Polarization dependent behaviors of biological nano-crystalline-structure studied with harmonic generation microscopies

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Abstract: The orientation and different levels of crystallization in biological nano-structures are revealed by polarization dependent studies under SHG and THG microscopies.

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Harmonic generation microscopy (HGM) of tissues has distinct advantages over fluorescence imaging on the lack of any staining preparation and eliminating the cytotoxic or phototoxic labeling as well as the "noninvasive" nature desirable for clinical imaging [1]. Similar to multi-photon fluorescence process, the nonlinear-dependence of HGs allows intrinsic optical sectioning. Nevertheless, the contrast in the HGM comes mainly from the local arrangement of underlied bio-nano-structures. Previous studies suggest that the polarization dependence of the SHG signal provides information about bio-photonic crystalline structure orientation and nonlinear susceptibility [2-3]. THG has been found to exhibit strong polarization dependence on the nanoscopic aggregation domains in conjugated polymer films [4] and also provides a powerful spectro-microscopic method for the study of ground-state electronic species once its photon energy coincides with the energy of a real molecular transition in sample. For example, in our experiment, the 410-nm wavelength of THG is very close to the fluorescence band of collagen and the absorption band of Flavin mononucleotide. Therefore, THG microscopy can be used to visualize these specific molecules and their orientations down to molecular levels. Here we present a thorough study of polarization dependence in

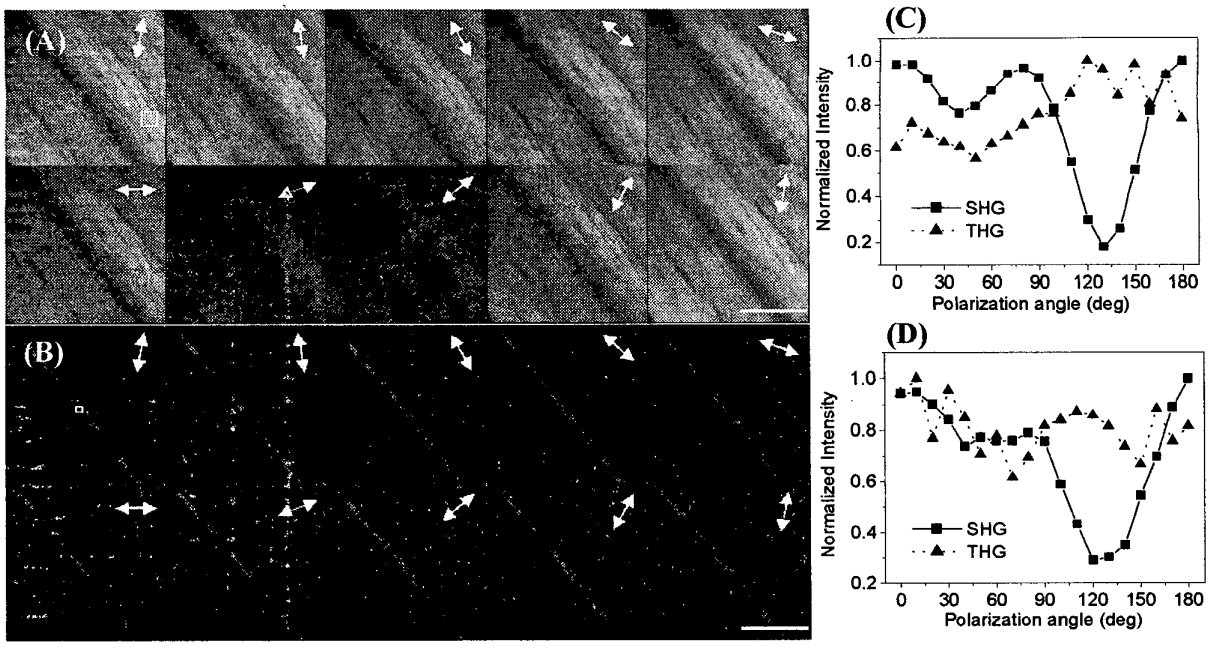

Fig. 1 (A) SHG and (B) THG scanning images showing the change of harmonic generation signal intensity as the laser polarization rotates along the imaging plane (shown by white arrow in each image). The focused spot size is ~530-nm, resulting in 285-nm and 220-nm radial resolution in SHG and THG microscopy, respectively. Scale bar: 50μm. (C) and (D) quantify the alternation of THG and SHG intensities in the region selected in (A) and (B), respectively. 0 degree is defined as the direction of laser polarization in the first image of (A) and (B).

biological nano-structure on the polarization relation between incident fundamental and the coherent emitted HG signals.

