

1300 nm Fiber Laser System for THG and 2PEF Bio-Imaging

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Abstract: We present a 1300 nm fiber laser source for bio-imaging applications. We demonstrate nonlinear imaging using nanocrystals as optical markers for THG microscopy and efficient 2-photon-excitation of fluorophores at 1300 nm in biological samples.

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1. Introduction

Nonlinear microscopy techniques have become an important tool in bio-imaging applications, offering advantages over traditional linear microscopy techniques and new possibilities for sample investigation [1]. Nonlinear microscopy offers an intrinsic 3-dimensional high resolution imaging capability and allows for the use of longer excitation wavelengths which enables deeper penetration into biological samples while causing less photo damage. Using excitation wavelengths between 700 and 1080 nm nonlinear microscopy can be performed using common Titanium:Sapphire laser systems, however, an extension to higher wavelengths requires expensive laser systems such as optical parametric oscillators (OPO) or optical parametric amplifiers (OPA). As a result, despite the advantage for bio-imaging, wavelength above 1080 nm are rarely used.

Here, we present a fiber laser system designed for bio-imaging at 1300 nm to provide cost-efficient access to the benefits of deeper sample penetration and reduced sample damage at longer excitation wavelength. We demonstrate bio-imaging of biological samples using different fluorophores and markers at 1300 nm excitation wavelength and low power levels.

2. Experimental Setup

For nonlinear bio-imaging the 1300 nm femtosecond pulses were free-space coupled into a commercial nonlinear microscope (LaVision BioTec, TRiMScope II) which allows for rapid multimodal imaging. The laser beam was focused onto the sample using a high NA microscope objective. For image acquisition the laser beam was scanned over the sample using a set of galvanometer scanning mirrors. The generated signal was collected in epi-direction by the focusing microscope objective and directed onto photomultipliers after spectral filtering. Image acquisition times were typically around 1 s for images of 512x512 pixels. At a second input port of the nonlinear microscope a femtosecond Titanium:Sapphire laser (tunable from 700 – 1050 nm) was coupled in for two-photon-fluorescence (2PEF) and second harmonic generation (SHG) microscopy providing multimodal imaging.

3. Results

The laser system is based on an ytterbium fiber laser (Orange, Menlo Systems) and a fiber-based frequency conversion unit. The fiber laser emits femtosecond pulses with an average power of up to 1 W at 1040 nm and at a repetition rate of 100 MHz. About a quarter of the power at 1040 nm is used for nonlinear frequency conversion generating 62 mW of average power at 1300 nm, of which a maximum power of 15 mW is available at the sample plane. The remaining power at 1040 nm is available at an optional second output port and can be used simultaneously to the 1300 nm emission. A wavelength tunability from 1200-1350 nm can be achieved by changing the pulse duration or power of the pump light at 1040 nm.

Figure 1(a) shows a projection of a 2PEF image z-stack acquired from a 15 μ m-thick p20 mouse brain cortex, where microtubule associated protein 2 (MAP2) was stained with Alexa Fluor 647 and excited at 1300 nm with the

fiber laser system. The MAP2 signal in this case is used to investigate neuronal dendritic processes at different stages of mouse brain development. Besides fluorophores we also used BFO nanoparticles as optical markers for bio-imaging as they show low cytotoxicity [2] and generate strong non-resonant SHG and THG signals over a wide range of excitation wavelengths [3,4]. They can be ingested by phagocytes, working as a tracking molecule to trace the phagocytes with nonlinear microscopy. Figure 1(b) shows images of BFO particles on a cover slide acquired with third harmonic generation (THG) microscopy at 1300 nm (top, green) and SHG microscopy at 840 nm (bottom, red). THG and SHG images were taken sequentially and detected by the same photomultiplier equipped with a bandpass filter centered at 420 nm. Despite the longer excitation wavelength, THG imaging with the fiber laser system at 1300 nm provided better spatial resolution than SHG imaging with the Titanium:Sapphire laser at 840 nm resulting in a much sharper image of the BFO nanoparticle (fig. 1 (b) top, green).

