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

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Introduction

Nonlinear microscopy techniques have become an important tool in bio-imaging applications. This technique 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. When using excitation wavelengths higher than 1080 nm, non linear microscopy used to require expensive laser systems (OPO or OPA) so, despite the advantage for bio-imaging, those wavelengths are rarely used.

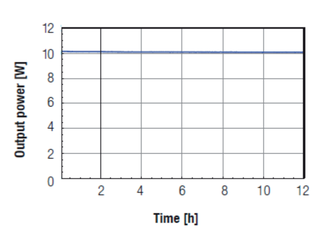
Here, we present a fiber laser system designed for bio-imaging at 1300 nm to provide cost-efficient access to the benefits of deeper sample penetration and reduced sample damage at longer excitation wavelength.

Experimental set-up

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 epidirection 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-photonfluorescence (2PEF) and second harmonic generation (SHG) microscopy providing multimodal imaging.

Ytterbium fiber laser + Conversion unit:
Wavelength 1040 nm
Average power: 1 W
Pulse duration < 400 fs
100 MHz repetition rate
Fiber-based frequency conversion unit, generating 62 mW of average power at 1300 nm

Amplitude noise
< 1% rms (over 12h)



Application1: 2PEF image z-stack acquired from a 15 µm-thick p20 mouse brain cortex

The 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 and generate strong non-resonant SHG and THG signals over a wide range of excitation wavelengths

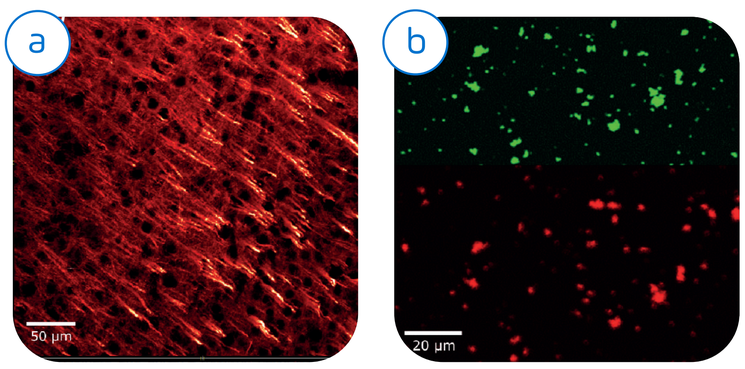


Figure 1: (a) 2PEF image of a 15 µm thick mouse brain slice excited at 1300 nm. (b) top: THG image of BFO nanoparticles excited by the fiber laser system at 1300 nm and (bottom) SHG image of the same sample excited with a Titanium:-Sapphire laser at 840 nm.

THG imaging with the fiber laser system at 1300 nm provided better spatial resolution than SHG imaging with the Titanium:Sapphire (840 nm)

Sharper image of the BFO nanoparticle (fig. 1 (b) top, green).

Application 2: H8N8 mouse mammary tumor cells which phagocyte BFO particles.

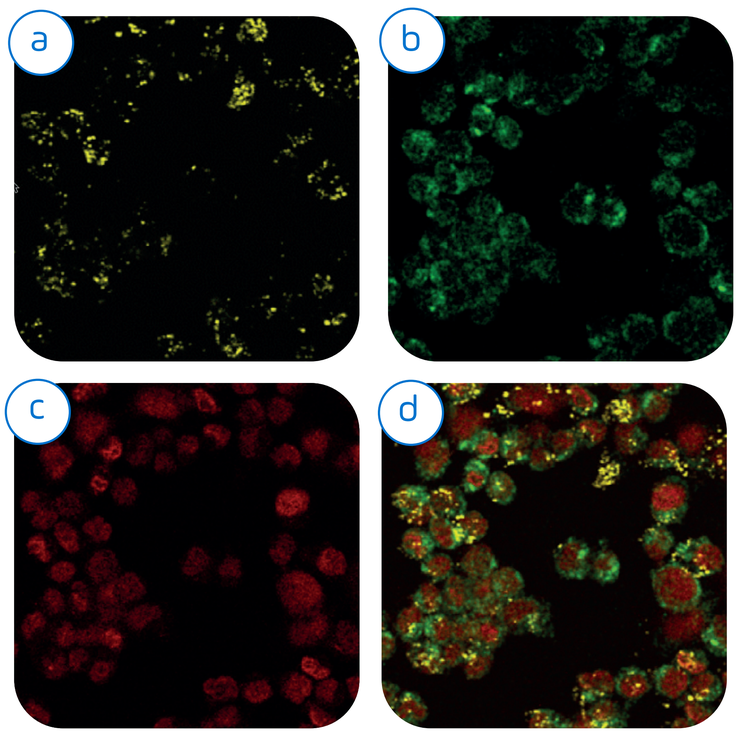


Figure 2: H8N8 mouse mammary tumor cells which phagocyte BFO particles. (a) THG image of BFO particles excited at 1300 nm. (b) 2PEF image of T-Antigen excited at 820 nm used for staining the cell membrane. (c) 2PEF image of DRAQ5 excited at 820 nm used for staining the nuclear. (d) Merged image of (a),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

References

[[1] C. Cleff, F. Ramos-Gomes, T. Bergmann, L. Bonacina, U. Weikert, M. Mitkovski, M. Schuette, F. Alves, M. Mei, Conference proceedings CLEO: Science and Innovations 2016, p. STh4G.3

Conclusions

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.