Nonlinear optical microscopy based on double-clad photonic crystal fibers

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Abstract: We report on a nonlinear optical microscope that adopts double-clad photonic crystal fibers for single-mode illumination delivery and multimode signal collection. It is demonstrated that two-photon fluorescence and second harmonic generation signals can be simultaneously collected in such a microscope with axial resolution of 2.8 μm and 2.5 μm, respectively. The delivery and detection efficiencies of the photonic-crystal-fiber-based microscope are significantly improved by approximately 3 times and 40 times compared with those in the single-mode fiber-optic microscope. The high resolution three-dimensional second harmonic generation images from rat tail tendon demonstrate the effectiveness of the system.

OCIS codes: (110.0180) Microscopy; (180.2520) Fluorescence microscopy; (110.2350) Fiber optics imaging

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1. Introduction

Nonlinear optical microscopy is based on the use of nonlinear optical effects such as two-photon absorption and second harmonic generation (SHG), which correspond to incoherent and coherent imaging processes, respectively [1,2]. These two imaging processes provide the cellular-level functionality and morphology information of a sample and exhibit advantages of an inherent sectioning ability, relatively deep optical penetration, and direct visualization of intrinsic indicators within biological tissue [1,2]. A combination of two-photon excited fluorescence (TPEF) and SHG enables complementary information regarding functionalities and structures in tissue environment, which is crucial for tissue morphology and disease diagnostics [1,2]. To achieve compact and miniature nonlinear microscopes for *in vivo* applications, micro optics or flexible fiber-optic components such as optical fibers and optical fiber couplers are usually integrated into the imaging system to replace complicated bulk optics [3-9]. Although single-mode fibers (SMFs) can deliver a high quality laser beam and provide an enhanced sectioning capability due to the effective pinhole effect compared with multimode fibers or fiber bundles, the lower numerical aperture (NA) and the finite core size of the SMF give rise to a restricted sensitivity of a nonlinear optical microscope system. Therefore, it is essential to construct a high performance fiber-optic nonlinear optical microscope that can collect images efficiently and maintain the flexibility as well.

Recently, the emergency of photonic crystal fibers (PCFs) has been a renaissance of fundamental research and development on optical fibers. Double-clad PCFs originally developed for fiber lasers [10] have attracted the research in the fields of biosensing and endoscopy for improvement in signal level due to its unique properties of the single-mode central core and the high NA multimode inner cladding [11,12]. However, the reported results [11,12] are not necessarily applicable in three-dimensional nonlinear optical microscopy for the following reasons. First, no imaging objective has been used [11,12] and thus the reported results do not hold for imaging a thick sample in which an optical sectioning property provided by an objective is necessary. Second, no measurement has been conducted for SHG which is a coherent signal rather than an incoherent signal such as TPEF. Third, due to the different NA of the central core and the inner cladding at different wavelengths, optimizing excitation delivery and emission collection with an objective for TPEF and SHG are different. In this paper, we report on a TPEF and SHG microscope by the use of a double-clad PCF that can play a dual role of the efficient delivery of a near infrared illumination beam and the efficient collection of visible signals. The strength of both TPEF and SHG signals can be significantly improved for three-dimensional imaging with axial resolution of 2.8 μm and 2.5 μm. Our measurements show that the signal collection efficiency in a nonlinear optical microscope based on a double-clad PCF is approximately 40 times higher than that in a microscope based on a standard SMF.

2. Double-clad PCFs and experimental setup

The double-clad PCF we used (Crystal Fiber A/S) is shown in the inset (a) of Fig. 1, having a core diameter of 20 μm (i.e., a 17 μm mode field diameter at wavelength 780 nm), an inner cladding with a diameter of 165 μm and NA of 0.6 at wavelength 800 nm. The fiber core is surrounded by air holes with a hole to hole pitch ratio of 0.26. Within the outer cladding region of 340 μm in diameter, a ring of air holes is used to efficiently guide and collect light in the pure silica multimode inner cladding. The background propagation losses are as low as 10 dB/Km at wavelength 800 nm. As the double-clad PCF in the nonlinear microscope is used to deliver a near infrared excitation laser beam and collect nonlinear signals in the visible range, it is important to understand the properties of the fiber under various operating conditions. Fig. 1(b) shows the coupling efficiency of the fiber for three given values of the NA of the coupling objectives over the wavelength range between 410 and 870 nm. Moreover, the output light patterns of the fiber at different wavelengths are depicted in Fig. 1(c)-(h). The laser

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beams at various wavelengths are obtained in the same way as described elsewhere [8]. It is shown that the double-clad PCF offers the robust single-mode guidance of near infrared light in the central core and the efficient propagation of visible light within the multimode inner cladding. Figure 1(i) illustrates an intensity profile across the output pattern of the fiber at 800 nm (Fig. 1(h)). In spite of the leakage from the core to the inner cladding and a triangular shape of the fiber core, the beam profile of the central core has a nearly Gaussian intensity distribution, indicating the single-mode operation in the fiber core at a near infrared wavelength.

