Novel fiber-based ultrafast platform for multimodal optical virtual skin biopsy

Hsiang-Yu Chung,[1,2,*] Wei Liu,[1,2] Rüdiger Greinert,[3] Franz X. Kärtner,[1,2,4] and Guoqing Chang[1,4,5]

[1]*Center for Free-Electron Laser Science, DESY, Notkestraße 85, 22607 Hamburg, Germany*
[2]*Physics Department, Universität Hamburg, Luruper Chaussee 149, 22761 Hamburg, Germany*
[3]*Skin Cancer Center Buxtehude, 21614 Buxtehude, Germany*
[4]*The Hamburg Centre for Ultrafast Imaging, Universität Hamburg, Luruper Chaussee 149, 22761 Hamburg, Germany*
[5]*Beijing National Laboratory for Condensed Matter Physics, Institute of Physics, Chinese Academy of Sciences, Beijing 100190, China*
[*]*hsiang-yu.chung@cfel.de*

Abstract: We demonstrate a fiber-based ultrafast platform generating energetic femtosecond pulses at 1250 nm and 775 nm simultaneously, which enables label-free second-harmonic generation, third-harmonic generation, and two-photon excitation fluorescence imaging in human skin.

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1. Introduction

Multiphoton microscopy (MPM) is one of the most important label-free techniques of optical virtual skin biopsy. It features submicron optical resolution, intercellular information, and intrinsic sectioning ability. Skin can be visualized via various interaction mechanisms between ultrafast pulses and tissues, such as two-photon excitation fluorescence (2PEF) [1,2], second-harmonic generation (SHG) [3–6], and third-harmonic generation (THG) [4–6]. These modalities are powerful bio-imaging tools for histopathology, morphology, and disease diagnosis.

Ultrafast lasers are the key to drive these modalities. Ti:sapphire lasers generating pulses in the range of 700-800 nm are the main 2PEF driving sources to excite endogenous fluorophores in human skin. Using femtosecond pulses in this wavelength range, the resulting SHG becomes another modality to visualize collagen and elastin fibers with non-centrosymmetry in dermis. THG from interfaces and optical inhomogeneity allows imaging cells and stratums in epidermis. However, the corresponding THG is ultraviolet under this excitation wavelength, which suffers from strong attenuation and absence of high-sensitivity detectors. To detect both SHG and THG efficiently, longer excitation wavelength within the transmission window (1150-1350 nm) [7] is highly desired. Conventionally, femtosecond pulses at this wavelength range are produced by Ti:sapphire laser pumped optical parametric oscillators/amplifiers (OPOs/OPAs) or Cr:forsterite lasers.

Compared with solid-state lasers, fiber lasers are robust and cost effective, but their wavelengths are restricted to certain spectra due to the limited gain materials. Recently we demonstrated a method to overcome this drawback and developed a series of fiber-based sources suitable for MPM [8–12]. The essence of this approach is to employ self-phase modulation (SPM) dominated nonlinearities inside optical fibers to broaden a narrowband input spectrum. The broadened spectrum features several well-isolated spectral lobes; filtering the leftmost/rightmost spectral lobes generates nearly transform-limited pulses. Such SPM-enabled spectral selection (SESS) allows us to demonstrate fiber-based widely tunable femtosecond sources.

In this submission, we demonstrate a fiber-based platform for multimodal skin imaging. The femtosecond source is derived from a 31-MHz Er-fiber laser followed by nonlinear wavelength conversion. We employ SESS in a DSF to generate 11.7-nJ, 47-fs pulses at 1250 nm and frequency doubling in a MgO:PPLN crystal [77] to obtain 6.7-nJ, 190-fs pulses at 775 nm. With these two excitation wavelengths, we carry out 3-channel imaging (SHG, THG, and 2PEF) of *ex vivo* human skin.

2. Experimental setup and results

Figure 1 illustrates the fiber-based ultrafast platform for multimodal MPM consists of a high-power Er-fiber laser pump source, two nonlinear wavelength converters, and a scanning microscope. The Er-fiber laser system operates at 31-MHz repetition rate and generates 290-fs pulses centered at 1550 nm with 160-nJ pulse energy.
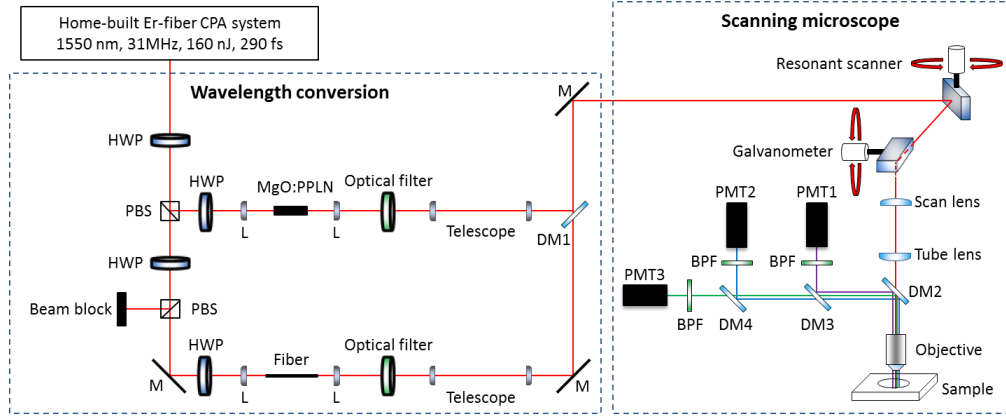
Fig. 1. Schematic setup of the multimodal microscope driven by a fiber-based ultrafast platform. CPA: chirped-pulse amplification, HWP: half-wave plate, PBS: polarization beamsplitter, L: lens, M: mirror, DM: dichroic mirror, BPF: bandpass filter.

