Microlensed dual-fiber probe for depth-resolved fluorescence measurements

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Abstract: We propose and demonstrate a compact microlensed dual-fiber probe that has a good collection efficiency and a high depth-resolution ability for fluorescence measurements. The probe is formed with a conventional fusion splicer creating a common focusing lens on two fibers placed side by side. The collection efficiency of the fabricated probe was evaluated by measuring the fluorescence signal of a fresh ginkgo leaf. It was shown experimentally that the proposed probe could effectively collect the fluorescence signal with a six-fold increase compared to that of a general flat-tipped probe. The beam propagation method was used to design a probe with an optimized working distance and an improved resolving depth. It was found that the working distance depends mainly on the radius of curvature of the lens, whereas the resolving depth is determined by the core diameters of the illumination and collection fibers. The depth-resolved ability of probes with working distances of ~100 μm and 300 μm was validated by using a two-layer tissue phantom. The experimental results demonstrate that the microlensed dual-fiber probe has the potential to facilitate depth-resolved fluorescence detection of epithelial tissue.

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1. Introduction

Light-induced fluorescence spectroscopy is an invaluable measurement tool for many biomedical, biological, and chemical applications [1–3]. In recent years, the interest in the use of fluorescence spectroscopy as an effective diagnostic technique for early detection of epithelial pre-cancer has been increasing [4–8]. However, analyzing fluorescence signals measured from the tissue surface is usually complicated because a typical fluorescence spectrum is a mixture of signals originating from different tissue layers. The epithelial tissue is mainly composed of a superficial epithelium and an underlying stroma. Hence, depth-resolved fluorescence spectroscopy with highly localized resolving depth is required for better understanding the origins of fluorescence signals and further improving the diagnosis of epithelial pre-cancer.

A fluorescence spectroscopic system is typically composed of a light source, a spectrometer, and a probe for delivering the excitation and collection beams. The design of the probe is important for both the implementation of depth-resolved spectroscopy and the realization of endoscope-based applications. Many systems used in fluorescence studies have incorporated fiber optic probes for these purposes because the use of optical fibers provides immense advantages such as a flexible probe, compact size, and easy coupling to spectroscopic instruments [9–18].

Currently available fiber optic probes can be categorized into two types: single-fiber [16–18] or multi-fiber [9–15]. Single fiber probes have the advantages of a small probe diameter, small beam spot size, and simple configuration. However, their usefulness is limited by a lower signal-to-noise ratio due to the autofluorescence background signal induced in the fiber.
itself. On the other hand, within a multi-fiber structure, various types of fiber optic probes are possible. Flat-tipped and beveled multi-fiber probes are the most common configurations for depth-resolved fluorescence spectroscopy. Flat-tipped multi-fiber geometries with varying excitation-collection fiber separations permit some depth discrimination of fluorescence [10]. Although flat-tipped probes offer better resolving depth than single-fiber probes for fluorescence measurements, flat-tipped probes are not sufficient for effectively isolating the relatively small signals of the superficial epithelium from the strong signals of the underlying stroma [13]. In the case of beveled type multi-fiber geometries, the overlapping area between the excitation and collection can be effectively reduced with large bevel angles [11–13] and the collection efficiency can also be improved by 1.5 times more than flat-tipped geometries [1]. However, targeting the epithelial layer requires a large bevel angle, which leads to a low collection efficiency. In addition, the bulky probe design of beveled multi-fiber probes makes them unsuitable for endoscope-based applications [19].

Recently, ball lens coupled fiber optic probes for depth-resolved fluorescence and Raman measurements targeting epithelial tissue were introduced [19–23]. This probe has some advantages, such as a compact size and a short working distance, for probe-tissue contact configurations. It was demonstrated experimentally that the ball lens coupled probe could effectively collect localized fluorescence signals close to the probe tip. However, for these configurations, the fabrication process of the ball lens assembly is relatively complex; requiring precise alignment of the fibers with respect to the center of the ball lens, and the probe size is also limited by the size of ball lens. In our recent work, we have proposed a lensed dual-fiber structure that can overcome these limitations by realizing a single-body lensed-fiber probe without using any bulk optics [24].

