Development of a double-clad photonic-crystal-fiber based scanning microscope

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Despite the fact that laser scanning confocal microscopy (LSCM) has become an important tool in modern biological laboratories, it is bulky, inflexible and has limited field of view, thus limiting its applications. To overcome these drawbacks, we report the development of a compact dual-clad photonic-crystal-fiber (DCPCF) based multiphoton scanning microscope. In this novel microscope, beam-scanning is achieved by directly scanning an optical fiber, in contrast to conventional beam scanning achieved by varying the incident angle of a laser beam at an objective entrance pupil. The fiber delivers femtosecond laser pulses for two-photon excitation and collects fluorescence back through the same fiber. Conventional fibers, either single-mode fiber (SMF) or multimode fiber (MMF), are not suitable for this detection configuration because of the low collection efficiency for a SMF and low excitation rate for a MMF. Our newly invented DCPCF allows one to optimize collection and excitation efficiency at the same time. In addition, when a gradient-index (GRIN) lens is used to focus the fiber output to a tight spot, the fluorescence signal collected back through the GRIN lens forms a large spot at the fiber tip because of the chromatic aberrations of the GRIN lens. This problem prevents a standard fiber from being applicable, but is completely overcome by the DCPCF. We demonstrate that this next generation scanning confocal microscope has an extremely simple structure and a number of unique features owing to its fundamentally different scanning mechanism: high flexibility, arbitrarily large scan range, aberration-free imaging, and low cost.

Keywords: Fiber optics imaging, Confocal scanning microscopy , Femtosecond laser, Photonic crystal fiber, Two-photon fluorescence

1. Introduction

Laser scanning confocal microscopy (LSCM) has attracted much attention and become an important research tool in modern biological laboratories in recent years [1,2]. Over four decades ago, Minsky filed a patent for a microscope that used a stage-scanning confocal optical system [3], which offers a higher resolution than a wide-field microscope and possesses sectioning capability to achieve 3-D images. However, the serious drawback that limits its application is its slow scanning rate, because it requires time to translate the massive stage precisely. In addition, a moving stage causes vibration problems to samples, especially liquid bathed biological samples. These problems were circumvented when laser scanning confocal microscope was developed into a practical instrument in the late 1980s, where beam scanning was controlled by two galvanometer mirrors that are imaged onto the entrance pupil of an objective lens [4,5]. In contrast to stage-scanning, the beam scanning provides much higher scanning rates, and has no vibrations to the samples under detection. However, the off-axis aberrations associated the beam scanning are inevitable, and the field of view is severely limited by the acceptable scanning angle of the objective lens. Although the invention of multiphoton confocal microscopes enhanced the detection efficiency by omitting the exit pinholes, the basic scanning mechanism remains the same as previous confocal microscopes [6].

In the present paper, we demonstrate a novel double-clad photonic crystal fiber (DCPCF) based scanning two-

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photon confocal microscope, which uses a new scanning mechanism distinguished from both normal stage- and beam-scanning configurations. It overcomes the drawbacks in the conventional scanning confocal microscopes, while combining the advantages of normal stage- and beam-scanning. In addition, this novel DCPCF based scanning microscope also has the other important new features, such as high flexibility and potentially low cost.

2. Experimental and discussion

Different from conventional beam scanning that changes the angle of an incident beam at an objective lens, we constructed a novel two-photon scanning microscope based on a new beam scanning mechanism by directly moving an optical fiber, which delivers laser beam for excitation and collects fluorescence signals back along the same fiber [7]. Femtosecond laser pulses generated from a Ti:sapphire oscillator (Coherent, Mira 900) have pulse duration of 50 fs and at the wavelength of 800 nm. A fiber coupler was used to couple the laser pulses into a DCPCF, whose output end was mounted on a XYZ scanning stage (Fig. 1). The hole-to-hole pitch ratio, d/Λ value, for this fiber is 0.4 which ensures endlessly single mode guidance down the centrally situated inner core which has a diameter of 6 μm. The photonic crystal inner cladding structure is then suspended in air by a silica web with large air holes, whose width, 230 nm, is less than half the wavelength of light. This forms a very high numerical aperture (NA) inner cladding (outer core) structure with a measured NA of 0.8 at 1-μm wavelength. The inner cladding (outer core) is 55μm across the hexagonal flats (Fig. 2). A gradient-index (GRIN) lens with O.D. of 1.8 mm and NA of 0.6 was connected to the fiber output end to focus laser onto a sample for two-photon excitation. A grating pulse stretcher was used to precompensate the dispersion resulting from the fiber and the other optical components in order to obtain the highest peak power at the laser focus. The fluorescence emission was collected back by the same GRIN lens and the same fiber, and separated from the excitation beam by a dichroic mirror. It then passed through a short-pass filter and was focused onto the entrance slit of a spectrometer. The wavelength-resolved output spectrum was detected by a photon counting photomultiplier tube. When the DCPCF moves in a raster scanning pattern across the sample of interest, a two-photon fluorescence image can be reconstructed.

