MULTIPHOTON IMAGING MICROSCOPY WITH A SUB-10-FS LASER SYSTEM

Francisco J. Ávila, Íñigo J. Sola*, Pablo Artal and Juan M. Bueno,
Laboratorio de Óptica, Universidad de Murcia, 30100 Murcia, Spain
***Dept. Física Aplicada, Universidad de Salamanca, 37008 Salamanca, Spain**
E-mail: franciscojavier.avila@um.es

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The performance of multiphoton (MP) microscopy techniques is limited by the presence of optical aberrations. Different adaptive optics approaches have been reported to minimize aberrations and optimize MP images [1,2]. However, the temporal properties of the illumination laser beam also deserve attention. During propagation, fs-pulses suffer temporal broadening what reduces the effectiveness of MP processes. To counteract this optical dispersion, pulse compression devices are used [3].

Ti:sapphire lasers often used in MP microscopes provide nominal pulses of 120 fs, that broaden when passing through the microscope optics. Although these beams can be pre-compensated by introducing negative group velocity dispersion, this operation has some experimental constrains [4,5]. In the last years a number of commercial lasers providing sub-10-fs pluses have been developed and successfully used in MPM experiments [6]. However, to our knowledge, direct comparisons between both types of laser devices are lacking in the literature.

A research MP microscope [7] has been modified to include a dual Ti:sapphire laser system illumination. This consisted of a tunable standard unit (Mira 900f, Coherent) and a fix-wavelength sub-10-fs laser (Octavius, Thorlabs). Both lasers provided the same central wavelength (800 nm) and similar repetition rate (76 and 85 MHz). The two beams covered identical optical pathways and the measured wavefront aberrations were also similar.

To compare the performance of both lasers MP images were acquired in a number of samples emitting two-photon excitation fluorescence (TPEF) and second harmonic generation (SHG) signal. Although MP images of fairly good quality were acquired with both lasers, the sub-10-fs images provided a significantly lower MP signal. This occurred for both TPEF and SHG signals. This reduction in MP efficiency might be directly related to their broadband (>100 nm) and to their higher sensitivity to dispersion. The averaged power of the illumination laser had to be increased x3-x4 to get equivalent MP images (in contrast and resolution), what might produce photo-damage and put the durability of biological specimens at high risk.

These results show that, compared to “regular fs-sources”, sub-10-fs lasers originate a decrease in SHG conversion processes and a TPEF excitation of multiple fluorophores simultaneously, possibly due to their higher sensitivity to the set-up dispersion and the spectral limits of the nonlinear effects. This leads to a limited MP effectiveness, indicating that this type of lasers might not be suitable for MP imaging for particular experimental conditions, especially those involving biological specimens.

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