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Reflectance spectroscopy for diagnosis of epithelial precancer: model-based analysis of fiber-optic probe designs to resolve spectral information from epithelium and stroma

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Abstract

Reflectance spectroscopy is a promising technology for detection of epithelial precancer. Fiber-optic probes that selectively collect scattered light from both the epithelium and the underlying stroma are likely to improve diagnostic performance of *in vivo* reflectance spectroscopy by revealing diagnostic features unique to each layer. We present Monte Carlo models with which to evaluate fiber-optic probe geometries with respect to sampling depth and depth resolution. We propose a probe design that utilizes half-ball lens coupled source and detector fibers to isolate epithelial scattering from stromal scattering and hence to resolve spectral information from the two layers. The probe is extremely compact and can provide easy access to different organ sites.

1. Introduction

Recent studies demonstrate the promise of reflectance spectroscopy in detecting early precancerous changes in many organ sites, including bladder,^{1,2} skin,³ breast,⁴ intestine,⁵ oral cavity,⁶ and cervix.^{7–10} This technique is based on the fact that tissue optical properties, such as absorption and scattering, change as tissue becomes dysplastic. For *in vivo* clinical studies, fiber-optic probes are widely used to deliver light to the tissue site of interest and to collect the reflected light.¹¹ The probability that photons reach a certain depth in the tissue before they are reflected back and detected is highly dependent on the source–detector geometry, indicating that the effective volume of the tissue visited by the detected photons depends on the specific arrangement of the source and detector fibers in the probe in addition to the tissue optical properties. Therefore fiber-optic probe design plays a crucial role in obtaining depth-resolved spectroscopic information.

Depth resolution is clinically important because spatial localization of information obtained during *in vivo* measurements aids in interpretation of tissue spectra. This is particularly important when multilayered tissues are being investigated. If different layers are selectively targeted, spectral information unique to each layer can reveal various aspects of dysplastic progression, increasing the diagnostic potential of optical spectroscopy.

Epithelial tissues, such as intestine, oral cavity, and cervix, are generally described as two-layer media consisting of a thin epithelium on top of an underlying stroma. These two layers have significantly different optical properties and undergo different changes as dysplasia develops. Dysplastic changes include increased scattering from epithelial cells, decreased

scattering from collagen fibers in the stroma beneath the epithelium, and increased absorption in the stroma owing to increases in hemoglobin concentration. Increased scattering from epithelial cells is due to increased nuclear size, increased DNA content, and hyperchromasia with coarse and irregular chromatin clumping.^{12,13} It is believed that the morphological structure of collagen fibers changes and the volume fraction of fibers decreases as dysplasia develops,^{14–16} leading to decreased scattering in the stroma. Increase in hemoglobin concentration and hence absorption is the result of increased microvessel density and angiogenic activity in dysplastic tissue.^{17,18} Such structural and biochemical changes are critical diagnostic indicators for development of preinvasive cancer. Reflectance spectroscopy is capable of providing information about all these changes non-invasively, and fiber-optic probe designs that can selectively collect light from the epithelium and the stroma are necessary to exploit the diagnostic information inherent in optical signatures specific to each layer.

Recent computational and experimental studies analyzing the influence of fiber-optic probe design parameters on depth sensitivity have focused mostly on fluorescence measurements. Results reported by Pfefer *et al.*^{19–21} provide extensive insight into the effects of numerical aperture, fiber diameter, source–detector separation, and probe-to-tissue distance on the origin of detected fluorescence. Zhu *et al.*²² and Skala *et al.*²³ have proposed probe designs that can provide enhanced sensitivity to epithelial fluorescence. The number of similar studies focusing on reflectance measurements is relatively limited. Patterson *et al.*,²⁴ Meglinskii and Matcher,²⁵ and Larsson *et al.*²⁶ have investigated the relationship between the source–detector separation and the sampling depth. The results of these studies show that as the source–detector separation is increased, the mean penetration depth of detected photons increases. Use of multiple source–detector separations is a common way to acquire depth-resolved reflectance signals.^{9,11,27} In epithelial tissues, however, scattering from epithelial cells is relatively low and mostly forward directed, and most of the reflected photons detected come from the highly scattering stroma beneath the epithelium. Even when the source and detector fibers are closely spaced, photons penetrate into the stroma and scatter many times before reaching the detector. Therefore, depth resolution that can be achieved with minimal source–detector separation is not sufficient for selectively targeting the epithelial layer. Note that using a single fiber for both light delivery and collection as suggested by Papaioannou *et al.*²⁸ is not likely to provide enhanced sensitivity to the weakly scattering epithelial layer either. Further, such a single-fiber probe geometry is difficult to implement because special care needs to be taken to eliminate the possibility of detecting photons that are specularly reflected off the fiber tip. Specular reflection can, in principle, be accounted for by adoption of a calibration scheme.²⁹ For tissue measurements, however, specular reflection at the fiber–tissue interface constitutes a dominant portion of the total detected reflectance.²⁸ More importantly, it is not possible to assess accurately the exact contribution of specularly reflected light to total reflectance signals for *in vivo* applications.

Collecting photons that scatter only in the epithelium and do not penetrate into the stroma is a challenging but nontrivial problem. Changes in the nuclei of epithelial cells are important indicators of preinvasive cancer.³⁰ The fact that these changes alter the scattering properties of the epithelium indicates that epithelial scattering contains significant diagnostic information. In addition, pathologists have traditionally used atypical nuclear features in the epithelium as one of the major diagnostic criteria for dysplasia. Thus, isolation of epithelial scattering from stromal scattering is likely to prove invaluable in correlating optical signatures with the corresponding tissue histopathology, which is currently the gold standard in assessment of the sensitivity and the specificity of optical technologies targeting noninvasive detection of precancerous changes.

In this paper we model two fiber-optic probe designs to evaluate their potential in providing depth-resolved reflectance measurements from epithelial tissues. In the first design, the source

and detector fibers are tilted with respect to the tissue surface, and the fiber tips are polished parallel to the surface. This geometry is based on a recent study by Nieman *et al.*³¹ that suggests the use of obliquely oriented fibers to control the penetration depth of detected photons more precisely. In the second design, the source and detector fibers are coupled to a half-ball lens such that the flat surface of the lens faces the tissue. We have implemented a multilayered Monte Carlo code that enables us to model tilted fibers and to incorporate photon propagation through the spherical lens interface. Simulations have been carried out to characterize the penetration depth of detected photons and to evaluate each probe with respect to the sampling depth. The emphasis of our study is on determining the potential of these two probe geometries in resolving spectral information from the epithelium and the stroma. We then use the simulation results to propose a probe design that can be used to acquire reflectance spectra unique to each tissue layer.