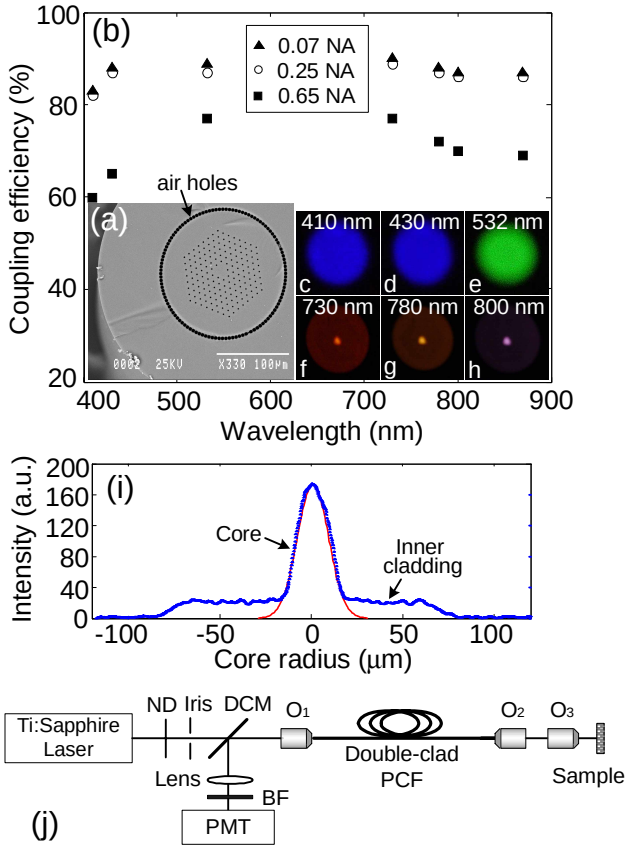

Fig. 1. (a) Scanning electron microscopy image of a double-clad PCF. (b) Coupling efficiency of the double-clad PCF in the wavelength range 410-870 nm for three values of the NA of coupling objectives (0.07, 0.25 and 0.65). (c)-(h) Digital camera photograph of the output pattern from a double-clad PCF between 410 and 800 nm. A microscope objective with NA 0.07 is used for coupling. (i) Gaussian fit of an intensity profile at the output of the fiber. (j) Schematic diagram of the nonlinear optical microscope based on a double-clad PCF. ND, neutral density filter. Other abbreviations defined in the text.

A coupling efficiency of over 80% with a maximum of approximately 90% in the wavelength range of 410-800 nm is achievable, if the NA of coupling objectives is 0.07 or 0.25 to match the lower NA of the central core. However, there is a 20% degradation in the coupling efficiency by using the coupling objective with a NA of 0.65 due to the mode leakage in the inner cladding. It is found that approximate 28% of the output power from the double-clad fiber is guided in the central core at 800 nm when a coupling objective of NA 0.07 is used, whereas only 10% and 8% are in the core for a coupling objective of NA 0.25 and NA 0.65, respectively. Consequently, the use of a coupling objective with a NA of 0.07 can optimize the coupling efficiency at both the near infrared and the visible wavelength

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ranges. In particular, the coupling efficiency at wavelength 532 nm is approximately twice higher than that obtained with the single-mode fiber coupler [8]. In addition, it has been found from our experiment that the degree of polarization of the output laser beam in the central core is 0.84 at wavelength 800 nm, demonstrating that the linear polarization state is almost preserved in the central core. This conclusion together Fig. 1 confirms the feasibility of a simultaneous improvement in TPEF and SHG imaging.

Based on above measurements we construct the microscope imaging system as shown in Fig. 1(j). A laser beam generated from a Ti:Sapphire laser (Spectra Physics, Mai Tai) with a repetition rate of 80 MHz and a pulse width of approximately 80 fs is coupled through an iris diaphragm and a microscope objective O_1 (0.65 NA, 40×) into the double-clad PCF with a length of approximately 1 meter. The size of the iris diaphragm is adjusted to achieve the maximum laser power guided in the central core. The output beam from the fiber is collimated by the objective O_2 of NA 0.07 before being launched into the imaging objective O_3 (0.85 NA, 40×). The coupling efficiency of the excitation laser beam to the double-clad PCF is approximately 88%, in which case 38% of the power after the objective O_2 is delivered by the central core. The backward nonlinear signal via the PCF is collected by objective O_1 to match the high NA of the PCF inner cladding. The choice of a low NA objective O_2 and a high NA objective O_1 maximizes the collection efficiency of the nonlinear signals. A dichroic mirror (DCM) reflects the TPEF and SHG signals which are further filtered by a bandpass filter (BF) and focused onto a photomultiplier tube (PMT).

