Depth-selective fiber-optic probe for characterization of superficial tissue at a constant physical depth

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Abstract: The in vivo assessment of superficial tissue has shown great promise in many biomedical applications. Significant efforts have been expended in designing compact fiber-optic probes with short tissue penetration depth targeting the superficial epithelium. In this paper, we present a compact and simple two-channel fiber-optic probe with superior depth selectivity for the superficial tissue. This probe employs a high-index ball-lens with an optimized illumination area and the maximal overlap between light illumination and collection spots, while maintaining sufficient light collection efficiency with minimized specular reflection. Importantly, we show that this probe allows the selection of a constant and shallow physical penetration depth, insensitive to a wide range of tissue-relevant scattering coefficients and anisotropy factors. We demonstrate the capability of this depth-selective fiber-optic probe to accurately quantify the absorber concentration in superficial tissue without the distortion of tissue scattering properties; and characterize the optical properties of superficial skin tissue.

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1. Introduction

The in vivo characterization of superficial tissue is of significant importance for many biomedical applications, such as cancer detection, and investigation of skin pigmentation and hydration. Due to the multi-layered structures of most biological tissue, the characterization of the avascular superficial epithelium is often confounded by the contribution of the scattering and absorption from the underlying connective tissue and blood vessels. To selectively assess the superficial tissue, the depth-selective measurement is crucial to distinguish photons originating in the superficial tissue from those propagating into the deeper tissue.

Reflectance spectroscopy has been widely used to probe tissue properties in vivo, often implemented via a compact fiber optic probe with well-controlled source and detection fiber separations, typically in the ranges from several hundred microns to a few millimeters. They generally assess the entire epithelium and the underlying connective tissue in conjunction with a variety of blood vessels such as capillaries, venules and arterioles. In order to achieve a short penetration depth for superficial epithelium, several strategies have been proposed, such as the reduction of fiber size [1–4], obliquely placed or beveled collection fiber [5–7] or with fiber-coupling ball lens and graded refractive index (GRIN) lens [5,8–11], polarization gating [11,12] and differential path length spectroscopy [13,14]. These approaches have effectively reduced the multiply-scattered photons from the deeper tissue layers and improved the sensitivity to the superficial tissue to some extent.

However, several challenges remain in the development of fiber-optic probes that can selectively characterize the superficial epithelium without being confounded by the underlying connective tissue and blood vessels, while still maintaining a high signal collection efficacy. First, since most epithelial tissues are relatively thin (ranging from 20 to 200 microns), it is difficult to achieve such a short penetration depth for most fiber-optic probe designs. The recent work by Kanick et al. has achieved a very short superficial depth of 100 microns [15]. Second, the efficacy of reflectance signal collection could be reduced for smaller sampling depth. Third, although the use of fiber-coupling optics such as gradient-index lens and ball lens could reduce the probing depth [10,11], if the source-detection fibers...
are positioned at a close distance, the fiber-coupling optics often introduces a significant specular reflection that strongly interferes with the collected signals from tissue.

Furthermore, the determination of optical properties of biological tissue is often complicated by the dependence of penetration depth and sampling volume on tissue scattering coefficient and anisotropy factor of biological tissue. In general, smaller scattering coefficient and higher anisotropy factor will result in a deeper penetration depth [3,12,16] and larger sampling volume, leading to inaccurate estimation of absorber content. In other words, the intrinsic variation of tissue optical properties could introduce significant uncertainties in measuring absorber concentrations in turbid media. Thus, it is highly desirable to design an optical probe whose penetration depth is independent of scattering properties of the medium. Although the choice of a “magic” source-detector separation distance of 1.7 mm has previously proposed for a nearly constant optical path length independent of scattering properties [16], its relatively deep sampling depth is unsuitable for assessing superficial tissue. The recent development of differential path length spectroscopy has achieved a very short penetration depth to probe superficial tissue without significant complication of optical properties [15], in which essentially three fibers are used by subtracting the diffuse scattered light from another collection fiber, and a polished probe tip at a certain angle to avoid specular reflection.

