FAST AND MINIMALLY-INVASIVE TUMOR MARGIN DETECTION USING A NOVEL MICROMACHINED “SIDE-VIEWING” OIDRS SENSOR PROBE

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ABSTRACT

In this paper, we report the design, fabrication and testing of a novel miniaturized optical sensor probe with “side-viewing” capability. Its unprecedented small size, unique “side-viewing” capability and high optical transmission efficiency enable the agile maneuvering and efficient data collection even in the narrow cavities inside the human body. The sensor probe consists of four micromachined substrates with optical fibers for oblique light incidence and collection of spatially resolved diffuse reflectance from the contacted tissues. The optical sensor probe has been used to conduct the oblique incidence diffuse reflectance spectroscopy (OIDRS) on a human pancreatic specimen. Based on the measurement results, the margin of the malignant tumor has been successfully determined optically, which matches well with the histological results.

INTRODUCTION

The American Cancer Society estimated that there were about 42,470 new cases of pancreas cancers with about 35,240 deaths in the U.S. in 2009 [1]. The survival rate of the pancreatic cancer is relatively low compared with many other types of cancers. The low survival rate is due to the fact that early detection of pancreatic cancer is very difficult. This is because the pancreas is deep inside the body and so far there are no tests or physical exam can detect the pancreatic cancer in its early stage. Currently clinical methods for diagnosing pancreas cancers include laparoscopy and biopsy. However, for the follow-up treatments, these detection methods lack the ability to quickly and precisely determine a safe margin for removing the malignant pancreatic tissues. Usually, the tissue in a suspicious region is removed and histologically analyzed. Based on the results, another suspicious cite for tissue removal is planned and performed. Multiple rounds of cut-and-analyze procedure are required to complete the tumor removal, which would end up with an extremely time-consuming surgical procedure. To address this issue, new techniques that can enable simple, fast, accurate and reliable tumor margin detection are necessary.

Recent studies have shown that the change in the optical absorption and scattering properties of human tissues are closely related to their states of the malignancy [2, 3]. Several different optical methodologies have been investigated for determining the malignancy of tumors [4, 5]. Among them, oblique incidence diffuse reflectance spectroscopy (OIDRS) is a unique approach, which utilizes a special fiber optic sensor probe to measure the diffuse reflectance of the tissue in contact [6]. The diffuse reflectance spectra can be further used to extract optical absorption and scattering properties of the tissue [7]. In our previous studies, a remarkably high accuracy of 95% in differentiating (pre)cancerous human skin lesions from benign ones with automated OIDRS data processing and classification [6]. However, to extend the application of OIDRS from skin to pancreas cancer which is inside the body, miniaturized fiber optic probes suitable for inner-body measurements are needed.

In this paper, we report the design, fabrication and testing of the first 2-mm OIDRS probe with side-viewing capability using micromachining technology. This miniaturized, high optical

transmission efficiency, and “side-viewing” configuration makes it compatible with medical endoscopes for effective and efficient *in-vivo* measurements even in the narrow cavities inside human body. The new OIDRS probe has been used to conduct diffuse-reflectance measurement on a human pancreas specimen. Based on the measurement and classification, the margin between the malignant tissues and the benign ones has been successfully identified, which matches well with the histological results.

THEORY

As shown in Fig. 1, when light is incident on the surface of an inhomogeneous medium (e.g. biological tissue), some of the incident light will be directly reflected (specular reflectance) and the remaining light will transmit into and interacts with the medium through scattering and absorption. After undergoing multiples times of interactions, part of the transmitted light will be scattered back to the surface and escape from the medium to form the diffuse reflectance.

Figure 1: Illustration of light absorption and scattering in an inhomogeneous medium.

The spatially resolved steady-state diffuse reflectance for a particular wavelength can be calculated by diffusion theory [8].

