Complex Susceptibilities of Collagen Imaged with Polarimetric Second-Harmonic Generation Microscopy

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Second-harmonic generation (SHG) microscopy is a non-invasive imaging tool that does not require labeling and provides optical sectioning. SHG is suitable for imaging and structural analysis of ordered samples such as collagenous tissues. The information about the microstructural properties of the sample can be obtained beyond the diffraction limit via extraction of the second-order nonlinear susceptibility tensor $\chi^{(2)}$ for each pixel of the image. The $\chi^{(2)}$ extraction is possible through analyzing polarization response of the emitted SHG signal from the sample. Recently, a polarimetric SHG microscopy using double Stokes-Mueller polarimetry (DSMP) formalism was developed [1], where a set of laser beam polarization states are prepared, and the resultant polarization of the outgoing SHG signal form the sample is measured. In this theory the sample is represented by a 4×9 double Mueller matrix whose elements are functions of the $\chi^{(2)}$ tensor components. Hence, this technique enables us to extract real and imaginary parts of six laboratory-frame susceptibility tensor components, $\chi^{(2)}_{XXX}$, $\chi^{(2)}_{XZZ}$, $\chi^{(2)}_{XXZ}$, $\chi^{(2)}_{ZXX}$, $\chi^{(2)}_{ZZZ}$, $\chi^{(2)}_{ZXZ}$ [2] of the sample, where *XZ* is the image plane and the light propagates along the *Y*-axis of the laboratory frame of reference.

This technique was utilized to extract the $\chi^{(2)}$ of collagen which is a structural protein. It is the most abundant protein in mammals, and its structural alteration is correlated with diseases such as cancer [3]. In analyzing the SHG responses from collagenous tissues, it is commonly assumed that the collagen fibers possess $C_{\infty v}$ symmetry. In addition, when the fundamental and SHG wavelengths are far from electronic resonance, the susceptibility tensor components are assumed to be real-valued. Here we investigate the conditions under which the chiral properties of collagen should be taken into account and consequently the $C_{\infty v}$ symmetry class cannot describe adequately the collagen structure. Since the chirality of collagen is associated with its 3-dimensional (3D) orientation in the image plane, the extraction of the chiral term enables us to elucidate on 3D organization of collagen fibers. Further, we extract the imaginary parts of $\chi^{(2)}$ of collagen that are imaged for the first time in a non-resonant SHG condition. It can be used to investigate the deviation from commonly assumed Kleinman symmetry condition in collagen.

Using this method the SHG circular dichroism (SHG-CD) and SHG optical rotatory dispersion (SHG-ORD) in ordered biological structures can be measured. They can extract ultrastructural information about the chirality of the sample from each pixel of the image. The technique is particularly powerful for studying extracellular matrix collagen and can be used for histopathology investigations and diagnostics of cancer tissue.

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