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AUTHORITY
USAMRMC ltr dtd 4 Jan 2000
A optical fiber needle based CD-ratiometer using native spectroscopic differences between diseased and normal tissues, has been designed, assembled, and tested for real time evaluation and diagnosis of breast tumor \textit{in vivo}.. A key point of the research project is using an optical fiber needle as a probe to deliver the excitation light to a tumor and collect the fluorescence from that region. The probe, with a small diameter of about 300 \( \mu \text{m} \), can penetrate through the organ and interrogate tumor. Cancerous, benign/normal breast tissues have been measured and distinguished by the CD-ratiometer \textit{in vitro}.. Simulated measurements of a tumor inside normal tissue were performed using layered samples. Multiple wavelengths have been introduced to the optic fiber needle based CD-ratiometer for increased capability to separate fat from cancer. The measurement results have indicated that the CD-ratiometer can identify different tissue types by reading fluorescence intensity ratios at specified wavelength pairs. This research demonstrates the technological and commercial potentials of the optic fiber needle based CD-ratiometer for \textit{in situ} spectroscopic analysis to reduce or replace breast biopsy.
AWARD NUMBER DAMD17-94-C-4081

TITLE: Fluorescence Optic Fiber Stereotactic Needle Ratiometer for Breast Tumor Diagnosis

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Fort Detrick, Maryland 21702-5012

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FLUORESCENCE OPTIC FIBER STEREOTACTIC NEEDLE RATIO METER FOR BREAST TUMOR DIAGNOSIS

I. Introduction

1. Research Subject

This Final Report documents the research work performed by Mediscience Technology Corp (MTC) on its project to develop instrumentation for doing spectroscopic analysis of breast tissue for the purpose of detecting malignancy. This work was performed under Grant DAMD17-94-C-4081. This report will describe the spectroscopic research performed on in vitro breast tissue which was formed the basis of developing the algorithms which will be used to distinguish cancerous from non-malignant tissue by fluorescence measurements. This report will also review the progress in instrument development. In this report, the results of spectroscopy measurements on in vitro breast tissue, and design and performance specifications of the optical detector instrumentation will be discussed.

2. Purpose of Research

It is well known that mammography screening of asymptomatic women can provide a method for early detection of breast cancer, with a subsequent reduction in mortality. Despite mammography’s ability to identify tumors, benign and malignant lesions may have similar morphology [1]. Often, biopsy is the only certain way to distinguish between benign and malignant tumors. Biopsy is a surgical procedure carried out in a hospital; it is tedious, time-consuming, and expensive. Most biopsies resulting from suspicious mammograms are found to be benign [2]. Without a visible lesion, the possibility for specimen sample error is considerable. Therefore, the need to develop a real time, in situ breast examination procedure is of critical importance. Fluorescence spectroscopy may play an important role in such an examination by monitoring tissue change, before, during and after chemo- and radiation therapy, and give information on images, without surgical procedures and side effects.

The object of this research is to develop spectroscopic instrumentation for breast tumor diagnosis. Successful implementation of this instrumentation depends upon both the identification of the spectral features which can distinguish cancerous from non-cancerous tissue, and the construction of a device which will allow these measurements to be performed in a clinical setting, with minimal discomfort to the patient. One of the aims of this project was to study the spectroscopy of in vitro breast tissue in order to determine the spectroscopic differences between normal, pre-malignant and malignant tissue. This work was then to be extended to clinical trials in collaboration with Dr. Daniel Kopans of the Department of Radiology at Massachusetts General Hospital. The needle probe CD-Ratiometer would be used during a stereotactic needle biopsy procedure to help characterize tissue prior to biopsy, and to better define the margins of tumorous regions.

The CD-Ratiometer is shown conceptually in Fig. 1. In a needle biopsy procedure, whenever, either, an ultrasound or X-ray mammogram indicates a suspicious mass, a stereotactic
imaging system guides a hollow needle to the mass. When the needle is properly position, cell samples are removed and sent for histopathology. If the pathology results indicate malignancy, the mass is then surgically removed. With the CD-Ratiometer, the fluorescence signature of the tissue will be measured as the needle is inserted inside the breast. The fluorescence results will be immediately analyzed, and the information relayed to the examining physician in real-time. In addition to providing immediate results as to whether a tissue is malignant, the spectroscopic information can also give details on the size of the tumor.

The preliminary research indicated that the most effective wavelengths for cancer detection were in the ultraviolet spectral region. Therefore clinical trials were put on hold until the potential risk from the UV exposure could be evaluated. The current status of Mediscience Technology Corp's (MTC) clinical trials and the Food and Drug Administration (FDA) decisions as to MTC's instrumentation will be discussed in detail in the section on CD-Scan, CD-Ratiometer and Potential UV Exposure Risk.

3. Scope of Research

Over the past several years, MTC has designed and developed prototype diagnostic optical spectroscopy based instrumentation. MTC has supported research aimed at determining spectroscopic signatures which will enable the discrimination of malignant from non-malignant tissue by spectroscopy. This research, performed at the New York State Center for Advanced Technology at The City College of the City University of New York (CCNY), Memorial Sloan Kettering Cancer Center (MSKCC), and New York Hospital – Cornell Medical Center, has investigated in vivo, ex vivo and animal tissue. MTC has a collaborative relationship with Dr. Daniel Kopans, Dept. of Radiology at Massachusetts General Hospital. Dr. Kopans is actively involved in stereotactic needle breast biopsy research and has a strong interest in adding spectroscopic measurements to the procedure.

Spectroscopic research on tissue

Native fluorescence spectroscopy offers new techniques for the detection and characterization of the physical and chemical changes which occur in either in vivo or in vitro diseased tissue. Tissue systems are made up of proteins, nucleic acids, lipids, and water with both fluorescing and non-fluorescing chromophores. Many systems are known to luminesce in the UV and visible fluorescence regions [3,4]. There are a number of natural fluorophors in cells and tissues fluorescing in the visible region such as flavins, tryptophan, tyrosine, NADH (nicotinamide adenine dinucleotide), collagen, etc. Flavin and NADH are known to exhibit spectral changes when transforming from the oxidized to reduced state. Riboflavin is known to fluoresce in the visible spectral region. The absorption and emission spectra of some of these fluorophores are shown in Figs. 2 and 3, respectively. Our goal is to develop native fluorescence spectroscopic techniques for cancer diagnosis. The use of native fluorescence will eliminate the invasiveness of injecting dyes into patients.

Two types of fluorescence measurements are possible. In emission spectrum measurements, the excitation light is held at a fixed wavelength, and the emitted intensity is measured over a range of emission wavelengths. In excitation spectrum measurements, the
emission intensity is held at a fixed wavelength, and this emitted intensity is measured over a range of excitation wavelengths. Excitation spectrum requires a broadband light source (i.e. a lamp) while an emission spectrum only needs a single excitation wavelength and thus either a lamp or laser can be used as an excitation source. A third type of measurement is also possible, referred to as "synchroscan" or diffuse reflective spectrum. In synchroscan measurements, the excitation and emission wavelengths are varied simultaneously, keeping both wavelengths equal. This type of scan gives information about the optical scattering and absorption properties of a sample. In complicated samples, which may contain multiple fluorophores such as tissue, the use of multiple measurements can improve the ability to differentiate samples.

