CIRCULAR DICHROISM SHG MICROSCOPY HIGHLIGHTS OUT-OF-PLANE COLLAGEN FIBRILS

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Collagen is the most abundant protein in mammals and the main component of connective tissues such as arteries, skin, bones or cornea. It is mainly organized as fibrils of various sizes and 3D distributions depending on the tissue. A change of collagen 3D organization or a defect in collagen synthesis can lead to pathologies or tissue malfunctions, including poor mechanical behavior. *In situ* imaging of these 3D structures is therefore a major biomedical concern.

The gold standard technique for *in situ* 3D visualization of collagen fibrils in intact tissues is second harmonic generation (SHG) microscopy [1, 2]. However, this technique is poorly sensitive to fibrils lying out of the imaging plane, which exhibit a lower SHG signal than fibrils lying in the focal plane. This may impede the determination of the collagen 3D organization in dense collagen tissues, where 3D or transverse reconstructions may be ambiguous.

To address this issue, we implement circular dichroism SHG experiments (CD-SHG) that measure the normalized difference in the SHG signal obtained upon excitation by left-handed versus right-handed circular polarizations. It reveals the chirality of collagen at molecular and tissular scales and is expected to be negligible for in-plane fibrils, thus highlighting out-of-plane fibrils [3-6]. However, like conventional CD signals, it is a low signal that is highly sensitive to experimental artefacts. It notably requires a careful calibration of the circular polarizations within the entire field of view to get rid of any linear dichroism contribution, and a perfect stabilization of the microscope stage to enable time-lapse measurements and increase the signal to noise ratio.

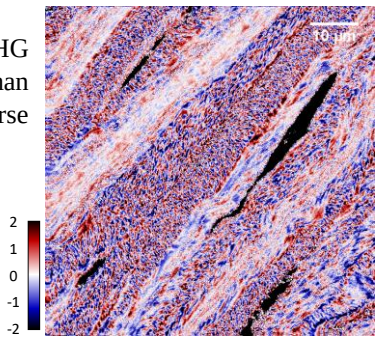

Figure 1: CD-SHG image of a human cornea transverse section.

After a thorough optimization of our experimental setup, we successfully record CD-SHG images in human cornea histological slides and in model collagen membranes prepared *in vitro*. We verify experimentally in these two types of samples with different 3D organization that CD-SHG vanishes for in-plane fibrils and highlights out-of-plane fibrils. It demonstrates that this technique is suitable for probing the 3D organization of collagen fibrils.

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