In this paper, we attempt to develop a fiber-optic probe that could address some of, if not all of the aforementioned challenges. We describe a compact and simple two-channel probe design optimized for superficial tissue. We refer to this probe as the depth-selective probe. The short depth-of-penetration is achieved by minimizing the spot size of illumination beam and maximally overlapping the illumination and collection areas through a high-index ball lens, while maintaining high signal collection efficiency without being affected by the notorious specular reflection. We validate the performance of the depth-selective probe by both numerical simulation and experimental studies. Our results showed that the depth-selective probe assesses a shallow physical penetration depth, insensitive to a wide range of tissue-relevant scattering coefficients and anisotropy factors. We further demonstrate the potential of this fiber-optic probe to accurately quantify the absorber concentration in superficial tissue without being distorted by tissue scattering properties. The importance of depth-selective probe for superficial tissue is also demonstrated in various human skin sites.

2. Materials and methods

2.1. Design of the depth-selective probe

The layout of the depth-selective probe is shown in Fig. 1. The probe consists of two 200μm-core-diameter fused silica fibers (NA = 0.22), one for light illumination and the other one for light collection. These two fibers are coupled by a high-index ball lens with 2 mm diameter (Edmund NT45-540, n = 1.85). The probe is housed in a stainless overtube for protection. We select the materials approved by the Food and Drug Administration. The probe is assembled by Fiberoptic Systems, Inc (Semi Valley, CA).

The use of the ball lens is to ensure the small illumination spot and the maximal overlap between light illumination and collection areas. The distances between the two fibers and between fiber tip and ball lens were numerically adjusted to maximize the light collection efficiency and minimize the specular reflection using optical design software – TracePro 3.22 (Lambda Research). This software uses Monte Carlo simulation to generate and trace light rays. To optimize the properties of both illumination and collection spots, we assume that each fiber is coupled with an independent light source and $10^5$ rays were launched for each light source.
In order to incorporate this depth-selective probe into a reflectance spectroscopy system, the illumination fiber of the probe was coupled to a 150W xenon lamp (Apex Fiber Illuminators, Newport). The collection fiber is directly connected to a spectrometer (380 nm to 710 nm, BTC112, BWtek). A custom-designed software using Labview 8.6 interface (National Instruments) is used to control the spectrometer for data acquisition.

2.2. Comparative fiber-optic probes

To benchmark the performance of the depth-selective probe, we experimentally compared its performance to a standard reflectance spectroscopy fiber-optic probe (QR200-7-UV-VIS, Ocean Optics, FL) that is commercially available and widely used in optical spectroscopy by many investigators [17]. This standard reflectance probe (SRP) consists of six illumination fibers and one collection fiber with 200 μm core diameter and the central distance between source and detection fibers to be approximately 250μm. In addition, to further compare the penetration depths of the depth-selective probe with other state-of-the-art probes that are reported to achieve a short penetration depth, such as probes with reduced fiber diameter and angled probes, we performed Monte Carlo simulations for the depth-selective probe with different fiber diameters, angled probes, SRP with different fiber diameters.

2.3. Monte Carlo simulation of penetration depth

We performed numerical simulations using the Monte Carlo code (Mcml) developed by Wang et al. [18] to determine the penetration depth of the depth-selective probe and other probes. The numerical experiments were designed to ensure that the light collection geometry modeled in the simulations emulated realistic experimental conditions and the output of the numerical simulations is analogous to those typically recorded in experiments.

The numerical experiments followed the protocol of saturation curve method described in our previous publications [12]. In brief, we varied the geometrical thickness \( D \) of the sample. For each \( D \), the reflectance signals were recorded for a specific collection radial ranges \( R \) according to the design of the fiber-optic probe. As \( D \) increases, the reflectance signal first increases and then reaches a plateau at a specific \( D_c \), known as saturation curve [12,19]. The penetration depth \( T \) is defined as the geometrical thickness \( D \) such that the saturation curve reaches 90% of its saturation value. In the simulation, the geometrical thickness \( D \) was varied from 20μm to 3000μm. A wide range of tissue-relevant scattering coefficient \( \mu_s \) and absorption coefficient \( \mu_a \) were used. We used \( 10^7 \) photons in our simulations.

