Two-photon fluorescence endoscopy with a micro-optic scanning head

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A major obstacle in the race to develop two-photon fluorescence endoscopy is the use of complicated bulk optics to transmit an ultrashort-pulsed laser beam and return the emitted fluorescence signal. We describe an all-fiber two-photon fluorescence microendoscope based on a single-mode optical fiber coupler, a microprism, and a gradient-index rod lens. It is found that the new endoscope exhibits an axial resolution of 3.2 μm and is capable of imaging transverse cross sections of internal cylindrical structures as small as approximately 3.0 mm in diameter. This device demonstrates the potential for developing a real-time diagnostic tool for biomedical research without the need for surgical biopsy and may find applications in photodynamic therapy, microsurgery, and early cancer detection.

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In recent years optical diagnostic methods founded on fluorescence spectroscopy have proved to be a key means of monitoring biochemical and morphological changes in tissue at the subcellular level.[1] This is largely attributable to the information that the fluorescence emission carries, which reflects not only its molecular origin but also the manner in which it interacts with its microenvironment. Analysis of particular fluorescence properties such as intensity and spectra provide exceptional insights into the biophysical properties of a sample with high molecular specificity and sensitivity.[1] These properties may be used to differentiate between malignant and healthy tissues through examination of the underlying differences in the physiology of the cells.[2] The application of two-photon excitation of fluorescence to laser scanning microscopy[3] is an important technique for three-dimensional imaging of biological specimens and has proven advantages over single-photon fluorescence microscopy. Among the most significant of these are the inherent optical sectioning effect and greater penetration depth into a sample. However, at the present time application of this technique to diagnostic imaging is limited to situations that involve excision of tissue via a surgical biopsy for examination on the bench top. Integration of fiber optics into an imaging system[4–7] overcomes this physical limitation and may provide the ability to image intact specimens within internal cavities of the body.

Recently, we reported a fiber-optic two-photon fluorescence microscope based on a single-mode optical fiber coupler.[8] The resolution performance of this system is significantly improved compared with that in conventional two-photon fluorescence microscopy with a large area detector because the small fiber aperture acts as an effective confocal pinhole.[7] Although it is a compact and self-aligning system, application of the instrument to endoscopy is not entirely feasible, primarily because of the large number of bulk components at the sample site, which include a conventional imaging objective, optics mounts, and a scanning stage. A key technology that is necessary for the application of two-photon fluorescence microscopy to imaging of internal organ systems is an endoscope that is capable of delivering, focusing, and scanning an ultrashort-pulsed laser beam for two-photon excitation. In addition to this requirement, the endoscope must be capable of collecting the emitted fluorescence with suitable efficiency. To meet these requirements, one can use gradient-index (GRIN) rod lenses and micro-optic components to miniaturize the imaging optics and realize a practical endoscopic tool.

In this Letter we report what is to our knowledge the first all-fiber two-photon fluorescence endoscope with a microscanning head and reveal the dependence of the imaging resolution on the illumination power. The experimental arrangement of the new two-photon fluorescence microendoscope is shown in Fig. 1. A turnkey Ti:sapphire laser (Spectra Physics, Mai Tai) generating ultrashort pulses at a wavelength of 800 nm and with a pulse width of 80 fs at a repetition rate of 80 MHz was used as the illumination source. This beam was coupled into port 3 of a 50/50 three-port single-mode optical fiber coupler (Newport) with an operating wavelength of 785 nm via an objective with a numerical aperture (NA) of 0.25. This coupler acted as a low-pass filter.[8] The power coupled to the system was controlled by rotating a neutral-density filter placed before objective.

To facilitate entry of the endoscope into internal channels of the body, the imaging optics were combined to form a single microscanning head, which is similar to that used in optical coherence tomography[9] and is depicted in inset (a) of Fig. 1. It consists of a 1.0-mm-diameter plano–plano, 0.25-pitch, 0.46-NA GRIN lens (Newport) designed for a wavelength of 830 nm used to focus incident illumination and a 1.0-mm BK7 right-angle microprism (Edmund Industrial Optics) used as a beam-directing element. The pitch of the lens was chosen to yield the required Gaussian beam parameters. The single-mode optical fiber [port 1 of the fiber coupler (Fig. 1)] was attached

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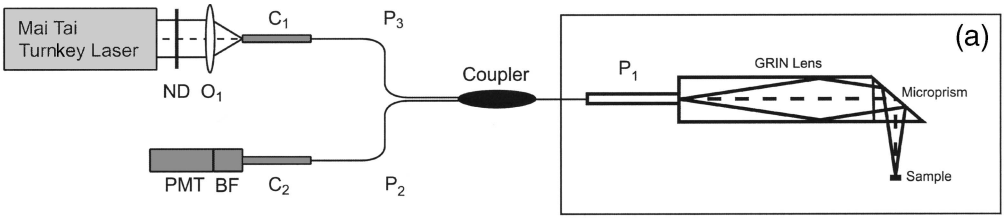

