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Abstract. Advancing the practical utility of nonlinear optical microscopy requires continued improvement in imaging depth and contrast. We evaluated second-harmonic generation (SHG) and third-harmonic generation images from ex vivo human skin and showed that a sub-40 fs, 1060-nm Yb-fiber laser can enhance SHG penetration depth by up to 80% compared to a >100 fs, 800 nm Ti:sapphire source. These results demonstrate the potential of fiber-based laser systems to address a key performance limitation related to nonlinear optical microscopy (NLOM) technology while providing a low-barrier-to-access alternative to Ti:sapphire sources that could help accelerate the movement of NLOM into clinical practice. © The Authors. Published by SPIE under Creative Commons Attribution 3.0 Unported License. Distribution or reproduction in any form or medium requires full attribution of the original publication, including its DOI. [DOI: 10.1117/1.JBO.20.12.120501]

Keywords: nonlinear optical microscopy human skin; third-harmonic generation microscopy; multiphoton microscopy; multimodal microscopy; optical biopsy; depth resolved imaging; Yb-fiber laser; adaptive phase-amplitude pulse shaper.

Fiber-based laser sources have been used for NLOM imaging of thin tissue cross-sections,26–28 mouse brain,29 and human skin tissue30 using fluorescence labeling. In this work, we evaluate the performance of a sub-40 fs, 1060-nm Yb-fiber laser for label-free NLOM imaging of human skin. The effect of excitation wavelength and pulse width on penetration depth in thick, turbid tissues is determined by comparing the fiber laser to an 800 nm Ti:sapphire laser source. We employ the depth-dependent decay of second-harmonic generation (SHG) signals as a standard metric for evaluating performance.

The excitation laser sources used were a Ti:sapphire oscillator (MIRA 900; Coherent Inc.; 220 fs, 76 MHz, 600 mW output power, tuning wavelength 720 to 980 nm) tuned to 800 nm for this study and a Yb-fiber laser (BioPhotonic Solutions Inc., 1060 nm, sub-40 fs, 39.2 MHz, 200 mW compressed output power). The prototype Yb-fiber laser, with self-similar pulse evolution,28 has an integrated adaptive phase-amplitude pulse shaper (MIIPS-HD, BioPhotonic Solutions Inc.) based on a 4f configuration with a two-dimensional spatial light modulator. The purpose of the pulse shaper was to control high-order phase distortions introduced by the high numerical-aperture (NA) objective and other dispersive elements in the beam path. The 1060-nm pulses were compressed to nearly transform limited duration using multiphoton intrapulse interference phase scan (MIIPS),31 and their full-width half maximum duration was measured by interferometric autocorrelation using the microscope detection unit (BioPhotonic Solutions Inc.) at the focal plane. Each of the two excitation beams (800 and 1060 nm) was directed toward our home-built laser-scanning microscope and focused into the sample by an Olympus objective (XLPL25XWMP, 25x/1.05 NA water). The nonlinear signals from the sample were epi-collected and directed toward two photomultiplier tubes (R3896, Hamamatsu) by a dichroic

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mirror (Semrock, Inc., 510 LP). The dichroic mirror was used to split the emission signal into two spectral channels defined by the emission filters: 440 SP; 375/110 BP and 720 SP; 535/150 BP (Semrock Inc.). We used discarded human skin tissue (fixed in formalin) to test the effect of sub-40 fs, 1060-nm excitation laser pulses on depth penetration in this sample. For each excitation wavelength (800 and 1060 nm), we acquired five stacks of images as optical sections of the sample, which shows that 1060 nm, sub-40 fs pulses can provide a low-barrier-to-access alternative to conventional Ti:sapphire lasers. They are of particular interest in applications where lack of tunability, short-pulse, and 20 mW for 1060 nm) such that the average intensity of the excitation laser pulses on depth penetration in this sample.

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related to *in vivo* imaging of human skin as they can deliver up to 80% improvement in SHG imaging depth compared to conventionally used Ti:sapphire lasers. An additional benefit for *in vivo* human skin imaging is related to the THG contrast mechanism which, unlike TPEF, does not involve absorption and might allow for the use of higher excitation powers. With continued development of expanded wavelengths, powers, and pulse characteristics, these systems are expected to increase in use, particularly in skin studies where assessment of 3-D morphology is important.

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![Fig. 2](https://ebooks.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics) Ex vivo imaging of human skin using 800 nm (Ti:sapphire laser) and 1060 nm (Yb-fiber laser). (a–c) Horizontal sections (x-y scans) at different depths corresponding to 800-nm excitation wavelength. The optical sections show images of the epidermal cells through the TPEF signal (magenta, \(z = 25 \mu m\)); collagen fibers (green; SHG signal) and elastin fibers (magenta, TPEF signal) (\(z = 100 \mu m; 140 \mu m\)). Vertical sections were obtained from three-dimensional reconstruction for (d) 800-nm and (e) 1060-nm excitation wavelengths (40 mW for 800 nm and 20 mW for 1060 nm). Horizontal sections (x-y scans) at different depths corresponding to 800- and 1060-nm excitation wavelengths are shown in (a–c), (f–h), respectively. The optical sections show images of the epidermal cells through the THG signal (magenta, \(z = 25 \mu m\)) and collagen fibers (green; SHG signal) (\(z = 100 \mu m; 140 \mu m\)). Scale bar is 50 \(\mu m\). The plot represents the SHG signal attenuation (logarithmic scale) with depth, for 800- and 1060-nm excitation wavelengths.
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