

Simultaneous usage of a 2-photon laser for two confocal microscope set-ups

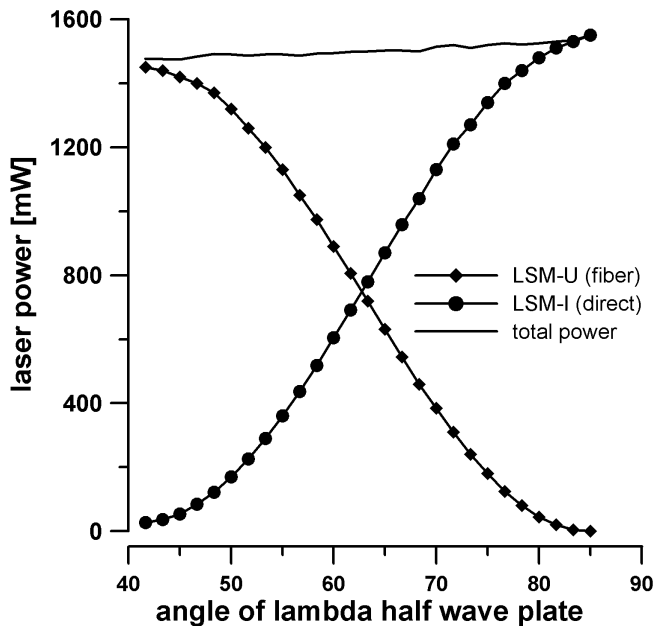
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The use of two-photon or multiphoton confocal microscopy has developed rapidly since the early 90s, when the first commercial set-up became available. The high cost for 2-photon lasers and the shorting of the grant budget calls for new technical solutions. 2-photon lasers have usually a mean power output between 0.4 – 1.6 W over a wavelength range from 700 – 950 nm (8 or 10 W pump laser). The power needed for imaging in the object plane must remain in ranges between 1–10 mW, in order to avoid thermal or photo damage of samples. The loss in power on the way to the object plane is in the order of 60 -90 %, still leaving enough unused excitation power.

We have built an optical set-up allowing variable and reproducible split of the output from a tunable 2-P laser (Mira 900F 10 W, Coherent) by using combinations of polarizing beamsplitters and



lambda/2 plates. The figure shows power measured in the splitted beams at different angles of the plate. The excitation can be used simultaneously on two independent confocal microscopes (upright and inverted Zeiss LSM 510 Meta) and in addition with a third beam path for optical manipulation on one set-up. The optical path to the upright microscope includes a single mode fiber and a modified grating dispersion compensator (GDC, Coherent), which allows easy, laser save, quick and repeatable adjustment of the GDC. The beam to the inverted microscope is directly coupled through another GDC (Coherent), which is scaled down to 1/6 of the ordinary GDC, but still allows optimal pulse-length in the sample. The third beam path, which is currently under construction can be used for 2-P bleaching or uncaging with a non scanning beam independent from the scanning beam. This path includes an acousto optic modulator, which is triggered, pixel accurate by the confocal microscope.

Data are shown for uncaging of caged-fluorescein and activation of photoactivatable-GFP in single cells with 2P-excitation using 20 % of the maximum laser output at 730 nm. The set-up we present is an ideal solution for imaging facilities, which need an upright and an inverted 2-P confocal or need to double their 2-P microscope capacity.