A fiber-based single-unit dual-mode optical imaging system: Swept source optical coherence tomography and fluorescence spectroscopy

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A R T I C L E  I N F O
Article history:
Received 26 August 2011
Received in revised form 22 December 2011
Accepted 28 December 2011
Available online 10 January 2012

Keywords:
Double-clad fiber coupler
Optical coherence tomography
Fluorescence spectroscopy

A B S T R A C T
We propose a fiber optic single-unit but dual-mode optical imaging system that can provide fast cross-sectional imaging capabilities of swept-source optical coherence tomography (SS-OCT) and functional capabilities of fluorescence spectroscopy (FS). By adopting a fiber optic FS system into a fiber-based SS-OCT system, a compact and effective multimodal single-unit SS-OCT-FS system is achieved. Here, the key element of the proposed multimodal imaging system is a specially designed fiber coupler based on double-clad fiber (DCF), which has only cladding-mode coupling capability. The DCF couplers are fabricated with home-drawn DCF by several fabrication methods; a twisting method, a side-polishing method and a fused biconical tapered (FBT) method. Experimentally, the FBT method provides rather flat cladding mode coupling efficiency over 40% in a wide wavelength range. With this specially designed DCF coupler, the OCT signal and the fluorescence signal is measured independently but with a single-unit system. The performance of the SS-OCT-FS system is confirmed by measuring the cross-sectional image and the fluorescence signal of a photosensitizer chlorin e6 injected in-vivo rat tumor model.

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1. Introduction

Recently, dual or triple optical imaging modalities have been merged into a single platform to ensure multiple types of information pertaining to cells or tissue. Specifically, several multimodal imaging systems combined with optical coherence tomography (OCT) have been demonstrated as effective tools for the simultaneous measurement of the internal morphological information in addition to the functional or biochemical information [1–5]. The OCT system combined with multiphoton microscopy provides high-contrast cell images [1–3]. Using the system combined with laser-induced fluorescence spectroscopy (LIFS), the complementary information of normal and atherosclerotic portions of aorta walls was extracted from the OCT images and the fluorescence spectra [4]. In addition, a dual-modal Raman spectroscopy (RS) and OCT system was shown to be capable of providing a biochemical and morphological evaluation of malignant human breast tissue and the wound healing of a human finger [5]. Although these combined systems have multi imaging capabilities, the combination of two or three imaging systems makes the system bulky and complex. Therefore, fiber optic techniques have been employed to overcome these problems.

A fiber optic OCT-LIFS dual-modality system based on an optical fiber bundle was recently proposed [6]. A single-mode fiber (SMF), located at the center of the bundle, was used for OCT imaging and three multimode fibers (MMFs), placed around the SMF, were used for the LIFS excitation and collection beam deliveries. The fiber optic bundle makes the combined system compact, but the fabrication of the bundle is hard work. Furthermore, the probing positions of the OCT mode and the FS mode are not exactly the same. To solve this problem, in our previous work, a single-fiber probe based on double-clad fiber (DCF) was employed [7,8]. DCF has two different guiding channels, core channel and inner cladding channel, by its low refractive index outer cladding layer. In the DCF system, the OCT beam and the FS excitation beam were guided through the small core channel of the DCF, while the weak fluorescence signal generated from a sample was independently guided to a detector through the relatively large inner cladding channel of the DCF. Although the concentric probe scheme was feasible for use with the multimodal system, the low coupling efficiency of the DCF coupler in the FS mode and the low scanning speed of the OCT mode inhibited the real-time monitoring of an in-vivo biological sample.

