STRUCTURED ILLUMINATION OPHTHALMOSCOPE FOR FLUORESCENCE IMAGING OF THE RETINAL PIGMENT EPITHELIUM

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KEY WORDS: structured illumination, ophthalmoscope, age-related macular degeneration

Age-related macular degeneration (AMD), the major cause of blindness in the elderly population of the developed world, is usually accompanied by a formation of characteristic autofluorescent (AF) structures or by changes in AF behavior in the ocular fundus (background of the eye). The AF is based on aggregation of broadband fluorescent lipofuscin compound in the retinal pigment epithelium between retina and choroid. It can be detected in vivo through the pupil of the intact eye when simultaneously the fundus is illuminated with blue or green light. An important tool to observe these AF structures in-vivo is provided by the method of scanning laser ophthalmoscopy (SLO). It is used for example to monitor AMD progression and effects of treatments. However the resolving power of existing SLO devices is insufficient to visualize the AF structures in detail. Efforts are made to use two-photon excitation and adaptive optics in order to improve SLO resolution.

In contrast to these scanning laser approaches our intent was to use wide-field (non-scanning) imaging to resolve the fine autofluorescent structures. Here we demonstrate a retinal camera setup using large distance structured illumination microscopy (SIM) to attain highly resolved fluorescence images in the intact eye. The device is using a stroboscopic series of flash-illumination SIM acquisitions with 532 nm excitation wavelength. After image acquisition the eye movement between the single images is corrected automatically using a tailored algorithm. Subsequently, SIM image reconstruction is applied in order to achieve an image of higher resolution and improved optical sectioning.

To study the setup's imaging capabilities, we used a human-type model eye containing RPE-choroid specimens. Enhanced lateral resolution and high contrast was achieved allowing us to resolve the fluorescence distribution in single cells. Our stroboscopic acquisition in combination with the a posteriori shift correction prevents motion artifacts caused by natural eye movements. The irradiance levels used by our method are far below maximum permissible values for ophthalmic instruments. Thus a future application at intact human eyes does not pose a hazard.

In conclusion structured illumination ophthalmoscopy offers an exciting new approach to provide a convenient autofluorescence imaging tool in the medical environment for research and diagnosis.