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POLARIZATION-RESOLVED SHG IMAGING: CHARACTERIZATION OF THE HUMAN CORNEA LAMELLAR STRUCTURE OVER ITS FULL THICKNESS

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The cornea is a unique tissue featuring several essential physiological properties, mainly transparency and refraction. These properties are closely related to the structure of the corneal stroma, which is composed of 1-3 µm-thick stacked lamellae made of collagen fibrils (around 30 nm in diameter) aligned and regularly packed to ensure cornea transparency. However, this anisotropic hierarchical organization and its physiological consequences on cornea biomechanics have been poorly characterized yet because of the limitations of conventional techniques.

Second Harmonic Generation (SHG) microscopy is nowadays the gold standard technique for *in situ* visualization of collagen three-dimensional (3D) organization in unstained biological tissues. Cornea SHG imaging is mainly performed using epi-detection because it is the only geometry compatible with usual corneal chambers that best reproduce physiological conditions, ie curvature and intraocular pressure. Unfortunately, epi-detected SHG images of the corneal stroma are mostly homogeneous because of the sub-wavelength size of stromal collagen fibrils.

In this study, we have implemented epi-detected polarization-resolved SHG (P-SHG) microscopy to measure the corneal lamellar structure over the whole depth of human corneas (500 to 600 µm). P-SHG indeed provides unique information about the hierarchical organization of collagen within the focal volume, mainly the mean orientation of collagen fibrils and their degree of alignment [1-2]. We have optimized our P-SHG imaging protocols to get reliable P-SHG data in depth and implemented an automatic workflow to process the P-SHG images and estimate the mean fibrils orientation in every voxel as well as the accuracy of this measure. Using this approach, we have obtained 3D reconstructions of the collagen orientation over the full depth of 10 intact human corneas. Slight artefacts are observed in the posterior region of the stroma due to degraded imaging conditions at depths larger than 350 µm. Further examination is on progress to address this issue. Nevertheless, these artefacts are symmetric along the two main axes of the cornea, so that they can be compensated by combining two P-SHG image stacks acquired with two orthogonal orientations of the cornea. This method thus provides a quantitative characterization of the lamellae orientations along the full thickness of the cornea, which cannot be accessed with conventional techniques.

These results represent the first quantitative characterization of the lamellar structure of the human cornea through its entire thickness with micrometric resolution. It also shows the unique potential of P-SHG microscopy for structural imaging of thick collagen-rich tissues.

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