WAVEFRONT-ASSISTED NON-LINEAR MICROSCOPY OF EX-VIVO OCULAR TISSUE

Emilio J. Gualda, Juan M. Bueno, Pablo Artal
Laboratorio de Óptica (CiOyN)
Universidad de Murcia
Campus Espinardo, 30071 Murcia, Spain
E-mail: egualda@um.es

KEY WORDS: Multiphoton microscopy, wavefront aberrations, polarization

The aim of this work is to investigate the influence of aberrations, wavelength and polarization on two-photon autofluorescence (TPEF) and second harmonic generation (SHG) imaging of ex-vivo retinal and corneal tissues. We have developed a multiphoton imaging system by adapting a wavelength-tunable ultrafast high-power laser, a scanning unit, a motorized Z-scan device and a photon-counting detector to a commercially available microscope. A custom-built Hartmann-Shack wavefront sensor [1] was included in the illumination pathway to measure wavefront aberrations (WA) of the incoming laser beam in real time (25 Hz). We recorded both TPEF and SHG images of ocular tissue samples at 1 Hz for different depth positions within the sample. A number of excitation wavelengths, polarization states, system magnifications conditions were tested. Moreover, images were recorded for different levels of low-order aberrations correction produced by means of passive optical elements placed in the illumination pathway.

The aberrations in the illuminating laser beam were fairly stable, with the main contribution being low-order aberrations, i.e., defocus and astigmatism [2]. Small changes are produced when modifying the wavelength, but stability keeps in the same range. The quality of the registered TPEF and SHG images of ex-vivo ocular tissues were affected by the beam aberrations. A static wavefront correction for the dominant terms, enhances the quality of the TPEF and SHG images. Examples of images corresponding to different levels of compensation will be shown. Moreover SHG signals were also affected by polarization and wavelength.

Non-linear microscopy techniques might be significantly improved when they are combined with wavefront-assisted approaches. This may reduce the required excitation power levels and minimize the side-effects of phototoxicity within the sample. In particular, this benefit may be important in the future diagnosis of ocular pathology or in the improvement of laser ablation surgery techniques.

REFERENCES

[1] P. M. Prieto, F. Vargas-Martín, S. Goelz, P. Artal, "Analysis of the performance of the Hartmann-Shack sensor in the human eye," *J. Opt. Soc. Am. A* **17**, 1388-1398 (2000).
[2] J. M. Bueno, B. Vohnsen, L. Roso, P. Artal, "Temporal wavefront stability of an ultrafast high-power laser beam," *Appl. Opt.* **48** (2009).

Acknowledgments

This work has been supported in part by the Ministerio de Educación y Ciencia of Spain, grants FIS2007-64765, CONSOLIDER-INGENIO 2010 , CSD2007-00033 SAUUL; and Fundación Seneca (Region de Murcia, Spain), grant 4524/GERM/06.