2.4. Experimental validation of penetration depth

To first validate the penetration depth of the fiber-optic probes determined by Monte-Carlo simulation and saturation-curve method [19], we performed the experiments with the 200μm-diameter depth-selective probe and tissue phantoms consisting of polystyrene microsphere suspension \((d = 4.3μm \pm 25\%, n = 1.59, Duke Scientific)\). The scattering coefficient \( \mu_s \) was calculated based on the concentration of microsphere suspension and Mie theory. To obtain the saturation curve, the probe tip is immersed in the microsphere suspension, and the distance between the probe tip and the bottom of the suspicion is gradually increased from 20μm to 3000μm via a micrometer-controlled translational stage. The reflectance spectroscopy
measurement of $I_s(\lambda)$ is taken at each step. The obtained spectrum $I_s(\lambda)$ is further normalized by using the following Eq. (1):

$$R(\lambda) = \frac{I_s(\lambda) - I_{bg}(\lambda)}{I_{ref}(\lambda) - I_{bg}(\lambda)}$$  \hspace{1cm} (1)

where $R(\lambda)$ denotes the normalized signal; $I(\lambda)$, $I_{ref}(\lambda)$ and $I_{bg}(\lambda)$ denotes the measured reflectance signals from the sample, reference and background, respectively. A reflectance standard (99% reflectivity, SphereOptics) is used as the reference. The background measurement is taken when the probe tip is immersed in pure water.

2.5. Calculation of hemoglobin content

A series of tissue phantoms with a range of similar scattering and absorption properties as the soft tissue were used. The human blood was used as the absorber and polystyrene microsphere suspension was used as the turbid scattering medium. A venous blood sample was collected from a healthy volunteer. The sample was collected in an ethylenediaminetetraacetic acid (EDTA)-treated Vacutainer® Blood Collection Tube and subsequently analyzed for hemoglobin concentration using a COULTER® LH 750 Hematology Analyzer (Beckman Instruments). We then added the base blood sample into the polystyrene microsphere suspension, yielding multiple samples of serially increasing hemoglobin concentration differing by about 1 g/L. To quantify the hemoglobin content in tissue phantoms, we used a modified Beer-Lambert law described as follows (Eq. (2)):

$$R(\lambda) = I_{scattering}(\lambda) \cdot \exp(-\alpha_{HbO_2} \cdot A_{HbO_2}(\lambda) - \alpha_{Hb} \cdot A_{Hb}(\lambda))$$  \hspace{1cm} (2)

where $R(\lambda)$ is the experimentally obtained reflectance spectrum as a function of wavelength $\lambda$; $I_{scattering}(\lambda)$ denotes the absorber-free scattering signal of the sample; $A_{HbO_2}(\lambda)$ and $A_{Hb}(\lambda)$ denote the absorption spectrum of the oxygenated and deoxygenated hemoglobin, respectively; and $\alpha_{HbO_2}$ and $\alpha_{Hb}$ represent the oxygenated and deoxygenated hemoglobin contents. The $I_{scattering}(\lambda)$ was obtained using the simulated curve based on Mie Theory. The $A_{HbO_2}(\lambda)$ and $A_{Hb}(\lambda)$ were obtained from the published sources [20]. To calculate $\alpha_{HbO_2}$ and $\alpha_{Hb}$, the Eq. (2) is fitted given $R(\lambda)$, $I_{scattering}(\lambda)$, $A_{HbO_2}(\lambda)$ and $A_{Hb}(\lambda)$. The fitting is optimized by minimizing the sum of square error over the wavelength range of 460-700 nm with the Matlab function $fminsearch$.

