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 sub-ps 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 bandwidth, peak intensity, etc.) were optimized for fiber delivery and low thermal load, *in vivo* imaging of the skin. Similar systems reported earlier [6, 7] applied hollow core photonics bandgap fibers in combination with a sub-100-fs, 76 MHz repetition rate Ti:sapphire laser and were sensitive for dispersive elements applied (e.g. Pockels cell, microscope objective, beam-steering mirrors [8]), operation wavelength of the laser [6], length and birefringence of the hollow core fiber [9]. Most of these problems might be solved by application of reversed dispersion slope hollow core photonic bandgap fibers [10], but they are not commercially available and might have a similar problem of birefringence resulting in double optical pulses at the fiber end in case of sub-100 fs pulses [9]. In this paper we report on a fiber-coupled, low repetition rate, sub-ps Ti:sapphire laser system which is free from all of these problems and allows fiber delivery with minimum temporal [6] and spatial [10, 11] distortion of the optical pulses.

2. Fiber-coupled, 20 MHz repetition rate, sub-ps Ti:sapphire laser

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 [12]. Its long cavity configuration laser comprising a Herriott-cell was described in details in Ref. 13. For our fiber delivery and imaging experiments, however, we had to make a few modifications regarding cavity dispersion and self-amplitude modulation in order to reduce the spectral bandwidth of the laser and to improve mode-locking performance, respectively. A lower spectral bandwidth results in higher chemical selectivity in