In vivo harmonic generation biopsy of human skin

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Abstract. The ability to in vivo image deep tissues noninvasively with a high resolution is strongly required for optical virtual biopsy. Higher harmonic generation microscopy, combined with second- and third-harmonic generation microscopies, is applied to 17 Asian volunteers’ forearm skin. After continuous observation for 30 min, no visible damage was found. Our study proves that harmonic generation biopsy (HGB) is able to satisfy the safety requirement and to provide high penetrability (∼300 µm) and submicron resolution all at the same time and is a promising tool for future virtual biopsy of skin diseases. In contrast to a previous study on fixed human skin specimens, a much improved penetrability and much reduced resolution-degradation versus depth are found in this in vivo examination. © 2009 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.3269676]

Keywords: harmonic generation microscopy; dermatology; noninvasive; in vivo; human skin; high penetrability; high resolution.

In this paper, real-time in vivo HHG imaging of Asian skin combined with epi-SHG and epi-third-harmonic generation (epi-THG) modalities is reported. To the best of our knowledge, this is also the first report on the clinical trial of THG imaging. In this study, a Cr:F laser is used, and a syringe-pump objective is designed to diminish the image blurring due to breathing and heart beating. Even with the image blurring, the submicron spatial resolution was preserved within the whole imaging depth (>300 µm) for all volunteers. The safety issue has also been preliminarily confirmed through a standard damage evaluation protocol for clinical trials. By measuring the Cr:F-excited spectra of live human skin (not shown), 2PF was found to be greatly diminished due to the low photon energy of the Cr:F excitation, while no three-photon fluorescence (3PF) can be observed, since the probability of the fifth-order nonlinear 3PF is several orders lower than that of the third-order nonlinear THG. In contrast to previous HHG imaging of fixed human skin, a higher penetrability with much reduced resolution-degradation versus depth was found. In addition, real-time dynamic information like blood flow can be provided. In this study, HHG imaging is found to satisfy the clinical safety requirement and to provide high penetrability and submicron resolution all at the same time and is an ideal virtual biopsy tool for assisting, minimizing, or even potentially replacing physical biopsy.

Based on the previous epi-HHG microscopy, some modifications were made for the in vivo harmonic generation biopsy (HGB) system. The in vivo HGB of human skin was performed on the ventral forearm skin of 17 volunteers (9 female and 8 male; 21 to 56 years). This clinical trial was approved by IRB of National Taiwan University Hospital. Before, during, and after the HGB, the tested site—ulnar, ventral, upper 1/3 forearm skin—of the volunteers was recorded by photographing. A medical doctor kept checking volunteers’ status during HGB. In this trial, the following protocols were applied: (1) the total exposure time was limited to 30 mins in the same area; and (2) two scanning modes were used: a slow mode (90 mW; 0.37 Hz) and a fast mode (120 mW; 2 Hz). The accumulated photon energy was around 180 to 200 J in one area. The tested area was evaluated by a dermatologist immediately, several hours, 24 hours, 3 days, and 1 week after HGB. During HGB, only one volunteer reported a possible stinging sensation for <1 s, which was claimed to be uncer-
There were no inflammatory symptoms, no skin color change, no pigmentation, no wound, no blister formation, and no ulceration reported. The collagen fibers in the tip of the papilla can be revealed by SHG microscopy [arrow in (e)]. The loose areolar connective tissue in the PD; and (b) the dense irregular connective tissue in the RD can be shown and distinguished by SHG, while the erythrocytes [arrowheads in (e), (f), and (h)] and fibroblasts [arrow in (g)] can be observed through THG. THG and SHG are represented by the magenta and green pseudo-colors. Scale bar: 50 μm. (Color online only.)

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**Fig. 1** (a) to (h) *In vivo* lateral epi-SHG and epi-THG images of human forearm skin (obtained with slow mode) at different depths inward from outside. In epidermis, THG can reveal the multilayer structure of the SC (arrow); (b) and (c) the SG cells at upper and deeper layers; and (d) and (e) the SS and SB cells at the epidermis–dermis junction. The collagen fibers in the tip of the papilla can be revealed by SHG microscopy [arrow in (e)]. (f) The loose areolar connective tissue in the PD; and (b) the dense irregular connective tissue in the RD can be shown and distinguished by SHG, while the erythrocytes [arrowheads in (e), (f), and (h)] and fibroblasts [arrow in (g)] can be observed through THG. THG and SHG are represented by the magenta and green pseudo-colors. Scale bar: 50 μm. (Color online only.)

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**Fig. 2** (a) Internuclear distances (INDs) versus depth analyzed from *in vivo* epi-THG images. The lateral resolution of THG microscopy right at skin surface can be analyzed from (b) multilayer stratum corneum (arrow), while the lateral resolution of SHG microscopy can be analyzed from (c) collagen fibrils (arrow; 200 μm deep). (d) The lateral resolution of THG microscopy versus depth. THG and SHG are represented by the magenta and green pseudo-colors. Scale bar: 50 μm.
pared with the previous analysis in fixed human skin, our in vivo study shows a much reduced resolution degradation versus depth, indicating much reduced point-spread function aberration of the 1230-nm excitation light in the live tissues versus fixed tissues.

In conclusion, in vivo real-time HGB of human skin has been demonstrated. Even with the image blurring resulting from vibrations, a submicron lateral resolution of THG microscopy was preserved at a depth of ~300 μm, achieved without increasing the PMT voltage. In contrast to the previous fixed skin imaging, the in vivo HGB of human skin showed much reduced resolution-degradation versus depth while providing real-time dynamic information in the live tissues. Through the damage evaluation, the noninvasiveness of this imaging tool has been preliminarily proved. Combined with the high spatial resolution, high penetrability, and various imaging capabilities, IR-based HHG imaging will be a promising tool for future noninvasive virtual biopsy of skin diseases.

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References