2.6. In-vivo measurement of reflectance spectra from human skin

We have also obtained the reflectance spectra from human skin using both the depth-selective probe and the standard reflectance probe. Three skin sites of an Asian volunteer were evaluated, including fingertip, lip and volar forearm. Six measurements were taken on each site and the averaged spectrum was used as the representative spectrum for each skin site. The final representative spectrum is fitted by the following Eq. (3) [11]:

$$R(\lambda) = \lambda^{-\beta} \cdot \exp(-\alpha_{HbO_2} \cdot A_{HbO_2}(\lambda) - \alpha_{Hb} \cdot A_{Hb}(\lambda))$$  \hspace{1cm} (3)

where $\beta$ denotes the scattering power, and $\alpha_{HbO_2}$ and $\alpha_{Hb}$ denote the oxygenated and deoxygenated hemoglobin coefficients, respectively. To derive the values of $\beta$, $\alpha_{HbO_2}$ and $\alpha_{Hb}$, we used a similar aforementioned fitting method.
3. Results

3.1 Depth-selective probe

In general, three major technical factors in a fiber-optic probe can affect its penetration depth:

1) Size of illumination and collection spots;
2) Separation distance between the illumination and collection spots; and
3) Angle between illumination beam and collection beam.

To achieve a short penetration depth, we need to maximize the overlap between illumination and collection spots while controlling the beam diameter to be as small as possible or adopt a large collection angle with respect to the illumination beam. In our depth-selective probe design, we optimized all of these three factors to result in a short penetration depth while maintaining sufficient light collection efficiency and minimizing the specular reflection. We employed a high-index ball lens ($n = 1.85$) that offers a large numerical aperture (NA $\approx 0.65$) and superior focusing capacity. Although the use of the high-index ball-lens could effectively couple the collection light for the high reflectance signal collection efficiency, it often accompanies significant specular reflection due to the multiple reflections at the ball lens-air and fiber-air interfaces that contribute to the background signals, resulting in reduced signal-to-noise ratio (SNR). We numerically adjusted the distance between the illumination and collection fibers as well as the distance between the fiber tip and ball lens to minimize such specular reflection in the background signal in our simulation using TracePro and optimizing a high collection efficacy of reflectance signal for smaller probing depth. Figure 2 shows the illumination and collection light intensity contour profile. The collection and illumination spots at the surface of the probe tip are controlled to be $\sim 300\mu$m in diameter, with a maximal overlap between the illumination and collection spots. Such configuration results in the smallest possible source-detection separation, essential for the short penetration depth, without a significant sacrifice of light collection efficiency and specular reflection. The size of the collection and illumination spots could be further reduced by reducing the fiber diameter. Additionally, the large angle between the illumination and the collection beam ($\theta = 37.4^\circ$ in air and $\theta = 27.8^\circ$ in water) further select those photons originating from a short tissue depth [3,6,21].

![Fig. 2. The illumination (blue) and collection (red) intensity contour profile of the depth-selective probe. The angle between the illumination and collection beam is calculated to be $\theta = 37.4^\circ$ in air and $\theta = 27.8^\circ$ in water.](image)

3.2 Characterization of reflectance signal collection efficiency

We quantified the SNR of the 200$\mu$m-diameter depth-selective probe both numerically and experimentally. Figure 3 shows the background and tissue reflectance irradiance map from the...
depth-selective probe. The tissue reflectance irradiance was simulated using a tissue model (i.e., tissue stratum corneum) selected from the sample library of the software. The SNR of our proposed depth-selective probe with 200μm fiber diameter is 15.1dB. In comparison, the previously reported ball-lens-coupled fiber-optic probe by Schwarz et al [10] has a SNR of about 3.9dB.

