Cancer is one of leading cause of death in developed countries. While the establishment brags that more cancer patients survive than ever before, the horrific side effects inflicted by conventional therapy often leave patients partially or severely debilitated, and set the stage for deadly secondary diseases. For those people suffering from cancer the dangerous mix of chemotherapy and surgery not only failed to cure the cancer, but destroyed their remaining quality of life. The laser treatment has the potential to destroy primary breast tumors. This unique treatment can also seek and destroy cancer cells that have spread (metastasized) from the original tumor to other parts of the body. This is especially critical as metastasized cells are the primary cause of cancer death. A laser beam operating in the near-infrared frequency of light heats tissue to a depth of several centimeters, allowing the beam to penetrate directly into a solid tumor with minimal damage to normal tissue because it can be focused directly through intact skin, no surgical incision is required.

In recent years there has been much progress in the use of optical diagnostics Methods in the detection & diagnosis of breast cancer. Early diagnosis of breast cancer affords early intervention and greatest chance to cure. Laser induced Fluorescence spectroscopy (LIF); Synchronous Luminescence (SL) Spectroscopy and Raman spectroscopy are some of the technique used to detect the cancer in early stage. In our diagnostic studies, we used the technique of Raman scattering. Raman spectroscopy is based on the interaction of photons with the target material producing a highly detailed biochemical ‘fingerprints’ of the sample. It can be appreciated that such a sensitive biochemical detection system could confer diagnostic benefit in a clinical setting. Raman spectroscopy has been successfully used as a key for the detection & diagnosis of breast cancer. Raman spectroscopy could confer a great to a patient with early, rapid and accurate diagnosis. This technique is almost labour free without the need for sample preparation. It could reduce the need for whole pathological specimen examination, in theatre it could help to determine margin status, and finally peripheral blood diagnosis may be an achievable target.

Keywords
Chemotherapy, surgery, Confer, Great, Raman spectroscopy.
1. Introduction.
Laser spectroscopic techniques are beginning to make an impact in the diagnosis of diseases. In particular the detection of dysphasia (or pre cancer) in many organ systems, atherosclerosis in cardiovascular disease etc have been widely studied using spectroscopic methods. Fluorescence, diffuse reflectance, elastic scattering and Raman scattering are the techniques employed in our diagnostic studies.
The fluorescence and diffuse reflectance studies rely on a fast EEM (Excitation-Emission Matrix) device. The instrument obtains fluorescence excited by 11 discrete Laser wavelengths ranging from 308nm to 600nm. Along with diffuse reflectance from a white light source, all acquired in 0.5 sec. modeling of the reflectance spectrum yields absorption and scattering constants of the tissue under consideration as well as the cellular nuclear size distribution. These parameters provide important information about the state of the tissue.
Analysis of the fluorescence spectra, in combination with information from the reflectance spectrum to remove distortions from scattering and absorption yields valuable information about changes that takes place in tissue biochemistry during the development of dysphasia. Extensive studies have been carried out in colon, bladder, esophagus and oral cavity and these results will be presented.
Raman spectroscopy is also ideally suited to study vulnerable plaques as the NIR radiations can penetrate the entire thickness of the artery and many of the biochemical constituents related to plaque stability can be identified, recently Scientist have been successfully carried out to assess plaque composition in femoral and carotid arteries from patients undergoing vascular surgery, Further work deals with real time data analysis and diagnosis.
Raman spectroscopy is an inelastic scattering in which photons incident on the samples transfer energy to or from molecular vibrational modes. Unlike conventional pathology, which is subjected to inter observer variation, Raman spectroscopy assesses these changes in an objective, reproducible manner without the need for tissue removal. Raman spectroscopy can be used to distinguish normal, benign and cancerous lesion with high sensitivities and specificities based on their biochemical composition.
Similarly Raman spectroscopy can be use to diagnose benign and malignant lesions in human breast tissue based on chemical composition. Data are fit by using a linear combination model in which some basic spectra represent the morphological and chemical features of breast tissue. The resulting fit coefficients provide insight into the chemical or morphological makeup of the tissue and are used to develop diagnostic algorithms. The fit coefficients for fat and collagen are the key parameters in the resulting diagnostic algorithm, which classifies samples according to their specific pathological diagnoses, attaining 94% sensitivity and 96% specificity for distinguishing cancerous tissues from normal and benign tissues. The excellent results demonstrate that Raman spectroscopy has the potential to be applied in vivo to accurately classify breast lesions their by reducing the number of excision breast biopsies that are performed.
Raman spectral peaks tend to be narrow, particularly in the fingerprint region of about 700-2000 cm⁻¹, and each peak can be associated with specific vibrations in molecular bonds. Thus, this technique provides biochemical information about a sample, including conformations and concentrations of constituents with the level of detail that is determined by the instrumentation and the need of the application.
Over the years, different forms of Raman spectroscopy have been developed and used for biological applications. The earliest of these is Fourier transform (FT) Raman spectroscopy, a method that measures Raman spectra with high signal-to-noise ratio (S/N) and minimal fluorescence interference and has been used for many in vitro applications. The typically long integration times and bulky instrumentation negate this technique for in vivo use. Ultraviolet resonance Raman (UVRR) spectroscopy can be used to target specific molecules by selecting excitation wavelength at their resonance, thus yielding strong Raman signals. The high excitation intensities and mutagenicity of UV light prevent the application of this technique for in vivo use. Surface-enhanced Raman spectroscopy (SERS) is an excellent technique that can detect molecular signatures in trace amounts and has been pursued for such applications as biochips. However, the use of silver and other such elements for enhancement prevents its implementation in vivo. Thus, near-infrared (NIR) dispersive Raman spectroscopy, in which NIR excitation minimizes fluorescence and absorption by tissue, has been the technique of choice for in vivo applications. Most in vivo Raman applications rely on fused silica-based optical fibers for remote sensing. However, the fibers themselves have a Raman signal, so this signal must be minimized using appropriate filters. A band pass filter between the delivery fiber and sample prevents Raman-scattered light from illuminating the sample, while a long pass or notch filter between the sample and collection fibers prevents reflected laser light and Rayleigh-scattered light from entering the collection leg and generating additional Raman signal (Figure 2). Based upon this fundamental concept, a number of different fiber-optic probe designs have been implemented for in vivo Raman studies. Each of these designs is optimized for increased S/N, targeted interrogation, and minimal background signal.
from within the probe. Some of these designs include obliquely polished fiber tips to increase the area of excitation and collection overlap, attaching a ball lens to the tip of the fibers to increase overlap or change the depth of focus, and side-firing fibers to get 360° of coverage (2). Some of these probes were developed by academic institutions. There also exist numerous commercial Raman fiber probes. However, these probes typically are designed for industrial applications and are not suitable for use on tissue. Thus, most in vivo probes are custom designed and built in-house or special ordered.