Figure 2 shows images of H8N8 mouse mammary tumor cells which were incubated overnight in a medium containing BFO particles of 100 – 150 nm size. In fig. 2(a) a THG image at 1300 nm excitation of the sample is shown only displaying the BFO particles. 2PEF images of the sample at 820 nm excitation are shown in fig. 2(b) and (c) showing the cell membrane (stained with T-Antigen in green) and nuclear (stained with DRAQ5 in red), respectively. Figure 2 (d) shows the merger of images (a)-(c), showing that the tumor cells are able to phagocyte the BFO particles successfully.

In conclusion, we have shown a cost-efficient femtosecond fiber laser source at 1300 nm presenting an alternative to currently available complex and expensive laser sources for 1300 nm emission. We demonstrated the suitability of our laser system for bio-imaging by acquiring high contrast images of biological samples at high speed and low power levels. In THG imaging we found strongly improved images using the fiber laser source at 1300 nm compared to SHG imaging with a Titanium:Sapphire laser.

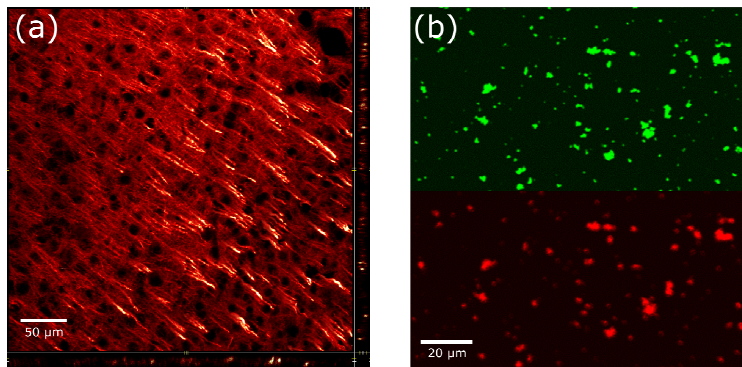


Figure 1: (a) 2PEF image of a 15 μm thick mouse brain slice excited at 1300 nm. (b) top: THG image of BFO nanoparticles excited by the fiber laser system at 1300 nm and (bottom) SHG image of the same sample excited with a Titanium:Sapphire laser at 840 nm.

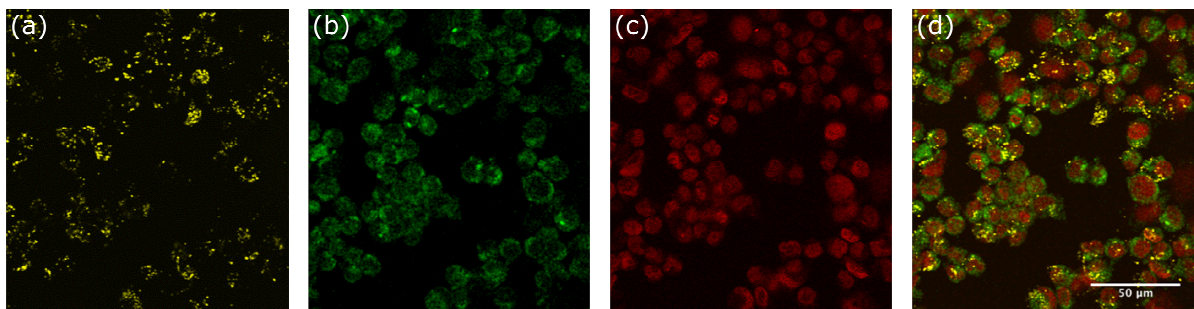


Figure 2: H8N8 mouse mammary tumor cells which phagocyte BFO particles. (a) THG image of BFO particles excited at 1300 nm. (b) 2PEF image of T-Antigen excited at 820 nm used for staining the cell membrane. (c) 2PEF image of DRAQ5 excited at 820 nm used for staining the nuclear. (d) Merged image of (a), (b), and (c).

4. References

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