# *FiberScope*: an Optical Fiber Laser Based, Handheld 3D Nonlinear Microscope System for *in vivo* Diagnostic Applications in Dermatology and Nanomedicine

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**Abstract:** A novel, Yb-fiber laser based, handheld 2PEF/SHG microscope imaging system is introduced being suitable for *in vivo* imaging of murine skin at an average power level as low as 5 mW at 200 kHz sampling rate.
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## 1. Introduction

Nonlinear microscopy, such as two-photon excitation fluorescence microscopy (2PEF) and second-harmonic generation (SHG) microscopy is being increasingly used to perform *in vivo* studies in dermatology. These techniques enable us to investigate the morphology and monitor the physiological process in the skin by the use of femtosecond pulse lasers (such as a broadly tunable Ti-sapphire laser [1]) operating in the near-infrared spectral range (680-1060 nm). Recent years brought revolutionary progress in development of femtosecond pulse, all-fiber laser oscillators [2] and amplifiers being suitable for nonlinear microscopy. Fiber lasers are of great interest not only because of their considerably lower price but the ease with which they can be combined with endoscopy [3], which would greatly increase the utility of nonlinear microscopy for pre-clinical applications and tissue imaging.

There are a few commercial 3D microscope systems that are presently being used for diagnostics purpouses in dermatology. Lucid's *VivaScope* is confocal, handheld 3D mircoscope system that utilizes a cw near infrared laser for imaging through a pinhole for optical sectioning [4]. Due to the low photon energy and the low intensitiy of cw laser radiation applied, it is not suitable for single photon excitation fluorescence (1PEF) or nonlinear imaging (2PEF, SHG), that is why it does not offer chemical selectivity for the different tissue components. JenLab's *DermaInspect* system [5] has the advantage of utilizing nonlinear imaging techniques (2PEF, SHG, CARS) for microscopic 3D tissue imaging, however, the extra price that a customer (i.e., a dermatologist) has to pay for chemical selectivity is very high: it is basically the price of a femtosecond pulse tunable Ti:sapphire laser applied.

A few years ago we reported on the first broadly tunable, long-cavity Ti:sapphire laser oscillator [6] being mode-locked in the net negative intra-cavity dispersion regime by Kerr-lens mode-locking, which laser delivered $\tau < 300$ fs pulses at 22 MHz repetition rate. Comparison of the reported laser with a standard, 76 MHz Ti:sapphire oscillator (such as used in [5]) regarding two-photon excitation efficiency in a laser scanning microscope showed that the 22 MHz laser generates the same fluorescence signal at considerably, 1.82 times lower average power, which results in a reduced photo-thermal damage probability of biological samples. This fact along with the broad tunability and a low pump power requirement made this cost-effective laser an ideal light source for nonlinear microscopy. In the same year, Baumgartl et al. reported on an all-fiber, two-wavelength laser source suitable for CARS microscopy, which laser delivered ~100 ps pulses at a few MHz repetition rate [7]. For high peak power of the (relatively long) laser pulses, which is crucial for nonlinear imaging, the repetition rate of the laser system was reduced by a programmable pulse picker, which was placed between the oscillator and the fiber amplifier unit operating at around 1030 nm. (Note that the nonlinear signal in a 2PEF or SHG imaging system scales inversely with the repetition rate as well as with the pulse duration of the laser as far as the average power is kept constant.)

In order to considerably cut the price that a dermatologist has to pay for 3D nonlinear microscopic imaging of the human skin, and to take the advantage of fiber optic delivery of the optical pulses to the (handheld) scanning microscope head, we decided to use highly efficient fiber lasers operating near infrared spectral range. The longer operation wavelength (compared to that of a Ti:sapphire laser) offers two main advantages: first, a lower scattering loss in the tissue, which allows deep tissue imaging without thermal damage of the skin; secondly, the longer wavelength (i.e., the lower photon energy) minimizes the risk of photochemical degradation (e.g., CPD formation) of the sample. This latter fact is extremely important from the point of view of human diagnostic applications, since in present 3D nonlinear microscope systems (e.g., in *DermaInspect*) Ti:sapphire lasers operating at 750 nm (for

