Evaluation of the degree of collagen crosslinking using polarization-resolved second harmonic generation microscopy

Chao-Wei Hung[1], Dan-Jae Lin[2], Ming-Che Chan[3], and Guan-Yu Zhuo[4,5,*]
[1]PhD Program for Biomedical Engineering, China Medical University, No.91, Hsueh-Shih Rd., Taichung 40402, Taiwan
[2]School of Dentistry, College of Dentistry, China Medical University, No.91, Hsueh-Shih Rd., Taichung 40402, Taiwan
[3]Institute of Photonic System, College of Photonics, National Chiao-Tung University, Tainan 71150, Taiwan
[4]Institute of New Drug Development, China Medical University, No.91, Hsueh-Shih Rd., Taichung 40402, Taiwan
[5]Integrative Stem Cell Center, China Medical University Hospital, No. 2, Yude Rd., Taichung 40447, Taiwan
E-mail: zhuo0929@mail.cmu.edu.tw

KEY WORDS: nonlinear optical microscopy, second harmonic generation, collagen crosslinking, second-order susceptibility tensor

ABSTRACT

Polarization-resolved second harmonic generation (P-SHG) microscopy has become a promising tool to identify the structure and function of bio-tissues in a label-free and non-invasive manner [1]. The contrast mechanism is from the structural specificity to the samples with a non-centrosymmetric molecular structure. Furthermore, the polarization-dependent SHG can be formulated by a second-order susceptibility tensor $\chi^{(2)}$, where the molecular structure is encrypted in $\chi^{(2)}$ tensor elements. Regarding the biomedical significance, it has been evidenced that changes in the ratios of $\chi^{(2)}$ tensor elements were found as comparing between normal and cancerous tissues, and associated with tissue age, tissue type, and mechanical tension [2, 3]. Therefore, the $\chi^{(2)}$ ratio is a key parameter and poses great possibilities to characterize different types of collagen-based tissues. In this work, various engineered bone-biomaterials are fabricated under different pretreatment methods and examined by P-SHG microscopy together with the tests of Fourier transform infrared spectroscopy (FTIR), swelling ratio, dissolution, and mechanical tension. Among the test items, the crosslinking degree mainly influences collagen properties, including the degree of folding of helical structure and degree of protein organization in tissues. It is expected that the tiny variation in collagen structure resulting in different functional performance can now be characterized and quantified by P-SHG microscopy.

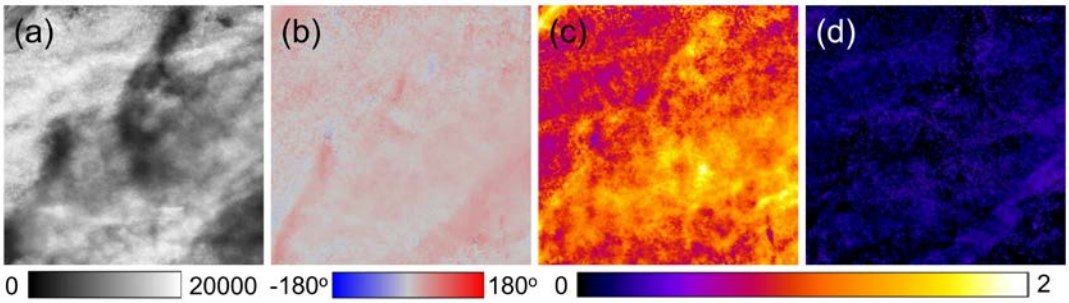

Figure 1: (a)-(d) are the images of engineered bone-biomaterial represented as SHG intensity, planner orientation of collagen molecules, χ_{33}/χ_{31}, and χ_{15}/χ_{31}, respectively. The corresponding color bar is shown below for each figure. Image size: 300 x 300 μm^2.

[1] S.-W. Chu, S.-Y. Chen, G.-W. Chern, T.-H. Tsai, Y.-C. Chen, B.-L. Lin, and C.-K. Sun, *Biophys. J.* **86**, 3914-3922 (2004).
[2] R. M. Williams, W. R. Zipfel, and W. W. Webb, Biophys. J. **88**, 1377-1386 (2005).
[3] A. Erikson, J. Ortegren, T. Hompland, C. d. L. Davies, and M. Lindgren, J. Biomed. **12**(4), 044002 (2007).