

Nonlinear chiral microspectroscopy of biological tissues

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ABSTRACT

Chirality plays a fundamental role in biomedical fields; many drugs, enzymes, and biomolecules cannot function unless their chiralities are correct. Since the conformation of a molecule, as well as the chirality, is very sensitive to the local microenvironment, it is vital to characterize molecular chirality without altering the surrounding conditions. To determine the chirality in materials, optical activity is the most common way. In linear optics, optical rotatory dispersion and circular-dichroism are the two well-developed methods for probing chirality. However, their weak contrast, poor optical sectioning, and low penetration depth constrain its application to study chirality in tissues and real bio-samples. Therefore, previous research has been mostly limited to surfaces or solutions.

In contrast to linear optics, several nonlinear optical activity effects can occur in chiral materials, such as vibrational circular dichroism, Raman optical activity, two-photon absorption circular dichroism, and second-harmonic generation circular-dichroism (SHG-CD). The last one is the most studied nonlinear optical activity effect since it shows significantly improved chiral contrast. An additional advantage of SHG-CD is its intrinsic optical sectioning due to nonlinearity. When combined with an infrared excitation, SHG-CD has been demonstrated to provide high penetration depth for three-dimensional imaging. However, in recent studies, the signal origin of SHG-CD in biological tissue has been found ambiguous, since not only molecular chirality, but also the anisotropy of molecules (macroscopic chirality) contributes to SHG-CD response. It is of great importance to find an experimental technique that can distinguish the contribution between these two mechanisms.

Here we studied SHG-CD responses of collagen, which is the most abundant protein in human body. Inspired by linear CD where resonant wavelength is required to reveal chirality, we have carried out nonlinear microspectroscopy measurement and shown that when the collagen is excited near resonance, chirality-induced SHG-CD is strongly enhanced and can be easily differentiated from the anisotropy-induced contribution. By slowly heating up the sample, we have further verified that there is a wavelength-independent anisotropy contribution of SHG-CD vanishing at around 40 – 50 degree Celsius. Interestingly, the resonance-enhanced chirality component of SHG-CD remains until temperature rise to 60 degree, after which the collagen protein starts denaturation. Our results feature the first quantitative identification of chirality-induced SHG-CD in an intact biological tissue, and will be a critical step toward nonlinear chiral microscopy.