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Multiphoton Imaging of Cornea and Retinal Tissues

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Abstract

Purpose: The importance of multiphoton imaging modalities, such as two-photon fluorescence and second-harmonic imaging, have become clear during the last decade by offering higher penetration depth and reduced radiation damage for many biological samples. In the context of the eye, we studied the potential applicability of multiphoton techniques for imaging of cornea and retinal tissues in vitro.

Methods:A multiphoton microscope has been constructed with a pulsed Ti:Sapphire femto-second laser as illumination source and highly sensitive photon-counting equipment for the measurement of nonlinearly-generated light at each point of an image. Scanning of the tissue is realized by means of two galvanometric mirror scanners that allow the focused beam to sweep the selected area of interest.

Results: The details of the experimental setup are presented and the potential of the technique explored. First results on the imaging of corneal and retinal tissue from porcine eyes are shown and discussed in relation to simultaneously acquired conventional linear optical images. For the cornea, it is found that nonlinear images can be of particular interest to examine the distribution of collagen, and that polarization contrast too can advantageously be used. Likewise, for the retinal images, nonlinear techniques offer advantages by an enhanced sensitivity to structural changes.

Conclusions: We explored the potential of nonlinear imaging as an alternative to more conventional imaging modalities for the study of ocular tissue. This has important implications for ocular surgery and may in the near future be further explored in real-life applications such as real-time monitoring of changes induced during corneal surgery.

Keywords: microscopy: confocal/tunneling • anatomy • imaging/image analysis: non-clinical