MTC has supported spectroscopic research on tissues of many different origins, including breast, oral cavity, digestive tract, gynecological tract, and colon. These measurements were performed at CCNY and MSKCC under grants from MTC. As part of this project, extensive optical measurements were performed on ex vivo breast tissues at CCNY. Analysis of this data led to the development of algorithms which could distinguish malignant from non-malignant breast tissue.

**Instrument development**

MTC has several generations of optical spectroscopy instrumentation under development. The first generation device, named the CD-Scan, is a lamp based fluorescence spectrometer with computer controlled data collection and data analysis software. A variety of fiber optic probes have been designed for different organs. This device is capable of performing emission, excitation and synchroscan spectral measurements in the visible and ultraviolet spectral regions from about 250 nm to 600 nm. This device can measure a series of complete spectra for a predefined range of excitation and emission wavelengths. The rich amount of data produced by the CD-Scan is as the basis for developing diagnostic algorithms. However, each spectral curve requires about 1 minute for data collection, including instrument overhead. If many sites must be scanned, with each site requiring multiple spectra, the examination time may become prohibitive long. Therefore MTC began the development of the next generation of diagnostic instrumentation, called the CD-Ratiometer. The CD-Ratiometer exploits the fact that the ratios of emitted intensity at key wavelengths is a very good diagnostic indicator. This eliminated the need to measure the entire spectra from a tissue site. The CD-Ratiometer is a dual channel optical detector, which, instead of measuring complete spectra, simultaneously measures and ratios the emission intensity at two discrete wavelengths. The excitation and emission wavelengths are tissue type specific, and chosen for their diagnostic potential. This reduces the measurement time from about one minute to about 2-4 seconds per ratio. Different emission and excitation combinations are available and can be selected by the physician during examination time. The CD-Ratiometer can be equipped with different fiber optic probes in order to deliver and collect light from different organs. Probes for the oral cavity, digestive tract and colon are available. Colon and digestive tract probes were engineered to fit in the working channel of an endoscope.

For this research project, a special fiber optic probe was developed which fits inside a hollow needle for insertion during stereotactic needle biopsy procedures. If a suspicious object is observed by mammography or ultrasound, a stereotactic imaging system guides the hollow needle, containing the fiber optic probe, through the breast to the suspicious area. Fluorescence
measurements are performed with the results displayed in real time. This unit is coupled to CD-Ratiometer. If the spectroscopic results indicate malignant tissue, the probe is removed and a needle forceps is inserted to remove a tissue sample for histopathology. Variations on this procedure are possible, including, performing continual fluorescence measurements as the needle is inserted. This may assist in accurately determining the margins of the tumor.

The small diameter of the fiber optic needle probe created signal to noise problems which required design modification to the CD-Ratiometer. These design changes are addressed in this report.

4. Background

Over the past decade, a large number of groups [5-23] have demonstrated that optical spectroscopy has the potential to diagnose pathological changes in human tissues. Major progress has been made in using fluorescence spectroscopy from native fluorophores emitting in the visible and ultraviolet spectral regions to detect disease. Some of the important fluorophores in tissue are tryptophan, collagen, elastin, NADH and flavins. Fluorescence spectroscopy studies were first performed on rat cancer and normal tissue [5] and later extended to human tissues using laser excitation. Spectral differences were observed [5,6]. These differences in the fluorescence spectra between cancerous and normal tissues were attributed to transformations in the local environment surrounding the fluorophores in normal and cancerous tissue [7]. The technique was extended further to distinguish between normal and neoplastic tissue [6] in the human breast and lung. The statistical rate of success [8] was around 86%. This work led to development of instruments by Mediscience Technology Corp, such as the CD-Scan™ for tumor tissue detection. Subsequently, excitation spectra [9], emission spectra under picosecond pulse excitation [10], and time resolved fluorescence decay spectra [9] from normal and cancerous human breast tissues were investigated by workers at CCNY. Later, this approach was used to distinguish malignant from non-malignant human breast tissue [11]. The reported sensitivity and specificity was over 90%. This work was extended to study malignant and nonmalignant gynecological tract tissue [12] with rate of statistical success of over 93%. Current measurements indicate that the combined use of emission, excitation, and scattering spectra, with multiple wavelengths, can increase diagnostic accuracy of breast cancer tissue to 95% [13-15].

Numerous other research groups have had similar success. Here, we will review some of these results. Fluorescence techniques have had success in distinguishing atherosclerotic plaque from the normal aorta [16]. The study was done on 91 normal and 91 atherosclerotic specimens with an overall classification accuracy of 95%. Human colonic mucosa was studied [17] with 325 nm laser excitation showing 100% satisfaction over the 34 normal specimens and 16 adenomatous polyps, and 94% accuracy for classifying hyperplastic polyps (non-adenomatous) as normal mucosa. A study on colonic tissues in vivo and in vitro [18] using 337-nm laser excitation has shown success a ratio of 77% to 86% for distinguishing between hyperplastic and adenomatous polyps. These rates are greater than evaluating polyps based on visual assessment and are nearly comparable to the clinical pathologist assessments of colonic polyps. It was reported [19] that fluorescence peaks at 630 nm and 690 nm from intrinsic porphyrin compounds could be used to diagnose tumors. This group studied tissues both in vitro and in vivo from the oral cavity, esophagus and stomach, reporting 89% accuracy from 100 oral tumor cases. A
research group from Sweden has done extensive work on native and dye fluorescence molecules in normal and malignant tissues [20-22]. They investigated fluorescence spectra from various rodent tissues and human skin tumor samples [21] and from human aorta [20,22]. A Canadian group studied autofluorescence of normal and malignant bronchial tissue [23]. Bladder cancer was also studied using the fluorescence technique [24]. Most recently, the fluorescence spectroscopic technique has been extended to \textit{in vivo} studies [25-26].

5. Experiment Basis of the Project

As part of Mediscience Technology’s ongoing research effort in developing technology for optical diagnosis of tissue, MTC has supported spectroscopic research on \textit{ex vivo} human breast tissue. This work was performed at the Mediphotonics Laboratory at The City College of New York. Although not part of this contract, this work provided the experimental data to demonstrate that fluorescence emission and/or excitation intensity ratios can reliably identify the state of tissue. The effectiveness of tissue diagnostics by using intensity ratios provided the basis for developing the CD-Ratiometer. These spectroscopic results will be reviewed in this section.

