

NON-LINEAR MICROSCOPY OF EX-VIVO OCULAR TISSUES: A COMPARATIVE STUDY FROM DIFFERENT SPECIES

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Non-linear imaging techniques have become important tools in the areas of medicine and cell and tissue biology, due to its advantages compared with classical imaging techniques. Two-photon emission autofluorescence (TPEF) and second harmonic generation (SHG) offer useful information that is not available with standard linear microscopy. These techniques offer the additional advantage of avoiding the use of staining substances. Moreover, the quadratic dependence of SHG and TPEF on the light intensity dramatically reduces the photobleaching and phototoxicity outside the focal plane and therefore diminishing tissue damage [1].

Ocular tissues are complex and vary among different species (human, bovine, porcine, fish). These are appropriate samples for a variety of studies where the use of non-linear microscopy offers significant advantages. In this work, we investigate the morphology of ocular tissues (samples from cornea, lens and retina) of different species by using a non-linear microscope with different configurations of wavelength and polarization. We used a custom built system with a tunable Ti:Sapphire fs-laser as excitation source. The system is fully computer controlled, via own-developed software, including a set of galvanometric mirrors for sample scanning, a photo-counting detection unit and a motorized focus control for 3D reconstructions. TPEF-SHG pairs of images are combined to build tomographic maps of the different tissues and to obtain complementary information of cell structures. In particular,

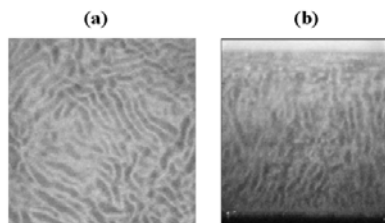


Figure 1. SHG image of a bovine cornea (a) and tomography (b).

while in the cornea SHG is measured from collagen (due to its characteristic triple helix molecular structure) [2], TPEF signal arise from autofluorescence of keratocytes, epithelial and endothelial cells (Figure 1). The comparison of normal tissue samples within different species may serve as a reference for future studies of pathological eyes. In this sense, a detailed information on the tissue organization and morphological changes could be used for future early detection of ocular diseases.

REFERENCES

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