COMPARING THE PERFORMANCE OF TWO FEMTOSECOND LASERS FOR MULTIPHOTON MICROSCOPY

Juan M. Bueno,[1] Francisco J. Ávila,[1] Matthias Handloser,[2]
Jan Schäfer[2] and Pablo Artal[1]
[1]Laboratorio de Óptica, Universidad de Murcia, 30100 Murcia, Spain
[2]TOPTICA Photonics AG, 82166 Graefelfing / Munich, Germany
E-mail: bueno@um.es

KEYWORDS: multiphoton microscopy, femtosecond laser system

Multiphoton microscopy (both two-photon excitation fluorescence -TPEF- and second harmonic generation -SHG-) is a well-established technique to image biological tissues at cellular level without fixation or labelling procedures and minimized photodamage. These imaging procedures are based on the quasi-simultaneous absorption of two infrared photons, followed by the emission of a single photon with higher energy. These nonlinear effects require a very large flux of photons which can be reached using mode-locked lasers providing pulses in the range of femtoseconds with a very high repetition rate (in the order of MHz). During more than two decades solid-state laser systems (mainly Ti:sapphire lasers) have been successfully used in TPEF and SHG microscopy imaging. Although they offer broad and tunable wavelengths in the near infrared, light in the range ~760-800 nm has been mostly used. More recent femtosecond lasers may offer advantages due to a reduced size allowing a better system integration.

We compared the performance of two available femtosecond laser systems when used as illumination sources in a custom multiphoton microscope [1]: a compact fiber laser (FemtoFiber ultra 780 laser, TOPTICA Photonics) and a standard Ti:sapphire laser (Mira 900f, Coherent). The two devices provided similar pulse duration and repetition rate. Moreover, the wavefront aberrations of both beams were also similar. We obtained multiphoton images of samples, providing both TPEF and SHG signals, to compare the performance of both lasers. As an example, Figure 1 shows the SHG images corresponding to a histological section of a fixed human cornea. A direct visualization reveals that the capabilities of both laser systems are comparable (similar results were obtained for TPEF images). This suggest that the compact fiber laser system can be used as multiphoton illumination source, since it provides images with similar quality as those obtained with the Ti:sapphire laser.

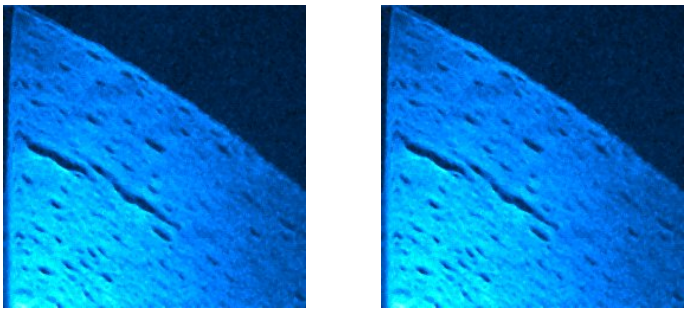

Figure 1. SHG images acquired with a femto-fiber (left panel) and a Ti:sapphire (right panel) laser source. Image size: 270x270 μm^2.

[1] Bueno et al., *J. Biomed. Opt.***15**, 0660042 (2010).

AKNOWLEDGEMENTS: This work has been supported by grants FIS2013-41237-R (SEIDI, Spain) and 19897/GERM/15 (Fundación Séneca, Murcia, Spain).