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# Adaptive Optics Multiphoton Microscopy of *ex-vivo* Ocular Tissues

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## Abstract

**Purpose:**To develop an improved version of a multiphoton microscope incorporating the control of the beam wavefront, wavelength and polarization. The instrument will be primarily applied to obtain two-photon autofluorescence (TPEF) and second harmonic generation (SHG) images of ex-vivo retinal and corneal tissues of fish, bovine and porcine eyes.

**Methods:**We have built a custom multiphoton microscope using a wavelength-tunable Ti:Sapphire femto-second laser for illumination, a scanning unit and a photon-counting detection device. A motorized z-scan allows recording of stacks of images to obtain 3D-reconstructions of the samples. A research-prototype Hartmann-Shack wavefront sensor was incorporated in the illumination pathway to measure the aberrations of the illuminating laser beam in real time. Appropriate correcting optics, both static and dynamic, is driven by the sensor data to correct the beam producing a highly focused spot on the sample. TPEF and SHG images of a number of ocular tissues of different species were registered at 1 Hz with different excitation wavelengths, polarization states, image magnification and different levels of wavefront correction.

**Results:**The wavefront aberrations of the illuminating laser beam were fairly constant over time and the main contribution is due to low order aberrations (defocus and astigmatism). This indicates that simple static correction of low order aberration produce a significant increase in the detected signal and in the resolution of the system (both axial and lateral). Additional dynamic wavefront correction of higher order aberration was also incorporated. Examples of TPEF and SHG images of ex-vivo corneal and retinal

tissues of different species were recorded for different beam wavefronts. Moreover SHG signals from collagen (stroma and sclera) were strongly affected by the polarization states of the light beam.

**Conclusions:** We showed that an accurate control of the illuminating beam wavefront increases the contrast and resolution in TPEM and SHG microscope images of ocular tissues. As an additional benefit, beam aberration correction allows the reduction of the required excitation power levels to generate non-linear effects, and hence, reducing unwanted side effects of photo-bleaching and phototoxicity. This work is the first step for possible future clinical applications of non-linear imaging techniques in the diagnosis of ocular pathologies, such as keratoconus or age-related macular degeneration, as well as to increase the accuracy of intra-tissue laser corneal ablation.

**Keywords:** imaging/image analysis: non-clinical • microscopy: light/fluorescence/immunohistochemistry • microscopy: confocal/tunneling