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Wavefront optimized two-photon microscopy of ocular tissues

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Two-photon (nonlinear) microscopy has the potential to non-invasively image biological structures. However, similar to linear microscopy, this technique is also affected by optical aberrations. These aberrations include those of the laser beam and the optical system itself, as well as others arising from the sample when in-depth imaging is required. Our objective has been to investigate and enhance the nonlinear signals (two-photon excitation fluorescence -TPEF-, and second harmonic generation -SHG-) of ex-vivo ocular tissues (both cornea and retina) using a customized wavefront optimized multiphoton microscope. The instrument works in backscattered mode and uses a Ti:Sapphire fs-laser as excitation source, a pair of galvanometric mirrors as a scanning unit and a photon-counting as detection device. A motorized Z-scan allows optical sectioning and recording of stacks of images to obtain 3D-reconstructions. It also includes an adaptive optics (AO) module (Hartmann-Shack sensor and deformable mirror) in the illumination pathway to measure and correct for the laser beam wavefront aberrations in real time. Firstly, the aberrations of the laser beam were minimized by realigning the laser cavity [1,2]. This operation produced a simply static correction of low-order aberrations leading to an increase in the detected nonlinear signal. As a second step the AO module was operating in closed-loop to correct for the remaining aberrations [3]. This significantly increased the efficiency of the nonlinear processes, improved contrast and enhanced lateral resolution in images of (non-stained) ocular tissues. For the cornea both epithelium and endothelium provided TPEF signals. However the only source of SHG was the stroma. Within the stroma, keratocytes were also observed when the TPEF filter was used. The retinal structures only presented TPEF signals: nerve fiber layer, blood vessels, ganglion cells, photoreceptors, retinal pigment epithelium,… We demonstrate the feasibility AO multiphoton microscopy for improved visualization of ocular structures which are relevant in Ophthalmology. In particular, wavefront optimization approaches improved multiphoton imaging without noticeable photo-damage and reduced excitation power levels. This is of huge interest to study the sources of nonlinear signal of ocular tissues, and to visualize ex-vivo retinal and corneal layers through volume rendering reconstructions with increased quality that might be useful in clinical environments.

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