In this paper, we describe a simply manufactured and highly miniaturized microlensed fiber probe for depth-resolved fluorescence measurements. We also demonstrate that the proposed probe offers many advantages such as simple fabrication, compact size, good collection efficiency, and highly localized resolving depth. For the depth-resolved spectroscopic system, the beam propagation method (BPM) was employed to design a probe with an optimized working distance, and a two-layer tissue phantom was used to evaluate the depth-resolving ability of the fabricated probes.

2. Fabrication and performance of microlensed dual-fiber probe

Figure 1(a) depicts the fabrication process of the proposed microlensed dual-fiber probe. The probe was fabricated using a fiber cleaving system, with two fibers for the excitation and collection channels, and a special fusion splicer. Two side-by-side located fibers, one is for excitation and the other for collection, are spliced together by using the fusion splicer (S183PM, FITEL Co.) having a wide fiber holder.

The fabrication process proceeds as follow: First, the protective resin coating on the fibers is removed. The naked fibers are then placed side by side and attached with an epoxy; this was required to keep the two fibers at their closest position and to cleave both fibers simultaneously. After the epoxy hardened, the dual-fiber was cleaved with a conventional cleaving system. The cleaving process is essential for forming a symmetric microlens. The degree of symmetry will influence the collection efficiency. The dual-fiber was then placed horizontally in the fiber holder of a fusion splicer and the probe was formed by arc discharging. The insets in Fig. 1(a) show that the dual-fiber end is well cleaved by the conventional cleaving system and the microlens is formed symmetrically with the arc discharging method. Two microlensed probes were constructed: one with 9/125 μm (core/cladding diameters) excitation and 50/125 μm collection fibers and another probe with 9/125 μm excitation and 105/125 μm collection fibers.
Figure 1(b) shows the detected fluorescence spectra of a fresh ginkgo leaf that were measured with three different probes at their optimal working distances. For comparison, these measurements were performed under the same experimental conditions. The ginkgo leaf is a well-known sample that has two fluorescence emission peaks in the red and far-red wavelength regions [25].

Initially, the flat-tipped dual-fiber probe, which was composed of 9/125 μm excitation and 50/125 μm collection fibers, was used without making a common fiber lens, and it produced the spectrum shown in blue. The intensity of the collected signal was extremely low. Next, after making the microlens on the tip (lower inset of Fig. 1(a)), we acquired the spectrum shown in red. The intensity of the collected signal increased significantly. The results indicate that microlensed probe can provide a six-fold increase in the collection efficiency compared to that of the flat-tipped fiber probe. Finally, in order to evaluate the influence of the core diameter, a microlensed dual-fiber probe with a large core diameter (105 μm) collection fiber was used to acquire the spectrum shown in black. We can see that the collection efficiency improves greatly (almost four-fold) by utilizing the large core collection fiber because the intensity of the collected fluorescence signal is, in general, proportional to the area of the collection fiber.

From this preliminary study, we can conclude that the configuration of the proposed probe offers two main advantages: a compact probe size and an excellent collection efficiency. Hence, this probe is an ideal candidate for realizing ultra-compact probes for endoscopic applications.

