Optical Fiber Needle to Probe Inside the Body Using Fluorescence Ratio Method

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ABSTRACT

An optical fiber needle probe was developed that can be inserted into a hollow metallic needle for tumor diagnosis using fluorescence at key wavelengths for breast, kidney, liver, and brain. The optical fiber needle probe is based on fluorescence ratio method which will allow to detect tumor in vivo for a real time evaluation. This method will be coupled with other current modalities such as X-ray, ultrasound and MRI.
Keywords: Spectroscopy, Optical Fiber, Breast Tumor, Diagnosis, Biopsy.

1. INTRODUCTION

Native fluorescence spectroscopy offers new techniques for detection and characterization of the physical and chemical changes that occur in diseased tissue, for either *in vivo* or *in vitro* applications. Tissue systems are made up of proteins, nucleic acids, lipids, and water with fluorescing and non-fluorescing chromophors. There are a number of natural fluorophores in cells and tissues fluorescing in the UV and visible region [1,2] such as flavins, tryptophan, tyrosine, nicotinamide adenine dinucleotide (NADH), collagen, and elastin. Native Fluorescence spectroscopy has been applied to study human tissues of different organs including breast [3-9], gynecological [10,11], colon [12-14], and esophageal tissues [15]. Differences in the fluorescence spectral profile and intensity from cancerous and normal tissues were identified, and attributed to either differences in the molecular environment, differences in structure, or differences in concentration of fluorophores in the different types of tissue specimens. There are several intrinsic fluorophores in tissues responsible for these fingerprints. The 340 nm emission, with 290 nm excitation is attributed most likely to the emission from tryptophan, the 380 nm emission band, with 340 nm excitation is from elastin and collagen, and the 460 nm emission band is from NADH.

Fluorescence emission and excitation spectroscopy of breast tissues has been studied for many years. It was found that when tissues are excited with UV or visible light, the native fluorescence spectra are significant difference among cancerous, benign or normal tissue [3,4,5,7,8]. When breast tissue is excited at 300 nm and the emission is observed in the region from 320 to 580 nm, differences between cancerous and normal tissue are clearly observed [7,9]. It is important to note that the spectrum from benign tumor appears similar to normal tissue rather than appearing malignant. As a result the fluorescence spectroscopy techniques have been developed to effectively distinguish malignant tumor from benign tissue.

Based on an analysis of spectral profile and relative intensities, it has been determined that the intensity ratios at certain crucial wavelengths is a highly accurate criterion for predicting the presence of malignancy [7]. The existence of these key wavelengths makes it possible to optically detect tumor, using a small set of optical filters to measure emission at the key wavelengths rather than measuring the entire spectrum.

In this paper, we will describe an optical fiber fluorescence ratiometer instrument which measures the fluorescence intensity ratios at key wavelengths. We have developed a suitable optical fiber needle probe that can be inserted freely into a hollow stereotactic needle system and probe tissues to deep to be interrogated by surface probes. The optical fiber needle based ratiometer technique allows for real time tissue evaluation and tumor detection *in vivo*. This technique will help target tissues for fine needle aspiration biopsy and reduce the number of false positive biopsies.

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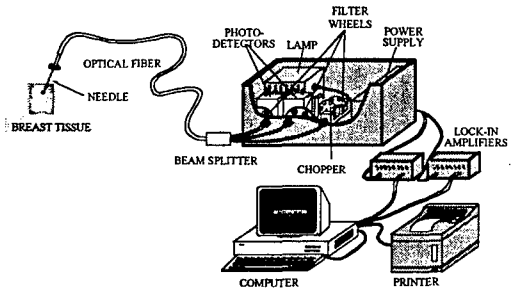
2. TECHNIQUES AND METHODS

The fluorescence spectroscopic ratiometer with an optical fiber needle is shown in Fig.1. The main components of the ratiometer are: (1) broadband xenon excitation lamp, (2) computer controlled filter wheels for selection of excitation and detection wavelengths, (3) UV transmitting optical fibers and collimators to deliver the excitation light to the tissue, and collect the emitted fluorescence, (4) photo multiplier tubes with dual channel phase sensitive detection, and (5) personnel computer to control instrumentation and analyze data.

The optical fiber needle probe consists of a 270 μm core fiber terminated with a 30 cm rigid metal jacket length for easy insertion into a hollow stereotactic needle. The front surface of the probe terminates with a normal fiber surface for directing excitation light and collecting fluorescence. The power density was about $0.13\mu W/cm^2$.