Fig. 1. Schematic diagram of the experimental two-photon fluorescence endoscope with a single-mode optical fiber coupler, a microprism, and a GRIN rod lens. O_1, 0.25-NA 10× microscope objective; ND, neutral-density filter; BF, bandpass filter; C_1, C_2, fiber chucks; P_1, P_2, P_3, coupler ports; PMT, photomultiplier tube. Inset (a), detailed component structure of the micro-optic scanning head.

to the microscanning head. The microprism, the GRIN rod lens, and the optical fiber were all attached with ultraviolet curing optical adhesive (Norland) to form a single unit, which had an approximate working distance of 2.0 mm. Image acquisition was achieved by circumferential scanning of a sample structure into which the microendoscope head was inserted. The fluorescence emitted from a sample was collected by the micro-optic scanning head and returned on the same optical path used for pulse delivery. The collected signal was delivered via port 2 of the fiber coupler to a photomultiplier tube masked with a bandpass filter with an operating wavelength of 550 nm (±20 nm).

To determine the magnitude of illumination power that can be delivered to a sample through the fiber coupler and the microendoscope, we measured the coupling efficiency for illumination of the 800-nm wavelength. The results are shown in Fig. 2, which depicts the measured output power at the focus as a function of the input power to port 3 of the coupler. The coupling efficiency of the system is reduced to approximately 28% compared with 35% obtained with the system by using bulk imaging components.[8] This decrease is caused by numerous factors, including the slight mismatch in the refractive indices of the fiber core, the GRIN rod lens, the microprism, and the optical adhesive. Further, it can be seen from Fig. 1 that a portion of the incident beam is transmitted through the microprism along the principal axis and lost. This portion was measured to be approximately 8% of the input power to port 3 of the fiber coupler.

The ability of the new imaging system to discriminate between axial depths was characterized by measuring the axial response to a fluorescent polymer sample, which has been detailed elsewhere.[8] The measured fluorescence intensity as a function of the axial distance perpendicular to the endoscope scanned through the micro-optic focus is shown in inset (a) of Fig. 2. The FWHM of the axial response [solid curve in inset (a) of Fig. 2] measured with the new two-photon fluorescence endoscope, Δz, is approximately 3.2 μm for an input power of 400 mW to port 3, which is increased by approximately 37% compared with that measured by a fiber-coupler-based bulk-optic microscope.[8] The increase in the FWHM indicates a degradation of axial resolution, which is attributed to two main factors. First, in this case the pinhole effect provided by the fiber tip aperture of port 1 is affected by the GRIN rod lens and microprism assembly. Even a small fabrication defect and mismatch between the refractive indices affects the alignment of the device. Second, the NA of the GRIN rod lens on the imaging side is effectively reduced. In inset (b) of Fig. 2 the dependence of the FWHM Δz on the incident power to port 3 is depicted, showing that the resolution is improved as the input power is increased. This feature is caused by the fact that the spectrum of the pulsed beam is broadened and blueshifted because of self-phase modulation and self-steepening, respectively, in a fiber.[7,10–12]

To demonstrate the feasibility of the microendoscope head for imaging an internal structure, we simulated rotation of the distal optics inside an internal body cavity by rotating a hollowed cylindrical sample holder around a fixed microendoscope head. The sample holder, as shown in Fig. 3, was machined from a 25-mm length of Perspex to form a hollowed cylindrical rod of 3-mm inner diameter and was mounted to the shaft of a variable speed 12-V dc rotary motor. The speed of rotation could be accurately controlled by the potential supplied to the motor. A two-dimensional image was constructed by progressively

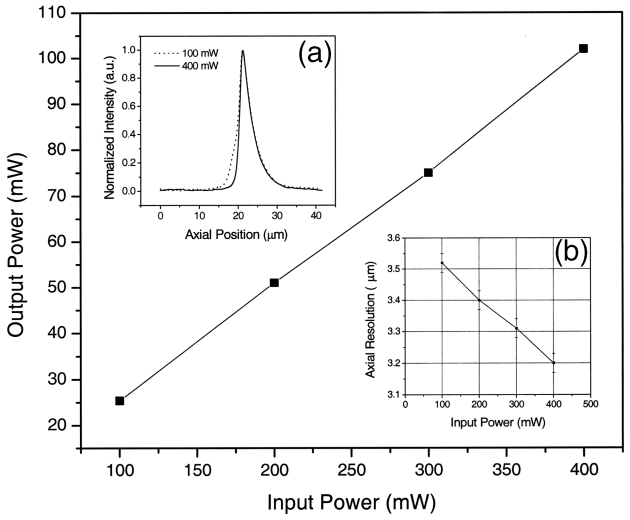

Fig. 2. Coupling efficiency of the two-photon fiber-optic microendoscope for the output power measured at the focus of the scan head and the input power measured at port 3 of the fiber coupler. Inset (a), axial responses to a fluorescent polymer block for an input power of 100 mW (dotted curve) and 400 mW (solid curve). Inset (b), dependence of the FWHM of the axial response as a function of the excitation power.