3. Probe design for depth-resolved fluorescence spectroscopy

The main aim of this study is to develop a probe configuration that can localize the fluorescence information to within a few hundred micrometers and separate the fluorescence signals emitted from the epithelial tissue. Intuitively, we can regard the effective collection depth of the detected fluorescence signals in dual-fiber configurations as being determined by the overlapping area of the illumination and collection regions. Hence, in order to measure the fluorescence spectra from multiple structures, the two main parameters, that is, the working distance of the lens and the full width at half maximum (FWHM) of the depth profiling curve, should be considered separately in the proposed probe design. For obtaining depth information of epithelial tissue, the sampling probe should have a working distance shorter than 300 μm. The working distance is defined as the distance at which the illumination light
crosses with the collection region on the optical axis. A distance shorter than 300 μm is beneficial because this is typically the thickness of the epithelium, and the ability to target this selectively is necessary for the detection of epithelial pre-cancer. Hence, the purpose of the microlens design is to realize probes that have a short working distance of less than 300 μm or a long working distance of over 300 μm.

![Fig. 2. Contour maps of the beam propagation simulated with BPM: (a), (b), and (c) correspond to flat-tipped dual-fiber probes constructed using SMF, MMF1 and MMF2 and (d), (e), (f), (g), and (h) correspond to the proposed microlensed dual-fiber probes with different fiber and radius of curvature (R) combinations. Design parameters: (a) flat-tipped probe, combination of SMF and MMF1; (b) flat-tipped probe, combination of MMF1 and MMF1; (c) flat-tipped probe, combination of SMF and MMF2; (d) 110 μm radius of curvature, combination of SMF and MMF1; (e) 110 μm radius of curvature, combination of MMF1 and MMF1; (f) 125 μm radius of curvature, combination of MMF1 and MMF1; (g) 125 μm radius of curvature, combination of MMF1 and MMF2; (h) 125 μm radius of curvature, combination of MMF1 and MMF2. The core/cladding diameters of SMF, MMF1, and MMF2 are 9/125, 50/125, and 105/125 μm, respectively. The working distance is indicated by the dotted blue line and FWHM is related with the dotted yellow lines in Figures.](image-url)
Table 1. Summarized Results of Working Distance and FWHM of Probes Simulated with BPM

|          | SMF-MMF1 | MMF1-MMF1 | SMF-MMF1 | MMF1-MMF1 | MMF1-MMF2 |
|----------|----------|-----------|----------|-----------|-----------|
| Working Distance | R=110 μm | R=110 μm | R=125 μm | R=125 μm | R=125 μm |
| FWHM     | 260 μm   | 260 μm    | 350 μm   | 350 μm    | 350 μm    |
|          | 60 μm    | 100 μm    | 60 μm    | 100 μm    | 120 μm    |

For this study, the beam propagation method (BPM) was used to design the microlensed dual-fiber probe. The most critical parameters for the probe design are the radius of curvature of the lens and the core diameters of illumination and collection fibers. Thus, we performed simulations to observe the changes in the working distance and resolving depth as a function of these parameters. The propagation performance of the microlensed dual-fiber probe with different simulation parameters are shown in Figs. 2(d)–2(h), and compared to that of general flat-tipped dual-fiber probes (Figs. 2(a)–2(c)). The important simulation parameters are the refractive index of cladding ($n_{cl}$), refractive index difference between core and cladding ($\Delta n$), wavelength of the source ($\lambda$), cladding size ($D$), refractive index of the propagation medium ($n_{air}$), radius of curvature of the microlens ($R$), and core size ($d$). In this work, the following values were used as common parameters: $n_{cl} = 1.444$, $n_{air} = 1$, $\lambda = 0.5$ μm, and $D = 125$ μm. Three different $\Delta n$ and $d$ values were defined: for a single-mode fiber (SMF), $\Delta n_{SMF} = 0.005$ and $d_{SMF} = 9$ μm; for a multi-mode fiber with core size of 50 μm (MMF1), $\Delta n_{MMF1} = 0.016$ and $d_{MMF1} = 50$ μm; and for a multi-mode fiber with size of 105 μm (MMF2), $\Delta n_{MMF2} = 0.022$ and $d_{MMF2} = 105$ μm. The microlenses were designed with short and long working distances, $R_1$ and $R_2$, of 110 and 125 μm, respectively.