2. Methods

A. Monte Carlo Modeling

Monte Carlo modeling provides a flexible approach to studying light propagation in biological tissues. This method traces three-dimensional random walk of photons in a medium, and the probability that a photon is scattered or absorbed after a given step size is determined by local optical properties.^{32,33} Internal reflection or refraction at the medium boundaries can be simulated. The implementation can be modified to account for different source–detector geometries.

The fixed-weight, multilayered Monte Carlo code used for the simulations presented hereafter has been implemented in C/C++ and is based on model descriptions given in several references.^{32,33} The layers are assumed to be infinitely wide and parallel to each other. Each layer is described by a thickness and several optical properties including refractive index n , absorption coefficient μ_a , scattering coefficient μ_s , and anisotropy factor g . The implementation is general enough to simulate clear media with no scattering or absorption. The simulations can be carried out with a source fiber centered at the origin and a detector fiber placed at a given distance from the source fiber. The code has been validated by use of single and multilayered tissue geometries and the corresponding reflectance results reported previously in the literature.^{32–34} Although most of these results have been obtained for infinitely narrow photon beams perpendicularly incident upon tissue surfaces, they collectively provide a standard against which to test the basic Monte Carlo implementation.

Modifications have been made to the basic Monte Carlo implementation to simulate two different fiberoptic probe geometries. In the first case, the source and detector fibers are tilted at an angle with respect to the tissue surface. In the second case, the fibers are coupled to a half-ball lens, and the rules of photon propagation need to be changed to simulate the refraction and reflection that take place at the curved surface of the lens. These two probe geometries are depicted in Figs. 1 and 2, respectively. The modifications that are necessary to account for tilted fibers or lens coupled fibers are explained below.

1. Modeling Tilted Source and Detector Fibers—In Monte Carlo modeling, each source or detector fiber is described by its radius, numerical aperture (NA), and refractive index. When the source fiber is oriented such that its axis is perpendicular to the tissue surface, the initial position of each photon is sampled from a circular region defined by the radius of the source fiber, and its initial trajectory is within the limits of a symmetric circular illumination cone determined by the fiber's NA and refractive index. Reflected photons are detected when they exit under the detector fiber and within a circular collection cone determined by the NA and refractive index of the detector fiber.

Figure 1 illustrates the probe geometry, in which the source and detector fibers are tilted toward each other and are polished parallel to the tissue surface. The tilt angle for both fibers is denoted β , and s represents the center-to-center source–detector separation. In this case, the initial position of each photon needs to be sampled from an elliptical contact area. Also, when the source fiber is tilted, the illumination cone is no longer symmetric about the z axis. One can implement photon launch from a tilted source fiber in Monte Carlo modeling by assuming circular symmetry about the fiber axis and then by transforming the direction cosines describing the initial photon trajectory through three-dimensional matrix rotation about the y axis. Rotation matrix $M(\beta)$ is given by

$$M(\beta) = \begin{bmatrix} \cos\beta & 0 & \sin\beta \\ 0 & 1 & 0 \\ -\sin\beta & 0 & \cos\beta \end{bmatrix}. \quad (1)$$

Rotating the direction cosines of each launched photon through β results in generation of an asymmetric elliptical illumination cone. Photons can then be propagated in the usual coordinate system in which the z axis is perpendicular to the tissue surface. Reflected photons are detected if their exit position falls under the elliptical contact area of the tilted detector fiber and their trajectory is within the limits of an asymmetric elliptical collection cone defined by the fiber's NA, refractive index, and tilt angle β . To determine whether a reflected photon is within the limits of the asymmetric collection cone, one again rotates the direction cosines at the exit point through β about the y axis. This three-dimensional matrix rotation, also performed with $M(\beta)$ as defined in Eq. (1), transforms the direction cosines into a coordinate system with circular symmetry about the detector fiber axis. Transformation among different coordinate systems by matrix rotation provides the easiest and the most direct way to generate asymmetric elliptical illumination and collection cones consistent with geometric optics principles.

2. Modeling Half-Ball Lens Coupled Source and Detector Fibers—Figure 2

illustrates the probe geometry in which the source and detector fibers are positioned over a half-ball lens of radius R . The fibers are symmetrically oriented about the central axis of the lens, and the center-to-center source–detector separation is again denoted s . The thickness of the air gap between the fiber tips and the top of the lens is represented by d . The figure also shows a window of thickness w placed between the flat surface of the lens and the tissue surface.

We have modified the basic Monte Carlo implementation originally developed for infinitely wide and parallel layers to account for light propagation through the spherical lens surface. We have adopted a modeling scheme in which the first two layers are allowed to have variable thickness. The first layer represents the air gap between the fibers and the half-ball lens, and the second layer represents the lens. Assignment of layer thickness is carried out reiteratively whenever a photon is about to encounter the spherical interface. Figure 3 shows a two-dimensional view of a photon incident upon a lens from above. The refractive index of the lens is n_2 , and the refractive indices of the layers above and below the lens are n_1 and n_3 , respectively. The first step is to compute the coordinates where the photon will intersect the lens surface. The z coordinate of the intersection point is then used to adjust the thickness of the first two layers and to create a simulation geometry with infinitely wide and parallel layers. Once the photon is moved to the boundary representing the lens interface, the decision for refraction or reflection needs to be based on the angle of incidence with respect to a local normal that characterizes the degree of curvature at the intersection point. As Fig. 3 demonstrates, the local normal is defined to be the vector pointing from the lens center to the intersection point. The direction of propagation after reflection or refraction is also determined with reference to the local normal.

A similar technique is applied when a photon approaches the spherical interface from within the lens. When the photon undergoes reflection and encounters the spherical boundary for a second time, however, the parallel layer geometry may no longer be valid. Depending on the z component of the propagation vector after an occurrence of internal reflection, problems may arise as to whether the parallel layer formulation can be used to describe accurately the lens boundary. An example of such a situation is depicted in Fig. 4. In this case, even though the photon should be considered to be incident from a medium with refractive index n_2 to a medium with refractive index n_1 , the parallel layer geometry assumption would incorrectly suggest just the contrary, as shown at the right in the figure. As the simulations described in this paper involve a high-index lens, the photon is likely to get trapped within the lens as a result of total internal reflection. It will undergo successive internal reflections and will bounce across different parts of the spherical interface until it hits the flat surface of the lens. Our model accounts for these multiple-reflection cases by assigning the photon a random location on the flat surface of the lens and a random propagation vector with a positive z component. Note that random sampling of photon position and propagation direction fits well within the context of Monte Carlo modeling. If a significant number of photons are simulated, such a statistical formulation for propagating multiply reflected photons provides a close approximation to spatial and directional randomization caused by successive occurrences of internal reflection off the spherical boundary.

