

# NON-LINEAR TOMOGRAPHIC IMAGING OF EX-VIVO CORNEAL TISSUES AS A FUNCTION OF TIME

**Emilio J. Gualda, Juan M. Bueno, Anastasia Giakoumaki, and Pablo Artal**  
**Laboratorio de Óptica (CiOyN)**  
**Universidad de Murcia**  
**Campus Espinardo, 30100 Murcia, Spain**  
**E-mail: [bueno@um.es](mailto:bueno@um.es)**

**KEY WORDS:** Multiphoton microscopy, second harmonic generation, corneal stroma, collagen.

Multiphoton imaging microscopy is a powerful technique to explore the internal arrangement of biological samples, in particular ocular tissues such as the cornea. The structure of the corneal stroma produces a strong second harmonic generation (SHG) signal useful to obtain valuable information in a minimal invasive way. Other corneal layers, such as epithelium and endothelium, provide two-photon excitation fluorescence (TPEF) signals. Although in previous studies the temporal dependence of corneal SHG imaging have not been widely considered, in some cases the temporal changes might be important for tissue analysis. In particular, some treatments and surgery procedures modify the corneal structure in a non-controlled manner, whose effects are still not fully understood. The aim of this work is to apply backscattered SHG imaging to study chemical or photo-induced morphological changes across the stroma as a function of time.

A research prototype non-linear (two-photon) microscope was used to image SHG signal of bovine and porcine cornea samples, both normal as control and with structural alterations after different treatments. The setup used a mode-locked Ti:Sapphire laser as illumination and a photon-counting unit as detector. A motorized stage allowed optical sectioning across the entire cornea. Image acquisition and post-processing were computer-controlled with custom-developed software. Specimens were placed up-side-down on a glass-bottom dish filled with saline solution. Two different scanning modes were used to image the samples. A TPEF-SHG XZ tomography imaging allows analyzing the changes in thickness as well as fast changes in morphology after corneal treatment or manipulation. Moreover XY imaging along the Z direction allowed obtaining 3D volume renderings of the corneas. SHG images presented sets of lamellae packed with singular undulations. This pattern changed in altered corneas, where some abnormal structures such as thicker collagen bundles appear in some localized areas.

These results demonstrate that non-linear microscopy is useful to reveal the changes with time of corneal collagen after chemical or surgical treatments. Tomography SHG imaging permits the fast testing of in-depth morphological changes. This complements the current biometric measurements that only provide data of corneal thickness. The optical sectioning capabilities render 3D reconstruction of the collagen arrangement through the whole corneal thickness.

## **Acknowledgments**

This work has been supported in part by the Ministerio de Educación y Ciencia of Spain, grants FIS2007-64765 and CSD2007-00033 SAUUL (CONSOLIDER-INGENIO 2010); and Fundación Seneca (Region de Murcia, Spain), grant 4524/GERM/06.