

CIRCULAR-DICHOISM SECOND HARMONIC MICROSCOPY: A NEW TOOL TO PROBE THE SUB-MICROMETER SCALE POLARITY DISTRIBUTION OF OUT-OF-PLANE COLLAGEN FIBRILS

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Second harmonic generation microscopy (SHG) is now the gold standard technique for in situ visualization of unstained fibrillar collagen in intact tissues [1]. Collagen is the most abundant protein in mammals and is found in many tissues, such as arteries, skin, bone and cornea. The size and the three-dimensional (3D) distribution of collagen fibrils are key distinctive features of every tissue that are crucial for its functional behavior, notably its mechanical properties. In situ characterization of collagen 3D distribution is therefore a major biomedical challenge.

However, usual implementation of SHG microscopy poorly reveals collagen fibrils oriented out of the imaging plane. Recently, circular dichroism SHG (CD-SHG) has been implemented to address this issue [2-5]. This advanced SHG modality measures the normalized difference of the SHG signals excited with left-handed versus right-handed circular polarizations. In this study, a careful implementation of artefact-free CD-SHG imaging of human cornea sections and other collagen-rich samples confirms that it specifically highlights out-of-plane collagen fibrils [5]. We further present a theoretical analysis of CD-SHG signals beyond the electric dipolar approximation to account for collagen chirality and we demonstrate that magnetic dipolar contributions are indeed mandatory for the analysis of CD-SHG images. We show that the sign of CD-SHG signals does not reveal whether collagen fibrils point upwards or downwards the imaging plane as tentatively proposed previously. CD-SHG instead probes the polarity distribution at the sub-micrometer scale of the out-of-plane fibril assemblies that is homogeneous polarity versus a mix of anti-parallel fibrils [6]. This makes CD-SHG a powerful tool for the characterization of collagen organization in tissues, specifically its degree of disorder. CD-SHG may thus serve to discriminate between healthy and diseased collagen-rich tissues.

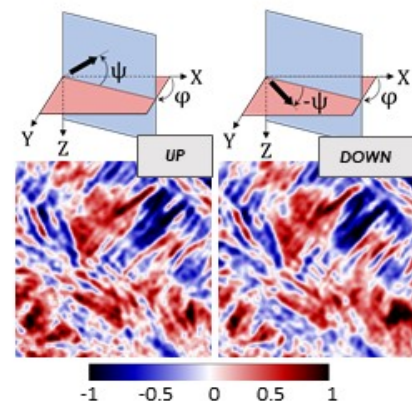


Fig. 1: CD-SHG images of a transverse section of a human cornea in the *up* and *down* configurations. The CD-SHG sign does not change with the out-of-plane orientation.

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