

WAVEFRONT CONTROL IN TWO-PHOTON MICROSCOPY OF EX-VIVO OCULAR TISSUES

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It has been recently reported that the aberration compensation of a femto-second laser beam enhances the quality of the two-photon excitation fluorescence (TPEF) and second harmonic generation (SHG) images. The aim of this work was to investigate the influence of the manipulation of different aberration terms in 3D multiphoton microscopy imaging of ex-vivo ocular tissues.

An adaptive optics (AO) multiphoton microscope was built using a mode-locked Ti:Sapphire laser as illumination source. The AO module combines a Hartmann-Shack (HS) wavefront sensor and a gold coated MEMS-type deformable mirror (DM) with 140 actuators. The beam passes the AO module before entering the microscope. A scanning unit composed of two galvanometric mirrors scans the sample in the XY plane and a motorized Z-scan device moves the microscope objective to record stacks of images at different depths within the sample to obtain 3D reconstructions. The backscattered SHG and TPEF signals travel back through the same objective, pass a dichroic mirror and the corresponding filter, to reach a photon-counting detection unit. The control of the image recording, as well as the post-processing, was performed via custom software. The aberrations of the laser beam were estimated from HS images and compensated or modified by the DM in closed-loop by appropriately changing its shape.

For a particular plane within the sample, examples of TPEF and SHG images of ex-vivo corneal and retinal tissues were recorded with and without AO. A significant increase in the recorded signal was obtained and more details were visible. 3D images were also reconstructed with the AO module in operation. The signal increase was not the same for every plane, although an overall improvement in the image quality was apparent. By inducing spherical aberration in the beam, the quality of the control imaged plane was reduced, but a higher signal was obtained from deeper planes within the sample. For similar amounts of asymmetric aberrations (such as coma) the decrease in image quality is more noticeable and the behaviour in depth does not follow a regular pattern.

3D multiphoton microscopy techniques might significantly be improved with adequate control of the aberrations of the illuminating beam. Lower energy levels can be used when AO is operating to record similar non-linear signals. Controlled amounts of spherical aberration increase the depth-of-focus within the sample independently of the specimen-induced aberrations, allowing improved in-depth imaging what is essential in the analysis of ocular tissues.

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