$$R(x) = \frac{1}{4\pi} \left[\frac{\Delta z(1 + \mu_{eff}\rho_1)\exp(-\mu_{eff}\rho_1)}{\rho_1^3} + \frac{(\Delta z + 2z_b)(1 + \mu_{eff}\rho_2)\exp(-\mu_{eff}\rho_2)}{\rho_2^3} \right] \quad (1)$$

where ρ_1 and ρ_2 are the distances between the positive and negative source points and the observation point on the medium surface (Fig. 2). Δz is the distance between the virtual boundary and the tissue depth, and z_b is the distance between the virtual boundary and the surface of the sample (Fig. 2). The distance from the point of incidence to the positive point source d_s is equal to $3D$. For oblique incidence, the diffusion coefficient is $D = (3(0.35\mu_a + \mu_s'))^{-1}$, where μ_a is the absorption coefficient and μ_s' is the reduced scattering coefficient. The effective attenuation coefficient $\mu_{eff} = (\mu_a/D)^{1/2}$. The shift of the point sources in the x direction $\Delta x = \sin(\alpha_t)/(3(0.35\mu_a + \mu_s'))$, and α_t is the angle of light transmission into

the medium. The absorption and reduced scattering coefficients can be calculated by [9]

$$\mu_a = \frac{\mu_{eff}^2 \Delta x}{3 \sin(\alpha_i)} \quad (2)$$

$$\mu_s' = \frac{\sin(\alpha_i)}{\Delta x} - 0.35 \mu_a \quad (3)$$

Figure 2: Schematic of the diffusion theory model for oblique incidence.

The assumption of the diffusion theory is that the reduced scattering coefficient is much larger than the absorption. The source and detector must also be separated in space so that the light is diffuse reflectance instead of specular reflectance. When the distance between the source and the detectors is comparable to the transport mean free path (~ 1 mm), diffusion theory does not apply. In this case, Monte Carlo simulation can be applied to extract optical properties from the diffuse reflectance [10].

The optical absorption of the human tissue is mostly related to the concentration of hemoglobin and its oxygen saturation. These parameters are believed to have close relationship with the disease state of lesions [11, 12]. On the other hand, the cell nuclei of the tissue can be considered as the major contributor for the scattering properties. Research has shown the diameter of the nuclei would increase with the degree of dysplasia in different lesions [13]. Therefore, the close relationship between the optical absorption and scattering properties can be used to differentiate the state of the malignancy of human tissue, which forms the physiological foundation for applying OIDRS for the cancer detection.

PROBE DESIGN AND FABRICATION

In the OIDRS measurements, light with particular wavelengths is delivered at a desirable oblique incidence angle on the tissue surface and the one-dimensional linear distribution of the diffuse reflectance $R(x)$ is collected. While this can be achieved by using a conventional front-viewing probe that consists of straight optical fiber bundles (Fig. 3(a)), it will not be convenient and feasible to conduct *in-vivo* OIDRS measurements inside the human body. This is due to the fact that internal organs mostly consist of long and narrow tubular cavities. To address this issue, we have investigated the idea of building a miniaturized side-viewing fiber optic probe suitable for measurements inside the human body (Fig. 3b). However, the side-viewing capability requires all the collection fibers to undergo a sharp 90° turn within a radius of curvature less than 2.5 mm. This inevitably would cause significant light loss and leakage and also possible mechanical fracture of the collection

fibers.

Figure 3: Schematic of micro OIDRS probes for *in-vivo* optical characterization of human tissues: (a) front-viewing configuration and (b) side-viewing configuration.

To solve this problem, we came up with a new probe design using optical-epoxy-filled waveguides. The probe consists of two micromachined devices assembled together. The first device functions as collectors of the diffuse reflection and contains five epoxy-filled waveguides with a 90° turn for side viewing, which are coupled to 100 μm diameter optical fibers (Fig. 4(a)). The waveguides are filled with optically transparent epoxy (Epotek 301) to increase the coupling efficiency with the fibers and protect them from possible contamination from the body fluids (e.g. blood). In the second device, two chips are assembled to hold and position one source fiber (200 μm in diameter) which delivers light precisely at an oblique angle to the tissue of interest (Fig. 4(b)).

Figure 4: Schematic design of the new "side-viewing" OIDRS probe: (a) Collection device and (b) Source device.

The detail of the probe fabrication process is described below. Four silicon substrates with patterned SU-8 channel are used. SU-8 is chosen as the structural material because it can easily form patterned thick layers with high aspect ratio. The fabrication of SU-8 structure fabrication is based on the recipe from the material provider [14]. The first and second substrates have five curved SU-8 channel (~ 75 μm deep, ~ 150 μm wide) on each of them and their patterns are mirror-flipped. When the two substrates are stacked together, tightly closed channels with cross section 150 $\mu\text{m} \times 150$ μm can be formed and serve as the waveguide and positioning

device for the connection fibers. Similarly, the third and fourth substrates each have one open channel ($\sim 125 \mu\text{m}$ deep, $250 \mu\text{m}$ wide) and mirror flipped patterns. When being stacked together, a close channel with $250 \mu\text{m} \times 250 \mu\text{m}$ cross section can be created as a position structure for source fiber.