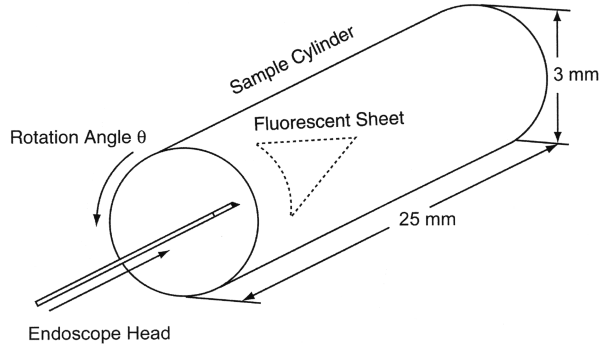

Fig. 3. Schematic diagram of the cylindrical sample.

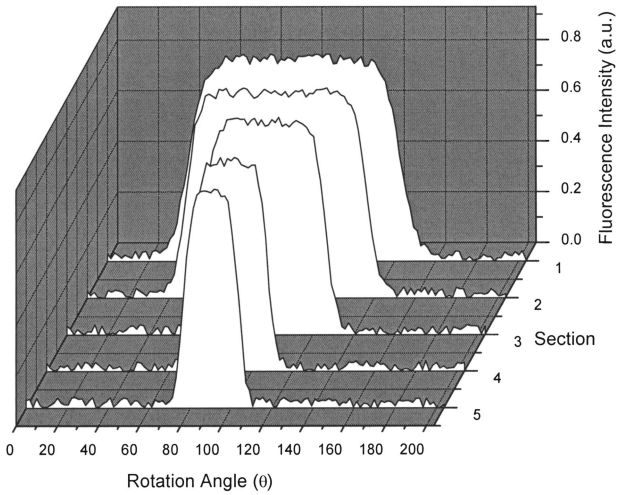

Fig. 4. Series of transverse cross-sectional images taken at 0.5-mm steps into a cylindrical channel with a 5-mm equilateral-triangle fluorescent sheet attached to the internal surface. The initial series (1) was acquired 1.0 mm into the cylinder relative to the base of the fluorescent triangle.

increasing the insertion depth of the microendoscope head into the cylinder with a step size of 0.5 mm and performing a single rotational scan. The sample consisted of a fluorescent paper sheet cut into the shape of a 5.0-mm equilateral triangle. The sheet was prepared by uniformly coating a piece of 75 g/m^2 paper with a mixture of AF-50 fluorescent dye and isopropyl alcohol.[8] Double-sided adhesive tape was used to secure the sample inside the cylinder oriented such that the base of the triangle was parallel with the circumference of the cylinder.

Figure 4 shows five acquisitions into the cylinder. The first series is obtained by rotating the cylinder with the focus of the microendoscope positioned 1.0 mm along the fluorescent sample. The trigonometric shape of the top constructed by the five curves reveals that the region of the detected fluorescence intensity as a function of the angular displacement of the cylindrical sample holder is consistent with the actual shape of the fluorescent material. It is clearly shown that as the microendoscope head is inserted further into the cavity the rotation angle range over which fluorescence is detected decreases accordingly.

It should be pointed out that for practical clinical implementation of the device it is clearly not feasible to rotate the sample around the endoscope. One method that may address this issue is close contact coupling of two optical fibers with a fiber ferrule.[9] In this manner it is possible for the scanning head at the distal end of the endoscope to be rotated independently of the fixed fiber coupler. Further, a high coupling efficiency of the incident illumination across the small air gap at the interface of the two fibers can be maintained.[9] For precision placement of the scanning head at internal lesion sites the fiber may be incorporated into an existing endoscope. Such an instrument usually consists of a white-light source for visual aid and other apparatuses, including magnification optics for targeting a lesion site within the viewing area.

In conclusion, we have reported on the design and implementation of a microendoscope head for two-photon fluorescence imaging. The instrument is founded on a single-mode fiber coupler, a microprism, and a GRIN rod lens, which result in a compact and self-aligning geometry. It provides not only access to internal specimens but also an optical sectioning property with an axial resolution of approximately 3.2 μm. The signal level of the instrument can be increased through dichromatic coating of the microprism surfaces.

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