In the modeling scheme described, we consider only photons that enter and exit the tissue through the flat surface of the lens. Photons that cross the interface representing this surface outside the circular contact area are terminated. Because the diameter of the half-ball lens determines the effective contact area of the probe, further propagation of photons exiting outside this region will not be relevant. In addition, photons impinging on the lens from above can reflect off the spherical surface and travel sideways and downward away from the lens. On a few occasions, photons hitting the spherical interface from within the lens can refract and also travel sideways and downward away from the lens. We can avoid further scattering of these stray photons by shielding the inside wall of the probe with an absorptive material. Therefore termination of such photons in Monte Carlo simulations does not lead to information loss.

To verify the part of the code related to light propagation through the spherical interface, we traced the path of a number of photons impinging on a half-ball lens with a given diameter and refractive index. In all cases, the computed trajectory was consistent with geometric optics.

B. Input for Monte Carlo Modeling

Simulations have been carried out at two different wavelengths, namely, $\lambda = 450$ nm and $\lambda = 650$ nm. Each simulation has been performed with 10^8 photons.

1. Tissue Parameters—Epithelial tissue was considered to be a two-layer medium with the epithelium on the top and the stroma underneath. The thickness of the normal epithelium was assumed to be 300 μm , which is an approximate average for epithelial tissues,^{35,36} and the stroma was modeled as a semi-infinite medium. Use of realistic and relevant optical properties is a crucial part of modeling. The values used in this paper to model normal epithelial tissue have been collected from various sources and were reported in a recent paper by Chang *et al.*³⁷ These are mostly based on measurements from cervical tissue or other tissues that have a similar architecture and are assumed to provide a reasonable characterization of epithelial tissues in general. Table 1 lists the optical properties for the epithelium and the stroma at $\lambda = 450$ nm and $\lambda = 650$ nm. Note that the two layers are index matched with $n = 1.40$. The refractive indices and the anisotropy factors are assumed to be independent of wavelength.

Measurements by Collier *et al.*³⁸ using confocal microscopy indicate that there is a threefold increase in epithelial scattering coefficient in highly dysplastic tissue compared to normal tissue. Ravazoula *et al.*¹⁷ and Lee *et al.*¹⁸ have shown that the microvessel density in the stroma increases by approximately a factor of 2 in the case of severe dysplasia. It is also known that collagen matrix in the stroma degrades with progression of dysplasia.^{14,15} Degradation of collagen matrix leads to decreased scattering.^{6,8} It has been observed that collagen fiber density decreases by approximately a factor of 2 in the case of severe dysplasia.¹⁶ Therefore, to model epithelial precancer we increased the epithelial scattering coefficient by a factor of 3 and the absorption coefficient in the stroma by a factor of 2, and we decreased the stromal scattering coefficient by a factor of 2. The epithelial absorption coefficient, the refractive indices, and the anisotropy factors were kept the same as for normal tissue. Epithelial thickness was again assumed to be 300 μm .³⁶

2. Fiber-Optic Probe Parameters—For all the simulations, both the source fiber and the detector fiber were assigned a 100 μm diameter and a numerical aperture of 0.11 (in air). The refractive indices of the fibers were set to 1.50. We also assumed that the material between the fiber tips was highly absorptive to prevent internal reflection of photons.

We chose sapphire as the high-index material for the half-ball lens. The refractive index of sapphire is 1.78 for $\lambda = 450$ nm and 1.76 for $\lambda = 650$ nm. In all the simulations described in this paper, radius R of the half-ball lens was set to 0.5 mm, and the thickness of the air gap, d , was 200 μm . Any window placed between the lens and the tissue was also made of sapphire. In addition to having significant refractive power, sapphire is extremely hard and resistant to scratching and abrasion. These characteristics make this substrate a suitable choice for optical applications in a clinical environment.

C. Output from Monte Carlo Modeling

Penetration depths of all the photons collected by using each fiber-optic probe geometry were recorded during the Monte Carlo simulations. Note that penetration depth as used here is defined to be the maximum z depth in tissue at which a photon undergoes a scattering event. To illustrate the depth-resolving characteristics of a given probe geometry, we present the penetration depth statistics in the form of histograms with a bin size of 10 μm up to a depth of 1000 μm . We also calculate the mean penetration depth for each simulation, which corresponds to the penetration depth averaged over all detected photons. Because our major goal in this paper is to evaluate the potential of each probe design in resolving spectral information from epithelium and stroma, another parameter of interest is the percentage of photons that scatter only in the epithelium without penetrating into the stroma. To provide better insight into the detection profiles of different probe geometries, we also recorded the number of scattering events that each photon undergoes before it reaches the detector fiber. For photons collected from the epithelium, we report the mean number of scattering events in the epithelial layer. For photons collected from the stroma, we report the mean number of scattering events in the epithelial layer and the stromal layer separately.

To establish model reliability, we carried out three simulations for every case of probe geometry considered. The histograms shown represent the averages over three simulations. All the parameters calculated have also been averaged, and the standard deviations have been determined based on the results from the three simulations. As we simulate rather subtle source–detector geometries, we report these standard deviations to provide strong evidence for convergence of Monte Carlo modeling results.

3. Results

A. Penetration Depth Statistics for Tilted Source and Detector Fibers

Figure 5 shows the penetration depth histograms obtained with the optical properties that correspond to those of normal tissue. Center-to-center source–detector separation s is set to 150 μm for all cases. This separation represents the minimum distance between the fibers that allows enough spacing for fiber cladding as well as for an extended fiber contact area resulting from tilting the fibers. The figure shows the penetration depth histograms for tilt angles $\beta = 15^\circ$ and $\beta = 30^\circ$. Results for $\beta = 0^\circ$ are also included for comparison purposes. Histograms for $\lambda = 450\text{ nm}$ are shown in Fig. 5(a), and the histograms for $\lambda = 650\text{ nm}$ are shown in Fig. 5(b). The corresponding results for the mean penetration depth, percentage of photons collected from the epithelium, and the mean number of scattering events are given in Table 2.

When $\beta = 0^\circ$, the penetration depth distributions are broad and flat. Almost all the photons collected come from the stroma. The fraction of detected photons that do not penetrate into the stroma is as small as 0.7% for $\lambda = 450\text{ nm}$ and 0.4% for $\lambda = 650\text{ nm}$. Tilting the two fibers toward each other by 15° results in narrower distributions with peaks shifted to the left. In this case, the penetration depth distributions peak at the junction of the epithelium and the stroma, and this geometry probes the superficial regions of the stroma. When $\beta = 30^\circ$, the majority of photons do not enter into the stroma and scatter only in the epithelium, but even though the peaks of the penetration depth distributions occur at $\sim 100\text{ }\mu\text{m}$ into the epithelium for both wavelengths, there is still considerable contribution from the stroma. The fraction of photons collected from the epithelium is only 74.9% for $\lambda = 450\text{ nm}$, and this fraction drops to 69.4% for $\lambda = 650\text{ nm}$. In fact, the mean penetration depth for $\lambda = 650\text{ nm}$ is greater than the epithelial thickness.

