PROBING THE STRUCTURE OF COLLAGEN IN TISSUES: FORWARD VERSUS BACKWARD POLARIZATION-RESOLVED SHG MICROSCOPY.

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In situ visualization of fibrillar collagen in biological tissues is a major biomedical concern, whether to study collagen accumulation, impairment or disorder in numerous pathologies, or to understand structure of organs and guide tissue engineering. To this goal, second harmonic generation (SHG) is a powerful technique to observe fibrillar collagen without any staining and with a good contrast. This is not possible with classic imaging modalities. More information about structure of collagen fibrils in tissues can be gained with polarization-resolved second-harmonic generation (P-SHG) microscopy [1]. Two quantitative parameters can be measured and are linked with structural information: in-plane orientation of fibrils in the field of view and SHG anisotropy, which is related to collagen’s molecular structure and 3D orientation inside the excitation volume (submicroscopic scale).

Nevertheless, observed tissues are heterogeneous and anisotropic media and strong focusing is required for effective imaging. Light propagation in those media is therefore complex and not thoroughly understood yet, preventing accurate and reproducible measurements. Moreover, imaging can be performed through the sample (forwards) or backwards for in vivo imaging, but different measured anisotropy have been observed for those two modalities [2].

In this study, we performed advanced nonlinear optics studies, taking focusing and properties of the media into account, to understand how this coherent SHG signal is built [3]. Theoretical analysis, vectorial numerical simulations and experiments in tendon and in cornea were implemented to understand how geometrical parameters, such as collection direction, focusing NA and collection NA, affect second harmonic anisotropy in homogeneous media. We obtained an excellent agreement between simulations and experiments, showing that P-SHG measurements are highly sensitive to detection geometry (see Fig. 1).

Fig. 1: Second harmonic anisotropy: numerical simulations and measurements in tendon