Much of the discussion to this point has been focused on macroscopic or volumetric measurement of Raman signals from tissue. Recently, there has been much interest in obtaining depth-resolved Raman signals from tissues where discrimination could be improved by filtering out signatures from above or below the lesion. One way to obtain depth resolution is with a con-focal setup, in which a pinhole (or a fiber) is used to reject out-of-focus light, allowing for depth separation. One such design is shown in Figure 3. In fact, confocality for Raman spectroscopy typically is incorporated in a microscope setting. These systems provide excellent spatial resolution and are used for biochemical mapping and spectral characterization of tissues. Although some of these systems have been used to obtain tissue spectra from easily accessible areas of the skin, there is a need for compact handheld con-focal probes for routine clinical use. Recent studies have demonstrated other methods for depth resolution such as Kerr-gated (3), spatially offset (4), and polarized (5) Raman spectroscopy. These methods show much promise for biomedical applications and will be worth tracking in coming years.

In all tissue Raman applications, various data-processing steps must be followed to extract the tissue Raman signal from the raw measured spectra (Figure 4). These include system calibration, fluorescence background subtraction, and noise smoothing. Perhaps the most challenging of these is the problem of fluorescence, the primary reason that most researchers in the field have moved to the NIR wavelengths. Current methods rely on mathematical techniques such as the use of second derivatives, Fourier filtering, and polynomial fitting to remove the fluorescence background. Once Raman spectra are extracted, classification algorithms are developed using a variety of multivariate statistical methods such as linear and nonlinear discrimination analysis, neural networks, genetic algorithms, and cluster analysis. The goal in each case is to obtain high sensitivity and specificity in the recognition of a target condition amidst a variety of tissue categories depending upon the application at hand.

