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MULTI-HARMONIC GENERATION BIOPSY OF SKIN

Cheng-Chi Chen, Shi-Wei Chu, I-Hsiu Chen, and Chi-Kuang Sun
Graduate Institute of Electro-Optical Engineering, National Taiwan University
Taipei 10617, Taiwan, R.O.C.
Phone:+886-2-23659703, Fax:+886-2-23677467, E-Mail: r91941018@ntu.edu.tw

Yung-Chih Chen, Bai-Ling Lin
Molecular and Cell Biology Division, Development Center for Biotechnology
Taipei 10672, Taiwan, R.O.C.

Bai-Ling Lin
Department of Physics, National Taiwan Normal University
Taipei 11650, Taiwan, R.O.C.

Abstract -- *Avoiding the on-focus photodamage and phototoxicity problem of two-photon-fluorescence excitation, harmonic generation biopsy based on a ~1300 nm light source provides a truly non-invasive and highly penetrative optical sectioning of skin.*

Multi-harmonic generation (including second and third harmonic generation) biopsy of skin based on a ~1300 nm Cr:forsterite femtosecond laser provides lower attenuation (Fig.1), scattering, dispersion, and photodamage than the commonly used 800nm Ti:sapphire lasers. This technique avoids the common problems encountered by two-photon fluorescence microscopy (2PFM) such as the photodamage, photobleaching, phototoxicity and dyeing toxicity because of the virtual transition of harmonic generations and the necessity of no fluorescence markers for tissues. Since the generated THG intensity depends on cubic of the incident light intensity, THG biopsy also provides higher intrinsic 3D sectioning capability than the 2PFM [1,2].

We have recently developed a multi-harmonic generation biopsy based on a highly penetrative Cr:forsterite laser at ~ 1300 nm. Fig. 2A and 2B shows the reconstructed 3D THG transverse and longitudinal images of a capillary with a ~10 μm diameter inside a mouse external ear taken with a transmission-type system, showing the excellent 3D resolution of our developed system. The unique ability of THG biopsy to visualize the subtle distribution of capillaries inside skin without any exogenous labeling is useful for the early diagnosis of skin cancer symptom such as the angiogenesis. For practical applications, optical biopsy for clinical diagnosis requires a reflective detection mode. We have also developed a multi-harmonic generation biopsy system based on reflective signals. Fig. 3 shows the sectioned paradermal scanning images of connective tissue septa from the adipose tissue inside a laboratory mouse skin using backward reflected SHG. Single collagen fiber can be readily observed. More preliminary harmonic biopsy study, including second harmonic and third harmonic sectioned images, of mouse, porcine, or even human skin will be presented in this talk.

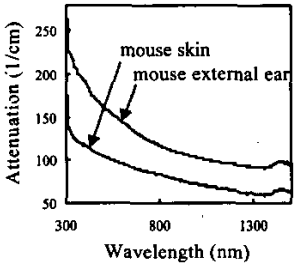
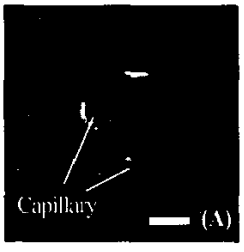


Fig. 1. Light attenuation spectra of the mouse skin and external ear.



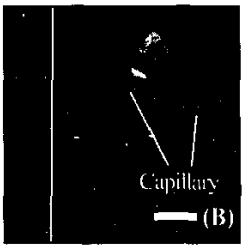


Fig. 2. the reconstructed 3D THG transverse image (A) and longitudinal image (B) of a capillary inside a mouse external ear. Scale bar: 12 μm.

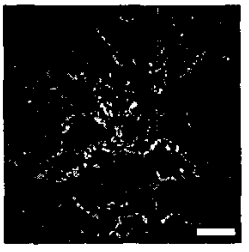


Fig. 3. Sectioned SHG images of connective tissue septa inside a mouse skin. Scale bar: 20 μm

[1] U. K. Tirlapur *et al.*, "Femtosecond Near-infrared Laser Pulses Elicit Generation of Reactive Oxygen Species in Mammalian Cells Leading to Apoptosis Death," *Exp. Cell Research*, **263**, pp. 88-97 (2001).

[2] I. H. Chen *et al.*, "Wavelength Dependent Damage in Biological Multi-photon Confocal Microscopy: A Micro-Spectroscopic Comparison between Femtosecond Ti:sapphire and Cr:forsterite Laser Sources," *Opt. Quantum. Electron*, **34**, pp.1251-1266 (2002).

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