![Fig. 3. The background and tissue reflectance signals from our depth-selective probe with 200μm diameter. The color bar represents irradiance (W/m²).](image)

To further compare the reflectance signal collection efficacy for the depth-selective probe that samples a smaller penetration depth with those that sample a much deeper depth, we experimentally quantified the SNR from the depth-selective probe and that from the commercially available standard reflectance probe (SRP) with the same fiber core diameter of 200 μm (QR200-7-UV-VIS, Ocean Optics, FL). The reflectance signals were obtained from the human fingertip. The average SNR for the depth-selective probe (500-700 nm) is ~11.2 dB, while the SRP with 200μm diameter has an average SNR of ~12.6 dB. Therefore, the SNR for our proposed depth-selective probe maintains about 73% of collection efficiency compared to a SRP with the same diameter. However, considering that the SRP has 6 illumination fibers and one collection fiber, while the 200μm-diameter depth-selective probe has only one illumination fiber and one collection fiber, the reflectance signal collection efficiency of the depth-selective probe is in fact similar to or even better than SRP with the same fiber diameter.

3.3 Characterization of penetration depth

The penetration depth of the depth-selective probe and other comparative standard reflectance probes were characterized using a series of Monte-Carlo based numerical simulations. First, to validate the accuracy of the numerical simulation approach in defining the penetration depth, we compared the saturation curve obtained from numerical simulations with that from the experiments using tissue phantoms consisting of polystyrene microsphere suspension, as shown in Fig. 4. It is evident that the saturation curve obtained from the numerical simulation shows a good agreement with the experimental result. We also note that both numerical simulation and experiments confirmed that our 200μm-diameter depth-selective probe selects a very short penetration depth of approximately 200 μm [12,19].
Fig. 4. Saturation curves of the depth-selective probe obtained from both numerical simulations and experiments for $\mu_s = 200 \text{ cm}^{-1}$.

We further evaluated the penetration depth of the depth-selective probe with different fiber diameters (50, 100, 150, 200μm) using the Monte-Carlo simulation in conjunction with the saturation curve analysis. As shown in Fig. 5, the penetration depth is approximately linearly proportional to the fiber diameter. If the fiber diameter size is reduced to 50 μm (one of the smallest available core diameters of multi-mode fibers), the penetration depth could reach approximately 50-60 μm.

Fig. 5. Dependence of sampling depth on the fiber core diameters of the depth-selective probe ($\mu_s = 200 \text{ cm}^{-1}, g = 0.87, \mu_a = 5 \text{ cm}^{-1}$).

3.4 Effect of scattering and absorption properties on the penetration depth

The optical properties of biological tissue can generally be described by three physical parameters: scattering coefficient $\mu_s$, anisotropy factor $g$ and absorption coefficient $\mu_a$. We evaluated the effect of optical properties on the penetration depth using Monte Carlo simulation and saturation curve method. The penetration depths of 7 different fiber-optic probes are compared: the depth-selective probe with 50, 100 and 200μm fiber core diameters, the standard reflectance probe (SRP) with 50 and 200μm fiber core diameters, and angled probes whose collection fiber is placed at 15° and 45° with 200μm fiber core diameter. Figure 6 shows the effect of scattering coefficient $\mu_s$, anisotropy factor $g$ and absorption coefficient $\mu_a$ in a wide tissue-relevant range.
Evidently, the depth-selective probes assess a smaller penetration depth compared to SRP and angled probes. The penetration depth of the depth-selective probe is insensitive to a range of tissue-relevant scattering coefficients (50-300 cm\(^{-1}\)) and anisotropy factors (\(g = 0.8\)-0.96), even in the case of highly forward-directed scattering medium (\(g > 0.9\)), in which a short penetration depth is especially difficult to achieve even for polarization-gating probes [12]. In contrast, the penetration depth of the SRP with 200\(\mu\)m and 50\(\mu\)m and the angled probes showed a stronger dependence on the scattering and absorption properties. As shown in Fig. 6(a), across a wide range of scattering coefficients (50-300 cm\(^{-1}\)), the depth-selective probe assesses a much shallower penetration depth than the SRP with the smallest available core diameter of multi-mode fibers (i.e., 50 \(\mu\)m). This result implies that the maximal overlap between the illumination and collection spots is a more important factor in reducing the penetration depth than the fiber diameter. Similarly, as shown in Fig. 6(b), the penetration depth of the SRP increases significantly with the increase of anisotropy factor \(g\). On the other hand, as shown in Fig. 6(c), when the absorption coefficient is small (\(\mu_a < 1 \text{ cm}^{-1}\)), the penetration depth of the depth-selective probe with 200\(\mu\)m diameter shows a slight dependence on absorption coefficient. Such effect is almost negligible if the fiber diameter is reduced to 100 and 50 \(\mu\)m or when the absorption level ranges from medium to strong (\(\mu_a < 2 \text{ cm}^{-1}\)). Therefore, the depth-selective probe with the smallest available core diameter could achieve the shortest penetration depth and have least sensitivity to a wide range of optical properties.

