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Advances in adaptive optics nonlinear microscopy for applications in medical and life sciences

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Abstract content :

In the last years, the combination of imaging microscopy and ultrafast laser sources to produce nonlinear signals has opened new research opportunities in Biology and Biomedicine. In nonlinear (multiphoton) microscopy two infrared photons are simultaneously absorbed by the tissue and one visible is emitted. Two-Photon Excitation Fluorescence (TPEF) and Second-Harmonic Generation (SHG) are well-established non-invasive methods used for imaging and mapping sub-cellular biological structures. This technique is auto-confocal, provides intrinsic optical sectioning and allows 3D analysis and volume rendering. However, multiphoton imaging is also affected by aberrations (laser beam, microscope optics and specimen itself). These reduce the quality of images, in particular those corresponding to deeper planes within the sample. In our lab, a custom-made multiphoton microscope combined with adaptive optics (AO) devices has been developed to improve multiphoton imaging of biological tissues, in particular those of the eye. A deformable mirror with a Hartmann-Shack sensor (working in closed-loop), and a liquid-crystal spatial light modulator (in sensor-less mode) have been used to investigate the effects of aberrations when visualizing corneal and retinal structures. Along this presentation the last advances in AO multiphoton imaging, both TPEF and SHG, will be shown. AO techniques revealed ocular features never seen before without using fixation or staining procedures. Multiphoton image quality was improved independently of the depth location of the imaged plane. It will be also shown how that deeper the imaged structure the more influence of higher order aberration terms. The influence of the field-of-view and the order of the Zernike modes in a modal correction will be also explored. Finally, different algorithms and image quality metrics used to optimize the final image will be also compared.

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