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In this paper, we demonstrated a compact fiber optic SSOCT-FS system with a fast OCT imaging speed and improved FS collection efficiency to evaluate malignant tumor tissue morphologically and biochemically. By adopting a fast scanning swept source for an OCT system, high-speed tomographic measurements were possible. The FS mode was achieved with a cladding-mode coupler fabricated with home-drawn polymer-coated DCF using several methods, specifically a twisting method, a side polishing method and a fused biconical tapered (FBT) method. The properties of the fabricated DCF couplers were experimentally evaluated. The best coupling efficiency of over 40% was obtained with the FBT method. With this coupler, the FS system can be easily combined into the SS-OCT system, thus achieving a single-unit dual-mode system. To confirm the performance of the implemented system, the fluorescence signals and the cross-sectional images of an in-vivo rat tumor model injected with a photosensitizer (PS) chlorin e6 (Ce6) were measured.

2. Methods

2.1. Combined SSOCT-FS system

A schematic diagram of the combined SSOCT-FS system is shown in Fig. 1. The fiber optic SS-OCT system based on single mode fiber (SMF) coupler and the FS system based on DCF coupler are combined through a wavelength-division multiplexer (WDM). The DCF coupler guides both the broadband swept source for OCT and the excitation light source for FS into a sample and collects the back-scattered OCT signal and the fluorescence signal generated from the sample. The DCF itself is used as the sample probe for the FS mode, and a 2-D Galvano scanner is additionally used for the OCT mode. The collected OCT signal and fluorescence signal are separated by the DCF coupler. The collected OCT signal guided via the core channel of the through port is coupled back to the SMF coupler and finally detected by a spectrometer. Due to the cladding-mode coupling characteristic of the DCF, the back coupled OCT signal interferes with the light returning from the reference arm and is detected by an InGaAs balanced photodetector (1817, New Focus). And then, it is digitized by an AD converter (NI PCI-5142, National Instruments) and the digitized interference signal is rescaled to the linear frequency domain using the rescaling parameters based on the phase-oriented fringe analysis technique [9]. This signal is Hamming-windowed and zero-filled from a 3200 point to 4096 point sample length and then digitally inverse Fourier-transformed to yield a single A-scan signal. By scanning the sample arm with a galvano scanner, 2-D OCT signals were measured and finally they are converted to 2-D OCT images and displayed by LABVIEW program. In this SS-OCT mode, 500 A-line 2-D OCT images were acquired with acquisition speed of 40 frames/second. The measured axial resolution and sensitivity were ~12 μm and 105 dB, respectively.

2.2. Swept-source OCT mode

Left part of Fig. 1 shows the SS-OCT system that is composed of a swept light source based on a polygon scanner (HSL-2000, Santec, scan rate: 20 kHz, center wavelength: 1330 nm, bandwidth: 110 nm), a 90:10 SMF coupler, another 50:50 SMF coupler, two circulators, a balanced photo-detector and some data acquisition electronics. The broadband swept source launched into the 90:10 SMF coupler is divided and guided to the sample arm (90%) and to the reference arm (10%). At the common DCF probe, the light is focused onto a sample by a focusing lens and the back-scattered OCT signal is collected and delivered back to the 50:50 SMF coupler. The OCT beam collected through the core channel of the DCF probe can be directly back coupled to the core of SMF and other beam collected through the inner cladding of DCF is decayed out when the beam is back coupled to cladding of SMF due to high refractive index of jacket. Therefore, the back coupled OCT signal interferes with the light returning from the reference arm and is detected by an InGaAs balanced photodetector (1817, New Focus). And then, it is digitized by an AD converter (NI PCI-5142, National Instruments) and the digitized interference signal is rescaled to the linear frequency domain using the rescaling parameters based on the phase-oriented fringe analysis technique [9]. This signal is Hamming-windowed and zero-filled from a 3200 point to 4096 point sample length and then digitally inverse Fourier-transformed to yield a single A-scan signal. By scanning the sample arm with a galvano scanner, 2-D OCT signals were measured and finally they are converted to 2-D OCT images and displayed by LABVIEW program. In this SS-OCT mode, 500 A-line 2-D OCT images were acquired with acquisition speed of 40 frames/second. The measured axial resolution and sensitivity were ~12 μm and 105 dB, respectively.