The working distance was first evaluated by increasing the radius of curvature of the lens, denoted by R in the figures. With the use of a small radius of curvature, the working distance tends to be shorter than that of a lens with a large radius of curvature. For instance, a radius of curvature of 125 μm resulted in a long working distance that exceeded 300 μm, whereas a radius of curvature of 110 μm resulted in a highly localized working distance located within 300 μm of the surface of the probe. Table 1 summarizes the working distance and FWHM of the simulated microlensed probes for the different design parameters. From the simulation results, we can conclude that the use of a probe with a small radius of curvature will be more effective for fluorescence measurements of superficial layers in tissue. It was also found that the core diameters of both the illumination and collection fibers do not affect the working distance of the probe. This is because the center-to-center spacing between the illumination and collection fibers is constant since their cladding diameters are equal.

The FWHM related to the resolving depth was also evaluated. The FWHM increased for all cases with increasing core size of the illumination and collection fibers (see Figs. 2(d), 2(e) and 2(f)). The FWHM was obtained to be approximately 60 μm for a probe with combination of a single-mode fiber (SMF: core size of 9 μm) and a multi-mode fiber (MMF1: core size of 50 μm), while it was more than 120 μm for the probe with a combination of MMF1 and MMF2 (core size of 105 μm). As a result of the BPM analysis, we can conclude that a probe with a smaller radius of curvature and fiber core size is the most beneficial for fluorescence measurement of a superficial layer (epithelium) in tissue. In addition, it is also anticipated that fluorescence signals emitted from deeper tissue layers (stroma) can be independently collected with a well designed probe.

4. Depth-resolved fluorescence measurements

4.1 Characterization of microlensed dual-fiber probes

Based upon the experimental results (Fig. 1(b)) and BPM analysis, two microlensed dual-fiber probes for depth-resolved fluorescence spectroscopy were fabricated. Probe 1 was designed to have a short working distance and a narrow FWHM; SMF and MMF1 were used for light illumination and signal collection, respectively (see Fig. 2(d)). Probe 2 was designed to have a...
long working distance and a broad FWHM; MMF1 fibers were used for both illumination and collection (see Fig. 2(g)). During fabrication the working distance can be controlled by using appropriate arc powers and duration times; the details can be found in our previous work [26]. Probe 1 and probe 2 were fabricated with arc powers of 180 and 160 mW, respectively, for the same duration time of 1600 ms. In general, the radius of curvature can be increased by using a lower arc power or shorter arc duration time. However, it is a non-reversible process since the arc power of a commercial fusion splicer is not stable enough to give highly repeatable results. In addition, the cleaving status is also an important parameter for both the working distance and collection efficiency.

To characterize the performance of the fabricated probes, the variation of the reflection power with respect to the distance between the probe end and a mirror used as the reflection target was investigated and compared to the results of a flat-tipped dual-fiber probe fabricated with a combination of SMF (9/125 μm) and MMF1 (50/125 μm). All measurements were made using a He-Ne laser source of 1 mW, and the results are presented in Fig. 3.

![Intensity variation of the reflected light measured as a function of distance between the probe and reflection target for (a) the microlensed dual-fiber probes and (b) the flat-tipped dual-fiber probe.](image)

We can clearly see that the collected intensity increases to a maximum and then, for the microlensed probes, decreases sharply as the distance between the probe tip and the mirror increases, whereas, in the case of the flat-tipped probe, the intensity decreases monotonically. For probe 1, the maximum signal was obtained at a distance of ~100 μm from the lens surface and the signal distribution was highly localized with a FWHM of ~50 μm (blue curve in Fig. 3(a)). For probe 2, the maximum signal occurred at a distance of ~300 μm and the signal distribution was broader with a FWHM of 150 μm (red curve in Fig. 3(a)). However, for the flat-tipped dual-fiber probe the normalized intensity drops to half the maximum value at a probe-to-target distance of approximately 1.5 mm and to 10% of the maximum at distance of ~3 mm (Fig. 3(b)). From these results, we can expect that the fabricated probes are sufficient for separating the relatively small fluorescence signal of a superficial layer from the strong signals of underlying layers.