NADH excitation) and at 800 nm (for collagen detection) are used. In these imaging systems, the typical laser irradiation (average power) is at around 50 mW, which value is measured directly above the skin. According to our recent measurements [8], this power level has the risk of CPD formation in the DNA (at the excitation wavelength of 750 nm) when using typical pixel dwell time values of 5 μsec and a water immersion objective with a numerical aperture of NA = 1.0 in a scanning nonlinear microscope.

In the followings, we introduce our novel, handheld nonlinear microscope system named *FiberScope* comprising a ~2MHz repetition rate Yb-fiber laser as a pulsed light source for nonlinear imaging. The system has the main advantages of the lower price of the fs laser applied, fiber optics flexibility, a relatively small, light-weight scanning and detection head, and no risk of thermal or photochemical damage of the skin. In order to mention some possible applications in dermatology, we show that our novel microscope system is capable of high quality, *in vivo* 3D SHG imaging of the collagen content of murine skin at average power levels as low as ~5 mW. Among others, using SHG imaging of the collagen, we could investigate and follow the effects of obesity on dermal collagen alterations [9]. For demonstration purposes in cosmetology and nanomedicine, we also used our 2PEF/SHG imaging system for *in vivo* visualization and monitoring the uptake of Alexa Fluor 546 labelled nanomedicine by Langerhans cells [10] for 24 hours.

## 2. Experimental setup

The block scheme of our *FiberScope* in vivo 3D microscope imaging system is shown in Fig. 1. The primary pulsed laser source is an all-fiber, all-normal dispersion ytterbium ring oscillator (*FiberSource*, product of R&D Ultrafast Lasers Ltd, Budapest, Hungary) [2]. It operates at a ~36.4 MHz repetition rate and delivers (positively chirped) 10-12 ps optical pulses at $\lambda_0$ ~1030 nm with spectral bandwidths in the $\Delta\lambda$ ~ 8-12 nm range. The average power of the oscillator is $P_{ml}$ ~ 10 mW. The repetition rate of the laser system is reduced by a fiber integrated, programmable pulse picker unit with controller electronics (product of *JenOptik*, Jena, Germany). The pulse picker is followed by a two-stage Yb-fiber amplifier (*FemtoCARS Stokes Unit*, product of R&D Ultrafast Lasers Ltd, Budapest, Hungary), which amplifies the rarefied optical pulses to an average power of ~200 mW. For our measurements, we typically set the repetiton rate of the amplified laser system in the 1 to 2 MHz range, which assures the highest peak powers without observable nonlinear distortion of the amplified pulses in the optical fiber amplifier. The low repetition rate, picosecond pulse Yb-fiber laser system has a FC/APC connector at the fiber output, which is introduced to an FC/APC fiber collimator (F220APC-1064, beam diameter: ~2.4 mm, product of Thorlabs Inc., USA) mechanically fixed in the imaging unit. Before entering the scanning head of our handhold microscope, the collimated laser beam passes through a small size transmission grating compressor, which reduces the pulse duration below 0.5 ps. The compressor unit, which includes a few mirrors and the grating pair, is fixed to the mechanical chassis of the imaging unit. Without any mechanical translation in the compressor unit (i.e., keeping the grating separation at a constant value), we are capable electronically control (practically minimize) the pulse duration of the compressed pulses, hence obtaining the highest signal to noise ratio at a certain average power level.