The two fibers are directed to the two emission filter wheels. The light exiting the fibers is collimated and passes through the filter wheels. The transmitted and selected wavelengths are incident on two photo multiplier tubes. Stepping motors rotate the filter wheels to the correct filter position. Each stepping motor is independently computer controlled by the ratiometer software.

The two photo multiplier tubes convert the optical signal to the electrical signal, with the current being proportional to the optical signal intensity. The electrical signals are directed to the two lock-in amplifiers. A lock-in amplifier is a phase sensitive detector at a specific frequency, while rejecting all other signals. A personal computer is used to control the instrumentation, to process the amplified signals, and to display and store the ratio data.

3. EXPERIMENTAL RESULTS

3.1. Simulation with layered tissue

A simulation of the ability to detect tumor tissue imbedded in normal tissue, such as a small tumor inside a human breast, was performed to investigate problems which may be arise during an in vivo examination. Some potential problem areas include deformation of the needle probe or deposition of body fluids on the probe surface. It may also be important to understand how the interface between tissue types could effect measurements. This simulation was created by "sandwiching" a cancer sample between two normal tissues, as displayed in Fig. 2. The optical fiber needle was mounted on a three-axis translation stage with 10 μm resolution. The holder is shown in Fig. 3. The fluorescence intensity ratios of 340 nm to 440 nm, with 300 nm excitation, were measured as a function of penetration depth. The total sample thickness was about 12 mm. Measurements were taken at 0.2 mm intervals. The intensity ratios are plotted as a function of penetration depth in Fig. 4. The sharp transition between the normal and malignant tissue regions is clearly evident in Fig 4 . The average value of the I_{340}/I_{440} ratio for the three tissue layers is shown in Fig. 5. From Figs. 4 and 5, one can clearly distinguish the cancerous and normal breast tissue layers. These results demonstrate the potential ability of the fluorescence ratiometer with an optical fiber needle probe to distinguish between normal and abnormal tissues in the human body.

3.2. Differentiating fat from tumor with multiple wavelength ratios

It was observed that adipose tissue also exhibited a high I_{340}/I_{440} ratio leading to the possibility that adipose tissue may falsely be classified as malignant. This issue has been investigated by Yang et. al.[8,9] in which multiple intensity ratios were used to correctly distinguish among normal, adipose and malignant tissue. In this study, spectral differences were distinguish adipose from malignant tissue. The first algorithm was based on the 340 nm emission intensity with 289 nm and 271 nm excitation and the second was based on the 460 and 520 nm emission intensities with 340 nm excitation.

The application of these algorithms for use with the ratiometer was tested by measuring the 340 nm emission for 271 and 289 nm excitation, and the 460 nm and 520 nm emission for 340 nm excitation at 18 different locations on the adipose sample, malignant, and normal sample. For the normal tissue, the average values of I_{289}/I_{271} and I_{460}/I_{520} were 0.509 ± 0.021, and 1.055 ± 0.025, respectively. For the fat sample, the corresponding average ratio values were 0.872 ± 0.097 and 1.314 ± 0.036;

and for the cancer samples, the average ratio values were 0.445 ± 0.051 and 1.655 ± 0.024. The I_{460}/I_{520} ratio from the cancer sample was significantly higher than the ratios from either normal or fat tissue while the I_{289}/I_{271} ratio from fat tissue was higher than the corresponding ratios from the normal and cancer tissues. For the normal specimens, the fluctuations in the ratio were less than 5%. For the cancer and fat samples, the fluctuations were less than 12%. These results are summarized in Table 1. These measurements indicate that the I_{289}/I_{271} and I_{460}/I_{520} ratios could effectively distinguish cancerous tissue from fat tissue.

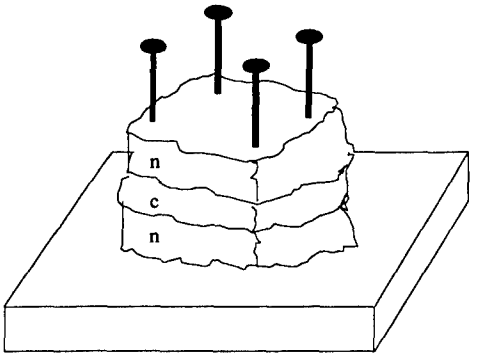


Fig. 2 Simulated tissue layer structure formed by "sandwiching" cancer tissue between two normal tissues.