To form the optical waveguides, all channels were coated with a thin layer of silver ($\sim 300 \text{ nm}$ thick) uniformly using electron-beam evaporation. Transparent optical epoxy is applied to fill the collection waveguides and the fibers are placed inside all the aligning SU-8 channels. The assembly of the probe was essentially stacking and aligning the four substrates and glued together. The proximal ends of the fibers are connected to the OIRS system and the light source via SMA connectors. The completely assembled probe is shown in Fig. 5. The probe has one 45° oblique incidence channel and five collection channels with an overall dimension of $8 \times 2.5 \times 2 \text{ mm}^3$. This small size provides the possibilities for endoscopic applications in the pancreatic duct.

Figure 5: Complete OIRS probe.

TESTING AND RESULTS

The OIRS measurement setup is shown in Fig. 6. It consists of a white light source, a fiber optic probe, an imaging spectrograph coupled with a CCD camera, and a personal computer. The source fiber of the optical probe is connected to the light source, while the collection fibers are connected to the spectrograph. During the OIRS measurement, the probe is gently placed on the surface of the tissue. The incident light is delivered at an oblique angle through the source fiber, and the spatial distribution of the diffuse reflectance ($R(x)$) is acquired by the collection waveguides and transmitted through the collection fibers to the spectrograph. The CCD camera captures the diffuse reflectance spectra (from 455 to 765 nm) from all the collection channels within about one second, which are then stored in the personal computer for future analysis. Before the OIRS measurement, the whole experimental setup was calibrated using a standard liquid phantom with trypan blue dye as the absorbers and polystyrene microspheres as the scattering elements [15]. After calibration, the OIRS probe was used to conduct *ex-vivo* measurements on a freshly excised human pancreas specimen with malignant tumors (Fig. 7). It should be noted that due to the extensive bleeding caused by the surgical excision, the entire pancreatic specimen appears in the same bloody color, which makes it almost impossible to visually differentiate the normal and malignant regions.

Figure 6: Experimental setup for OIRS measurement.

Figure 7: A human pancreas specimen with malignant tumors.

Fig. 8a and 8b show the representative diffuse reflectance spectra (five channels) measured from both the normal and the malignant regions in the pancreas specimen, which clearly show the difference in their optical signatures. To achieve a better understanding of the physiological origin of these differences, the absorption and scattering parameters were extracted from the measured diffuse reflectance spectra. In human tissues, hemoglobin is the major absorber within the visible spectrum.

Figure 8: Diffuse reflectance spectra of human pancreas tissue from *ex-vivo* OIRS measurement: (a) Normal tissue; and (b) Cancer tissue.

As shown in Fig. 7, due to the extensive bleeding caused by the surgical excision, the concentration of the hemoglobin and thus the absorption parameters of both the normal and malignant regions in the pancreatic specimen must have changed significantly from their original values. However, the scattering parameter, which is mainly due to the cell density and the size of the cell nuclei, will not be affected by the bleeding. Previous studies have shown that malignant tissues usually manifest higher optical scattering due to increased cell density and enlarged cell nuclei. Therefore, the relative value of the scattering parameter could serve as a good indicator to differentiate the malignant tissues from the normal ones. To test the feasibility of using OIDRS for malignant tumor margin detection, we made a series of measurements along the opened pancreatic duct (where the pancreatic cancers usually start to develop). Fig. 9 shows the extracted scattering parameters for different locations along the pancreatic duct, which clearly indicate the existence and location of the tumor region. This result matches well with the histological reading of the pancreas specimen.

Figure 9: Average scattering coefficient along the pancreatic duct

CONCLUSIONS

A new miniaturized OIDRS optical sensor probe with side-viewing capability has been successfully developed utilizing micromachining technology. Our preliminary results show that the probe, combined with OIDRS can provide both functional and structural information of tissue malignancy and thus can be a useful tool for rapidly determining a safe margin for the surgical treatment of cancers. The micromachining technology plays a critical role in enabling probe miniaturization and performance enhancement for the clinical application of OIDRS.

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