2.3. Fluorescence spectroscopy mode

As a fiber optic FS system, we employed a DCF coupler capable of separating the excitation and the collection channels via the cladding-mode coupling [7,8,10]. As shown in the right part of Fig. 1, a laser diode (OZ-1000-405, OZ Optics, center wavelength: 405 nm) as an excitation light source is coupled to the DCF coupler by the WDM and is directed to the core channel of the DCF coupler. The fluorescence signal collected by the DCF probe is guided through the inner cladding of the DCF, and coupled to the cross port of the DCF coupler. Due to the cladding-mode coupling characteristic of the DCF coupler, only the fluorescence signal is directed to the FS detector, a spectrometer (QE65000, Ocean Optics, Inc.), after being long-pass filtered.

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**Fig. 1.** Schematic of the SSOCT-FS combined system. The DCF-based FS system is adapted into a SMF-based SS-OCT system. As the sample probe, only the DCF itself is used in the FS mode while a 2-D scanner is inserted in the OCT mode. WDM: wavelength-division multiplexer.
filtered at 450 nm. The tip of the DCF probe was placed ~500 μm above the sample surface and the integration time was 200 ms. Fluorescence spectra were measured with a spectral resolution of 3.5 nm. The wavelength-dependent signal detection efficiency of the system was corrected with a standard tungsten halogen lamp (LS-1-CAL, Ocean Optics, Inc.), and the dark current noise of the detector was subtracted before the spectral measurements.

In the proposed FS system, the DCF coupler is a key device. In the fabrication process, we should consider few functional role of DCF coupler to combine with SMF based OCT system. Firstly, the core mode area of DCF coupler should be well matched with that of SMF coupler to remove unwanted interference signal by mode mismatch and to reduce coupling loss. To fulfill the requirements, we fabricated the DCF coupler using home-drawn DCF that is drawn from a conventional SMF preform but with a low-index polymer coating [8]. Because a SMF preform was used, the core size of the DCF was well matched with the conventional SMF and it could reduce the coupling loss between the SMF and DCF. Secondly, DCF coupler should have high enough cladding mode coupling efficiency keeping low enough fluorescence background noise for efficient measurement of weak fluorescence signal. One of main advantage of DCF coupler as a fluorescence probe is that the fluorescence background noise by fiber itself can be dramatically reduced by cladding mode coupling performance [10]. The fluorescence background noise generated by high power excitation beam is guided through the core channel. Therefore, the fabrication condition should be optimized not only for better cladding mode coupling performance but also for no core mode coupling performance. To obtain best cladding mode coupler, we tried several fabrication methods including a twisting method, a side polishing method and a fused biconical tapered (FBT) method.

The twisting method is the simplest method of fabricating couplers with low-index polymer-coated DCF. The DCF coupler was realized by simply allowing physical contact between the inner cladding regions of two pieces of DCF with the polymer jacket removed [8]. As shown in Fig. 2(a), a cladding-mode coupling ratio exceeding 30% was obtained at a twisting length of ~16 cm. This fabrication method is very simple and does not require complex fabrication process. Also, only cladding mode coupling was induced through the contact region. However, the contact length is rather long and tight contact is required for better coupling efficiency, which requirements make the coupler fragile and difficult to package.

The side polishing method is a conventional coupler fabrication method [11]. By polishing and mating the cladding areas of two DCF pieces, cladding-mode coupling can be obtained [10]. In this scheme, the cladding mode coupling ratio is controlled by adjusting the polishing depth at inner cladding region. The calculated coupling depth

![Fig. 2. The measured coupling efficiencies of the DCF couplers fabricated by the twisting method (a), the side-polishing method (b), and the fused biconical tapered (FBT) method (c).](image)

![Fig. 3. (a) The fluorescence spectrum measured from a photosensitizer (Ce6) solution. The main peak was at ~660 nm. (b) The fluorescence spectra measured from tumor tissues at doses of 5 mg/kg, 10 mg/kg and 20 mg/kg, respectively.](image)
was about 40 μm at a coupling length of ~12.5 mm in this experiment. Fig. 2(b) shows the cladding-mode coupling efficiency maximally extracted from the DCF coupler fabricated by the side polishing method. The efficiency was approximately 30% in a 600 nm–900 nm wavelength range. Although, the coupling ratio is tunable in this scheme, there is appreciable wavelength dependency. Also, this method has low reproducibility.