To generate pulses at 1250 nm, we employ SESS in 9-cm optical fiber, which has a 10-µm mode-field diameter and -10 fs^2/mm group-velocity dispersion at 1550 nm. Figure 2(a) shows the output spectrum of 85-nJ pulses after propagating in 9-cm fiber. The filtered spectral lobe peaking at 1250 nm [Fig. 2(b)] has an average power of 365 mW (11.7-nJ pulse energy and 14% conversion efficiency). Figure 2(c) shows the measured intensity autocorrelation trace (red curve) of the filtered pulses at 1250 nm. The full-width at half-maximum (FWHM) duration of the autocorrelation trace is 72 fs. The pulse duration is estimated to be 47 fs, assuming a hyperbolic-secant pulse with a deconvolution factor of 1.54. The femtosecond pulses at 775 nm are generated by frequency doubling using 0.3-mm thick MgO:PPLN crystal. Figure 2(d) shows the filtered spectrum peaking at 775 nm with an average power of 208 mW (6.7-nJ pulse energy). The measured FWHM duration is 292 fs (red curve), and the pulse duration is estimated to be 190 fs [Fig. 2(e)]. The black dashed curves in both Fig. 2(c) and 2(e) are the calculated autocorrelation traces of the transform-limited pulses allowed by the filtered spectra, showing that the pulses at 1250 nm and 775 nm are close to be transform-limited.
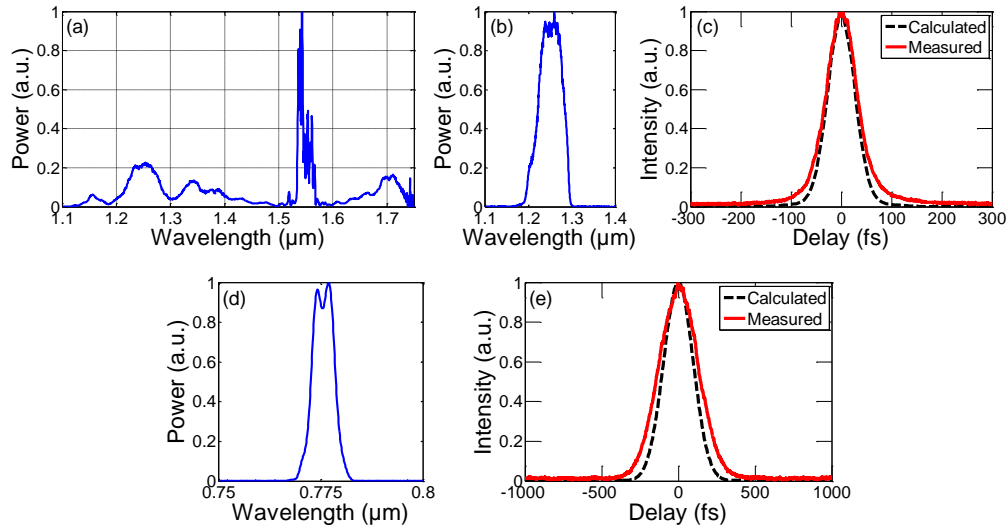

Fig. 2. (a) Spectral broadening from 9-cm optical fiber. (b) Filtered spectrum centered at 1250 nm. (c) Measured autocorrelation trace of the filtered pulses at 1250 nm (red curve) and calculated autocorrelation trace (black dashed curve) of the transform-limited pulse allowed by the filtered spectrum (black dashed curve). (d) Frequency doubled spectrum centered at 775 nm. (e) Measured autocorrelation trace of the pulses at 775 nm (red curve) and calculated autocorrelation trace (black dashed curve) of the transform-limited pulse allowed by the spectrum.