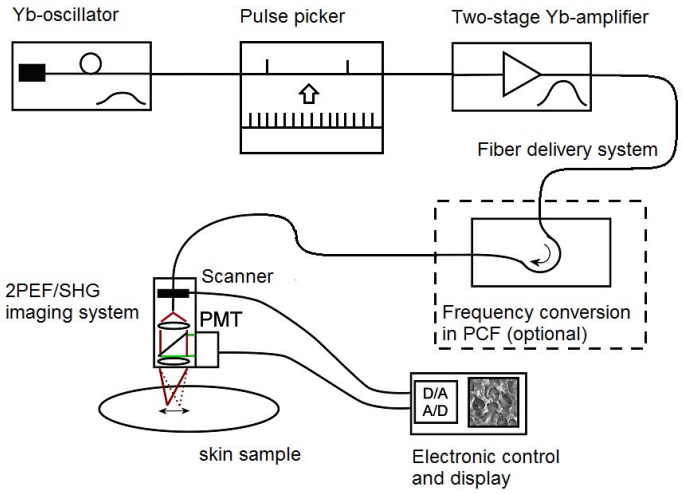


Fig. 1. Bloch scheme of our novel 3D 2PEF/SHG microscope imaging system.

The handheld scanning 2PEF/SHG imaging head comprises an x-y scanner unit (Cambridge Technologies), a 1:3 telescope system custom designed for an *EC Plan-Neofluar 40x/0.75* objective (Carl Zeiss, Germany). The physical length of the whole 3D microscope imaging system is at around ~150 mm including the scanner unit as well as the microscope objective. The 2PEF/SHG signal is detected by two PMT detectors (Hamamatsu, Japan) supplied with properly chosen dichroic and bandpass filters. The analogous electronic signals of the PMT detectors are amplified and digitized by some home-built electronics.

## 3. Results

We tested the *FiberScope* system for different *in vivo* murine skin samples. The SHG image of the collagen (shown in Fig. 2, left) was recorded at ~5 mW average power. The pixel dwell time was set to 5 µs, which corresponds to our 200 kHz sampling rate. On the right side of Fig. 2, we show the result of an *in vivo* penetration measurement of Alexa-546 labelled nanoparticles (AF546-DV) in murine skin using the same device optimized for combined 2PEF/SHG measurements.

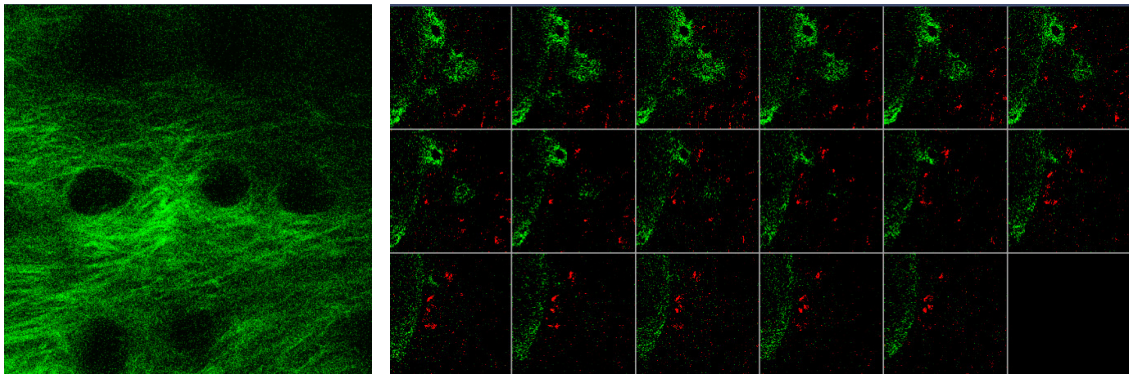

Fig. 2 Left: SHG image of collagen recorded at 5 mW average power (measured directly above the skin) from and Yb-fiber laser operating at a 1.89 MHz repetition rate. Right: *in vivo* penetration measurement of Alexa-546 labelled nanoparticles (AF546-DV) in murine skin. Excitation wavelength: ~1030 nm, z-stack image (128x128 pixels/frame). Green: SHG signal of collagen. Red: fluorescence signal of AF546-labelled nanoparticles 1 hour after of the topical treatment by nanomedicine of the skin. One can observe that the Langerhans cells, which are a part of the immune system, accumulate the AF546-labelled nanoparticles [10].

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