The FBT method is commonly used for the core-mode coupler fabrication [12]. In this work, the cladding-mode coupler is fabricated by this FBT method. A pair of jacket-removed DCFs was twisted around each other and elongated while being heated with a ceramic heater. The cladding-mode coupling ratio was controlled via the pulling length which was shorter than that of the conventional core-mode coupler. At a pulling length of around 6 mm, coupling efficiency over 40% was obtained as shown in Fig. 2(c) in a wavelength range of 600 nm–900 nm. The experimental results show that the coupling ratio is quite flat in over the whole measured wavelength range. Moreover, the coupling efficiency is much higher than those obtained by other methods, in this case the twisting method and the side polishing method. Theoretically, cladding mode coupling ratio can be improved up to 50% but core mode coupling is also induced in that case. In the FBT method, the core mode coupling efficiency was almost negligible when the cladding mode coupling efficiency was around 40%. This means that the maximum cladding mode coupling efficiency

Fig. 4. Fluorescence spectrum measured from rat tumor tissue without a Ce6 injection (a) and with a Ce6 injection of 5 mg/kg (b); 2-D OCT images, without a Ce6 injection, of normal rat skin and subcutaneous tissue (c) and tumor tissue (d); 2-D OCT images, with a Ce6 injection, of normal rat skin and subcutaneous tissue (e) and tumor tissue (f); histologic view of normal rat skin with subcutaneous tissue (g) and rat tumor tissue (h). The scale bar is 1 mm. SC: subcutaneous tissue, M: muscle layer, T: tumor.
without bothering core mode coupling is around 40%, experimentally. For better sensitive measurements, a DCF having larger inner cladding area should be utilized.

The preparations and applications of specialty fiber couplers are well summarized in the literature [13].

2.4. Sample preparation

To demonstrate the dual-mode measurement performance of the combined SSOCT-FS system, we measured both the fluorescence spectrum and an OCT image from an in-vivo rat tumor model that was administered by PS Ce6. PS is a photosensitive molecule that is localized to a target cell or tissue. It is involved in tumor localization and the destruction of tumor cells in clinical photodynamic diagnoses and therapy [14,15]. As a PS, we used a Ce6 with high yield and high level of purity from chlorophyll α of seawater live chlorella (Chlorella ellipsoidea) belonging to green algae [15]. For a tumor model, the RK3E-ras cell induced rat tumor model was employed since it is an easy-to-use and cost-effective animal model as regards invasion and metastasis of cancer [16]. RK3E-ras cells, rat kidney epithelial cells transformed with the K-ras gene, were maintained in DMEM medium containing 5% fetal bovine serum, and 100 U/ml penicillin-streptomycin (Invitrogen, CA, USA) and subsequently incubated at 37 °C in an atmosphere containing 5% CO₂ [15,16].

Four three-week-old male Sprague-Dawley (SD) rats (Samtaco, Osan, Korea) were inoculated in the subcutaneous tissue of the right flank with 1 × 10⁷ of RK3E-ras cells. After four days, three of the animals were administered i.v. with Ce6 at a dosage of 5 mg/kg, 10 mg/kg, and 20 mg/kg, respectively. All experiments were performed under protocols approved by the Animal Care and Use Committee of the Chosun University School of Dentistry, Korea. The tumor regions of the four animals were monitored initially by measuring the fluorescence signal at 24 h after the Ce6 injection. 2-D cross-sectional images were then taken around the area where the FS measurement had been made to monitor the morphological change. The animals were sacrificed immediately after obtaining the 2-D cross-sectional images. The tumor with overlying skin was removed carefully and fixed in 10% formalin for 24 h. The tissues were then dehydrated in an alcohol–xylene series and embedded in paraffin wax. From paraffin blocks, sections 2 μm thick were prepared and stained with hematoxylin and eosin for histological examination.

