

## MULTIMODAL MULTIPHOTON IMAGING OF INTACT EYE TISSUES

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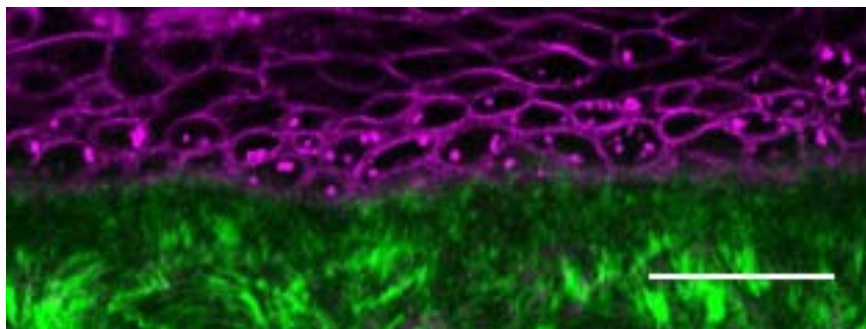
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We evaluated three combined modalities of multiphoton microscopy [1], second-harmonic generation (SHG), third-harmonic generation (THG), and two-photon-excited fluorescence (2PEF) for imaging the anterior segment [2] of intact human eye tissue.

The three imaging modalities provide complementary information on intact tissue over the entire thickness of the cornea (see figure 1). THG imaging reveals the tissue morphology, including the epithelium and endothelium structure with sub-cellular resolution. SHG imaging probes the distribution of stromal collagen lamellae organization. 2PEF imaging reveals the elastic component of the extra-cellular matrix and the distribution of fluorescent organelles (mitochondria etc) in stromal, epithelial and endothelial cells. Images of the trabecular meshwork show the three-dimensional organisation of the trabecular lamellas. THG and SHG signals are predominantly forward directed, but in some cases, SHG images can be recorded in the epi-direction.

To conclude, the combined imaging modalities of SHG, THG, and 2PEF microscopy are effective methods to evaluate corneal microstructures in situ. This imaging approach should prove particularly appropriate for assessing corneal and glaucoma physiopathologies.



*Figure 1: Combined THG (purple) and SHG (green) image of the epithelial-stromal junction in freshly excised human eye tissue. Scale bar: 50  $\mu$ m.*

[1] Débarre et al, Nat. Methods 3, 47 (2006).

[2] Nuzzo, Plamann et al, J. Biomed. Optics 12, 064032 (2008).