Quadband Dichroic