1.1 Experimental set up:
Different samples of tissues were collected and they were recorded using the laser Raman instrumentation. In this set up, a diode laser 785 nm, 100 mw) was used for excitation and scattering was detected by HR320 spectrograph + spectrum one liquid N2 cooled CCD. A holographic filter was used to filter the excitation source. A notch filter was used for removing the Raleigh scattering. Baseline corrected, smoothened, calibrated and normalized spectra (to the highest peak) were subjected to multivariate statistical analysis PCA for objective classification of normal, malignant and benign tissues. (Figure 5)

1.2 Raman Spectroscopy for Cancer Diagnosis:
The phenomenon of Raman spectroscopy makes it ideal for probing tissue because numerous biological molecules undergo some Raman scattering, allowing one to recognize subtle changes in tissue biochemistry. Many biomedical applications of Raman spectroscopy exist today, including characterization of human atherosclerotic plaque (6), evaluation of skin composition (7), quantification of blood analytes (such as glucose, cholesterol, and urea) (8), estimation of secondary protein structures (9), and cell viability after exposure to toxic agents (10). Raman spectroscopy is particularly suited for diagnosing cancer because of its sensitivity in detecting small molecular changes that are associated with cancer, such as an increased nucleus-to-cytoplasm ratio, disordered chromatin, higher metabolic activity, and changes in lipid and protein levels (11) (Figure 5). Many researchers and clinicians believe that Raman spectroscopy can thus provide real-time, noninvasive or minimally invasive, differential diagnosis of cancer. As a result, there has been a concerted effort in applying Raman spectroscopy for the diagnosis of cancers in the skin, breast, gastrointestinal tract, and cervix, among others.

1.3 Breast:
Breast cancer is more common in female. It is common in U.S.A because they neglect on feeding... In the United States nearly 216000 new case of breast cancer are diagnosed each year, and 40000 women die from the disease. Mammography is the most common technique for detecting non palpable, highly curable breast cancer, employs X-rays to quantitatively probe density changes in breast tissue, because these density changes are not uniquely correlated with breast cancer, mammography serves as a screening technique rather than a diagnostic tool. Mammography is always biopsies and it may take a couple of months and multiple biopsies and elaborates sample preparations. Optical spectroscopy methods like Raman fluorescence and FTIR have been shown as potential alternatives. Raman fluorescence did not require any biopsy. They are very convenient for periodic monitoring and with accumulation of spectra from each class [Inflamatory, pre malignant and malignant.] can be used for correct prognosis enabling early detection and therapy.
Raman spectroscopy can provide detailed chemical information about a tissue sample and thus insight into the chemical changes that accompany breast cancer, in contrast to fluorescence, there are a large number of Raman active molecules in breast tissues, and their spectral signatures are sharp and well delineated.

Near infrared (NIR) Raman spectroscopy is employed to study the biochemical composition of atherosclerosis in arteries and breast tissue in vitro samples. Breast tissue studies indicate that Raman spectroscopy can be used to distinguish normal, benign and cancerous lesions with high sensitivities and specificities based on their biochemical composition.

Raman spectroscopy is an inelastic scattering in which photons incident on the samples transfer energy to or from molecular vibrational modes. Unlike conventional pathology, which is subjected to inter observer variation, Raman spectroscopy assesses these changes in an objective, reproducible manner without the need for tissue removal. Similarly, Raman spectroscopy can be used to diagnose benign and malignant lesions in human breast tissue based on chemical composition. Data are fit by using a linear combination model in which some basic spectra represent the morphological and chemical features of breast tissue. The resulting fit coefficients provide insight into the chemical or morphological makeup of the tissue and are used to develop diagnostic algorithms to probe density changes in breast tissue.

Because it is the most common cancer in women, several groups have examined Raman spectroscopy for rapid breast cancer diagnosis. Infiltrating ductal carcinoma (IDC) is the most common malignancy, so it has been a prominent condition to study. There also has been much interest in lobular carcinoma and benign or precancerous conditions like fibrosis, cyst formation, and ductal carcinoma in situ (DCIS).

Alfano and colleagues (20) were the first to look at the ability of Raman spectroscopy to distinguish normal from malignant breast tissue. Later, Redd and colleagues (21) demonstrated the advantages of NIR excitation particularly in the context of breast cancer. Although no rigorous algorithm was attempted, they saw results similar to those of Alfano—namely, a decrease in lipid contributions to spectra from IDC samples, as well as an increase in the collagen contributions in benign and malignant samples. More recently, Feld and colleagues (22) have done extensive work on using Raman spectroscopy for breast cancer diagnosis. An early study showed comparable spectra, but multivariate statistical algorithms improved diagnosis (22). Over the past several years, this group has developed a system that classifies breast Raman spectra according to the modeled contributions of individual component spectra from materials like fat, collagen, and DNA to discriminate malignant from normal and benign tissues with 94% sensitivity and 96% specificity (23). Specificity indicates the ability to correctly recognize normal tissue and is defined as the number of true negatives divided by the total number of negatives.