### 3.5 Determination of hemoglobin content in turbid media

To demonstrate the potential of the depth-selective probe to accurately determine the absorber concentration in the turbid media, we quantified the absorption coefficient of hemoglobin in tissue models with three different scattering coefficients: 80, 130 and 200 cm\(^{-1}\). For each model, the concentration of total hemoglobin was varied from 0 g/L to 15 g/L, with the
corresponding absorption coefficient $\mu_a$ ranging from 0 to 13 cm$^{-1}$ at the wavelength of 570 nm.

Figure 7(a) shows the reflectance spectra obtained with the depth-selective probe and the corresponding fitted spectra using the method described in Section 2.5. A good fit can be achieved for a wide range of hemoglobin concentration. The calculated hemoglobin coefficient is then compared with the actual hemoglobin concentration, as shown in Fig. 7(b). Evidently, the calculated hemoglobin coefficient is linearly related to the actual hemoglobin concentration, independent of a wide range of tissue-relevant scattering coefficients. In comparison, the determination of hemoglobin coefficient is strongly confounded by the scattering coefficients if the SRP with 200 μm core diameter is used, as shown in Fig. 7(c).

Essentially, the derived hemoglobin coefficients from both probes represent the hemoglobin content within the sampling volume. The depth-selective probe assesses a relatively constant optical path length, (or sampling volume); while the assessing optical path length from the SRP probe is very sensitive to the scattering coefficient that results in inaccurate estimation of hemoglobin concentration. Our results demonstrate the potential of the depth-selective probe in unambiguous determination of absorber concentration in turbid media.

![Fig. 7.](image)

3.6 In vivo reflectance spectra from human skin

We further explored the capability of the depth-selective probe to evaluate the reflectance spectra of the multi-layered tissue. Three human skin tissue sites were evaluated: fingertip, lip, and volar forearm. These three skin sites are representative of distinct tissue properties at different tissue layers: Fingertip consists of a very thick (a few hundred microns) and highly-scattered superficial layer of stratum corneum with a highly dense vascular network underneath the epidermis. Lip is also a highly vascular site but with a very thin layer (only a few microns) of stratum corneum. The volar forearm has approximately 20 μm thickness of stratum corneum underneath which the blood vessels are less dense than those in the lip and fingertip [22,23]. Figure 8 shows the representative reflectance spectra from these three skin tissue sites, obtained with both depth-selective probe and SRP (200 μm diameter). The reflectance spectrum from fingertip obtained with the depth-selective probe shows very little hemoglobin absorption features, in distinct contrast to the strong hemoglobin absorption shown in the reflectance spectrum obtained with the SRP, as shown in Fig. 8(a). This result implies that the depth-selective probe indeed assesses a very short penetration depth, approximately within the thickness of stratum corneum and epidermis of ~200 μm. In addition, in the spectral range of 630-700 nm, the reflectance spectrum with the depth-selective probe is nearly flat, but the spectrum with the SRP shows a strong declining slope in part due to the contribution of the hemoglobin absorption from the deeper tissue. On the other hand, lip has a much thinner stratum corneum of only several microns. Although the reflectance spectra from the lip obtained with both probes show the hemoglobin absorption characteristics, the spectrum with the depth-selective probe shows a significantly less amount of hemoglobin absorption. Furthermore, the reflectance spectra from the volar forearm show
almost no hemoglobin absorption when the depth-selective probe is used, due to the small amount of vasculature in the superficial skin.

