A 20 MHz, sub-ps, Tunable Ti:sapphire Laser System for Real Time, Stain Free, High Contrast Histology of the Skin

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Abstract: A 20 MHz repetition rate, sub-ps Ti:sapphire (Ti:S) laser system is proposed for real time, high chemical contrast dual vibration resonance frequency (DVRF) CARS imaging of the skin suitable for *in vivo* histology.
OCIS codes: (140.7090) Ultrafast lasers; (180.4315) Nonlinear microscopy.

1. Introduction

Coherent anti-stokes Raman scattering (CARS) [1] microscopy is widely used in label-free biomedical imaging applications. For *in vivo* diagnostic use of CARS microscopy, wide field detection is preferred to descanned configurations [2]. Chemical selectivity poses a major difficulty when femtosecond (fs) pulse lasers are applied, as their spectral bandwidth is typically significantly higher (~5-10 nm) than the optimum value (~ 1 nm) matching the bandwidth of molecular vibrations. This fact leads to the appearance of an enhanced non-specific background and the decrease of spectral sensitivity in CARS imaging. Two years ago we proposed a fast spectral modulation technique for sub-100 fs pulse Ti:S lasers [3], which allowed us to modulate the laser spectrum on ms time scale with the use of a piezo-driven Michelson interferometer. In one of the settings we used, we modulated the laser spectrum of our laser in such a way, that CARS imaging for CH_2 bonds in "lipids" and CH_3 bonds "proteins" did not require any tuning of the pump (Ti:S) laser or any readjustment of the delay between the pump and Stokes (Yb-amplifier) pulses, which allowed us to record stain-free histological images [4] of brain slices. In this paper we report on a newly developed, ~20 MHz, sub-ps Ti:sapphire laser system, which supports real time, two-channel, high chemical contrast, DVRF CARS imaging, i.e. histology of the skin by a commercial LSM 7 MP microscope (Carl Zeiss, Jena, DE) without any modification of its ZEN software or postprocessing of the images like in case of our previous CARS setups used for histology [3,4].

2. Experimental setup

For our comparative studies, we used two different CARS imaging setups, as shown in Fig.1. In our setup at the University Szeged (USZ) [3,5], we used a ~80 MHz, ~80 fs Ti:S laser (*Mai Tai*, Newport Spectra-Physics, USA) as a pump laser (for details, see Refs. 3 and 5). In the setup at Wigner RCP, Budapest [1,4], we replaced our ~76 MHz, ~150 fs Ti:S laser by a newly developed, ~ 20 MHz repetition rate, sub-ps Ti:S laser (*FemtoRose 300 TUN LC*, R&D Ultrafast Lasers Ltd.). The long cavity laser configuration was similar to that was published in Ref. 6, with a few modifications, among them the most critical was the following: we replaced the SF10 prism pair by a Gires-Tournois interferometer, which provided considerably higher intracavity dispersion than the prism pair previously used. Beside a birefringent tuning element, fine tuning of the Ti:sapphire laser was obtained by the piezo controlled GTI. In our new setup, the spectral bandwidth of the pump (Ti:S) laser was reduced from 6-8 nm to ~2 nm. Accordingly, the pulse duration increased from ~150 fs to ~ 600 fs, or slightly above. This four-fold reduction in the peak intensity was compensated by the lower repetition rate of our long cavity Ti:sapphire laser comprising a Herriott-cell and a ~2W average power, 532 nm pump laser [6]. Pulse duration of the ~20 MHz laser was characterized by a PulseCheck autocorrelator (APE GmbH, DE). Depending on the intracavity dispersion set by the mirror spacing of an intracavity GTI, the pulse duration could be set in the 0.6-1 ps range. Spectral bandwidth of the ~20 MHz Ti:sapphire laser (pump) was measured $\Delta\lambda < 2$ nm allowing high spectral resolution DVRF-CARS imaging. For higher spectral contrast between the anti-Stokes signals generated by „lipids" and „proteins", we placed a Michelson interferometer similar to that was used in Ref. 3 into the beam path of our Stokes (Yb) laser. By spectral modulation, we obtained a double peaked spectrum with a peak separation of 5-6 nm at around 1030 nm. DVRF CARS imaging was performed by two NDD detectors of our microscope: the anti-Stokes signals for „lipids" and „proteins" were separated by a dichroic mirror with a long pass edge at around 645 nm, while two bandpass filters with central wavelengths at 641 nm and 650 nm were respectively placed in front of the NDD-s.