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Communication

A 20 MHz Repetition Rate, Sub-Picosecond Ti–Sapphire Laser for Fiber Delivery in Nonlinear Microscopy of the Skin

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Abstract: Nonlinear microscopy (NM) enables us to investigate the morphology or monitor the physiological processes of the skin through the use of ultrafast lasers. Fiber (or fiber-coupled) lasers are of great interest because they can easily be combined with a handheld, scanning nonlinear microscope. This latter feature greatly increases the utility of NM for pre-clinical applications and in vivo tissue imaging. Here, we present a fiber-coupled, sub-ps Ti–sapphire laser system being optimized for in vivo, stain-free, 3D imaging of skin alterations with a low thermal load of the skin. The laser is pumped by a low-cost, 2.1 W, 532 nm pump laser and delivers 0.5–1 ps, high-peak-power pulses at a ~20 MHz repetition rate. The spectral bandwidth of the laser is below 2 nm, which results in a low sensitivity for dispersion during fiber delivery. The reduction in the peak intensity due to the increased pulse duration is compensated by the lower repetition rate of our laser. In our proof-of-concept imaging experiments, a ~1.8 m long, commercial hollow-core photonic bandgap fiber was used for fiber delivery. Fresh and frozen skin biopsies of different skin alterations (e.g., adult hemangioma, basal cell cancer) and an unaffected control were used for high-quality, two-photon excitation fluorescence microscopy (2PEF) and second-harmonic generation (SHG) z-stack (3D) imaging.

Keywords: nonlinear optical microscopy; second-harmonic generation; two-photon excitation fluorescence; skin cancer; adult hemangioma

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1. Introduction

Nonlinear microscopy, such as two-photon excitation fluorescence microscopy (2PEF) [1,2], second-harmonic generation (SHG) microscopy [3,4] and coherent anti-Stokes Raman scattering (CARS) microscopy [5,6], is increasingly used to perform non-invasive, in vivo studies in life sciences. These techniques enable us to investigate the morphology [7–12] or monitor the physiological processes (e.g., monitoring drug delivery) of the skin [13–15] through the use of ultrafast pulse lasers. Recent years have brought revolutionary progress in the development of sub-ps pulse, all-fiber laser oscillators and amplifiers that are suitable for nonlinear microscopy. Fiber (or fiber-coupled) lasers are of great interest because they can easily be combined with endoscopy [16–20]. 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, ~2 MHz repetition rate Yb–fiber laser system [21], which was suitable for the in vivo imaging of murine skin at an average power level of as low as 5 mW at a 200 kHz sampling rate (corresponding to a 5 µs pixel dwell time value). The whole nonlinear microscope imaging system had the main advantages of a low price for 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 [22,23].