Fig. 8. Reflectance spectra from three skin tissue sites: (a) fingertip (b) lip and (c) volar forearm.

Table 1. Major Tissue Properties of Three Skin Sites

| Depth-selective probe | SRP with 200μm diameter |
|-----------------------|-------------------------|
| β        | αHbO2 | αHb | β        | αHbO2 | αHb |
| Finger tip | 0.50  | 1.6e-6 | 2.8e-7 | 1.69  | 2.2e-6 | 1.7e-6 |
| Lip       | 0.93  | 2.2e-6 | 7.3e-7 | 1.24  | 8.8e-6 | 5.4e-6 |
| Volar forearm | 0.88  | 1.1e-6 | 2.1e-6 | 1.22  | 1.1e-6 | 2.1e-6 |

We further quantified major tissue properties by fitting Eq. (3) in the spectral range from 520nm to 700nm as described in Section 2.6, and determined the scattering power β, oxygenated hemoglobin coefficient [αHbO2] and deoxygenated hemoglobin coefficient [αHb] with the depth-selective probe and SRP, and tabulated in Table 1. Apparently, the hemoglobin coefficients assessed with the depth-selective probe are significantly less than those from the SRP, due to its small sampling volume and avascular nature of the superficial skin. We also note that the significantly distinct results of scattering power β using two different types of probes. With the depth-selective probe, the fingertip shows the smallest value of β, but this value is the largest with the SRP. The smaller value of β has been previously related to a larger scatterers’ size [24]. The smaller value of β with the depth-selective probe is consistent with the presence of large cells in the stratum corneum of fingertip. The results shown in Table 1 underlie the importance of evaluating superficial skin tissue properties in a depth-selective manner.

4. Discussions and conclusions

We have developed a compact and simple two-channel fiber-optic depth-selective probe for in vivo assessment of superficial tissue. Our depth-selective probe efficiently incorporates three approaches to limit the penetration depth: reducing separation distance between illumination and collection spots, reducing fiber diameter and enlarging collection angle, via a fiber-coupling high-index ball lens and appropriate positioning of fibers and fiber-lens distance, while maintaining sufficiently high reflectance signal collection efficiency similar to the standard reflectance probe with the same fiber core diameter. Since this probe only uses two fibers, the physical dimension of this depth-selective probe could be further reduced to less than 1 mm-diameter. Using both experiments and numerical simulation, we demonstrated that this probe selects a very short physical penetration depth for superficial tissue, with approximately similar penetration depth to the fiber diameter. We showed that to reduce the penetration depth, the maximal overlap between source and collection spots (not fibers) is a more important factor than smaller fiber diameter. The most important characteristic of this depth-selective probe is its ability to assess a constant physical depth that is insensitive to a wide range of scattering coefficient and anisotropy factor. Furthermore, we showed that with
the depth-selective probe, the determination of the absorber concentration in turbid media is not confounded by a wide range of tissue-relevant scattering coefficients. The capability of the depth-selective probe for in vivo tissue assessment was further demonstrated in multi-layered skin tissue with three distinct tissue sites. We found that the reflectance spectra obtained with the depth-selective probe provide dramatically different information about tissue properties compared to those obtained with a standard reflectance spectroscopy probe. The quantification of absorption and scattering properties of the superficial tissue could be significantly biased without the depth-selective evaluation. In summary, the major advantages of this depth-selective probe include a compact and simple two-fiber design, very short penetration depth, high reflectance signal collection efficacy for small penetration depth, and independence of a wide range of optical properties. This depth-selective probe could find a broad range of biomedical applications such as endoscopic tissue assessment, characterization of superficial skin pigmentation, and evaluation of the effect of therapeutic agents on superficial tissue.

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