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Tunable, low-repetition-rate, cost-efficient femtosecond Ti:sapphire laser for nonlinear microscopy

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Abstract We report on a broadly tunable, long-cavity Ti:sapphire laser oscillator being mode-locked in the net negative intracavity dispersion regime by Kerr-lens mode-locking, delivering $\tau_{FWHM} < 300$ fs pulses at 22 MHz repetition rate. The wavelength of the laser can be tuned over a 170 nm wide range between 712 nm and 882 nm. Having a typical pump power of 2.6 W, the maximum pulse peak power is 60 kW. Comparison of the reported laser with a standard, 76 MHz Ti:sapphire oscillator regarding two-photon excitation efficiency in a laser scanning microscope shows that the 22 MHz laser generates the same fluorescence signal at considerably, 1.82 times lower average power, which is expected to result in a reduced photothermal damage probability of biological samples. This fact along with the broad tunability and a low pump power requirement makes this cost-effective laser an ideal light source for nonlinear microscopy.

1 Introduction

Nonlinear laser scanning microscopy is a novel 3D biomedical imaging technique with sub-micrometer resolution. There are several nonlinear optical effects that can be used for imaging, such as two- or three-photon absorption fluorescence and second-harmonic generation. To obtain the light intensity sufficient for nonlinearities, ultrashort (fs or ps) pulse mode-locked lasers are used as light sources, most often femtosecond pulse, tunable Ti:sapphire lasers having a repetition rate at around 80 MHz.

Multiphoton transitions of intracellular fluorophores used in microscopy can be efficiently excited in the 700–1200 nm spectral region, which wavelengths penetrate deeper in tissues and are much less harmful for living specimen than direct UV illumination in single-photon fluorescent microscopy. Photochemical damage mechanisms, which can cause oxidative stress or direct DNA damage, only occur in the focus of the objective lens, where multiphoton absorption takes place. Single-photon absorption of the NIR radiation can cause photothermal damage, though this is not significant in most cells, where water is the major NIR absorber [1]. However, in cells where other efficient NIR absorbers (e.g., melanin, hemoglobin, chlorophyll) are present, photothermal damage can be a problem. Since one of the most important noninvasive diagnostic applications of nonlinear microscopy is based on in vivo multiphoton imaging of skin tissues [2, 3] containing a significant amount of melanin, photo-thermal damage effects should be taken care of. When the pulse repetition rate of the exciting laser radiation is not very low ($\nu_{rep} > 5$–10 MHz), the dominating effect responsible for temperature rise and thermal damage is the cumulative heating effect of the consecutive pulses, which is proportional to the time averaged power of the pulse train incident on the sample [4]. Thus, thermal damage can be mitigated by decreasing the average power, which can be achieved by the reduction of the pulse energy or by the reduction of the repetition rate. Since two-photon absorption rate, and thus the two-photon fluorescence signal are quadratic functions of the pulse energy and linear functions of the repetition rate, mitigating thermal damage by reducing the repetition rate is preferable [4]; in [4] that was

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