Multiphoton microscopy based on 1GHz-1MHz energetic tunable ultrafast fiber sources

Shih-Hsuan Chia[1,2], Hsiang-Yu Chung[1], Wei Liu[1], Franz X. Kärtner[1,2], and Guoqing Chang[1,2]

[1]Center for Free-Electron Laser Science, DESY, and Physics Department, University of Hamburg, Luruper Chaussee 149, 22761 Hamburg, Germany
[2]The Hamburg Center for Ultrafast Imaging, Luruper Chaussee 149, 22761 Hamburg, Germany
E-mail: shih-hsuan.chia@desy.de

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Multiphoton fluorescence and optical harmonics, using near infrared driving sources with low tissue attenuation, have been widely applied to image deep-tissue morphology/micro-activities at a cellular level (*e.g.*, 3-photon-fluoresonce for subcortical neural imaging in mice brain, harmonics generation for *in vivo* skin assessment). However, enough nonlinear optical contrast, obtained by increasing driving pulse energy and laser repetition rate, is limited by the photo damage probability. Similar discussion for optimizing second harmonic generation signals has been addressed using 800 nm pulses [1], but it is hampered by the driving wavelength of Ti:sapphire lasers to provide comprehensive discussions regarding imaging penetration depth and optical induced damage between different nonlinear processes. As a result, energetic femtosecond sources, capable of tuning the driving wavelength to the optical penetration window, with various repetition rates are required to evaluate the optical-induced damage in tissues from both thermal heating and multiphoton ionization, in order to improve the imaging frame rate, signal-to-noise ratio, and penetration depth. Although successful demonstrations of tunable sources using optical parametric oscillators (OPOs), optical parametric amplifiers (OPAs), and soliton self-frequency shift (SSFS) sources have been implemented, these approaches are limited by either pulse energy (*i.e.*, typically <10 nJ with OPO) or the average power (*i.e.*, <100 mW with less-than-few-MHz repetition rates using OPA and SSFS). In this submission, we demonstrate sub-Watt-level femtosecond sources with various repetition rates, enabled by optical Kerr nonlinearity with a broad tuning range from 800 nm to 1300 nm. The demonstrated ultrafast source with a high repetition rate (*e.g.*, >100 MHz) benefits harmonic generation imaging with a high SNR, and lower-repetition-rate sources are ideal for multiphoton fluorescence imaging without saturating the upper fluorescence level.

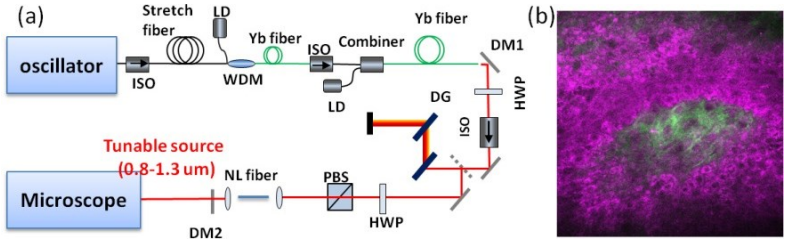

Fig. 1. (a) Schematic of novel fiber-sources based on Yb-fiber oscillators with various repetition rates. DM: dichroic mirror, ISO: isolator, LD: laser diode, DG: diffraction grating, NL fiber: nonlinear fiber, HWP: half-wave plate, PBS: polarization beam splitter. (b) Harmonics imaging of *ex vivo* human skin using 1200nm femtosecond pulses with 50MHz repetition rate. Magenta: THG; Green: SHG; Imaging size: 200μm ×200μm

References

[1] S.-W. Chu, *et al.*, *Opt. Express* 11, 933-938 (2003)