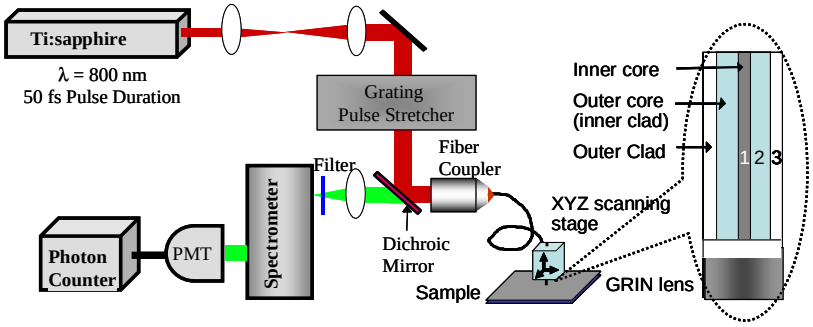

Fig.1. Schematic diagram of the DCPCF scanning microscope.

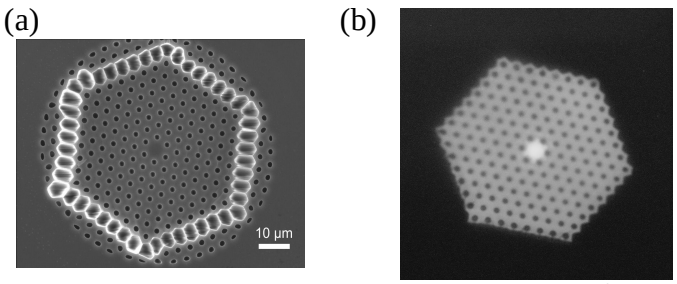

Fig. 2. (a) SEM image of a DCPCF tip, and (b) Excitation through the center core while fluorescence collection through both the core and inner cladding.

Despite this fiber scanning microscope has an extremely simple configuration, the DCPCF is a unique component that is critical to make the fiber scanning microscope practical. Note that conventional fibers, both a single-mode fiber (SMF) and a multimode fiber (MMF), are not suitable for this detection configuration. Although a SMF may also have an excitation rate as high as a DCPCF, its small numerical aperture (NA) (typically only about 0.1) results in a very inefficient signal collection. On the other hand, a MMF may have a large NA for fluorescence collection, but it has an inefficient excitation and low resolution because its output multi-mode beam is hard to be tightly focused and the multimode propagation also severely deforms an ultrashort laser pulse [8,9].

This trade-off problem has been ultimately solved with our DCPCF, which allows one to independently adjust the fiber parameters to have single-mode propagation of excitation light in the center core to achieve high two-photon excitation rate while having a large NA of the inner cladding for efficient fluorescence collection [10]. The NA of the inner cladding of the DCPCF we used is as large as 0.8, which is comparable with most high magnification objective lenses. In addition, when a gradient-index (GRIN) lens focuses the laser from the fiber output to a tight spot, the fluorescence signal collected back through the GRIN lens forms a large spot at the fiber tip because of the chromatic aberrations of the GRIN lens. This chromatic aberration is especially severe for two-photon excitation, since the wavelength of two-photon fluorescence is widely separated with that of the excitation light. Our simulation results using Zemax are shown in Fig. 3. This problem prevents standard fiber from being applicable, because the single core of a conventional fiber is impossible to efficiently couple the two-photon fluorescence collected by the GRIN lens. However, this problem is completely overcome by the DCPCF, which has not only its core but also the large first cladding area to collect the fluorescence efficiently. We observed 120-fold enhancement of fluorescence detection efficiency using a DCPCF comparing with the best commercially available SMF.

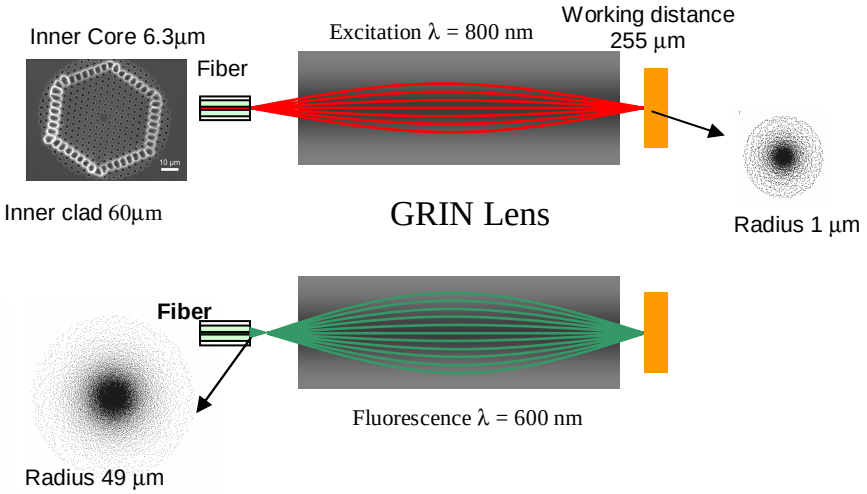

Fig. 3. Zemax simulation of a laser beam from a fiber focused with a GRIN lens and fluorescence collected back through the same system. The wavelengths of the excitation laser and the two-photon fluorescence were assumed to be 800 and 600 nm, respectively. Although the laser can be tightly focused with the GRIN lens for efficient excitation, the fluorescence collected back form a big spot at the fiber tip plane. Thus, a DCPCF is necessary for efficient fluorescence collection.