Breast tissue is a complicated system consisting of adipose, glandular and fibrous tissues and, potentially, undesired tumors with underlying muscle. Since the spectral features are expected to vary from different tissue types found in the breast, the spectroscopic research covered a large range of excitation and emission wavelengths. The goal of this work was to develop algorithms based on multiple spectral measurements which would allow an accurate determination of malignancy as well as classification of the different types of breast tissue.

Another issue to address in developing diagnostic algorithms is the effect of blood absorption on the fluorescence spectra. Blood content can vary significantly in a breast, increasing sharply when bleeding is present. Blood does not fluoresce, however, its strong, broad band, absorption can reduced signal strength by absorbing the excitation light (photons absorbed by blood do not excite tissue) or distort the spectrum by preferentially absorbing different wavelengths of emitted light. The effects of variation in blood content can be minimized by measuring emission ratios at wavelengths where blood absorption is nearly equivalent. For example, blood absorption is about the same at 340 nm and 440 nm. Thus, when the 340 / 440 nm wavelength pair is selected, the effects of blood concentration on the their ratio is minimized.

Typical \textit{ex vivo} emission spectra from cancerous and normal human breast tissue are shown in the left side of Fig. 4. The samples were excited at 300 nm and scanned from 320 to 580 nm. The curves were normalized to be equal height at 340 nm. Referring to Fig. 4, a dominant fluorescence band centered at 340 nm and a weaker band at about 450 nm are visible. The 340 nm fluorescence is most likely attributed to emission from tryptophan, while the 460 nm band may be emitted from a combination of NADH, elastin, and collagen. The intensity of the 450 emission band, relative to the 340 band, is significantly lower for cancerous tissue than for non-cancerous tissue. It has been found, experimentally, from a large number of samples with different origins, that the emitted intensity at 440 nm relative to the emission at 340 nm is significantly lower for cancerous tissue than non-cancerous tissue. This would imply that the emission intensity ratio at 340 nm to 440 nm could be an effective classifier between cancerous and normal tissue with the cancer tissue having a significantly higher ratio of 340 nm to 440 nm emission. The results of spectral measurements on 40 malignant and 47 non-malignant (either
normal or benign tumor) samples is summarized in the histograms shown in Fig. 4. The average value for the ratios for cancerous tumor tissues was about 12.6, while for normal and benign tumor tissues, the ratio was about 4.8. Using a cutoff value of 9, 37 of 40 malignant and 46 or 47 non-malignant tumors were successfully identified for a sensitivity of 92.5% and a specificity of 98%. All tissue diagnoses were confirmed by histopathology. These results clearly show a separation between cancerous and non-cancerous tissues, at least in vitro.

Although the 340 /440 nm ratio is effective in distinguishing cancerous breast tissue from normal, it is not as effective in correctly separating cancer from fat tissue. Extensive emission, excitation and synchronous scan measurements performed on large numbers of ex vivo breast tissue have identified additional wavelength ratios which could effectively separate fat tissue from cancer, as well as provide further corrections for blood content [9, 14, 15].

For example, the ratio of the synchronous scan signal at 520 nm to the signal at 460 nm, is about twice as large for fat tissue than for normal or cancerous tissue. Another useful measurement is the excitation spectrum scanned from 250 nm to 320 nm, with the emission fixed at 340 nm [15]. Fig. 5 shows the averaged excitation spectra for 63 benign and 103 malignant breast tissue samples. The ratio of the 340 nm emission intensity, when excited at 289 nm, to the 340 emission intensity when excited at 268 nm accurately separates malignant from benign breast tissue. The capability to measure both of these ratios has been added to the CD-Ratiometer.

II. Body of Report

1. CD-Ratiometer Design

In this section, the technical design of the instrument and experimental methods in the project are described.

The objective of the research program is to develop a clinically useful diagnostic instrument using fluorescence technology to detect and monitor breast tumor characteristics in vivo, in real time. This instrument, called The Optical Fiber Needle Based CD-Ratiometer, can measure the ratio of fluorescence intensity at pre-defined wavelengths using an optic fiber needle probe inserted into in vivo or in vitro tissue.

The CD-Ratiometer is shown in Figs. 6. The main components are:

A personnel computer to control the instrumentation and collect, analysis, display and store data.

A broadband light source to provide the excitation light. The CD-Ratiometer uses a 175 Watt xenon lamp with UV transmitting optics. The lamp was selected to maximum the coupling of light into the excitation fiber.

A mechanical chopper to provide a low frequency modulation to the excitation light.

Rotating filter wheels for wavelength selection. Three rotating filter wheels are used to provide wavelength selection. Each wheel can contain up to eight interference filters. One wheel is placed in the excitation light path to allow selection of excitation wavelength. The other two
filter wheels are positioned in front of the two optical detectors. These filter wheels select the emission wavelength intensities to be measured. The filter wheel positions are computer controlled and predefined wavelength combinations are provided for ease of use. The three filter wheel combinations are shown in Fig. 7.

Optical fibers and collimators for delivering the excitation light to the sample and for collecting the emitted light.

Optical beam splitter assembly for directing the excitation light to the sample site and directing the emitted light to the photo detectors.

The optical detectors consist of two photomultiplier tubes for detection of the emitted light.

Dual channel lock-in amplifiers for data processing and analysis. The lock-in amplifiers are mounted inside the controlling computer. A lock-in amplifier is a phase sensitive detector and will be tuned to the modulation frequency of the excitation light. The lock-in amplifier digitizes and integrates the optical signal. All lock-in amplifier functions and data processing are controlled via software on the host computer.

The CD-Ratiometer functions as follows:

Light from a broadband xenon source is focused into the first fused silica delivery fiber with a core diameter of 400 μm. The output of this fiber is collimated and passed through the excitation filter wheel. Interference filters on the excitation filter wheel transmit only the selected excitation wavelength. The full width at half maximum (fwhm) band pass of these filters is 10 nm. The beam is then passed through the mechanical chopper, which imparts a low frequency (400 Hz) modulation to the excitation light. A reference signal conveys the frequency and phase information of the modulation to both channels of the lock-in amplifiers. The beam is focused into a second fused silica fiber (400 μm diameter core) and transmitted to the beam splitter assembly. The beam splitter assembly consists of two beam splitters and four fiber input/output ports. One fiber port is for the excitation light, two fiber ports are for each detector and the fourth fiber port connects to the needle probe. The output of excitation fiber is recollimated and reflected by the forward beam splitter. It is then focused and directed to the needle probe.

The 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. Two types of probes have been tested. The front surface fiber probe terminates with a normal fiber surface for direct illumination and collection. The side firing probe terminates with a right angle prism for side illumination. The fiber jacket and needle are cut with a groove to allow transverse fluorescence measurements as the needle is inserted into the tissue. These front firing and side firing probe designs are shown in Figs. 8 and 9, respectively.

The light emitted by the tissue is collected by the needle probe and redirected back to the beam splitter assembly. The emitted light is recollimated and transmitted through the first beam splitter. At the second beam splitter the light is split into two beams. Each beam is refocused into an optical fiber and routed to each of the photo detectors. Broadband beam splitter coatings are used to provide excitation and emission wavelength tunability.