Note that the mean number of scattering events for photons collected from the epithelium decreases with increasing tilt angle. In the case of tilted fibers, photons undergo fewer scattering events in the epithelium before they reach the detector fiber. For photons collected from the stroma, the mean number of scattering events in both layers decreases in going from $\beta = 0^\circ$ to $\beta = 15^\circ$ but then increases for $\beta = 30^\circ$.

Figure 6 and Table 3 show the results obtained with the optical properties corresponding to those of dysplastic tissue. Center-to-center source–detector separation s is again 150 μm for all cases. In the case of dysplasia, the mean penetration depths are smaller, and the percentage of photons collected from the epithelium increases for all tilt angles. Photons tend to undergo more scattering events in the dysplastic epithelium than in the normal epithelium and fewer scattering events in the dysplastic stroma than in the normal stroma. For $\beta = 30^\circ$, the mean penetration depths for both wavelengths are well below the epithelial thickness, and the percentage of photons collected from the epithelium is 96.4% for $\lambda = 450\text{ nm}$ and 94.6% for $\lambda = 650\text{ nm}$.

B. Penetration Depth Statistics for Half-Ball Lens Coupled Source and Detector Fibers

1. Using Half-Ball Lens Coupled Fibers for Improved Depth Resolution—Figure 7 shows the penetration depth histograms obtained for window thickness $w = 0\text{ }\mu\text{m}$ that represents the case when the flat surface of the half-ball lens is in direct contact with the tissue. The optical properties used correspond to those of normal epithelial tissue. The figure shows the penetration depth histograms for three source–detector separations, namely, $s = 500\text{ }\mu\text{m}$, $s = 700\text{ }\mu\text{m}$, and $s = 900\text{ }\mu\text{m}$. Table 4 lists the results for the mean penetration depth, percentage of photons collected from the epithelium, and the mean number of scattering events.

Increasing the source–detector separation and thus moving the fibers farther to the opposite edges of the lens results in narrower distributions. As s increases, the histogram peaks shift to

the left and become more localized within superficial depths in the stromal layer. Photons tend to undergo fewer scattering events in the stroma before reaching the detector fiber, which is well correlated with increased depth localization.

The results for $w = 0 \mu\text{m}$ demonstrate that coupling the source and detector fibers to a half-ball lens significantly improves depth resolution for photons detected from the stroma. When the lens is in direct contact with the tissue, however, this probe geometry cannot exclusively probe the epithelial layer. Even for $s = 900 \mu\text{m}$, the percentage of photons collected from the epithelium is negligibly small.

Simulation results indicate that incident photons that are specularly reflected at the lens–tissue interface account for approximately 3%–4% of total detected reflectance for $s = 500 \mu\text{m}$ and $s = 700 \mu\text{m}$, and 1% for $s = 900 \mu\text{m}$. The penetration depth of these photons is not defined because they do not undergo scattering in the tissue. In calculating the parameters given in Table 4, we have excluded specularly reflected photons from consideration.

2. Using Half-Ball Lens Coupled Fibers to Selectively Target the Epithelial Layer

—Simulations have been carried out to analyze the effect of placing a window between the flat surface of the lens and the tissue surface. Figure 8 and Table 5 show the results for $w = 300 \mu\text{m}$, obtained for the optical properties that characterize normal epithelial tissue. It is apparent that the presence of the window decreases the mean penetration depth of detected photons and increases the percentage of photons collected from the epithelium. When $s = 900 \mu\text{m}$, the mean penetration depth is $119 \mu\text{m}$ for $\lambda = 450 \text{ nm}$ and $187 \mu\text{m}$ for $\lambda = 650 \text{ nm}$, both values being well within the limits of the epithelial thickness. For $\lambda = 450 \text{ nm}$, 93.8% of photons collected do not enter the stroma and scatter only in the epithelium. This percentage for $\lambda = 650 \text{ nm}$ is 87.7%.

Photons collected from the epithelium tend to undergo fewer scattering events with increasing s . In contrast, photons collected from the stroma undergo a greater number of scattering events in both layers as s is increased.

The penetration depth histograms and the penetration depth statistics that correspond to dysplastic epithelial tissue for $w = 300 \mu\text{m}$ are shown in Fig. 9 and Table 6, respectively. Comparison of these results and those for normal epithelial tissue reveals significant changes in mean penetration depth and percentage of photons collected from the epithelium for all source–detector separations. When $s = 700 \mu\text{m}$ or $s = 900 \mu\text{m}$, there is a prominent increase in sensitivity to the epithelial layer. Especially for $s = 900 \mu\text{m}$, almost all the photons collected do not penetrate into the stroma and scatter only in the epithelium. The percentage of photons collected from the epithelium is 99.1% for $\lambda = 450 \text{ nm}$ and 98.2% for $\lambda = 650 \text{ nm}$.

An important observation to make is that, with $w = 300 \mu\text{m}$, no specular reflection is detected. All the photons detected scatter in the tissue, and the detected reflectance is entirely diffuse.

C. Probe Design to Selectively Target the Epithelial and Stromal Layers

Based on the simulation results for tilted and half-ball lens coupled fibers presented above, we propose a probe design that can be used to selectively target both the epithelium and the stroma. The proposed probe design is based on the geometry involving the sapphire half-ball lens. The design consists of two pairs of source and detector fibers positioned over the lens, and the window thickness is $300 \mu\text{m}$. The fibers that belong to the first pair are oriented perpendicular to the tissue surface and are characterized by a source–detector separation of $900 \mu\text{m}$. The fibers that belong to the second pair are characterized by a source–detector separation of $500 \mu\text{m}$, but, instead of being positioned perpendicular to the tissue surface, they are tilted slightly away from each other, in a direction opposite that shown in Fig. 1. The tilt angle for both the

source and the detector fiber is specified to be -5° , the negative sign being a reminder of the direction in which the fibers are tilted. The fiber tips are polished parallel to the tissue surface.

The penetration depth histograms and the penetration depth statistics for the proposed probe design are shown in Fig. 10 and Table 7, respectively. Although the results for the first fiber pair, with $s = 900 \mu\text{m}$ and $\beta = 0^\circ$, were already presented in Subsection 3.B, we include those results as well to facilitate assessment of the depth selectivity characteristics of the probe. This fiber pair can selectively target the epithelium both at $\lambda = 450 \text{ nm}$ [Fig. 10(a)] and at $\lambda = 650 \text{ nm}$ [Fig. 10(b)]. The second fiber pair, with $s = 500 \mu\text{m}$ and $\beta = -5^\circ$, is sensitive to the superficial stroma both for $\lambda = 450 \text{ nm}$ [Fig. 10(c)] and for $\lambda = 650 \text{ nm}$ [Fig. 10(d)]. Note that the number photons detected from the epithelium increases with progression of dysplasia, whereas the number of photons detected from the stroma decreases.

