

# Imaging Human Cornea by Second Harmonic Generation Microscopy and Confocal Reflectance Microscopy

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In this work, we demonstrate a combined application of reflected confocal and SHG (Second Harmonic Generation) microscopy [1, 2] on ex vivo human cornea. We show that back-scattered second harmonic generation signal combined with confocal reflectance signal can be employed to study the cellular and stromal collagen in cornea. This microscope modality can be used for obtaining useful information about the corneal collagen structure and function without any staining or preparation of the tissue. This method has the potential of being used in in-vivo imaging of cornea.

A mode-locked fiber laser, with 100 femto-second pulses at a wavelength of 800 nm and average power of 110 mW, is used to excite the collagen in the stroma of human cornea to image the back-scattered SHG signal. The weakly back-scattered SHG signal was collected with a sensitive PMT. Longer wavelength helps in deep tissue imaging and nonlinear contrast comes from the asymmetry of collagen matrix in the stroma. The cellular epithelium and keratocytes were also imaged with the confocal reflectance signal at a wavelength of 543 nm in a laser scanning microscope ( Fig. 1). The two imaging modalities are combined to obtain images at different layers of the tissue. In reflected confocal images contrast originated from variations in the refractive index at the edges of epithelial cells and cell nuclei. The contrast obtained with SHG signal comes from the stromal collagen.

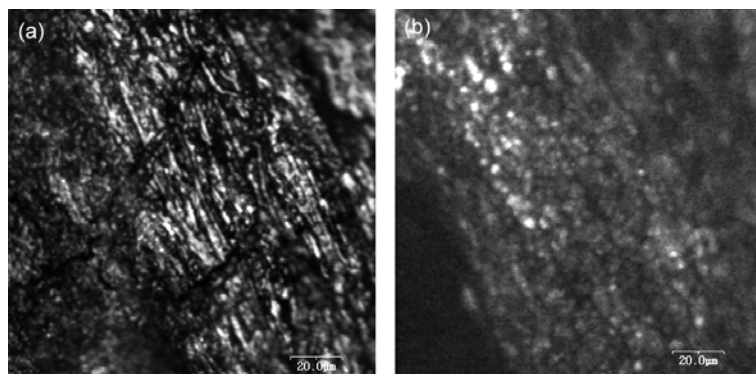


Fig.1 The confocal reflectance (a) and back-scattered SHG signal (b) from cornea.

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