These 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 wavelengths are incident on two photo multiplier tubes. Each filter wheel has positions for up to eight filters. Stepping motors rotate the filter wheels to the correct filter position. Each stepping motor is independently computer controlled by the CD-Ratiometer software.

The two photo multiplier tubes convert the optical signal to an electrical signal, with the current being proportional to the optical power. The electrical signals are directed to the two lock-in amplifiers. Each lock-in amplifier consists of external pre-amplifier and a printed circuit board designed to fit in an ISA slot of a Personnel Computer. A lock-in amplifier is a phase sensitive detector designed to amplify signals at a specific frequency and phase, while rejecting all other signals. The lock-in amplifiers receive the phase and frequency information from a reference signal generated by the optical chopper in the excitation beam path. This is a very effective tool for blocking noise signals generated in the detection system or by stray light. However, it can not eliminate spurious signals generated by the excitation beam since these would carry the same modulation as the desired signal. The lock-in amplifier parameters, including gain, pre-amplifier time constant, integration time and processing of error messages is all handle by the controlling software. The analog to digital conversion and signal integration is handled on the PC board.

In addition to the two lock-in amplifier mounted on PC boards, the CD-Ratiometer contains a custom designed control board which positions the filter wheels and activates an excitation beam shutter assembly.

Software was developed to integrate control of all CD-Ratiometer functions. This software was designed to be user friendly (i.e. can be operated by a physician or technician with a minimum amount of training). While the software offers full control of the unit, it is not necessary to fully understand all features of the unit in order to operate the unit. The developed software contains calibration functions, error correction routines and a system self test. The current software is MS-DOS® based, but will operate in an MS-DOS® prompt under a Windows® environment.

The CD-Ratiometer design allows measuring both emission and excitation spectral ratios. For emission spectra, each detector filter wheel is tuned to one of the desired wavelengths and the emission intensities are simultaneously measured. This procedure makes the measurement insensitive to fluctuations in excitation lamp power output or movement of the probe. For excitation measurements, one of the detector filter wheels is tuned to desired emission wavelength and the excitation filter wheel is rotated to the first excitation wavelength and the emitted intensity measured and stored. Next the excitation filter wheel is turned to the second excitation wavelength and the emitted intensity is measured. Since the spectral output of the lamp depends on wavelength, the output power at each excitation wavelength must be previously measured and stored in the computer. Using these values, the excitation spectral ratio is computed.

2. CD-Ratiometer Performance Characteristics

The CD-Ratiometer was extensively tested on standard samples, animal tissue and ex vivo human tissue. The standard samples were chosen which have absorption and emission
characteristics similar to tissue but which have spatial uniformity and do not degrade over time. The animal tissues (beef and chicken) were acquired from a local supermarket and were selected for their availability and structure. Chicken tissue is highly uniform, from a spectroscopic perspective, which allowed for testing the effects of geometric factors and for comparison of results with other instrumentation. This uniformity alleviated the need for precise geometric alignment when comparing measurements made with different types of instruments. Beef tissue provided a layered structure (mostly muscle and fat) which is a good model to study how the CD-Ratiometer will perform when the needle probe passes through different types of tissue.

Power levels

Excitation power levels were measured at 300 nm and summarized in Table 1 for a 400 μm core fiber and the 270 μm core fiber used in the stereotactic needle fiber designed for this project. Note that the duty cycle of the chopper is 50% and therefore the average power is ½ the peak power. Power density measurements assume that the exposure area is equal to the fiber aperture.

<table>
<thead>
<tr>
<th>CD-Ratiometer (300 nm Narrow Band Filter)</th>
<th>400 μm Core Fiber</th>
<th>270 μm Core Needle Fiber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Power μW</td>
<td>0.168</td>
<td>0.072</td>
</tr>
<tr>
<td>Power Density μW/cm²</td>
<td>0.13</td>
<td>0.126</td>
</tr>
</tbody>
</table>

Table 1. CD-Ratiometer excitation power at 300 nm.

Signal-to-noise ratio and background levels

The signal-to-noise ratio and background levels from the CD-Ratiometer were measured using a single layer of parafilm as a sample. The signal strength from parafilm is comparable to the signal levels from tissue. The signal-to-noise ratio and background levels were measured at 340 nm and 440 nm emission under 300 nm excitation. These wavelengths were chosen based on the significance of the 340/440 ratio as a cancer diagnostic marker as determined by prior research. The measurements were performed with a time constant of 33 milliseconds and an integration time of 12 seconds. The background levels are primarily the result of leakage through the interference filters and fluorescence generated in the fibers. Both of these spurious signals will have the same modulation as the desired signal and therefore can not be eliminated by the lock-in amplifier. Excitation light leakage can consist of both 300 nm light leaking through the detector filters and/or lamp light at the emission wavelengths leaking through the excitation filter. The use of multiple filters can reduce light leakage at the cost of reducing total overall signal. At 340 nm, the signal-to-noise ratio is 350 while the signal-to-background ratio is 19. At 440 nm the signal-to-noise ratio and signal-to-background levels are both 9. The higher leakage level at 340 nm is expected, since it is closer in wavelength to the 300 nm excitation. The poorer signal-to-noise and signal-to-background ratios at 440 nm are the mostly the result of the weaker signal level at 440 nm. The results are summarized in Table 2. To improve the S/N, a new design
concept was implemented.

<table>
<thead>
<tr>
<th></th>
<th>340 nm Emission V ± σ (mV)</th>
<th>440 nm Emission V ± σ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background (No Sample)</td>
<td>2.02 ± 0.02</td>
<td>0.06 ± 0.04</td>
</tr>
<tr>
<td>Single Layer Parafilm (300 nm excitation)</td>
<td>38.57 ± 0.11</td>
<td>0.54 ± 0.06</td>
</tr>
<tr>
<td>Signal/Noise</td>
<td>350</td>
<td>9</td>
</tr>
<tr>
<td>Signal/Background</td>
<td>19</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 2. Signal-to-noise and background levels of CD-Ratiometer.

3. New CD-Ratiometer Design

The CD-Ratiometer was initially developed for applications in the aerodigestive tract and gynecological tract. As such the units were designed to work with a relative large diameter, trifurcated fiber optic probe. In addition to being larger diameter, the trifurcated probe provided one fiber channel for excitation and two for emission, without the need for beam splitters. The needle based probe introduced considerably larger losses into the system from both the smaller diameter and the use of beam splitters. This substantially degraded system performance. It was decided that the most effective method for compensating for the increased losses was to improve the optical coupling between the xenon lamp and the excitation fiber. A breadboard CD-Ratiometer was constructed and testing using a xenon lamp with a smaller gap between the electrodes. This allowed tighter focusing of the excitation light and hence better coupling into the fiber. The new CD-Ratiometer breadboard design is shown in Fig. 10. Most of the other components were not changed. This new lamp improved the output power by a factor of about 30. The power output of the old and new CD-Ratiometers are shown in Table 3 for 300 nm excitation.