4. Discussion

One can estimate the depth range in the tissue to which a given probe geometry is most sensitive and the corresponding depth resolution by considering the location and extent of the geometric overlap between the illumination and collection cones. It is important to remember, though, that the specific penetration depth profile of detected photons depends not only on this geometrical overlap but also on the optical properties of the tissue under consideration. With this fact in mind, we carried out simulations at $\lambda = 450 \text{ nm}$ and $\lambda = 650 \text{ nm}$ to reveal wavelength-dependent changes in penetration depth profiles. We have also tried to simulate changes in optical properties associated with severe dysplasia, and we have incorporated these changes into our modeling to provide some insight into differences in penetration profiles caused by dysplastic progression.

A. Tilted Source and Detector Fibers

When the source and detector fibers are oriented perpendicular to the tissue surface, the overlapping region of the illumination and collection cones extends to large depths within the tissue. In this case, photons detected exhibit a wide range of penetration depths, as demonstrated in Figs. 5 and 6. When the fibers are tilted, however, the illumination and the collection cones are slanted toward each other, and the overlapping region moves closer to the surface and is more localized. This trend results in improved depth selectivity and collection of photons from more superficial depths within the tissue.

For $\beta = 15^\circ$, the simulation results for normal epithelial tissue imply that the illumination and collection cones overlap over the superficial stroma and then diverge from each other, leading to increased sensitivity to this region. The fact that photons undergo fewer scattering events in the stroma before they reach the detector fiber is also consistent with this localization. Photons collected from the epithelium undergo fewer scattering events as well. As the fibers are tilted toward each other, photons no longer need to backscatter through large angles to reach the detector. Because they enter the tissue at an angle, there is increased probability that they can reverse their path through fewer numbers of scattering events and travel toward the detector. The detector, which is also oriented at an angle, favors collection of photons that backscatter through smaller angles.

The results for $\beta = 30^\circ$ indicate that the illumination and collection cones overlap over the epithelial layer and then diverge from each other more rapidly, with little or no overlap over the stromal layer. The probe geometry is mostly sensitive to epithelial scattering. Photons collected from the epithelium undergo fewer scattering events, again consistent with an even higher probability of backscattering toward the detector. It is important to note that photons collected from the stroma undergo a greater number of scattering events in the stromal layer than for the $\beta = 15^\circ$ case. Because of the increased tilt angle and the more rapidly diverging

illumination and collection cones over the stromal layer, photons from the stroma need to scatter more to alter their path and enter the collection cone where there is increased probability of detection.

Comparison of Figs. 5 and 6 and Tables 2 and 3 reveals significant differences between penetration depth profiles of normal and dysplastic epithelial tissue. There is a noticeable increase in sensitivity to the epithelial layer for dysplastic tissue, and this trend is consistent with increased epithelial scattering, decreased stromal scattering, and increased stromal absorption that accompany dysplasia. Increase in epithelial scattering and decrease in stromal scattering associated with dysplastic progression are also reflected in statistics regarding the number of scattering events.

There are practical limits on the maximum tilt angle that can be achieved. If the overall dimension of the fiber-optic probe is to be kept small, the fibers cannot be tilted extensively. There is also an upper limit on the extent of bending that a fiber can tolerate. Tilt angles greater than 30° are difficult to construct in a compact package and have not been considered in this paper. The results given in Table 2 for normal epithelial tissue indicate that, even when the fibers are placed close to each other and are tilted to the maximum extent compatible with design limitations, a significant percentage of detected photons still comes from the stroma. Even though the illumination and collection cones overlap over the epithelium, the angle at which the cones diverge from each other is not sufficient to eliminate detection of photons from the highly scattering stroma. Therefore we conclude that this probe geometry does not have the potential to provide adequate separation of epithelial scattering from stromal scattering in normal tissue. The results for $\beta = 30^\circ$ given in Table 3, however, demonstrate that the same probe geometry provides significant separation of epithelial scattering from stromal scattering in dysplastic tissue. Such a contrast in penetration depth profiles of normal and dysplastic tissue is not desirable because we would like to be able to compare spectra that are unique to each of the two layers. We need a probe design that demonstrates comparable performance in isolating epithelial scattering from stromal scattering for both normal and dysplastic tissue.

B. Half-Ball Lens Coupled Source and Detector Fibers

Instead of physically tilting the fibers, we can make use of the refractive power of a high-index spherical lens to obtain slanted illumination and collection cones. Figure 7 illustrates the depth selectivity potential of a sapphire half-ball lens in direct contact with normal epithelial tissue. As the source and detector fibers are moved to the outer edges over the lens, the angle at which the illumination and collection cones intersect within the tissue increases. The cones overlap over more superficial tissue depths and then diverge rapidly in opposite directions, giving rise to increased depth selectivity.

When the lens is in direct contact with the tissue, however, the overlapping region of the illumination and collection cones occurs in the stroma. Simulation results also indicate that, for $w = 0 \mu\text{m}$, specular reflection accounts for a few percent of the total detected reflectance. For the three source–detector separations used in the simulations, the illumination and collection paths do not intersect on the flat surface of the lens. Therefore incident photons that are specularly reflected at the lens–tissue interface are not directed toward the detector. Detected specular reflection is attributable to incident photons undergoing internal reflection at the flat surface of the lens, bouncing back from around the central part of the spherical interface, and undergoing reflection at the flat surface of the lens a second time. Owing to spherical symmetry, some of these photons reach the detector fiber within the limits of the numerical aperture.

The epithelial layer can be selectively targeted by insertion of a sapphire window between the flat surface of the lens and the tissue surface. The extended path length within the window

brings the illumination and collection cones closer together on the surface of the tissue. Comparison of Figs. 7 and 8 suggests how the penetration depth profiles are affected by the presence of the window. Table 5 shows that photons collected from the epithelium tend to undergo fewer scattering events as the source–detector separation increases, similar to the effect caused by increasing the tilt angle of the fibers. Also, photons collected from the stroma undergo a greater number of scattering events in the stromal layer. This is again due to more rapidly diverging illumination and collection cones over the stromal layer, and photons need to scatter more to alter their paths and reach the detector. Table 5 indicates that, with $s = 900\ \mu\text{m}$, which represents the case when the source and detector fibers are placed at the very edges of the lens, a significant percentage of detected photons comes from the epithelium at both wavelengths. It can be concluded that placing the fibers far apart over the lens is an effective way of creating illumination and collection cones that are steep enough to eliminate collection of photons from the stroma. Simulation results in Fig. 9 and Table 6 imply that $s = 900\ \mu\text{m}$ also provides excellent sensitivity to the epithelial layer in the case of dysplastic tissue. Therefore, this probe geometry is capable of providing sufficient separation of epithelial scattering from stromal scattering for both normal and dysplastic tissue.

