Abstract

Nonlinear microscopy techniques such as 2-photon excitation fluorescence (TPEF), coherent Raman scattering (CRS) and second harmonic generation (SHG) have presented themselves as a powerful alternative to conventional wide field (WF) and confocal microscopy. Most importantly, the listed above nonlinear microscopy approaches offer intrinsic 3D sectioning, high penetration depth due to the use of IR excitation beams and don't require specific and often invasive tissue labelling. The information about cellular organization and bio-chemical content of tissue samples provided by the nonlinear contrasts is very valuable as it can be used for different biological studies and medical applications.

Many tissues, however, have thicknesses superior to the light penetration depth, and thus can only be inspected with the introduction of thin optical elements such as endoscopes. However, developing fiber probes suitable to perform nonlinear imaging, faces several challenges such as the delivery of ultra-short pulses at the sample plane, collecting the signal and performing imaging.

In this work, we present a miniature, flexible endoscope for nonlinear imaging. This endoscope consists of a hollow-core fiber which transmits a broad spectral range of excitation beams with low losses and very low group velocity dispersion (GVD). This optical fiber features a pure silica double-clad for an efficient signal collection generated by the sample. Scanning on the sample plane is executed with help of a piezotube to which the hollow-core fiber is attached to. The piezotube drives the free-standing tip of the fiber at mechanical resonance, leading to high deflection amplitudes and large fields-of-view. The hollow-core fiber, the piezotube and a custom-made mini-objective for light focusing and collection have been integrated into a small (38 mm rigid length) and thin (2.2 mm outer diameter) portable probe, capable of performing TPEF, SHG and CARS.

To demonstrate the potential of the developed endoscope, we have performed CARS imaging of CH chemical bonds highly concentrated in human colon sample and in mouse myelin sheaths covering the spinal cord axons. Finally, we have demonstrated the applicability of the developed fast scanning endoscope to record individual calcium bursts from GFP-labelled neurons in live mouse brain hippocampus.

Key-words: microscopy, fluorescence, piezotube, endoscope, nonlinear imaging.