4.2 Preparation of single- and two-layer tissue phantoms

In order to confirm the depth sectioning ability of the fabricated probes, fluorescence measurements were performed using two-layer tissue phantoms with approximately 300-μm-thick layers. The phantoms were prepared using a mixture of agarose powder (Type VII; Sigma Aldrich Co., St. Louis, Missouri, USA), quantum dot solutions (Invitrogen; Qdot 655 and Qdot 565), and a 0.5x tris-acetate-EDTA (TAE) buffer. The agarose was slowly combined with TAE and the solution was heated in a microwave oven for 30 s and then stirred. Stirring was repeated until the solution became entirely transparent. For the bottom layer, a small
amount of agarose solution was mixed with the quantum dot solution Qdot 655, and then the solution was poured into a PVC mold (cylindrical: 13 mm diameter, thickness of 300 μm). A thin microfilm was placed on top of the mould to ensure a flat surface, and the Qdot-agarose solution was solidified to form the bottom layer. After the bottom layer was prepared, the top layer, consisting of a Qdot 565-agarose mixture, was then formed by repeating the above procedure. Single layer phantoms of each Qdot-agarose mixture were also prepared for measurements of the pure fluorescence spectra generated from each individual layer.

4.3 Fluorescence measurements in phantoms

Figure 4 shows a schematic of the overall system employing the fabricated compact probes. For the fluorescence measurements, a standard optical fiber based fluorescence measurement system was used. The excitation light coming from a fiber pigtailed laser diode (OZ-1000-405, OZ Optics; 405 nm center wavelength) was delivered to the sample through the illumination fiber, and the fluorescence signal excited at the sample was subsequently detected by a broadband spectrometer (QE6500, Ocean Optics, Inc.) via the collection fiber. A long pass filter (LPF at 450 nm) was employed in the detector to block the fundamental excitation light that was elastically backscattered from the sample surface. The probe was held with a fiber holder mounted with a 3-axis translation stage in order to keep and control the distance between the probe and the sample surface. The surrounding medium was taken as air.

The excitation beams were adjusted to have the same power (200 μW) in all measurements, and the spectra were taken with an acquisition time of 50 ms. In addition, all measured spectra were corrected with regards to the wavelength dependent signal detection efficiency of the spectrometer by using a standard tungsten halogen lamp (LS-1-CAL, Ocean Optics, Inc.). First, the wavelength dependent correction curve was determined by dividing the standard lamp spectrum by the lamp spectrum measured in the proposed system. Next, the calculated collection curve was multiplied by each of the measured fluorescence emission curve. The dark current noise of the detector was also subtracted before the spectral measurements were taken.

![Fig. 4. Fluorescence measurement system based on the fabricated compact probe. LPF: Long pass filter. The fiber holder mounted on 3-axis translation stage was used to control the distance between the probe and sample surface.](image)

Figure 5 shows the results of fluorescence measurements obtained for the single layers and the two-layer tissue phantoms. As was mentioned, the two layer phantom was made by stacking the two single layer phantoms. The measured spectra were normalized to facilitate the comparison. Both spectra in Fig. 5(a) were measured by microlensed probe 1 under the same experimental conditions to evaluate the intensity ratio of the fluorescence signals generated from the individual top and bottom phantom layers. Spectra measured by the flat-tipped probe and microlensed probe 2 are not presented in Fig. 5(a) because the difference was minimal. Figure 5(a) shows that the fluorescence intensity of the single-layer phantom of (Qdot 655) is 4 times greater than that of the phantom of (Qdot 565), due to the intrinsic properties of the quantum dot solutions used in preparation of these phantoms. Hence, the
intensity ratio value, defined as the ratio of the collected fluorescence intensity from the bottom layer to the top layer \( \left( \frac{I_{\text{bottom}}}{I_{\text{top}}} \right) \), is approximately 4 [20].