2. Results and Discussion:
It can be seen that the differences between normal and malignant spectra are quite significant. Normal spectra show the presence of structural proteins like collagen (850 and 930 cm⁻¹) whereas malignant tissues show this to a lesser extent with more of nucleic acids and lipids (1080 c-1 C=C chain 1440 cm⁻¹ CH₂ and sharp C=C at 1650 cm⁻¹). Raman spectroscopy can provide an objective and fast diagnostic for screening and early detection, prognosis periodic monitoring and surgical boundary demarcations without any need for biopsy and sample preparation.

In case of oral cancer several bands seen in the normal spectra indicate the presence of lipids and malignant spectra is predominantly due to protein contributions. A broad amide 3 and amide1 are noticed in inflammatory and pre malignant condition. Classification by scores of factor 1 is not yield good clusters in all cases. Hence Mahalanobis distance and spectral residual were the other parameters used for discrimination.

In case of ovarian tumors good classification between normal and malignant tissues was achieved using scores of factors as the discriminating parameters. However this approach failed to solve benign and malignant conditions. To improve the classification, other parameters such as Mahalanobis distance and spectral residuals are being pursued. In these methods, standard sets for each category were developed and constituent spectra compared with standard sets for match or mismatch. This study indicates that Raman spectroscopy can be used for discrimination of normal and malignant ovarian tissues. With possible in situ applications, this approach can be an alternative to frozen.

3. Conclusions:
We employ Raman spectroscopy to diagnose benign and malignant lesions in human tissue based on chemical composition.

Raman spectroscopy can be used to discrimination of normal and malignant ovarian tissues. Raman spectroscopy is very convenient for periodic monitoring and with accumulation of spectra from each class. Raman spectroscopic technique is used for discriminating normal and malignant tissues with sensitivities and specificity > 90%.
Several other cancers also have been studied with Raman spectroscopy, such as those in the ovary, brain, and lung, with similar results. Thus, many researchers have applied NIR Raman spectroscopy in vitro, ex vivo as well as in vivo for the diagnosis of cancer with varying degrees of success. Despite this research for more than 20 years, the technique has not been incorporated into routine clinical care for several reasons. There is a continued tendency by researchers to use empirical or several different classification techniques after many spectra are recorded demonstrating varying degrees of success. But for successful widespread implementation, a robust, multi-class discrimination algorithm is needed. Perhaps the single most critical need that will lead to the applicability of this technique in a real-time diagnostic setting is one of independent validation via large clinical trials. The inherent intra-patient as well as inter-patient variability in the spectra is a limitation that confounds the development of these algorithms and thus, implementation of clinical trials. Raman instruments with the sensitivity for in vivo application must be assembled for implementation because there are few suitable commercial instruments available. Interest by companies to commercialize the technique would facilitate great strides in solving many of these problems. There is every indication that Raman spectroscopy is poised to follow through on its potential to provide real-time, noninvasive, automated diagnosis of various cancers. Although the process of developing automated classification algorithms has proven arduous, new cutting-edge classification algorithms suggest that, with enough data, this type of diagnosis will soon be possible. Some groups have begun to undertake large-scale validation studies, although most of these are conducted by or with the help of industry. Researchers also are beginning to understand the biological basis of Raman spectral differences between normal and cancerous tissues with the help of techniques like spectral mapping and using tissue model systems. This would not only further the diagnosis of the disease but also would enable improved understanding of the underlying biochemistry associate with the progression of cancer. Although the end goal of Raman spectroscopy being used routinely in the operating room and the doctor's office is still a few years away, clinical studies at medical centers around the world have shown promise, and exciting new discoveries like improved algorithms and reliable tissue model systems can only help. Early detection of cancer and delineation of tumor boundaries are critical in patient care. Raman spectroscopy is a tool that provides the information necessary to make a difference toward this process.

Fig 1: Block diagram to study the Raman Effect.           Figure 2: A typical system for macroscopic Raman Spectra of tissue.
Figure 3: Reflected laser light and Rayleigh scattered light from entering the collection leg and generating additional Raman signal.

Figure 4: A typical confocal Raman system used together depth resolved data with white light imaging capability colocalization.

Figure 5: Raman spectra for three tissue types. Shown are (a) raw and (b) processed spectra.
References


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