<table>
<thead>
<tr>
<th>System</th>
<th>400 μm Core Fiber</th>
<th>270 μm Core, 24 Gauge Needle Fiber</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Power (μW)</td>
<td>Power Density (μW/cm²)</td>
</tr>
<tr>
<td>Old CD-Ratiometer</td>
<td>0.168</td>
<td>0.13</td>
</tr>
<tr>
<td>New CD-Ratiometer</td>
<td>5.73</td>
<td>4.56</td>
</tr>
</tbody>
</table>

Table 3. Power output of old and new CD-Ratiometer designs at 300 nm.

The increase in output power also increased the signal-to-noise ratio and signal-to-background ratio. These results are summarized in Table 4. Lock-in time constant was 33 milliseconds with a 12 second integration time. The sensitivity of the new unit should be about 7 times better than the old unit, and the precision should be about 20 times better. We are currently placing the new CD-Ratiometer in a casing.
<table>
<thead>
<tr>
<th></th>
<th>Old CD-Ratiometer</th>
<th>New CD-Ratiometer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>340 nm</td>
<td>440 nm</td>
</tr>
<tr>
<td></td>
<td>V ± σ (mV)</td>
<td>V ± σ (mV)</td>
</tr>
<tr>
<td>Background (No Sample)</td>
<td>2.02 ± 0.02</td>
<td>0.06 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>4.93 ± 0.05</td>
<td>1.36 ± 0.14</td>
</tr>
<tr>
<td>Single Layer Parafilm (300 nm excitation)</td>
<td>38.57 ± 0.11</td>
<td>0.54 ± 0.06</td>
</tr>
<tr>
<td>Signal/Noise</td>
<td>350</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>953</td>
<td>242</td>
</tr>
<tr>
<td>Signal/Background</td>
<td>19</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>128</td>
<td>64</td>
</tr>
</tbody>
</table>

Table 4. Signal levels from old and new CD-Ratiometers.

4. CD-Scan, CD-Ratiometer and Potential UV Exposure Risk

The first generation instrument developed by MTC for fluorescence diagnosis of tissue was the CD-Scan™. MTC has applied to the Food and Drug Administration (FDA) for, and received, an Investigative Device Exemption (IDE) for this unit. The IDE allows MTC to perform clinical trials for evaluating the CD-Scan™. This instrument is capable of measuring emission, excitation and synchronous scan spectra in the visible and ultraviolet, and is the device used in a significant portion of MTC’s tissue fluorescence research. This device has been used for ex vivo and in vivo measurements. The in vivo measurements were performed at the Department of Head and Neck Services at Memorial Sloan Kettering Cancer Center (MSKCC). This work was done under Institutional Review Board (IRB) approval prior to the submission to the FDA. The FDA raised concerns about the danger of UV exposure levels of the CD-Scan™. Measurements of the UV exposure levels from the CD-Scan™ indicated that the total UV exposure for the five scans used at MSKCC was approximately 2% of the Minimal Erythemal Dosage (MED) or equivalent to less than 1 minute exposure under a tropical sun. Additionally, a toxicology study was performed at MSKCC using a hamster cheek pouch model. The results of this study demonstrated that the CD-Scan™ posed no detectable risk to normal tissue of scanned subjects. The FDA has requested that a second toxicology study be performed to determine if the CD-Scan™ posed a threat to tissue already at risk. However the FDA has given MTC permission to perform the second toxicology study in parallel with CD-Scan™ clinical trials. The FDA has also indicated that they feel that the hamster cheek pouch results would be applicable to all mucosal tissue. The issue of whether it would apply to breast tissue has not been determined.

The CD-Scan™ performs a series of measurements, which typical take from 30 seconds to one minute to complete, while the CD-Ratiometer typically takes from 2 to 4 seconds to complete a measurement. The output power levels of the CD-Ratiometer is comparable to that of the CD-Scan™ but the intensity (power per unit area) is higher for the CD-Ratiometer since the excitation area is significantly smaller. However the shorter measurement times required for the CD-Ratiometer results in fluence (energy per unit area) levels slightly higher than the CD-Scan™. Table 5 summarizes the UVB (280 to 320 nm) and UVC (< 280 nm) fluence for the CD-Scan™ and the estimated level for the 300 nm measurement with the CD-Ratiometer. The CD-Ratiometer measurement assumes 2 seconds of exposure. UVA (320 – 400 nm) exposure is not included because it is considered to be significantly less hazardous than either UVB or UVC radiation.
Based on the FDA’s classification of the CD-Scan a significant risk device (with may change based on the results of the second toxicology study), it was decided that the CD-Ratiometer clinical trials should not commence until completion of the appropriate UV toxicology study and FDA approval. Note, this work was not performed under this contract.

### 5. Ex vivo Breast Tissue Measurements

To test the CD-Ratiometer, spectral ratios were measured using the optical fiber needle probe on about 20 *ex vivo* breast tissue samples. Samples were acquired from either Memorial Sloan Kettering Cancer Center under IRB approval, or from the National Disease Research Interchange (NDRI). Pathology, available for all tissue samples, was used as the standard for classifying tissue. The tissue samples varied from 5 mm to 1 cm in size and each sample was measured at several different locations.

In the first series of measurements, the emission intensities at 340 and 440 nm with 300 nm excitation, and background levels were measured for multiple locations on three cancer breast tissue samples. At 340 nm, the tissue signal levels were about 45 mV while the background signal level was about 15 mV, a signal to background ratio of 3. At 440 nm, the tissue signal levels exhibited considerably more variation, but averaged about 20 mV. These background levels are the result of leakage through the interference filters and some fluorescence emitted by the optical fibers. The leakage level is fairly uniform and thus can be subtracted from further measurements. Note that the leakage caused by scattered excitation light at the sample can not be subtracted out since the intensity of scattered light will vary with sample type and location. The 440 nm background level was 2.5 mV, a signal to background ratio of about 8. The 340 and 440 nm emission data are shown in Figs. 11 and 12, respectively.

The 340/440 nm ratio, with 300 nm excitation, was measured for 7 cancerous, 3 normal and 1 adipose (fat) tissue samples. These measurements were performed on multiple sites on each sample. Consistent with previous work, the ratios for the normal tissues were lower than for the malignant and fat samples. Measurements were also performed on 2 chicken tissue samples (acquired from a local supermarket) using both the CD-Ratiometer and the CD-Scan. The CD-Ratiometer and CD-Scan ratios from the relatively uniform chicken samples were used to calibrate the spectral response of the CD-Ratiometer relative to the CD-Scan. This calibration factor was then applied to the human tissue measurements. The corrected results are shown in Fig. 13. In Fig. 13, the cancer tissue, (numbered 1-7) are marked by an open circle (O), the normal samples (numbered 8-10) are marked by a bar (-), the fat sample (number 11) is marked
by a square ($\square$) and the two chicken tissues (numbered 12 and 13) are marked by triangle ($\triangle$). The average value of the 340/440 ratio for normal tissue is $4.1 \pm 0.89$ and for cancerous tissue is $18.6 \pm 8.0$. These results are summarized graphically in Fig. 14.

