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Large band multiphoton microendoscope with single-core standard graded-index multimode fiber based on spatial beam self-cleaning

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ABSTRACT

We demonstrate the feasibility of multiphoton fluorescence imaging with high spatial resolution using commercially available single-core 50/125 multimode graded-index fiber. Light propagating forward inside the endoscopic fiber undergoes a non-reciprocal propagation exhibiting a robust nonlinear spatial self-cleaning process. Whereas fluorescence from nonlinear interactions with biological samples linearly propagates backward along the same fiber. The scanner head, located at the distal end of the endoscope and suited for multimode fibers, is based on a ceramic tube where the fiber end follows a spiral course to explore the sample. No knowledge of the fiber transfer matrix is required.

Keywords: Multimode fiber, endoscopy, multiphoton fluorescence, spatial self-cleaning

1. INTRODUCTION

Multimode fibers are particularly interesting media to study the complex interactions between light and matter, by involving a large number of modes and by introducing different spatial beam localizations, modal group velocities and chromatic dispersions. Among all of the new effects observed during the last 10 years [1-10], the process of spatial Kerr self-cleaning [2,3,5,7-10] appears as particularly interesting, because of its unconventional dynamics which can be described either by numerical simulations or by a more mathematical approach, using a thermodynamic concept [11-12]. This nonlinear mechanism, based on spatiotemporal modulation of the internal electromagnetic field because of the combined actions of Kerr and self-imaging processes, has the potential to transfer the initial energy coupled on high-order modes toward the fundamental mode of the fiber, improving the beam output brightness without significant spectral broadening. Thus, the M² coefficient can be seriously reduced, facilitating the emission of coherent light, which is easily usable in many applications, ranging from telecommunications to micro-spectroscopy and LIDAR systems based on multispectral analysis. This process has been observed for pulses ranging from the femtosecond to the nanosecond domains, and it has been applied to laser emission involving complex spatio-temporal dynamics in mode-locked and Q-switched mode-locked configurations [13, 14]. The combination of spatial cleaning and nonlinear frequency conversion by means of geometric parametric instability in a single laser cavity, integrating multimode fibers, has been developed to demonstrate a single spatial mode and multifrequency source [15].

One of the main advantages of using multimode fiber lies in its ability to guide high powers, despite mechanical constraints linked to curvatures and fiber manipulation. The possibility of having a large fundamental mode can also be used in endoscopy configurations, in order to improve the spatial resolution of the system when the focusing lenses, placed at the fiber output, have limited focal lengths. In other words, the improvement of the spatial resolution of an endoscope can be

obtained by enlarging the initial diameter of the beam, without inducing degradation of spatial coherence. Endoscopic imaging using multimode fibers has already been widely reported. Spatial light modulators have been used to suppress deleterious beam spreading during multimodal propagation. In this approach, an initial deep learning process is required in order to determine the transfer matrix of the fiber [16-19]. The major drawback of this method is the deleterious impact of random coupling between modes, which considerably modifies the output beam as a consequence of any manipulation of the optical fiber. It is then essential to re-learn, before continuing the imagery analysis. Thus, the deep learning time limits the speed of the image rate.

In our case, spatial shaping is achieved through the Kerr self-cleaning process, which acts as a sort of spatial light modulator which is insensitive to fiber manipulation, and increasing in strength even when random mode coupling becomes larger [11-12]. This effect is also obtained with a moderate beam power, and transforms the symmetric multimode fiber into an unsymmetrical component, thus facilitating the delivery of a powerful quasi-single mode beam in the forward direction, and allowing for the recording of incoherent fluorescence in the backward direction. Thus, a standard multimode graded-index (GRIN) fiber can be used for nonlinear fluorescence imaging in an endoscopic setup. The spatial resolution of our system has been measured to be as small as 0.9 µm for a working wavelength of 1030 nm.

2. EXPERIMENTAL SETUP

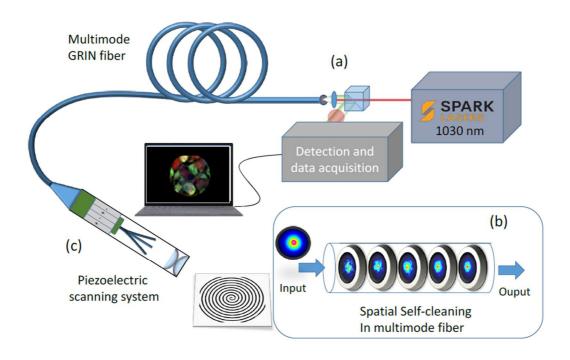


Figure 1: Experimental setup; (a) Laser source@1030 nm, detection and data acquisition, multimode GRIN optical fiber (50 μ m of core diameter, L=2.5 m); (b) Illustration of spatial self-cleaning in multimode fiber; (c) Scanning head composed of piezoelectric scanner and convergent lenses (focal length: 4 mm), scanning area: 0 to 500 μ m diameter.

The experimental setup is presented in figure 1. It is composed of a Spark Laser source delivering tunable pulses from 250 fs to 100 ps at 1030 nm. The repetition rate is adjustable from 0 to 2 MHz, with a peak power ranging from 0 to 6 MW. The output Gaussian beam is coupled to the 50 μ m/125 μ m GRIN multimode optical fiber by means of a polarizer, a half-wave plate, and a converging lens (focal length: 5 cm) with anti-reflection coating covering 700 nm to 1100 nm.