As an illustration of our micro-polarization study, figure 1 shows the change of HG signal intensity as the laser polarization rotates along the imaging plane in the mouse skeletal muscle. It is obvious, though somewhat surprising, that there are two SHG maxima, and both of them occur as the laser polarization is ~40 degrees away from the direction of muscle fiber. When laser polarization and muscle fibers are aligned to each other (40 degrees in figure 1C), there is a local minimum. Nevertheless, the global minimum falls as the laser polarization and muscle fibers are perpendicular to each other. Note the SHG intensity at global minimum is less than 20% of that at the maximum, indicating highly crystallized structures inside muscle fibers as well as a strong orientation selecting power of SHG and a tolerable de-polarizing effect of our high-NA objective. On the other hand, in the capillary between two adjacent muscle fibers (the rectangle in figure 1B), it seems that the maximum of THG falls at the same angle of SHG global minimum. Another noteworthy observation is the second peak of SHG in muscle (~80 degrees in figure 1C) is suppressed to nearly flat in the capillary (figure 1D).

Beside from the intensity dependence on input laser polarization, another important parameter is the polarization of the emitted nonlinear coherent signals relative to the incident fundamental polarization, whose analysis is shown in figure 2 as an example. In figure 2C, the emitted SHG polarization is parallel to that of fundamental, which agrees with a recent work [3]. However, in the other three figures, the maxima of SHG occur at 100, 70, and 30 degrees respectively, indicating complicated phase transition process inside. Moreover, the polarization of THG seems to be randomly distributed, resulting from the collective behavior of myofibril and fibroblast with different orientations inside. More results regarding to the polarization dependence in tendon, collagen tissue in skin and teeth will be given and discussed in the conference.

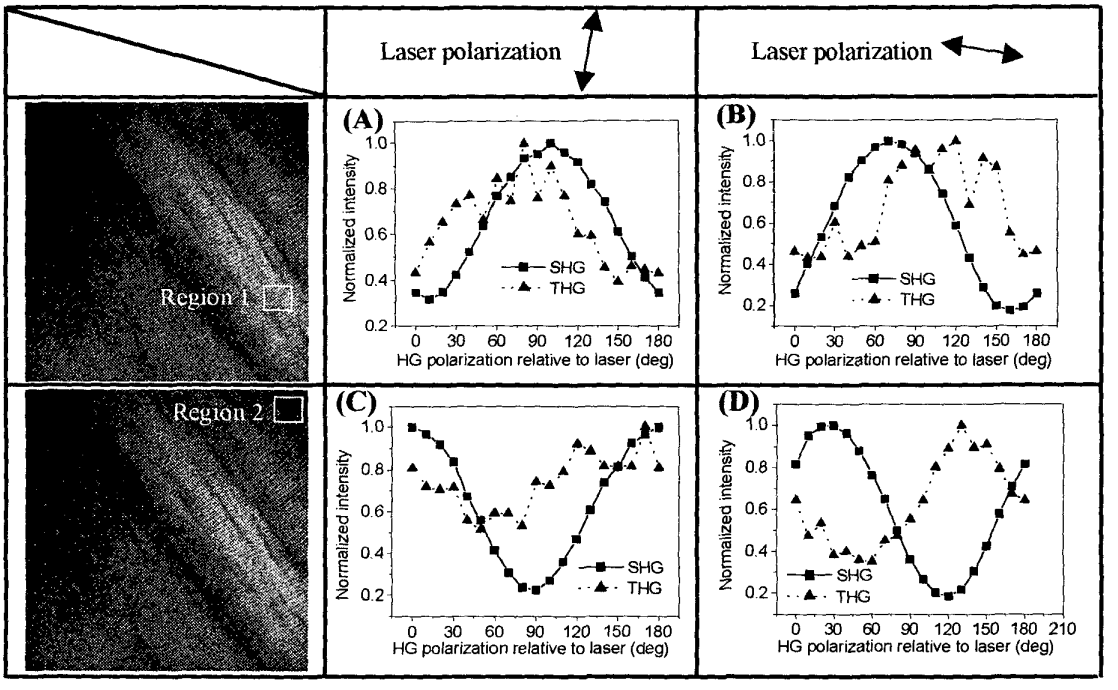

Fig. 2 Analysis of the polarization of emitted harmonic generations versus that of incident fundamental. (A) and (B) show the integrated response of harmonic generations in region1 with different laser polarizations. (C) and (D), on the other hand, shows that in region2.

References

[1] S.-W. Chu *et al.* "Multi-modal Nonlinear Spectral Microscopy Based on a Femtosecond Cr:forsterite Laser." *Opt. Lett.* **26**, 1909-1911 (2001).

[2] S.-W. Chu *et al.* "Nonlinear bio-photonic crystal effects revealed with multi-modal nonlinear microscopy," to be published in *J. Microscopy* Dec. issue 2002.

[3] P. J. Campagnola *et al.* "Three-Dimensional High-Resolution Second-Harmonic Generation Imaging of Endogenous Structural Proteins in Biological Tissues" *Biophys. J.*, **81**, 493-508 (2002)

[4] R. D. Schaller *et al.* "Nanoscopic interchain aggregate domain formation in conjugated polymer films studied by third harmonic generation near-field scanning optical microscopy" *J. Chem. Phys.*, **117**, 6688-6698 (2002)