For one sample each of normal, fat, and cancer, first the emission intensities at 340 nm was measured for excitation at 289 nm and 271 nm. Next the emission intensities for the three samples was measured at 460 nm and 520 nm for excitation at 340 nm. These measurements were repeated 18 times at the same location. The 289/271 nm and 460/520 nm ratios were calculated. The ratios are displayed in Figs. 15 and 16, for 289/271 nm and 460/520 nm, respectively. For the normal tissue, the average values for the 289/271 and 460/520 ratios were $0.509 \pm 0.021$, and $1.055 \pm 0.025$, respectively. For the fat sample, the corresponding average ratio values are $0.872 \pm 0.097$ and $1.314 \pm 0.036$; and for the cancer samples, the average ratio values are $0.445 \pm 0.051$ and $1.655 \pm 0.024$. The 460/520 ratio from the cancer sample was significantly higher than the ratios from either normal or fat tissue while the 289/271 ratio for fat tissue was higher than the corresponding ratios from normal and cancer tissue. For the normal tissue sample the fluctuations were less than 5%. For the cancer and fat samples, the fluctuations were less than 12%. These measurements were taken using a two second integration time. The results are summarized in Table 6. The data indicates that the 289/271 and 460/520 ratios could effectively distinguish cancerous tissue from non-cancerous tissue. The combination of the three ratios may increase diagnostic accuracy.

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Wavelength Ratio (nm)</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Excitation 289/271</td>
<td>0.509</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>Emission 460/520</td>
<td>1.055</td>
<td>0.025</td>
</tr>
<tr>
<td>Fat</td>
<td>Excitation 289/271</td>
<td>0.872</td>
<td>0.097</td>
</tr>
<tr>
<td></td>
<td>Emission 460/520</td>
<td>1.314</td>
<td>0.036</td>
</tr>
<tr>
<td>Cancer</td>
<td>Excitation 289/271</td>
<td>0.445</td>
<td>0.051</td>
</tr>
<tr>
<td></td>
<td>Emission 460/520</td>
<td>1.655</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Table 6. Summary of the 460/520 emission ratio (excitation at 340 nm) and the 289/271 excitation ratio (emission at 340 nm) for a normal, cancer and fat sample.

**Simulation with layered tissue**

The effectiveness of using fluorescence as a diagnostic tool has been extensively demonstrated and documented in the references listed in this report. However, these primarily involve surface measurements, therefore it was necessary to test the needle CD-Ratiometer with the probe inserted inside tissue. Some potential problems in this area include deformation of the probe, or the depositing of body fluids on the probe surface. It was also desired to learn how interfaces between tissue types could effect measurements.
A simulated model of a tumor in a breast was created by “sandwiching” a cancer sample between two normal tissues, as displayed in Fig 17. The optical fiber needle was mounted on a micrometer controlled three-axis translation stage with 10 μm resolution. This holder is shown in Fig. 18. The fluorescence intensity ratios at 340 nm and 440 nm 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. 19. The sharp transition between the normal and malignant tissue regions are clearly evident. The average value of the 340/440 ratio for the three tissue layers is shown in Fig. 20.

The 340 nm / 440 nm measurements were repeated with a second simulated “sandwich”, made by inserting a normal tissue sample between two malignant samples. The 340/440 ratio at 0.2 mm steps is plotted in Fig. 21 and the average ratio value for the three tissue types are shown in Fig. 22. The sharp transition between the malignant and normal tissue regions is evident in Fig. 21. These measurements demonstrate that the CD-Ratiometer can successfully identify transitions from one tissue type to another and could be useful in defining the size and position of a tumor.

During the course of these measurements, the needle probe experienced some deformation. The deformation was greater from harder (i.e. tumor) tissue than soft tissue. This deformation will make it harder to accurately determine the depth of the needle penetration.

**Measurements with new breadboard CD-Ratiometer**

In order to test the new CD-Ratiometer design, a small number of ex vivo breast tissue samples were excited at 300 nm and the emission intensities at 340 nm and 440 nm were measured at different depths. The 340/440 ratios were calculated and corrected for instrument response. The results, along with CD-Scan ratios are shown in Table 7. The 340/440 ratio for the carcinoma samples were significantly higher than the normal tissues. Since the geometry changes between measurements, one can not compare absolute intensities.

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Signal</th>
<th>Ratio (Corrected)</th>
<th>CD-Scan Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$V_{340}$ (mV)</td>
<td>$V_{440}$ (mV)</td>
<td></td>
</tr>
<tr>
<td>Normal fibrous tissue</td>
<td>133</td>
<td>22</td>
<td>3.0</td>
</tr>
<tr>
<td>Normal fatty tissue</td>
<td>73</td>
<td>29</td>
<td>7.3</td>
</tr>
<tr>
<td>Infiltrating ductal carcinoma</td>
<td>263</td>
<td>14</td>
<td>22.7</td>
</tr>
<tr>
<td>Mucinous ductal Carcinoma</td>
<td>265</td>
<td>11</td>
<td>29.2</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>106</td>
<td>11.0</td>
<td>13.0</td>
</tr>
</tbody>
</table>

The 340 and 440 nm emission signal (300 nm excitation) was measured as a function of penetration depth in three of these tissue samples. The mucinous ductal carcinoma sample was a very homogeneous piece of tumor. The depth profile for 340 nm, 440 nm, and the 340/440 ratio
is shown in Fig. 23. The measurements were taken, starting approximately 0.8 mm from the sample surface and penetration 6 mm into the samples. Outside the sample, the signals show a steady increase as the probe approaches the sample. This is a purely geometric effect. As the probe nears the sample, more of the fluorescence is collected. Note that in this range, the ratio is constant. Immediately inside the sample, the 440 nm signal increases while the 340 signal decreases resulting in a steep drop in the 340/440 ratio. Further into the tissue at about 0.8 mm, the 340 signal stops decreasing and starts increasing while the 440 nm signal continues to increase, resulting in a slow increase in the 340/440 ratio at greater penetration depths. The sharp change in signals at the tissue surface reflects biochemical changes taken place on the surface, including oxidation of NADH by atmospheric oxygen and from proteolysis due to lysosome breaking.

The depth profile for the fatty breast tissue sample is shown in Fig. 24. The sample increase in signal is noted as the probe approaches the tissue surface. However, once inside the sample, the 340 nm and 440 nm intensities remain constant. The 340/440 ratio also remains constant both inside and outside the sample. The sample surface was located 0.2 mm from the starting position of the probe. The total penetration distance was about 6 mm.

