

HIGH-RESOLUTION CORNEAL IMAGING BY SECOND HARMONIC GENERATION AND THIRD HARMONIC GENERATION MICROSCOPY

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KEY WORDS: second harmonic generation microscopy, third harmonic generation microscopy, tissue imaging, cornea.

Advances in femtosecond laser technology have resulted in significant progress in corneal refractive surgery. However, further progress requires a non-invasive imaging solution that offers high spatial resolution and that can also cover the whole depth of the cornea [1].

For applications in ophthalmology, second harmonic generation (SHG) and third harmonic generation (THG) microscopy are attractive imaging techniques. Because of the presence of collagen I, SHG microscopy can characterize the stromal layer, while third harmonic can also be detected at the level of epithelial and endothelial layers, allowing high-resolution imaging of the natural structures of the epithelium, stroma and endothelium (e.g. cells, collagen fibres). Moreover, previous studies have shown that SHG and THG imaging can be detected in backward (B) as well as the forward (F) direction [2].

In this work, we show that SHG and THG can be used in combination to perform high-resolution imaging of full thickness *ex vivo* pig corneas. We show that one could obtain both SHG and THG signals in epi-detection from fresh eyes. For example, we have imaged endothelial cell structure using F-THG (Fig. 1), while B-THG provides detailed structures of epithelial cells (Fig. 2). The results may find promising applications as control tool for femtosecond refractive surgery.

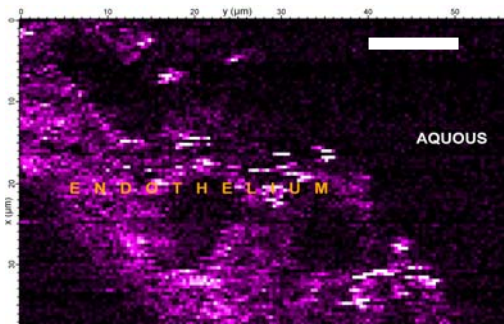


Fig. 1: F-THG image in (x, y) plane of endothelial cells in *ex vivo* porcine eye. Scale bar: 10 µm.

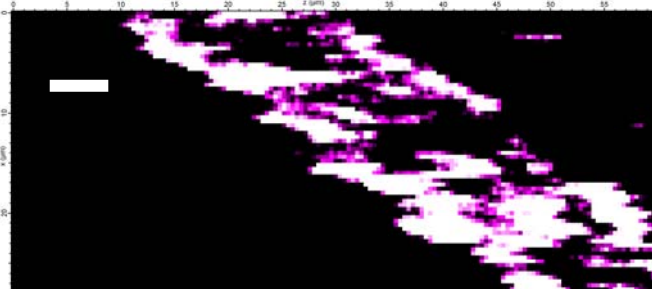


Fig. 2: B-THG image in (x, z) plane of epithelial cells in *ex vivo* porcine eye. Scale bar: 5 µm.

[1] Lubatschowski H. et al, "Application of ultrashort laser pulses for intrastromal refractive surgery", *Graefe's Archive for Clinical and Experimental Ophthalmology* **238**, 33-39 (2000)

[2] Tai S.P. et al, "Optical biopsy of fixed human skin with backward-collected optical harmonics signals", *Optics Express* **13**, 8231-8242 (2005)