3. Axial resolution and signal level

An experiment investigation into the axial resolution of the system for characterizing the three-dimensional imaging performance of the nonlinear microscope is executed by scanning a thin layer of AF 50 dye in the z direction [4,5]. The result is shown in Fig. 2, where the full width at half maximum (FWHM) of the axial responses of TPEF and SHG at an excitation wavelength of 800 nm is 2.8 μm and 2.5 μm, respectively, obtained by placing a 510/20 nm bandpass filter or a 400/9 nm bandpass filter before the PMT. It reveals a degradation of axial resolution of approximately 33% in the double-clad-PCF-based microscope, compared with that in a microscope which uses a single-mode-fiber-coupler [8]. This may result from the centrally localized light distribution before the imaging objective (similar to Fig. 1(h)), which effectively decreases the NA of the imaging objective, and the large area of the inner cladding, which effectively increases the pinhole size.

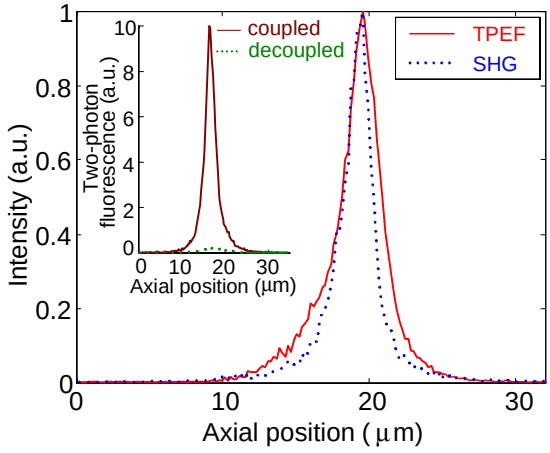

Fig. 2. Axial responses of the TPEF and SHG signals from a thin layer of AF-50 dye at an excitation wavelength of 800 nm. Inset shows the axial responses for TPEF in the case of the well-coupled illumination in the central core and the decoupled illumination in the inner cladding of the fiber. The power on the sample is approximately 1.5 mW.

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It should be pointed out that the laser beam from the inner cladding experiences a stronger effect of modal dispersion than that from the central core [13]. As a result, the laser beam delivered outside the central core contributes little to the nonlinear excitation. This feature is confirmed by the TPEF axial response depicted in the inset of Fig. 2, where the peak TPEF intensity when the excitation beam is well coupled in the central core is approximately 39 times as high as that when the excitation beam is decoupled transversely in the inner cladding.

To investigate the signal level of the double-clad PCF-based nonlinear microscope, we compare the strength of the axial responses of TPEF and SHG from the double-clad PCF and a standard SMF (Newport, F-SBA). The fused-silica SMF has an operation wavelength of 820 nm, a core/cladding diameter ratio of approximately 4/125 and NA 0.16 [6]. The coupling efficiency of the SMF of a 1-m length is approximately 30% at 800 nm [6]. The FWHM of the axial response with the two types of the fibers is kept the same for a given excitation power. Although an alternative comparison could be performed based on the given pulsewidth on the sample, our treatment is of importance in practical imaging when the excitation power from laser is given. The peak intensity of the axial responses from the two fibers as a function of the power before the imaging objective is shown in Fig. 3 on a log-log scale, where the slope of two demonstrates the quadratic dependence of the TPEF and SHG intensity on the excitation power. It is clearly observed that the detected intensity of the nonlinear signals from the double-clad PCF is approximately 6.8 times stronger than that from the SMF in the case of the same excitation power delivered to the sample. As a result, if one considers that the excitation beam in the central core of the two types of the fibers actually result in the nonlinear process, an enhancement of approximately 40 times in the nonlinear signal intensity detected through the double-clad PCF is achieved.

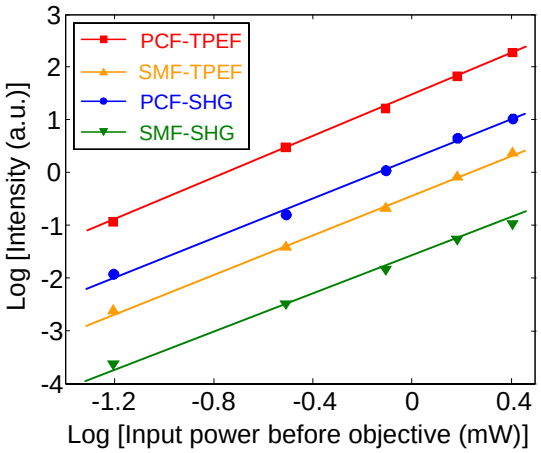

Fig. 3. Detected intensity of TPEF and SHG from the double-clad-PCF-based and a standard SMF-based microscope as a function of the power before the imaging objective.