The scanning head is made by means of a piezoelectric tube (2.2 mm in diameter), capable of vibrating the distal end of the fiber and forcing it to draw a spiral on the sample surface [20-21]. The cleaned beam at the fiber output is then focused

on the sample by using converging lenses with a focal length of few millimeters (4 mm). The rate of spiral scanning is between 0 to 8 images per second for an area of $500*500~\mu\text{m}^2$ made up of more than 250,000 pixels. The strength of the piezoelectric system can be controlled, in order to reduce the image collection aperture down to $10~\mu\text{m}^2$ without any spatial distortion of the image.

The collected fluorescence is sent to the data acquisition module by means of a dichroic mirror (DMLP 800 nm), and selected using four different filters: SP 720 nm, BP 641/75 nm, BP 509/22 nm, Bp 447/60 nm, in order to record the fluorescence coming from: the second harmonic at 515 nm, Alexa 568, Alexa 488 nm and Dapi, respectively. The average fiber output power focused on the sample is adjustable from 20 mW to 140 mW, for a laser repetition rate of 2 MHz and a pulse duration of 5 ps. Under these conditions, the measured spatial transverse resolution is 0.9 μ m (Full With at Half Maximum in Intensity - FWHMI) (see figure 2) for two-photon fluorescence imaging, and 18 μ m (FWHMI) for the longitudinal dimension.

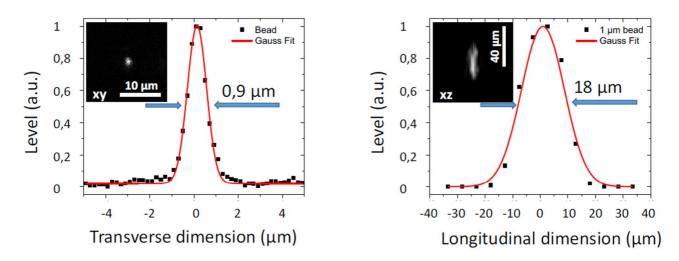


Figure 2: Spatial transverse and longitudinal resolution of our endoscopic system measured with polystyrene beads.

3. RESULTS

In order to demonstrate the capability of our system, we recorded fluorescence imaging from three different samples. The first consists of cellulose microfibrils, which can spontaneously produce nonlinear autofluorescence at 570-585 nm when illuminated at 1030 nm. Due to their noncentrosymmetric nature, the microfibrils can also produce a second harmonic signal which is cumulated with the two-photon autofluorescence. The image has 500×500 pixels and is displayed in black and white in figure 3(a).

Secondly, we performed a nonlinear fluorescence imaging of mouse kidneys labeled with Alexa 568 for actin detection, Alexa 488 for tubules, and Dapi for nuclei. Thus, actin and tubules imaging was achieved with a two-photon absorption process, while Dapi was identified in imaging by three-photon fluorescence. All figures are displayed in figure 3(b) with a spatial field of $250 \, \mu m$.

As a third step, we performed autofluorescence imaging of biological samples. We used mouse muscle capable of producing both harmonic generation from tendon due to collagen and two-photon fluorescence through proteins such as myosin and actin when illuminated at 1030 nm. Images with a field of 200 μ m with 500×500 pixels are shown in figure 4.

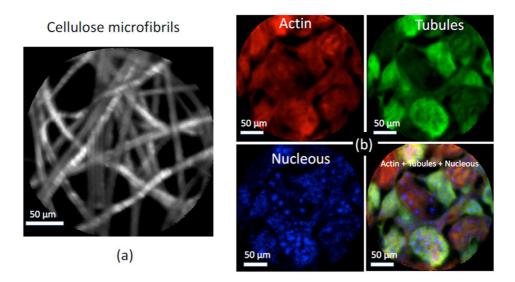


Figure 3: Nonlinear fluorescence imaging obtained with our endoscopic system integrating a standard multimode GRIN fiber (incident wavelength: 1030 nm); (a) Cellulose microfibrils emitting SHG (515 nm) and two-photon autofluorescence (570-585 nm), $500\times500 \text{ pixels}$; (b) Two-photon fluorescence images of actin, tubules and three-photon fluorescence for Dapy (nucleus), $500\times500 \text{ pixels}$; Pulse duration: 5 ps, repetition rate: 2 MHz, average power between 20 mW and 140 mW.

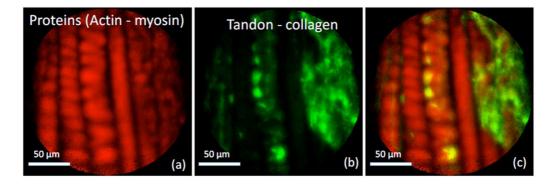


Figure 4: Nonlinear autofluorescence images of mouse muscle obtained with our endoscope integrating multimode standard GRIN fiber (incident wavelength: 1030 nm); (a) Proteins (641 nm / 75 nm); (b) Tendon - collagen (509 nm / 22 nm); (c) Superposition of both images

4. CONCLUSIONS

We used a large-core multimode GRIN optical fiber capable of supporting high average powers for the realization of an endoscopic nonlinear fluorescence imaging system. The shape of the beam, delivered to the end of the fiber, is controlled by means of the Kerr spatial self-cleaning effect, which is highly resistant to fiber manipulation and bending. We set up an endoscopic system by using a piezoelectric scanner, able of providing images of 500×500 pixels every 120 ms. In our setup, the fiber is used as a non-reciprocal device, in order to force the light to self-organize as it travels towards the sample, and thus produce a near single-mode beam at the end of the fiber. In the opposite direction, the optical fiber is used in the linear mode of propagation, in order to capture all of the fluorescence emission. Images of autofluorescence samples in the form of cellulose microfibrils and mouse muscle were shown. Fluorescence images of labeled elements were also obtained.

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