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Fiber-coupled, 20 MHz Repetition Rate, sub-ps Ti:sapphire Laser for *in vivo* Nonlinear Microscopy of the Skin

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Abstract: A fiber coupled, sub ps Ti:sapphire laser suitable for *in vivo,* stain free, 3D imaging of skin alterations is introduced. It is pumped by a low cost, 2.1 W pump laser and delivers 0.6 1 ps high peak power pulses optimized for fiber delivery.

1. Introduction

Nonlinear microscopy, such as two-photon excitation fluorescence microscopy (2PEF), second-harmonic generation (SHG) microscopy and Coherent anti-stokes Raman scattering (CARS) microscopy is increasingly used to perform non-invasive, *in vivo* studies in life sciences. These techniques enable us to investigate the morphology or monitor the physiological processes (e.g. monitoring drug delivery) in the skin [1] by the use of ultrafast pulse lasers. Recent years brought revolutionary progress in the development of sub-ps pulse, all-fiber laser oscillators and amplifiers being suitable for nonlinear microscopy. Fiber (or fiber coupled) lasers are of great interest because they can easily be combined with endoscopy. This latter feature greatly increases the utility of nonlinear microscopy for pre-clinical applications and tissue imaging. In 2016, we reported on a novel, handheld 2PEF/SHG microscope imaging system comprising a sub-ps Yb-fiber laser system [2], which was suitable for *in vivo* imaging of murine skin at an average power level as low as 5 mW at 200 kHz sampling rate. The whole nonlinear microscope imaging system had the main advantages of the low price of the fs laser, fiber optics flexibility, a relatively small, light-weight scanning and detection head, and a very low risk of thermal or photochemical damage of the skin samples.

In principle, 2PEF microscopy can visualize endogenous fluorophores, such as elastin, keratin, NADH, FAD, etc., while the morphology of collagen fibers can be assessed by SHG microscopy. Due to the limited photon energy of our Yb-fiber laser system operating at around 1030 nm, however, we could not efficiently excite a few of these endogenous fluorophores (such as elastic fibers, NADH, FAD) with our handheld our 2PEF imaging system. This fact considerably limited its applicability in case of rear skin diseases (such as Ehlers-Danlos syndrome (EDS) [3], pseudoxanthoma elasticum (PXE) [4]) or in case of basal cell carcinoma (BCC) [5], the latter one being the most common malignancy in Caucasians. In order to overcome this problem, we replaced our Yb-fiber laser by a fiber coupled Ti:sapphire laser operating at around 810 nm, whose physical parameters (repetition rate, spectral bandwith, peak intensity, etc) were optimized for fiber delivery and low thermal load, *in vivo* imaging of the skin.

2. Results

The ~ 20 MHz repetition rate (long cavity), sub-ps Ti:S laser used in our fiber delivery and nonlinear microscope imaging experiment is similar to that we used for real time histology of the skin by simultaneous CARS imaging of lipids and proteins [6]. The basic configuration of this long cavity was described in details in Ref. 7. For our imaging experiments, however, we had to make a few modifications in order to reduce the spectral bandwidth of the laser. It results in higher chemical selectivity in DVRF-CARS imaging [6] and lower sensitivity of the optical pulses for dispersive effects during fiber delivery. To this end, we replaced the SF10 prism pair by a piezo controlled Gires-Tournois interferometer (GTI), which provided considerably higher intracavity dispersion than a prism pair. A birefringent filter (BRF) is used for tuning of the laser. Owing to the enlarged intracavity dispersion, spectral bandwidth of the Ti:sapphire laser has been reduced below 2 nm. Accordingly, the pulse duration of the laser increased above 600 fs. 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 [7]. The laser is pumped by a 2.1 W average power, 532 nm laser (*Opus*, product of Laser Quantum, UK). Final setup of the 20 MHz repetition rate, sub-ps Ti:S laser used for fiber delivery and imaging experiment is shown in Fig. 1. 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 (see Fig. 2.a, black dots). The $\Delta\lambda < 2$ nm spectral bandwidth of the laser allows distortion free fiber delivery of the optical pulses through a