Placement of a sapphire window between the flat surface of the lens and the tissue surface not only facilitates selective targeting of the epithelial layer but also eliminates detection of specularly reflected photons. With the window in place, the incident photons reach the base of the window at a location closer to the central axis of the lens. If these photons are reflected, they will no longer hit the region around the tip of the spherical interface. This will effectively break the spherical symmetry that has led to collection of specular reflection when $w = 0\ \mu\text{m}$. Note that the window thickness needs to be adjusted carefully to prevent overlap of illumination and collection paths over the base of the window. As long as the source–detector separation is not smaller than $500\ \mu\text{m}$, an upper limit of $300\ \mu\text{m}$ on the window thickness ensures that no such overlap will occur and that photons that are specularly reflected at this interface will not travel directly toward the detector.

We have observed that the penetration depth profiles are not strongly dependent on the thickness of the air gap between the tip of the fibers and the top of the lens. Changing the thickness of the air gap to 100 or $300\ \mu\text{m}$ has a minimal effect on parameters such as the mean penetration depth and the percentage of photons collected from the epithelium.

C. Proposed Probe Design

Whereas the epithelial layer can selectively be targeted by use of half-ball lens coupled fibers that are oriented perpendicular to the tissue and of a window with $w = 300\ \mu\text{m}$, the ability to selectively target the stromal layer has been compromised. Even though $s = 500\ \mu\text{m}$ can be used to selectively probe the stroma in the case of normal epithelial tissue, the results given in Table 6 indicate that the same is not true for dysplastic epithelial tissue. With $w = 300\ \mu\text{m}$, a source–detector separation that is smaller than $500\ \mu\text{m}$ leads to collection of specularly reflected photons and cannot be used to obtain increased sensitivity to the stromal layer.

It is possible to overcome this problem by keeping the source–detector separation at $500\ \mu\text{m}$ but using tilted source and detector fibers. Tilting the fibers slightly away from each other brings the overlapping region of the illumination and collection cones over to the superficial stroma. The proposed tilt angle is only 5° , and such a small tilt angle can easily be implemented within the $500\ \mu\text{m}$ space.

The results compiled in Fig. 10 and Table 7 show that the depth selectivity achieved by the two fiber pairs is sufficient for selectively targeting both the epithelium and the stroma. Note that the penetration depth profiles for both fiber pairs are consistent across the two diagnostic categories, evidenced by histogram peaks localized within similar tissue depths. The mean

penetration depths and the percentages listed in Table 7 for normal and dysplastic tissue are also comparable for all four cases. This consistency in depth selectivity is a crucial advantage that can benefit the interpretation of tissue spectra.

An important consideration in the design of a fiberoptic probe is the efficiency of the optical setup. Because the fibers are in contact with air, specular reflection off the fiber tips is of the order of 5%. Also, sapphire has a high refractive index, and specular reflection off the spherical interface is likely to degrade the efficiency of the proposed probe. Monte Carlo simulation results indicate that, for the tilted fiber pair with $s = 500 \mu\text{m}$, the fraction of incident photons lost to specular reflection off the lens surface is $\sim 10\%$ for $d = 200 \mu\text{m}$. For the fiber pair with $s = 900 \mu\text{m}$, this loss increases to $\sim 15\%$. At the same time, the edges of the fibers separated by $900 \mu\text{m}$ are aligned with the edges of the spherical lens. Some of the photons leaving the source fiber will miss the lens and hit the inside wall of the probe. As the NA of the source fiber is only 0.11, the fraction of incident photons that will miss the lens is $\sim 10\%$ for $d = 200 \mu\text{m}$. Similarly, the full NA of the detector will not be utilized to collect photons exiting the tissue. Simulation results show that signal levels obtained with lens coupled fibers are indeed lower than those obtained with tilted fibers in contact with the tissue. Note, however, that the collection efficiency of a given source–detector geometry cannot be characterized simply by the total number of photons detected. A more appropriate way to assess collection efficiency in the context of this paper is to consider signal magnitude in conjunction with depth selectivity. To give a specific example, results for normal epithelial tissue at $\lambda = 450 \text{ nm}$ indicate that the total number of photons detected with lens coupled fibers with $s = 900 \mu\text{m}$ is $\sim 30\%$ less than the total number of photons detected by using tilted fibers with $\beta = 30^\circ$. Approximately 20% of this decrease in signal magnitude is in fact due to the ability of the lens coupled fiber pair to limit the sampling depth and to successfully eliminate detection of photons from the highly scattering stroma. Hence, coupling the source and detector fibers to a sapphire lens does not lead to considerable reduction in the number of photons collected from the epithelium, which, in this particular case, is the target layer. Comparison of the penetration depth histograms for the proposed probe design with those for tilted fibers in contact with the tissue also reveals comparable signal levels from the respective tissue layers. Therefore we can conclude that the focusing power of the lens compensates for a significant portion of optical losses associated with fiber–lens coupling. Specularly reflected photons or incident photons that miss the lens surface are sources of stray light within the probe. If the inside wall of the probe is shielded with an absorbing material, further scattering of these stray photons will be avoided.

D. Implications

Many techniques have recently been employed to selectively target the epithelial layer and isolate epithelial scattering from stromal scattering. Light-scattering spectroscopy and polarized reflectance spectroscopy have been shown to be sensitive to precancerous changes in the epithelium.^{6,8,31,39,40} Other methods proposed to target the most superficial depths within epithelial tissues include differential path-length spectroscopy⁴¹ and low-coherence backscattering spectroscopy.⁴² Our approach is based on improvement of depth selectivity simply through optimization of the fiber-optic probe design.

Ball or half-ball lens coupled fibers have been widely used for medical applications including laser surgery and photodynamic therapy.⁴³ The purpose of using a lens element in these applications is to increase light intensity at the tip of the probe. Motz *et al.*⁴⁴ recently described a probe design for biomedical Raman spectroscopy in which a sapphire ball lens inserted to the tip of the probe provides signal enhancement and increased collection efficiency. In this paper we have shown that a sapphire half-ball lens is capable of providing depth selectivity and improved depth resolution. Specifically, the simulation results for epithelial tissues indicate

that it is possible to use lens coupled source and detector fibers to isolate epithelial scattering from stromal scattering.

The proposed probe design provides a simple and efficient means to collect light selectively from both the epithelium and the stroma and facilitates acquisition of reflectance spectra unique to each of the two layers. As a result, optical trends from both tissue layers can be monitored for any changes associated with precancer progression. Spectra unique to the epithelium will be sensitive to dysplastic changes in epithelial cells, whereas spectra unique to the stroma will be sensitive to dysplastic changes in collagen fibers and microvessel density.

