Multiphoton Imaging of Porcine Eye: Cornea, Limbus, Conjunctiva, and Sclera

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Abstract: This purpose of this study is to demonstrate the feasibility of using multiphoton microscopy in imaging eye surface. Specifically, the cornea, limbus, conjunctiva, and sclera were imaged using multiphoton induced fluorescence and second-harmonic generation (SHG) imaging.

INTRODUCTIONS

Without extrinsic fluorescent molecules, we were able to image different parts of the eye surface. Our results show that individual corneal epithelial cells (autofluorescence) are visible as are the well organized corneal fibers (SHG). The histological morphology of the limbus also correlates well with the multiphoton image. With increasing depths, multiphoton limbus imaging reveals the surface epithelial cells followed by joining of the corneal and sclera collagen fibers. The conjunctiva and sclera are also visible under multiphoton excitation.

METHODS AND RESULTS

Shown in Fig. 1 is the multiphoton microscope used in this study. Figs. 2(a), 2(b) are, respectively, large area multiphoton images of the limbus, detailed image of the limbus. Autofluorescence (green) and second harmonic generation (blue) signals can be used to identify morphological features of the limbus and sclera.

Figure 1

THP-28(T5)

Figure 2(a)

Figure 2(b)

CONCLUSIONS

Our demonstrate that without the introduction of extrinsic fluorescent molecules, multiphoton induced fluorescence and SHG signals can be used to obtain structural information of intact porcine eyes. Autofluorescence from the epithelia and SHG from collagen fibers can be acquired for structural analysis of intact eyes. We have demonstrated that with additional developments, multiphoton microscopy may be used for in vivo investigation of ophthalmologic disease diagnosis and tissue physiological studies.

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