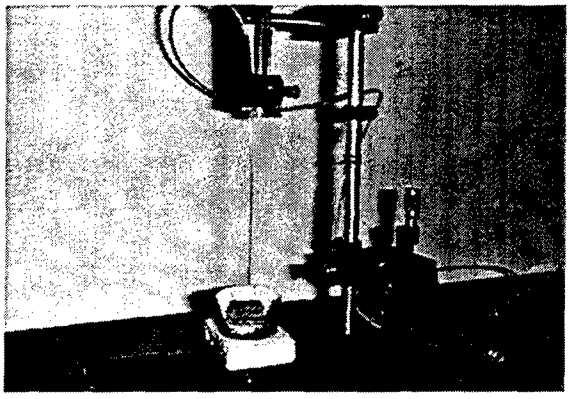


Fig. 3 Fluorescence ratios at wavelengths 340 and 440 nm for depth measurements of tissues.

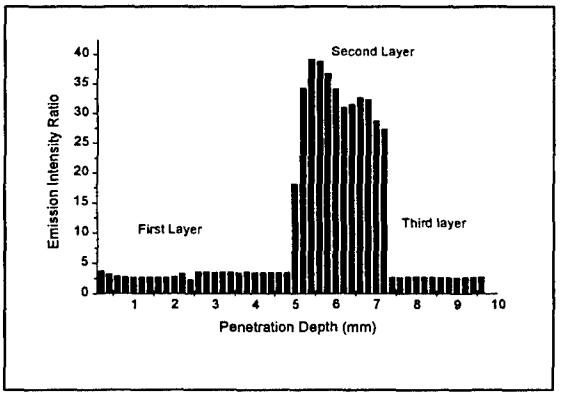


Fig. 4 Fluorescence ratios at wavelengths 340 and 440 nm for depth measurements of tissues.

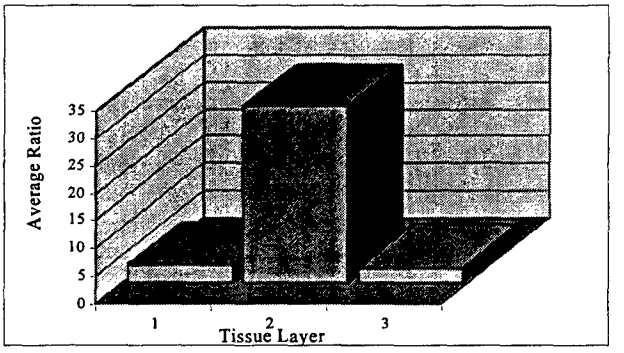


Fig. 5 Average value of 340/440 ratios for the three layers in the normal-cancer-normal sample.

Table 1 Summary of the 289/271 excitation ratio (emission at 340 nm) and the 460/520 emission ratio (excitation at 340 nm)

	289/271	460/520
Normal	0.509 ± 0.021	1.055 ± 0.025
Fat	0.872 ± 0.097	1.314 ±0.036
cancer	0.445 ±0.051	1.655 ± 0.024

4. DISCUSSION

Since diameter of the optical fiber is relatively small, the interrogated area was small and therefore the measured ratios were subjected to local variations in tissue state. The interrogated area was on the order of 300 to 500 μm. The variation in ratio

measurements may be related to the concentration of cancer cells, with higher ratios coming from locations with a higher concentration of malignant cells.

The fluorescence intensity ratio of 340 nm to 440 nm was a criterion to identify malignancy from benign and normal tissues. A multiple wavelength detection method has been introduced into the ratiometer to increase the identification capability of cancer from the benign and normal, specifically to separate cancer from fat. The ratiometer not only can distinguish between cancer and the normal, but also can identify cancer from fat.

In conclusion, there is a need to develop methods to probe deep into organs, such as a tumor inside breast, for a real time evaluation and diagnosis of tumor in vivo. An optical fiber needle based ratiometer, which is based on native spectroscopic differences between diseased and normal tissues, has been designed, assembled, and tested. A key point of the research project is using an optical fiber needle as a probe to delivery excitation light on tumor and collect the fluorescence from that tumor region, with a small diameter of about 300 μm, which can go through the organ and arrive at the inside tumor. Cancerous, benign/normal breast tissues have been measured and distinguished by the ratiometer *in vitro*. The simulation measurements of a tumor inside breast were performed using layering samples. Multiple wavelengths have been introduced to the optical fiber needle based ratiometer for increasing the capabilities to separate fat from cancer. The measurement results have indicated that the ratiometer can identify different tissue types reading fluorescence intensity ratio at two specified wavelengths. This research demonstrates the technological and commercial potentials of the optical fiber needle based ratiometer and *in situ* spectroscopy to reduce or replace breast biopsy and to obtain information from other in assemble body organ locations without surgical biopsy.

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