The depth scan for the normal glandular sample, shown in Fig. 25, shows an increase in signal as the probe approaches the sample. Once inside the sample, the 340 nm signal showed a rapid decline for the first ½ mm penetration and then leveled off for the most of the remaining 15 mm depth. The 440 nm remained constant while entering the sample but exhibited some large changes inside the sample. These changes correspond to different structure within the tissue. These changes in 440 nm signal are also reflected inversely in the 340/440 ratio.

This work clearly shows the importance of this optical needle technology to spatial information inside tissues.

6. Recommendations for Future Work

Although this research project, demonstrated that the CD-Ratiometer can successfully distinguish malignant from benign ex vivo breast tissue by fluorescence intensity ratios, there are areas for improvement in instrumentation, further ex vivo testing, integration into a clinical setting and clinical trials.

These areas are discussed below:

Hardware improvements

The major area for improvement in hardware and design is to continue development of new breadboard ratiometer with improved optical coupling between the xenon lamp and the excitation fiber. Alternative excitation light sources such as hydrogen lamps should be investigated. Hydrogen lamps are richer in UV output and produce significantly less infrared and visible light, reducing the problems associated with heating. The current xenon lamp produces substantial ozone, and the current CD-Ratiometer uses an external “ozone eater” to reduce ozone emission. Since ozone is produced by optically exciting oxygen molecules, blocking wavelengths shorter than 230 nm will eliminate most of the ozone production. This can be accomplished by either using lamps whose windows do not transmit wavelengths below 230...
nm, or by adding short wavelength blocking filters to the current lamps.

An alternative design of the CD-Ratiometer which uses boxcar (gated) integrators instead of lock-in amplifiers for detection. The boxcar integrator also allows use of a pulsed xenon lamp. The pulsed lamp is smaller in size and operates at a much lower Wattage. The boxcar integrator does not need any external preamplifiers. This factors combine to reduce the total size of the CD-Ratiometer. UV exposure from the pulsed system may be significantly lower than the lock-in version. Measurements of output power and integration time will allow a comparison of UV exposure levels from both types of units.

Further ex vivo testing

The CD-Ratiometer should be tested extensively with the additional diagnostic wavelength ratios developed at the Mediphotonics Laboratory at The City College of New York. In particular, the CD-Ratiometer performance should be tested more thoroughly with excitation and synchroscan measurements, since these measurements can not be made simultaneously. The effects of lamp power fluctuations should be studied in greater detail. Additional studies should be made to determine if the differences in spectral resolution between the CD-Scan and CD-Ratiometer require modifications in the choice of wavelength pairs. Different tissues of the breast should be measured with needle unit

Integration of CD-Ratiometer into Stereotactic Needle Biopsy System

Prior to clinical testing, the CD-Ratiometer should be integrated into a Stereotactic Needle Biopsy System and tested extensively on either ex vivo, animal or simulated tissue. These tests will help determine the spatial resolution of the CD-Ratiometer and reveal any potential problems which may arise during clinical trials. During these tests, it can be determined if the CD-Ratiometer measurements can be integrated into an examination without creating additional discomfort for the patient or unnecessary distractions for the examining physician. As a result of these tests, modifications to the CD-Ratiometer software may be necessary to improve integration into the examination process.

Clinical trials

The ultimate goal of this project is clinical testing of the CD-Ratiometer. However, due to the potential hazards from UV exposure, clinical trials must wait for FDA approval. FDA approval will only be given after a toxicology study is completed, and the results show that the CD-Ratiometer does not pose a risk to patients. Therefore a toxicology study using an appropriate animal model, should be a prerequisite to future work. A request and application to FDA will be then made to start clinical trails with the needle unit.

III. Conclusion

There is a need to develop minimally invasive methods to probe deep into organs, such as a tumor inside breast. The goal of this research project was to develop an optic fiber needle based fluorescence ratiometer for real-time evaluation and diagnosis of breast tumor in vivo. An optical
fiber needle ratiometer, based on native spectroscopic differences between diseased and normal tissues, has been designed, assembled, and tested. The spectroscopic optical biopsy technology developed during this work, could be implemented in a device suitable for clinical applications. It has been demonstrated that the CD-Ratiometer can be coupled to a stereotactic needle based fiber optic probe to deliver the excitation light and collect the fluorescence from the tumor region. The small, 300 μm diameter needle can probe inside an organ with minimal invasiveness and adequate signal to record diagnostic information from tissue in situ for breast, brain, and prostate.

During this work, ex vivo cancerous and benign/normal breast tissue samples have been measured and distinguished by the CD-Ratiometer. Measurements on human organs were simulated by using tissue samples created by layering different types of tissue. Multiple wavelength ratios have been introduced to the CD-Ratiometer for better diagnostic accuracy, specifically, improving the capability of separating fat tissue from cancer. This research demonstrates the technological and commercial potential of the optic fiber needle based CD-Ratiometer for in situ spectroscopic analysis in order to both improve the targeting of tissue for biopsy, and the reduction in the number of false biopsies performed.

IV. References


V. Figure Captions

Fig. 1 CD-Ratiometer with optical fiber needle probe for detection of breast cancer.
Fig. 2 Schematic absorption spectra of several intrinsic fluorophores.
Fig. 3 Schematic emission spectra of several intrinsic fluorophores.
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Fig. 5 Normalized average excitation spectra obtained from ex vivo breast cancerous, normal, and fat tissue. Emission wavelength was 340 nm.
Fig. 6 Diagram of optic fiber needle based ratiometer.
Fig. 7 Filter wheel combinations for CD-Ratiometer.
Fig. 8 Front firing optical fiber needle probe.
Fig. 9 Side firing optical fiber needle probe.
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Fig. 11 340 nm emission signal and background level.
Fig. 12 440 nm emission signal and background level.
Fig. 13 The 340/440 emission ratio for normal, fat and malignant human tissue, and chicken samples (used for instrument calibration).
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Fig. 17 Simulated tissue layer structure formed by “sandwiching” cancer tissue between two normal tissues.
Fig. 18 Photograph of three-axis positioning stage and fiber needle probe for depth measurements of tissues.
Fig. 19  The 340/440 ratio for depth measurements from simulated normal-cancer-normal “sandwiched” sample.

Fig. 20  Average value of the 340/440 ratio for the three layers in the normal-cancer-normal sample.

Fig. 21  The 340/440 ratio for depth measurements from simulated cancer-normal-cancer sample consisting of a normal tissue “sandwiched” between two cancer samples.

Fig. 22  Average value of the 340/440 ratio for the three layers in the cancer-normal-cancer sample.

Fig. 23  Depth profiles of spectral intensities and ratios for mucinous ductal carcinoma breast tissue using new CD-Ratiometer design.

Fig. 24  Depth profiles spectral intensities and ratios for fatty breast tissue using new CD-Ratiometer design.

