Multiphoton Microscopy / Fiber Laser

Multiphoton microscopy using a femtosecond fiber laser system

Juan M. Bueno, Francisco J. Ávila

Universidad de Murcia, Laboratorio de Óptica,
Campus de Espinardo (Edificio CiOyN), 30100, Murcia, Spain

Matthias Handloser, Jan Schäfer

TOPTICA Photonics AG, Lochhamer Schlag 19, 82166 Graefelfing, Germany

Nowadays multiphoton microscopy (both two-photon excitation fluorescence -TPEF- and second harmonic generation -SHG-) is one of the leading techniques to image biological tissues at cellular level without fixation or labelling procedures and minimized photodamage [1,2]. 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).

Fig. 1: TOPTICA's FemtoFiber ultra 780 femtosecond fiber laser system.

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 range, light of ~800 nm has been mostly used.

The main disadvantages of this type of lasers have been their size and price, and its complicated handling in terms of alignment. Although these issues have significantly been reduced in the last years, prices do not still fit the budget of many research groups interested in multiphoton techniques.

Here we report on a research multiphoton microscope equipped with a compact and cost-effective laser system (FemtoFiber ultra

780 laser, TOPTICA Photonics). This combination permits to obtain high quality multiphoton images of samples providing both TPEF and SHG signals.

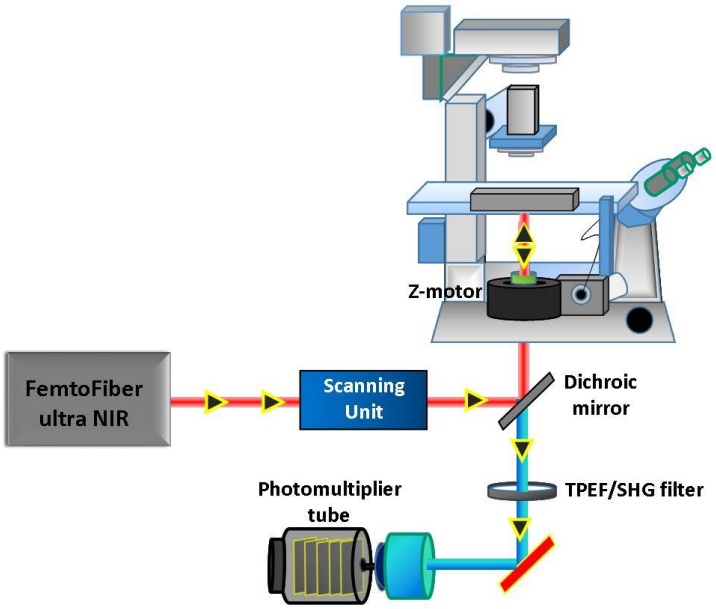

Fig. 2: Simplified schematic diagram of the multiphoton microscope.

Experimental setup

The FemtoFiber ultra 780 (Fig. 1) is combined with a commercially available inverted microscope (Fig. 2). The laser provides pulses with a wavelength of 780-nm with a nominal duration of 150 fs and a repetition rate of 80 MHz. This beam is firstly expanded with by a Galilean telescope before passing the scanning unit and entering the microscope to overfill the objective' aperture. A non-immersion long-working distance objective (20x, NA=0.5) is used to focus the light onto the investigated sample. The focus is rasterscanned in x-y-z direction by motorized motions of the objective. The nonlinear signal emitted by the sample is detected by a photomultiplier tube in epi configuration sharing the excitation beam path after appropriately filtering by a dichroic mirror and the corresponding spectral filter (broadband for TPEF and narrow-band for SHG). The average output power of the laser system (>500 mW) is high enough to generate nonlinear signals from the different specimens investigated throughout the present work. Acquisition time is around 1 s (for images of 250x250 pixels). Images will be presented in false color: green for TPEF and blue for SHG (see results section).

Results

Fig. 3 shows a TPEF and a SHG image corresponding to a piece of cellulose and a histological section of a fixed human cornea. For direct comparisons, Fig. 4 presents the images corresponding to the same samples and areas, but acquired with a Ti-sapphire laser.

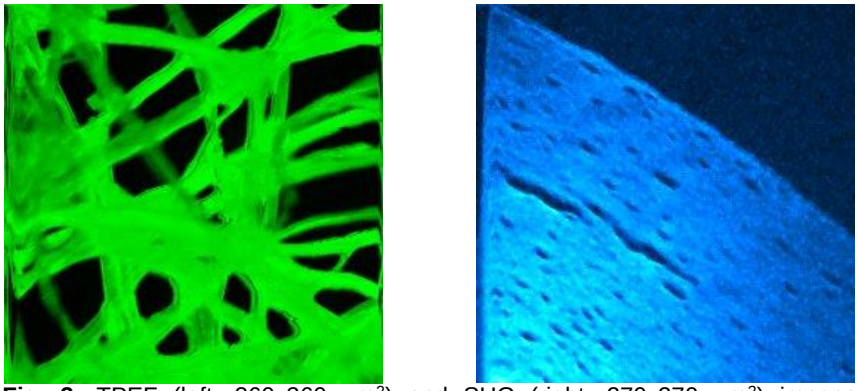

Fig. 3: TPEF (left, 360x360 µm^2) and SHG (right, 270x270 µm^2) images acquired with a multiphoton microscope that uses a FemtoFiber ultra 780 laser.

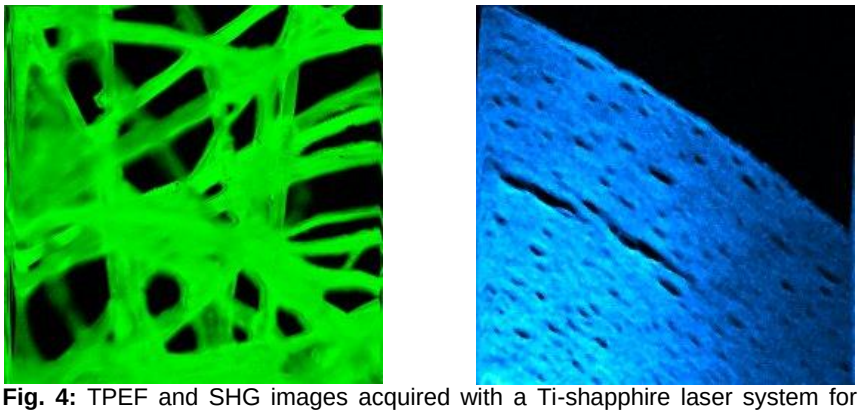

Fig. 4: TPEF and SHG images acquired with a Ti-shapphire laser system for comparison. Imaged areas are the same as those in Fig.3

Conclusions

Multiphoton microscopy using a cost-effective and compact (20x38x11 cm^3) laser system has successfully been demonstrated here. The laser source produces a highly stable output and is controlled via a user-friendly interface. To proof the performance of the FemtoFiber ultra 780 it was compared to a state of the art Ti-sapphire oscillator. These results show that this type of devices represents an excellent alternative to “classical” pulsed laser systems and can be used as an efficient tool in different multiphoton applications.

References

[1] W. R. Zipfel, R. M. Williams, and W. W. Webb, “Nonlinear magic: multiphoton microscopy in the biosciences,” Nature Biotechnol. 21, 1369-1377 (2003).

[2] P. J. Campagnola, H. A. Clark, W. A. Mohler, A. Lewis, and L. M. Loew, “Second-harmonic imaging microscopy of living cells,” J. Biomed. Opt. 6, 277-286 (2001).