In addition to providing resolution of spectral information from epithelial and stromal layers, the proposed design is extremely compact. The overall dimension of the fiber-optic probe is roughly determined by the lens diameter, which is as small as 1 mm. Such a small probe can provide easy access to epithelial tissues including the cervix and the oral cavity and can also be integrated into an endoscope for detection of dysplasia in otherwise inaccessible organ sites such as the intestine.

The principle of depth selectivity by using lens coupled fibers can also be applied to target epithelial fluorescence in fluorescence spectroscopy. In as much as sapphire has no autofluorescence, the design can possibly be extended for use in fluorescence measurements as well. In fact, initial results reported by Schwarz *et al.*⁴⁵ are promising, but modeling studies are required for characterizing depth selectivity specific to fluorescence measurements.

5. Conclusions

The diagnostic performance of *in vivo* biomedical optical spectroscopy is strongly dependent on the ability of the fiber-optic probe to target diagnostically significant features. Computational studies are necessary for investigation of the relationship between probe geometry and origin of a detected optical signal. The goal in this paper was to use Monte Carlo modeling to suggest a probe design for reflectance-based detection of precancer in epithelial tissues. Simulations have been performed to evaluate different probe geometries with respect to the sampling depth. The results provide a guideline for design and optimization of a fiber-optic probe that can resolve spectral information from the epithelium and the stroma. Selective targeting of these two distinct layers will facilitate simultaneous assessment of dysplastic changes unique to each layer and will improve the ability of reflectance spectroscopy to differentiate precancerous epithelial tissue from normal epithelial tissue.

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Fig. 1. Fiber-optic probe geometry with tilted source and detector fibers. The fiber tips are polished parallel to the tissue surface.

Fig. 2. Fiber-optic probe geometry with half-ball lens coupled source and detector fibers. The fibers are symmetrically oriented about the central axis of the lens.

Fig. 3. Modeling light propagation across the spherical lens interface through creation of a simulation geometry with infinitely wide and parallel layers.

Fig. 4. Situation in which the parallel layer formulation fails to describe accurately the spherical lens boundary. When the photon encounters the spherical boundary for a second time, it should be considered to be incident from a medium with refractive index n_2 to a medium with refractive index n_1 , but the parallel layer geometry assumption incorrectly suggests the contrary.

Fig. 5. Penetration depth histograms for normal epithelial tissue at (a) $\lambda = 450$ nm and (b) $\lambda = 650$ nm obtained with tilted source and detector fibers.

Fig. 6. Penetration depth histograms for dysplastic epithelial tissue at (a) $\lambda = 450$ nm and (b) $\lambda = 650$ nm obtained with tilted source and detector fibers.

Fig. 7. Penetration depth histograms for normal epithelial tissue at (a) $\lambda = 450$ nm and (b) $\lambda = 650$ nm obtained with half-ball lens coupled source and detector fibers ($w = 0$ μm).

Fig. 8. Penetration depth histograms for normal epithelial tissue at (a) $\lambda = 450$ nm and (b) $\lambda = 650$ nm obtained with half-ball lens coupled source and detector fibers ($w = 300$ μm).

Fig. 9.

Penetration depth histograms for dysplastic epithelial tissue at (a) $\lambda = 450$ nm and (b) $\lambda = 650$ nm obtained with half-ball lens coupled source and detector fibers ($w = 300$ μm).

Fig. 10.

Penetration depth histograms for the proposed probe design with two pairs of half-ball lens coupled source and detector fibers and a window with $w = 300 \mu\text{m}$. The fiber pair with $s = 900 \mu\text{m}$ and $\beta = 0^\circ$ can selectively target the epithelium at (a) $\lambda = 450 \text{ nm}$ and (b) $\lambda = 650 \text{ nm}$, and the fiber pair with $s = 500 \mu\text{m}$ and $\beta = -5^\circ$ can selectively target the stroma at (c) $\lambda = 450 \text{ nm}$ and (d) $\lambda = 650 \text{ nm}$.

Table 1
Optical Properties of Normal Epithelial Tissue^a

Layer	μ_a (cm ⁻¹)	μ_s (cm ⁻¹)	g
$\lambda = 450$ nm			
Epithelium	2.5	39.6	0.95
Stroma	4.3	248.5	0.88
$\lambda = 650$ nm			
Epithelium	1.2	27.4	0.95
Stroma	0.9	172.0	0.88

^aThe two layers are index matched with $n = 1.40$ in all cases.

Penetration Depth Statistics for Normal Epithelial Tissue Obtained with Tilted Source and Detector Fibers

Table 2

Case	Mean Penetration Depth (μm)	% Photons Collected from Epithelium	Mean Number of Scattering Events for Photons Collected from Stroma		
			Mean Number of Scattering from Epithelium	Epithelium	Stroma
$\lambda = 450 \text{ nm}$ $\beta = 0^\circ$ $\beta = 15^\circ$ $\beta = 30^\circ$	503 ± 3	0.7 ± 0.2	3.8 ± 0.4	2.4 ± 0.0	16.1 ± 0.3
	375 ± 0	11.4 ± 0.3	2.7 ± 0.1	2.2 ± 0.0	7.5 ± 0.0
	232 ± 2	74.9 ± 0.8	2.1 ± 0.0	3.0 ± 0.2	28.9 ± 0.4
$\lambda = 650 \text{ nm}$ $\beta = 0^\circ$ $\beta = 15^\circ$ $\beta = 30^\circ$	608 ± 4	0.4 ± 0.0	3.2 ± 0.4	1.6 ± 0.0	17.5 ± 0.5
	410 ± 3	10.5 ± 0.1	2.2 ± 0.0	1.5 ± 0.0	7.8 ± 0.3
	330 ± 12	69.4 ± 1.4	1.7 ± 0.0	2.0 ± 0.0	39.1 ± 0.8

Table 3
Penetration Depth Statistics for Dysplastic Epithelial Tissue Obtained with Tilted Source and Detector Fibers

Case	Mean Penetration Depth (μm)	% Photons Collected from Epithelium	Mean Number of Scattering Events for Photons Collected from Stroma		
			Mean Number of Scattering from Epithelium	Epithelium	Stroma
$\lambda = 450\text{ nm}$ $\beta = 0^\circ$ $\beta = 15^\circ$ $\beta = 30^\circ$	442 ± 4	14.1 ± 0.1	7.9 ± 0.5	7.2 ± 0.1	6.8 ± 0.1
	291 ± 3	55.9 ± 1.4	5.8 ± 0.1	6.8 ± 0.1	4.3 ± 0.1
	126 ± 1	96.4 ± 0.1	4.2 ± 0.0	9.2 ± 0.4	8.7 ± 0.8
$\lambda = 650\text{ nm}$ $\beta = 0^\circ$ $\beta = 15^\circ$ $\beta = 30^\circ$	597 ± 6	7.6 ± 1.2	6.0 ± 0.2	4.9 ± 0.1	8.3 ± 0.2
	342 ± 4	47.8 ± 1.0	4.4 ± 0.0	4.7 ± 0.1	4.7 ± 0.2
	147 ± 5	94.6 ± 0.3	3.2 ± 0.1	6.5 ± 0.1	14.5 ± 2.1