Figure 3 shows the THG/SHG imaging of *ex vivo* human skin from the trunk part excited by 1250-nm. The field of view (FOV) is 270 µm × 270 µm. As we increases the imaging depth, different layers in epidermis can be visualized. In Fig. 3(a) at 25-µm depth we can find three structures close to the skin surface: stratum corneum (SC), stratum lucidum (SL), and stratum granulosum (SG). SC can be found at the top left, middle left, and right of Fig.

3(a), while SL is at the top of Fig. 3(a). SL is a thin stratum composed of only a few (3-5) layers of keratinocytes. Another thin structure is SG. It consists of cells with their cytoplasm containing keratohyalin granules. Both SC and SL are known as their nucleiless feature, but SC is much thicker than SL. In Fig. 3(b) at 30-μm depth, SC can still be found at the top left corner, whereas SL vanishes. The main structures here are SG and stratum spinosum (SS). Spinous cells are the most common cells in epidermis [Fig. 3(b-d)]. Collagen fibers start to appear as the imaging depth reaches 55 μm [Fig. 3(c)]. The fibrous structure surrounded by basal cells is also called dermal papilla, which locates at the junction of epidermis and dermis [Fig. 3(c-d)].
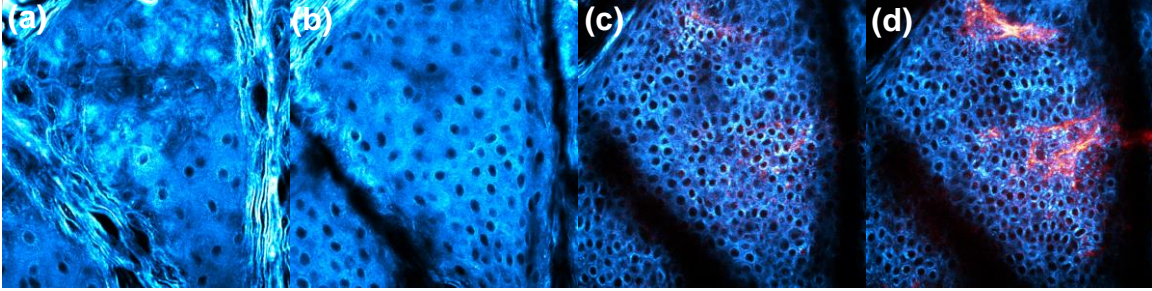

Fig. 3. THG/SHG imaging of *ex vivo* human skin from the trunk part at different penetration depth. (a) 25 μm. (b) 30 μm. (c) 55 μm. (d) 65 μm. THG is colored in cyan hot; SHG in red hot.

Figure 4 shows the *ex vivo* imaging of SG [Fig. 4(a,b)] and SS [Fig. 4(c,d)] visualized by different modalities. The contrast agent in Fig. 4(a) and 4(c) is 2PEF excited by 775-nm pulses, whereas the contrast in Fig. 4(b) and 4(d) originates from THG. Both modalities can differentiate stratums in epidermis.
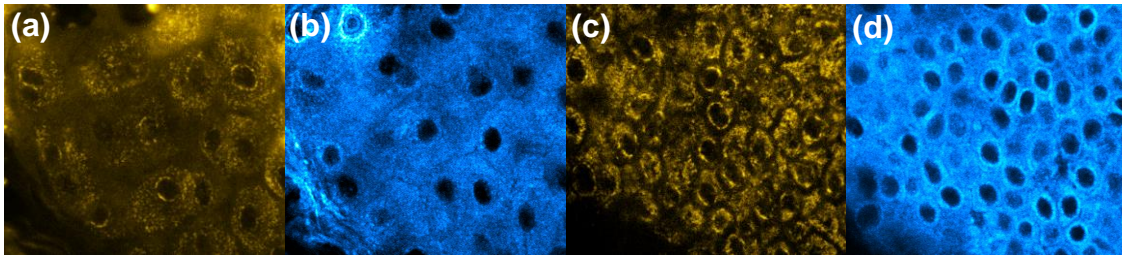

Fig. 4. *Ex vivo* human skin from the trunk part visualized by different modalities. (a) 2PEF of SG excited by 775-nm pulses. (b) THG of SG excited by 1250-nm pulses. (c) 2PEF of SS excited by 775-nm pulses. (d) THG of SS excited by 1250-nm pulses. 2PEF is colored in yellow hot.

3. Conclusion

We report a fiber-based ultrafast platform for multimodal label-free skin imaging. Femtosecond pulses required for driving three modalities—at 1250 nm for SHG/THG and at 775 nm for 2PEF—are generated by a single Er-fiber laser employing SESS and frequency doubling. Our proposed configuration achieves pulses at 1250 nm and 775 nm simultaneously, which allows us to demonstrate 3-channel imaging of *ex vivo* human skin. We believe that such a versatile ultrafast platform constitutes a relatively simple and practical solution to achieve multimodal optical virtual biopsy for clinical applications.

4. References

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