

# PROBING TISSUE STRUCTURE BY SECOND HARMONIC GENERATED SIGNALS

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## 1. INTRODUCTION

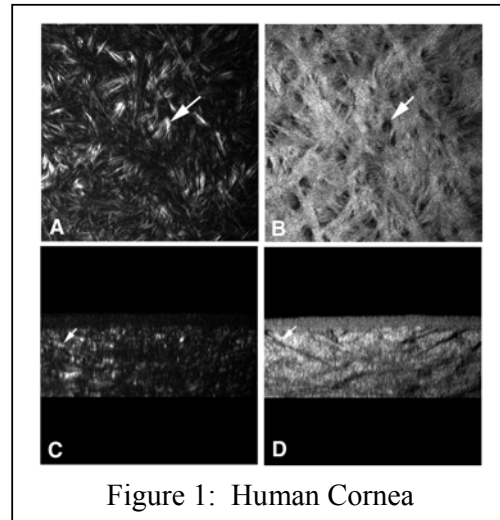
It is well known from many electron microscopic studies that the collagen fibrils in the cornea have a highly ordered pattern and are arranged in orthogonal layers forming collagen bundles or lamellae. The purpose of this study was to determine whether the structural organization of lamellae could be analyzed using second harmonic generated signals (SHG) from a femtosecond Ti:Sapphire laser.

## 2. METHODS

We have used a Zeiss 510 Meta NLO and a Coherent Chameleon laser (800 nm) to generate SHG signals from fresh and fixed whole corneas of chicken, mouse, rabbit and human origin. Both forward and backscattered signals were collected throughout the tissue sample.

## 3. RESULTS

The SHG from unstained chicken cornea showed a highly ordered, orthogonal arrangement of collagen bundles forming a lattice network that rotated 200 degrees within the anterior 50% of the cornea. In contrast, mammalian corneas had an anterior interwoven pattern of narrow collagen bands and a posterior orthogonal pattern of broad parallel bands. This pattern was most prominent in the human cornea where backscatter signals showed an amorphous pattern from the very anterior cornea representing Bowman's layer and a complex interwoven pattern underlying Bowman's layer. Interestingly, collagen lamellae with strong forward scattering (Fig. 1A, arrow) showed weak backscattering that was detected as spaces between collagen bundles (Fig. 1B, arrow). Many of these strong forward scattering lamellae appear to run tangential to the corneal surface and were detected as voids within the backscatter image (Fig. 1C and 1D, arrows).



## 4. CONCLUSION

SHG provides a sensitive technique to analyze macromolecular structure within biologic tissues.

