Harmonics Optical Biopsy of Human Skin

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Abstract: Avoiding the in-focus photodamage and phototoxicity problem of two-photon fluorescence excitation, noninvasive harmonics optical biopsy of human skin is demonstrated based on a 1200-1300-nm femtosecond light source.

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Traditional biopsy requires the removal, fixation, and staining of tissues from the human body. Its procedure is invasive and painful. Non-invasive *in vivo* optical biopsy is thus required, which should provide non-invasive, highly penetrative, three-dimensional (3D) imaging with sub-micron spatial resolution. Optical biopsy based on scanning two-photon fluorescence microscopy (TPFM) [1,2] is a good method for biopsy of skin due to its high lateral resolution, low out-of-focus damage, and intrinsic three-dimensional (3D) section capability. Previous two-photon fluorescence biopsy of skin [1,2] based on a 780 nm femtosecond light provides high resolution imaging from the skin surface through the epidermal-dermal junction. However for future clinical applications without surgery, current 700-850 nm based laser scanning technology still presents several limitations including low penetration depth, in-focus cell damages, and multi-photon phototoxicity due to high optical intensity in the 800 nm wavelength region, and toxicity if exogenous fluorescence markers were required.

Here we study the harmonics optical biopsy of a human skin sample using a femtosecond Cr:forsterite laser centered at 1230 nm [3]. According to previous studies, light attenuation (including both absorption and scattering) in human skin [4] reaches a minimum around 1200-1300nm due to the combination of diminishing scattering cross-section with wavelength and avoiding the resonant molecular absorption of common tissue constituents such as water, melanin, and hemoglobin. Besides, moving the excitation wavelength longer than 1200 nm makes second harmonic generation (SHG) and third harmonic generation (THG) possible due to visible SHG and THG wavelength that can avoid strong UV region absorption in human-tissues. Higher harmonics generation is known to leave no energy deposition to the interacted matters due to their energy-conservation characteristic. This energy-conservation characteristic provides the "noninvasive" nature desirable for clinical imaging. Due to its nonlinear nature, the generated SHG and THG intensities depend on square and cubic of the incident light intensity. Similar to multi-photon induced fluorescence process, this nonlinear dependency allows localized excitation and is ideal for intrinsic optical sectioning in optical biopsy applications.

The study of the harmonics optical biopsy (HOB) of human skin was performed using a femtosecond Cr:forsterite laser centered at 1230 nm with 140 fs pulsewidth at a 110MHz repetition rate. The thickness of the human skin sample was ~1mm. Figure 1 shows example vertically sectioned (x-z) HOB images taken from the human epidermis and dermis corresponding to forward propagating multi-harmonic generation intensities. From the vertically sectioned HOB images, the general histological structures in both epidermis and dermis layers can be identified through the THG modality due to its sensitivity to local optical inhomogeneity [5-7]. For example, the coverglass-formalin interface, stratum corneum, and basal layer can all be easily picked up through the THG modality (for example, see Fig.1 (b)). SHG, on the other hand, mainly reflects the distribution of connected tissues in the dermis layer (bottom of Fig. 1(a)) due to strong SHG generation in collagen fibers [8-10]. Through the SHG image (for example, see Fig.1 (a)), other structures like the keratin layer in the epidermis and the capillaries surrounded by connected tissues can also be clearly identified.

Figure 2 shows a horizontally sectioned (x-y) HOB image (THG modality) taken from the basal layer inside a normal human skin at a depth of 90 μ m from the skin surface. Detailed sub-cellular structures in basal cells can be clearly resolved through THG. Figure 3 shows another horizontally sectioned HOB image (SHG modality) taken from the dermis layer at a depth of 136 μ m from the skin surface. While SHG displays the rich collagen fibril structures in the dermal layer, other histological structures like capillaries can also be identified (also revealed in THG image, not shown). More HOB images in human skin showing different histological structures, including blood cells, will be presented in this talk.

In summary, current 800nm based two-photon optical biopsy technology still presents several limitations including finite penetration depth, in-focus cell damages, and phototoxicity due to the required high optical intensity and multi-photon absorption processes. Harmonics generation, however, can provide a truly non-

invasive biopsy tool with excellent three-dimensional spatial resolution due to their virtual transition and nonlinear characteristics. Combining with a 1200-1300-nm light source, our preliminary study suggests that this technique could provide sub-micron resolution deep-tissue non-invasive biopsy images in human skins without using fluorescence and exogenous markers. By improving the detection geometry (backward propagating), this technique could be useful for non-invasive clinical biopsy of human skin diseases and even useful for the early diagnosis of skin cancer symptom such as the angiogenesis. This work is sponsored by National Health Research Institute of Taiwan, R.O.C. through grant NHRI-EX92-9201EI.

Fig. 1. Vertically sectioned HOB images taken from the human epidermis and dermis corresponding to emitted (a) SHG and (b) THG. Image size: 240x380 μm . In the figures, A-F are keratin layer, capillary, connective tissue, coverglass-formalin interface, stratum corneum, and basal layer.

Fig. 2. Horizontally sectioned HOB image from the basal layer in the dermo-epidermal junction corresponding to the forward propagating THG modality. Image size: 60x60 μm

Fig. 3. Horizontally sectioned HOB image from the dermal layer corresponding to the forward propagating SHG

modality. Capillaries (A) surrounded by connected tissues can be clearly identified. Image size: 240x240 μm .

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