3. Experimental results

Before measuring the fluorescence spectrum from the in-vivo tissue, we measured the fluorescence spectrum from Ce6. The main peak of the Ce6 was at ~660 nm, as shown in Fig. 3(a). To confirm the minimal dose of Ce6 at the tissue level, we measured the fluorescence signals at different doses. Fig. 3(b) shows the fluorescence spectra measured from tumor tissues at doses of 5 mg/kg, 10 mg/kg, and 20 mg/kg, respectively. The main fluorescence peaks were also observed at ~660 nm, indicating that the Ce6 had accumulated in the tumor tissue. The intensity of the fluorescence peak was proportional to the dose level; however, the small dose of 5 mg/kg gave a strong enough signal to detect.

Fig. 4 shows the fluorescence spectra and the OCT images measured with the proposed SSOCT-FS system. The tumor tissue was investigated by the FS mode and the morphological changes between normal and tumor tissues were measured by the OCT mode. Fig. 4(a) and (b) show the fluorescence spectra measured from the tumor tissue without (a) and with (b) Ce6 injection at a dose of 5 mg/kg, respectively. As shown in Fig. 4(b), the tumor region could be easily identified by the spectral signal from the Ce6. Fig. 4(c) and (e) are OCT images of normal SD rat skin and subcutaneous fat tissue without and with a Ce6 injection, respectively. Fig. 4(d) and (f) are OCT images of SD rat tumor tissue without and with a Ce6 injection, respectively. The sizes of the OCT images were 4.0 mm × 1.8 mm in the transverse and longitudinal directions. Fig. 4(g) and (h) are historical views of normal rat skin with subcutaneous tissue and the rat tumor tissue, respectively. Fig. 4(g) shows the normal skin epidermis with the dermis containing skin appendages and well-delineated muscle layer, and subcutaneous fat tissues. Fig. 4(h) reveals solid and diffuse infiltration of malignant tumor cells with penetration of muscle layer overlying the subcutaneous tissue. The morphological changes between the normal and tumor tissue are also observable in the OCT images. The unclear layered structure in OCT images of tumor tissues may be caused by destruction of the muscle layer architecture by the tumor. Although the simultaneous measurements could not be made in this measurement, we confirmed that the SSOCT-FS system can provide both the fluorescence spectral information and the internal morphological information that are required for diagnosing malignant tissues of in-vivo samples with compact system configuration. As a future work, we plan to synchronize both modalities by newly designed dual-mode co-register probe.

4. Conclusion

This study presents a fiber optic SSOCT-FS dual-mode system that provides both the biochemical and the morphological information of in-vivo biological samples in a single-unit configuration. By employing DCF and DCF coupler, a compact but efficiently combined system could be achieved. The coupling efficiency of the DCF coupler was improved to over 40% by utilizing the FBT method. In addition, a fast imaging speed was achieved by utilizing a fast swept light source in the OCT mode. The feasibility of the proposed SSOCT-FS system was confirmed by measuring both the OCT and the FS signals of a biological sample—i.e., the PS Ce6 injected rat tumor model. From the experimental results, we can confirm that the combined SSOCT-FS system provides multiple types of information necessary to identify tumor regions. Therefore, we hold that the combined SSOCT-FS system has great potential as a promising multimodal diagnostic tool and may allow early detection of disease or cancer.

Acknowledgements

The RK3E-ras cells used in this experiment were kindly provided by Dr. Eric Fearon (University of Michigan Medical School, Ann Arbor, MI). This study was supported by a grant of the Korean Health Technology R&D Project (No. A100490), Ministry for Health, Welfare & Family Affairs, Korea.

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