It should be emphasized that Fig. 3 is physically different from that reported elsewhere in which case no imaging objective was used and the imaging system can not be used for three-dimensional nonlinear optical imaging [12]. Figure 3 is measured from the peak intensity of the axial responses and is thus applicable for imaging a thick sample when an optical sectioning property [1,2] is critical. Figure 3 also reveals that the double-clad PCFs can support efficient propagation for the incoherent TPEF signal as well the coherent SHG signal.

To further confirm the enhancement of the three-dimensional imaging efficiency by using double-clad PCFs, SHG optical sections are collected from a scale of black tetra fish with the PCF-based microscope and the fiber-coupler-based microscope [8], which are shown in Figs. 4(a) and (b), respectively. Figure 4(a) is obtained with a illumination power of 3.4 mW from central core and a PMT voltage of 670 V, while Fig. 4(b) is with 15 mW and 815 V,

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respectively. If a comparison is drawn between Figs. 4(a) and 4(b) by considering the NA and magnitude of the temporal broadening in two cases, the signal level of the PCF-based microscope system is increased by a factor of approximately 65.

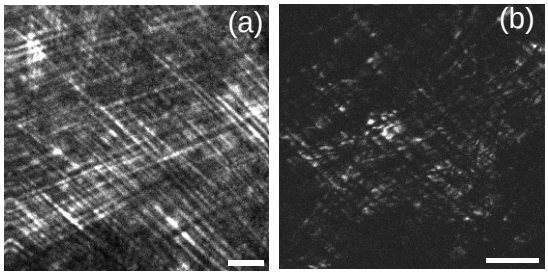

Fig. 4. SHG images from a scale of black tetra fish with (a) PCF-based microscope and (b) fiber-coupler-based microscope. Scale bars represent 20 μm.

Figure 5 is a series of SHG images of rat tail tendon with 2-μm-depth steps, demonstrating the three-dimensional imaging capability of the nonlinear optical microscope based on the double-clad PCF through a thick tissue medium. The tendon is obtained from an 8-weeks old Sprague-Dawley rat tail, attached to the coverslip directly, and imaged within 2 hours of extraction. The image sections displayed in Fig. 5(a)-(i) clearly resolve the morphology of mature, well organized collage fibrils even at an imaging depth of 20 μm, showing the pronounced optical sectioning property of the system. The result implies that the efficient PCF-based nonlinear microscopy could be a potential tool for direct visualization of collagen-related diseases.

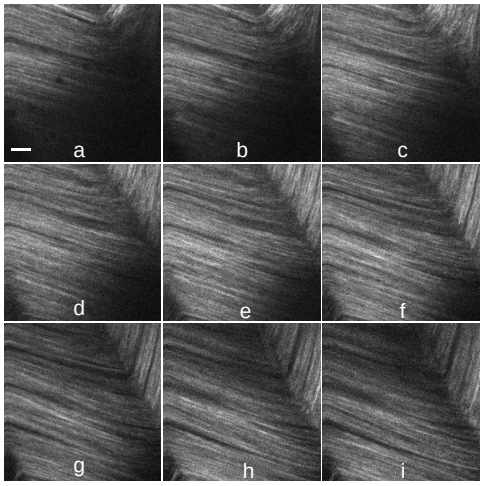

Fig. 5. (a)-(i) A series of SHG images sections from the rat tail tendon in the nonlinear optical microscope using a double-clad PCF. The image section spacing is 2 μm and the excitation power through the fiber core is approximately 5 mW. The scale bars in (a) represents 10 μm.

4. Conclusions

In conclusion, we have presented a nonlinear optical microscope by using the double-clad PCF. The new system exhibits a degree of polarization of approximately 0.84 as well as a delivery efficiency of up to 90% through the core and the inner cladding. Both the TPEF and SHG signal levels in the new system that has an optical sectioning property for three-dimensional imaging can be significantly improved by approximately 40 times in comparison with those in an SMF-based microscope. This feature is confirmed by nonlinear optical

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imaging of a scale of black tetra fish as well as by three-dimensional high resolution nonlinear optical imaging of rat tail tendon. Such a double-clad PCF-based microscopy system holds a promising future for application in nonlinear optical endoscopy.

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