Having optimized the fluorescence excitation and detection using the DCPCF, we proceeded to demonstrate the practical operation of the DCPCF scanning microscope. Some preliminary results have been obtained. The first samples we tested were a 10-μm fluorescent bead (Fig. 4a) and a green fluorescent protein (GFP)-transfected MCA207 mouse sarcoma cell (Fig. 4b). Both gave strong fluorescence signals as expected. We then imaged a cultured KB cell line (a sub-line derived from the cervical carcinoma HeLa cell line), targeted using a novel dendrimer-based drug delivery agent. Dendrimers are a novel class of polymer macromolecules, whose size, shape and functionality can be controlled at a molecular level. The dendrimers used in this experiment are conjugated both to a fluorescent dye to enable optical imaging of the presence of dendrimers in the cells, and to folic acid (FA), which enables the dendrimers to be selectively taken up by KB cells, which overexpress the folate receptor.

Specifically, we imaged a FA-receptor positive KB cell targeted with G5-FI-FA [generation-5 poly (amidoamine) (PAMAM) dendrimers (G5) conjugated with fluorescein isothiocyanate (FI) and FA] [11], as shown in Fig. 4c.

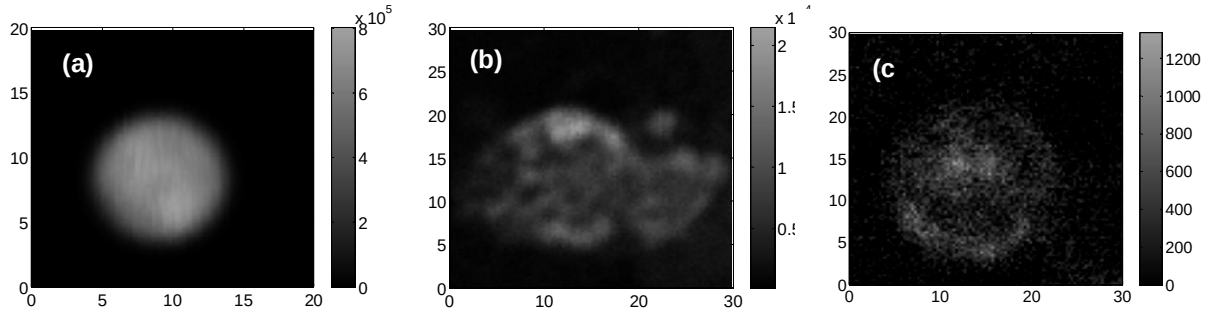

Fig. 4. Fluorescence images taken with the DCPCF scanning microscope, (a) a 10-μm fluorescent bead, (b) a GFP--transfected MCA207 mouse sarcoma cell, and (c) KB cells targeted with G5-FI-FA.

We will further develop this novel DCPCF scanning microscope, which potentially has a number of advantages over conventional scanning microscopes. First, it allows an extremely large scanning range, which can be as large as a few centimeters and is only limited by the scanning stage used, while still keeping a high resolution. Such large area scanning is impossible for a conventional beam-scanning confocal microscope limited by the field of view of an objective lens. This unique feature opens up the possibility for imaging a solid tumor by scanning across the whole tumor at a time. Note that this fiber scanning microscope is distinguished from conventional stage-scanning confocal microscopes, because the light weight of the fiber allows rapid scanning, while the sample remains stationary and avoids disturbing by the vibrations in the sample stage scanning. Second, this DCPCF scanning microscope will have excellent flexibility, because the scanning head can be freely adjusted without affecting the excitation source and the detection. Thus, scanning imaging can be performed in either upright or inverted configuration, or even at any arbitrary angle if needed. Third, the scanning of excitation beam with a flexible DCPCF fundamentally solved the problem of aberrations associated with the conventional beam scanning. Each scanned point of a sample is equally illuminated and signal collection remains the same all through the whole scanning range. This aberration-free scanning ensures a high quality image of a large sample of interest. In addition, expensive objective lens with off-axis aberration correction is not required in this DCPCF scanning microscope, and we expect much lower cost for constructing such a microscope.

3. Conclusion

We have demonstrated a novel mechanism for constructing a new generation of scanning microscopes based on a double-clad photonic crystal fiber. This microscope has an extremely simple structure, but it overcomes the limitations of conventional stage- and beam-scanning microscopes, and possesses many advantages as described above, i.e., excellent flexibility, large scanning range, fast scan rate, quiet scanning, aberration-free scanning, and low cost. With all these merits integrated into one microscope, we expect a wide range of potential applications.

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