Fig. 5. (a) Fluorescence spectra of the single layer phantoms corresponding to the top (green) and bottom (red) layers of the two-layer tissue phantoms. Fluorescence spectra of the two-layer tissue phantom collected by the use of (b) the flat-tipped dual fiber probe, (c) microlensed dual-fiber probe 1 with a short working distance, and (d) microlensed dual-fiber probe 2 with a long working distance. Note that all spectra were normalized to facilitate comparison.

Figure 5(b) shows the fluorescence spectrum collected from the two-layer tissue phantom using the flat-tipped dual-fiber probe. As anticipated, the measured spectrum is almost identical with the sum of the signals collected from the single-layer phantoms. The ratio of the collected fluorescence intensities from the bottom layer to the top layer is approximately 4. This ratio was not appreciably affected with the location of the probe along the depth, which indicates that it is not suitable as a probe for depth-resolved spectroscopy. Note that all measured results in Figs. 5(a) and 5(b) were taken at distances where the maximum intensity was recorded.

Figures 5(c) and 5(d) show the results obtained with the two-layer phantom for the proposed microlensed dual-fiber probes. For probe 1, with a working distance of \( \sim 100 \mu \text{m} \) (Fig. 5(c)), we can clearly see that only the fluorescence signal originating from the top layer is dominant, while the signal from the bottom layer is not registered at all. Even though the probe was placed in contact with the phantom surface, no signal was observed from the bottom layer. Hence, it is expected that probe 1 may be beneficial as an effective tool to selectively detect fluorescence signals generated from the epithelium layer only. It should be emphasized that, to the best of our knowledge, a probe that can provide highly localized depth information on par with the proposed probe in this work has not been reported.
The fluorescence spectra collected with probe 2 from the two-layer phantom are shown in Fig. 5(d). The measurements were performed for front focusing, corresponding to a separation of ~200 μm between the probe and sample, and back focusing, corresponding to the contact case. The intensity ratio is almost 0.1 in case of front focusing, and ~5 for the back focusing case (higher than the flat-tipped probe).

The experimental results suggest that a probe design using fibers with a small core size is more efficient for the examination of a superficial layer, while a probe design using fibers with a larger core size is necessary for detecting the fluorescence signals generated from deeper layers. These preliminary results are noteworthy since it provides evidence that the proposed microlensed dual-fiber probes have the ability for depth-resolved fluorescence measurements, and hence, they can be used as effective diagnostic tools for epithelial tissue. It should also be emphasized that the main advantages of the proposed probe are as follows: (i) its manufacturing simplicity, (ii) good collection efficiency and high resolving depth, and (iii) compact probe design which is important in endoscopy based fluorescence measurements.

5. Conclusions

We described a simply manufactured and highly miniaturized microlensed fiber probe that can be used for depth-resolved fluorescence measurements with good collection efficiency. We have comprehensively evaluated the effects of fiber selectivity and lens curvature in the proposed probe design by using simulations and experiments. Measurements performed with tissue phantoms suggest that a microlensed dual fiber probe with a narrow FWHM and a short working distance can be used as an effective tool for the diagnosis of epithelial pre-cancer, while a probe with a broader FWHM and a longer working distance is more efficient for detecting the fluorescence signals generated from deeper layers. Therefore, the proposed probe with a long working distance can be used for depth-resolved measurements in thick samples. The working distance of the proposed probes could be increased easily by optimizing the arc conditions as well as using fibers having large diameters. Our future work will focus on the measurements of depth-resolved spectroscopy of thick tissues by using probes with various working distances. It is also expected that the proposed scheme for depth-resolved fluorescence measurements will significantly reduce the probe size. Hence, we believe that the proposed probe will be used in realizing an ultra-compact probe for endoscopic applications.

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