Fig. 25  Depth profiles spectral intensities and ratios for normal glandular breast tissue using new CD-Ratiometer design.
CD-Ratiometer - Needle Version

Fig. 1. CD-Ratiometer - Needle version for detection of breast tumors.
Figure 2. Absorption spectra for some tissue chromophores.
Fig. 3. Fluorescence Spectra of some tissue fluorophores.
Fig. 4. Left - Typical spectra from benign and cancerous breast tissue. Right - Histograms showing statistical accuracy.
Fig. 5. Normalized excitation spectra from *ex vivo* human breast tissue. 
Emission intensity measured at 340 nm.
Fig. 6. Diagram of CD-Ratiometer
Fig. 7. Filter wheel combinations for Needle CD-Ratiometer
Front-firing fiber probe

1) Fiber faces are polished to a scratch-free finish at 40x magnification
2) Fiber type UV enhanced multimode 0.3 mm
3) Fiber Probe is freely inserted in 20 gauge hypodermic needle

Fig. 8. Front firing fiber needle probe
Side-firing fiber probe

24 gauge needle tubing (SS, 0.559 mm O.D.)

25 mm

PVC Tubing

SSTL Junction

1/4 in Dia

SMA905

Optic fiber UV enhanced, 0.25 mm O.D.

Light

S.S. Needle tubing (24 gauge, 0.559 mm O.D.)

45 degree mirror plug

Optical fiber probe inserted into hollow needle with groove for side excitation

1) Fiber faces are polished to a scratch-free finish at 40x magnification
2) Fiber type UV enhanced multimode 0.3 mm
3) Fiber Probe is freely inserted in 20 gauge hypodermic needle

Fig. 9. Side firing fiber needle probe
Fig. 10. The optical diagram of the new spectral CD–Ratiometer system for optical biopsy with a thin needle fiber probe.
Fig. 12. 440 nm emission signal and background level from three cancerous tissue samples.
Fig. 13. The 340/440 emission ratio for normal, fat and malignant human tissue, and chicken samples (used for instrument calibration).
**In-Vitro**

**Average Ratio for Cancerous and Normal breast Tissue**

- Cancer
  - $18.6 \pm 8.0$
- Normal
  - $4.1 \pm 0.89$

![Bar graph showing comparison between Cancer and Normal tissue](image)

*Fig. 14  Summary of 340/440 ratio from normal and cancer tissue*
Fig. 15. Ratios of 340 nm emission intensity with 289 nm and 271 nm excitations. Points represent a series of readings at one site each on normal, fat and cancer tissue.
Fig. 16. Ratios of 460 nm emission to 520 nm emission with 340 nm excitation.
Points represent a series of readings at one site each on normal, fat and cancer tissue.
Fig. 17 Simulated tissue layer structure formed by "sandwiching" cancer tissue between two normal tissues
Fig. 18 Photograph of three-axis positioning stage and fiber needle probe for depth measurements of tissues.
Fig. 19. The 340/440 ratio from simulated normal-cancer-normal sample consisting of a cancer tissue "sandwiched" between two normal samples.
Fig. 20. Average value of the 340/440 ratio for the three layers in the normal-cancer-normal sample.
Fig. 21. The 340/440 ratio from simulated cancer-normal-cancer sample consisting of a normal tissue "sandwiched" between two cancer samples.
Fig. 22. Average value of the 340/440 ratio for the three layers in the cancer-normal-cancer sample.
Fig. 23. The depth profiles of fluorescence intensities at 340 nm and 440 nm and their ratios for a mucinous ductal carcinoma breast tissue using the new CD-Ratiometer design.
Fig. 24. The depth profiles of the fluorescence intensities at 340 nm and 440 nm and their ratios for a normal human breast fatty tissue sample.
Fig. 25. The depth profiles of the fluorescence intensities at 340 nm and 440 nm and their ratios for a normal human breast glandular tissue.
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VII. BIBLIOGRAPHY OF ALL PUBLICATIONS AND MEETING ABSTRACT

PUBLICATIONS:

Fluorescence Optic Fiber Stereotactic Needle Ratiometer for Breast Tumor Diagnosis. 
In Proceeding of the Defense Breast Cancer Research Program Meeting --- Era of Hope, 

Optic Fiber Needle Endoscope Based Ratiometer for Breast Tumor Targeting. 
OSA Trends In Optics and Photonics Series, Vol. 22, Biomedical Optical Spectroscopy and 

MEETING ABSTRACT:

Please see next two pages.
Optic Fiber Needle based Ratiometer for Tumor Targeting
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Abstract

Fluorescence spectroscopy has long been used as a valuable tool for analyzing the structure and composition of materials. The different colors and intensity of light emitted or absorbed can give identifying information indicating the presence or absence of various types of molecules.

Over the past decade, optical biopsy techniques based on fluorescence spectroscopy, have been developed which can distinguish tumor tissue from normal tissue. Native tissue fluorescence spectroscopy (without the use of extrinsic dyes) have been demonstrated to be an accurate method for distinguishing malignancy from normal tissue. Fluorescence measurements have been applied to breast, GYN tract, colon and aerodigestive tract in-vitro tissue samples. Sensitivities of better than 90% have been achieved.

Optical biopsy for tissue diagnosis provides some significant advantages:

- Spectroscopy is less invasive. It does not require removal of tissue.
- Spectroscopic measurements utilize native fluorescence - no need to use extrinsic dyes.
- Results are available in real time.
- Spectroscopy may be able to identify pre-cancerous or early stage cancer conditions before a tumor can be visibly identified by a physician.

As part of this research, instrumentation is being developed which would allow in-vivo spectroscopic measurements of tissue. The CD-Ratiometer is a dual channel detector which can measure the intensity of emitted light at key pairs of wavelengths. The ratio of the emitted intensities, which are displayed to the physician in real time, indicate whether the tissue is normal or malignant. A special fiber optic probe has been developed for the CD-Ratiometer. This probe, when inserted in the type of needle used for fine needle aspiration biopsies, can be used for in situ examination of the breast, prostate or other organs. Additional optical probes have been developed for use in endoscopes and laparoscopes.
OPTIC FIBER NEEDLE ENDOSCOPE BASED RATIOMETER FOR TUMOR TARGETING


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Abstract

A optical fiber, needle-based, ratiometer for breast tumor targeting has been developed as an optical biopsy technique based on fluorescence spectroscopy. In vitro breast cancerous and normal tissues were measured using the ratiometer. Simulated experiments of detecting tumor in a breast were performed using a “sandwich” model made up of three tissue layers. Fluorescence measurement from these samples would be consistent with detection of cancerous tissue surrounded by normal breast tissue.
MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-OCA, 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for the attached Grants. Request the limited distribution statements for Accession Document Numbers listed be changed to "Approved for public release; distribution unlimited." This report should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by email at Judy.Pawlus@amedd.army.mil.

FOR THE COMMANDER:

PHYLIS M. RINEHART
Deputy Chief of Staff for Information Management

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completed 1-13-00 am