Table 4
Penetration Depth Statistics for Normal Epithelial Tissue Obtained with Half-Ball Lens Coupled Source and Detector Fibers ($w = 0 \text{ }\mu\text{m}$)

Case	Mean Penetration Depth (μm)	% Photons Collected from Epithelium	Mean Number of Scattering Events for Photons Collected from Stroma		
			Mean Number of Scattering Events for Photons Collected from Epithelium	Epithelium	Stroma
$\lambda = 450 \text{ nm}$ $s = 500 \text{ }\mu\text{m}$ $s = 700 \text{ }\mu\text{m}$ $s = 900 \text{ }\mu\text{m}$	422 ± 2	0.6 ± 0.0	4.3 ± 0.3	2.2 ± 0.0	9.2 ± 0.1
	395 ± 1	0.5 ± 0.1	4.5 ± 0.4	2.3 ± 0.0	7.7 ± 0.0
	329 ± 1	11.4 ± 0.5	3.9 ± 0.0	2.7 ± 0.0	4.6 ± 0.1
$\lambda = 650 \text{ nm}$ $s = 500 \text{ }\mu\text{m}$ $s = 700 \text{ }\mu\text{m}$ $s = 900 \text{ }\mu\text{m}$	467 ± 1	0.3 ± 0.0	3.3 ± 0.4	1.5 ± 0.0	8.8 ± 0.1
	429 ± 2	0.2 ± 0.0	3.6 ± 0.6	1.6 ± 0.0	7.2 ± 0.2
	346 ± 1	6.1 ± 0.1	3.2 ± 0.0	1.8 ± 0.0	4.3 ± 0.1

Table 5
Penetration Depth Statistics for Normal Epithelial Tissue Obtained with Half-Ball Lens Coupled Source and Detector Fibers ($w = 300\text{ }\mu\text{m}$)

Case	Mean Penetration Depth (μm)	% Photons Collected from Epithelium	Mean Number of Scattering Events for Photons Collected from Epithelium	Mean Number of Scattering Events for Photons Collected from Stroma	
				Epithelium	Stroma
$\lambda = 450\text{ nm}$					
$s = 500\text{ }\mu\text{m}$	379 ± 0	18.4 ± 0.4	2.5 ± 0.1	2.4 ± 0.0	9.8 ± 0.2
$s = 700\text{ }\mu\text{m}$	330 ± 5	55.4 ± 1.0	2.5 ± 0.0	2.9 ± 0.1	22.0 ± 0.8
$s = 900\text{ }\mu\text{m}$	119 ± 2	93.8 ± 0.2	2.1 ± 0.0	3.5 ± 0.1	33.1 ± 4.6
$\lambda = 650\text{ nm}$					
$s = 500\text{ }\mu\text{m}$	427 ± 1	14.2 ± 0.2	2.1 ± 0.1	1.7 ± 0.0	9.9 ± 0.2
$s = 700\text{ }\mu\text{m}$	417 ± 9	50.3 ± 0.7	2.1 ± 0.1	2.1 ± 0.1	25.0 ± 1.4
$s = 900\text{ }\mu\text{m}$	187 ± 12	87.7 ± 1.0	1.7 ± 0.0	2.6 ± 0.1	44.0 ± 5.4

Penetration Depth Statistics for Dysplastic Epithelial Tissue Obtained with Half-Ball Lens Coupled Source and Detector Fibers ($w = 300\text{ }\mu\text{m}$)

Table 6

Case	Mean Penetration Depth (μm)	% Photons Collected from Epithelium	Mean Number of Scattering Events for Photons Collected from Epithelium	Mean Number of Scattering Events for Photons Collected from Stroma	
				Epithelium	Stroma
$\lambda = 450\text{ nm}$					
$s = 500\text{ }\mu\text{m}$	256 ± 5	67.6 ± 1.0	5.6 ± 0.1	7.2 ± 0.1	4.4 ± 0.3
$s = 700\text{ }\mu\text{m}$	190 ± 2	90.3 ± 0.2	5.4 ± 0.0	8.3 ± 0.0	6.7 ± 0.6
$s = 900\text{ }\mu\text{m}$	87 ± 1	99.1 ± 0.1	4.1 ± 0.0	10.6 ± 1.4	10.4 ± 1.3
$\lambda = 650\text{ nm}$					
$s = 500\text{ }\mu\text{m}$	322 ± 4	55.1 ± 1.0	4.3 ± 0.1	4.9 ± 0.0	5.0 ± 0.2
$s = 700\text{ }\mu\text{m}$	236 ± 3	85.5 ± 0.8	4.2 ± 0.0	6.0 ± 0.1	8.9 ± 0.3
$s = 900\text{ }\mu\text{m}$	108 ± 3	98.2 ± 0.3	3.3 ± 0.1	7.1 ± 0.4	18.8 ± 4.7

Table 7

Penetration Depth Statistics for the Proposed Probe Design

Target	% Photons Collected from Epithelium	Mean Penetration Depth (μm)	Mean Number of Scattering Events for Photons Collected from Stroma	
			Epithelium	Stroma
Epithelium ^a $\lambda = 450\text{ nm}$ Normal Dysplastic	93.8 $\pm$ 0.2 99.1 $\pm$ 0.1	119 $\pm$ 2	2.1 $\pm$ 0.0	33.1 $\pm$ 4.6
		87 $\pm$ 1	4.1 $\pm$ 0.0	10.4 $\pm$ 1.3
		187 $\pm$ 12	1.7 $\pm$ 0.0	44.0 $\pm$ 5.4
		108 $\pm$ 3	3.3 $\pm$ 0.1	18.8 $\pm$ 4.7
Stroma ^b $\lambda = 450\text{ nm}$ Normal Dysplastic	0.8 $\pm$ 0.1 15.7 $\pm$ 0.1	406 $\pm$ 1	3.9 $\pm$ 0.4	7.9 $\pm$ 0.1
		384 $\pm$ 2	8.1 $\pm$ 0.0	4.4 $\pm$ 0.1
		452 $\pm$ 0	3.2 $\pm$ 0.1	8.0 $\pm$ 0.2
		452 $\pm$ 1	6.1 $\pm$ 0.3	4.6 $\pm$ 0.1

^aThe epithelium is targeted by the fiber pair with $s = 900\text{ }\mu\text{m}$ and $\beta = 0^\circ$.

^bThe stroma is targeted by the fiber pair with $s = 500\text{ }\mu\text{m}$ and $\beta = -5^\circ$.