Accurate polarization-resolved second harmonic generation (P-SHG) microscopy - A new model for interpreting polarization-dependent SHG

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ABSTRACT

Developing a promising imaging tool for precise diagnosis has drawn considerable attention in biomedical science because the tiny variation of collagen triple-helix is usually followed by collagen-related diseases. It has been proven that the degree of folding of helical molecules and organization of proteins in the tissue are correlated with the tensor elements of second-order susceptibility $\chi^{(2)}$ extracted by polarization-resolved second harmonic generation (P-SHG) microscopy. In principle, one can analyze the specific polarization dependency of a series of P-SHG images to isolate two species of SHG-active molecules or determine the pathological condition of tumor progression, wherein dissimilar values of $\chi^{(2)}$ tensor elements can be found. Therefore, changes in tensor elements provide a contrast mechanism for P-SHG microscopy on collagenous tissues. Despite the fact that type-I collagen has been extensively studied, the ratios of $\chi^{(2)}$ tensor elements exhibit a large deviation as comparing various literatures, which overlap between other collagen types [1]. It is thus questionable whether P-SHG microscopy is universal to all the cases for deciphering the nanostructure of SHG-active molecules.

In P-SHG imaging, it is common to assume that the collagen fibrils are laid nearly parallel to the glass substrate with the molecular tilt angle δ (with respect to the image plane) close to zero [2]. Thus, the deviation of $\chi^{(2)}$ tensor elements is relatively small and can then be ignored. However, in the cases of type-II collagen, esophagus tissues, ovarian tissues, and oral mucosa, in which the collagen fibrils are crimped and randomly organized in three dimensions (3D), this hypothesis might be failed. In addition, the effect from molecular chirality has a non-vanishing contribution in SHG signal when $\delta \neq 0$, leading to the fact that the model for C_6 symmetry could be a better fit than C_{6v} symmetry to characterize collagen structure and orientations [3]. To address the above issue, we propose a new model based on C_6 symmetry and split SHG signal into s- and p-polarized image components as gradually changing the angle of incident laser polarization for $\chi^{(2)}$ analysis. We expect that our method will make P-SHG microscopy a sensitive technique with unparalleled diagnostic precision to analyze 3D molecular structure of collagen.

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