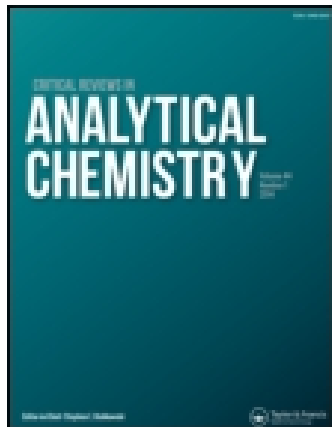


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Applications of FT-IR Spectrophotometry in Cancer Diagnostics

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This review provides a brief background to the application of infrared spectroscopy, including Fourier transform-infrared spectroscopy, in biological fluids. It is not meant to be complete or exhaustive but to provide the reader with sufficient background for selected applications in cancer diagnostics. Fourier transform-infrared spectroscopy (FT-IR) is a fast and nondestructive analytical method. The infrared spectrum of a mixture serves as the basis to quantitate its constituents, and a number of common clinical chemistry tests have proven to be feasible using this approach. This review focuses on biomedical FT-IR applications, published in the period 2009–2013, used for early detection of cancer through qualitative and quantitative analysis.

Keywords: biomedical analysis, clinical diagnosis, early detection of cancer, FT-IR spectroscopy

Introduction

Infrared (IR) spectroscopy is one of the most important analytical techniques available to scientists. One of the great advantages of IR spectroscopy is that any sample can be studied in virtually any state. As a consequence of improved instrumentation, a variety of new sensitive techniques have been developed in order to examine formerly intractable or difficult samples in biomedical research (Ellis and Goodacre, 2006; Lewis and Mcelhaney, 1996; Petibois and Deleris, 2006; Wang et al., 1996).

Vibrational spectroscopy offers a unique opportunity to investigate the composition of unknown substances on a molecular basis. Although this fact was discovered long ago, it is the recent technical advances that have generated strong and increasing interest in the application potential of molecular vibrational spectroscopy.

Biostructure disorders (e.g., uncontrolled cell division, invasive cell growth into adjacent tissue, and metastatic implantation to other body sites) are called “cancer.” Cancer is becoming the leading cause of death all around the world. Cancer-related diseases affect people in all age ranges, but the risk tends to increase with age. The highest death rates

are recorded for lung, colon, breast, and prostate cancers. Developments in cancer treatment are reported daily with serious attention paid to diagnostic methods. It is well known that a precise, accurate diagnostic report is very helpful for drawing up strategies for treatment.

There are several risk factors such as culturally determined habits and socio-economic conditions, physical and industrial processes, infectious agents, genetic predisposition, and the environment that affect an individual’s susceptibility to cancer. The risk of cancer in humans is increased by a wide spectrum of factors that range from exposure to an identified agent, such as environmental chemicals or a virus, to culturally determined behavior, such as smoking, to socio-economic conditions. We are today able to intervene on some of these factors. Only progress in understanding the mechanisms by which these factors act can lead to specific means of cancer prevention.

The main diagnostic method for cancer is histological confirmation provided by pathological examination of tissue samples, which can be obtained from biopsy or surgery (Kendall et al., 2009; Luna, 1968). Usually in the first step, a piece of tissue is fixed by formaldehyde solution in order to preserve it physically. In the next step, a paraffin-embedding procedure is performed to make a hard block of sample, which can be sectioned by a microtome. The sliced sections are then glass mounted and stained by hematoxylin and eosin to provide different colors for each biochemical structure of the tissue cells, prior to being investigated by pathological microscopic evaluation. The pathological inspections are based on certain criteria, according to the cell size and visual morphology, that are different between normal and

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cancerous cells (e.g., larger cell nucleus in cancer, multiple nuclei, irregular condition in cancer cases, and neoplastic invasion of malignant structures into normal ones). All the main diagnostic criteria in these cases are qualitative. The standard histopathological procedure involves tagging and visualizing the distribution and structure of cellular components in tissue sections using light microscopy. Although used in clinical routines, this common approach is not free of pitfalls and is associated with environmental contamination by agents used for sample preparation (Mordechai et al., 2004). The biopsy-based approach is time consuming and depends on the professional capabilities of the pathologist.

Innovative diagnostic methods that provide indications complementary to conventional histopathology, in particular the early biomolecular alterations under malignant conditions, are under scrutiny. One such candidate method is infrared (IR) spectral imaging, which has the potential to provide, in a nondestructive and label-free manner, a biochemical fingerprint of cells and tissues (Khanmohammadi and Garmarudi, 2011; Martin et al., 2010). As such, its potential has been exploited in various IR spectroscopic studies applied to cells and tissues from different organs. In comparison to the conventional hematoxylin and eosin (HE)-stained reference histological images, the cluster images (related to principal component analysis) permit retrieving specific IR spectral signatures representative of the nontumoral and tumoral epithelial components.

Cancer includes several different diseases originating from a defect in any cell in the body that generates progeny unrestricted by constraints imposed on their growth, division, and differentiation by normal regulatory mechanisms of the body (Tomatis, 1988). The goal of early detection and screening is the diagnosis and treatment of cancer before it spreads beyond the organ of origin, perhaps even in its pre-invasive stage. This approach is preferable to trying to control cancer by systemic therapy. However, available early detection and screening techniques pick up many tumors at a relatively late stage in their natural history (Bishop, 1987). As a result, reduction in mortality with the currently available detection modalities is likely to be modest.

Optical techniques have been studied extensively for the diagnosis of cancer because instead of using an approach based on morphological changes, as currently occurs in histopathological studies, the analysis is automated and relies on the detection of biochemical changes that occur in tumor tissues (Sidransky, 1995). One of the optical spectroscopy techniques that can effectively provide information concerning the structure and chemical composition of biological materials at the molecular level is Fourier transform-infrared spectroscopy (FT-IR).

Infrared spectroscopy (IR) not only differentiates cells and tissues based on their characteristic spectral properties, reflecting the chemical composition and structure, but also has the potential to serve as a diagnostic tool for detecting and discriminating different diseases or disease progression due to the induced changes of chemical composition and structure.

IR spectroscopy has emerged in recent years as the analytical method of choice in an enormous variety of applications (Bunaciu et al, 2010; Shaw and Mantsch, 1999). Molecular

structure and function are strongly correlated. This aspect is particularly relevant in the case of proteins, which play important roles in cell biochemistry. Changes of structure can be easily detected in an IR spectrum, and a cellular molecular marker may in fact be used to address a pathological status of tissue. Furthermore, comparison of spectra between healthy and cancerous tissue may improve understanding of pathogenesis of morbid processes. The clearest advantage is that no specific reagents are required. Automated, repetitive analyses can therefore be carried out at very low cost. The appeal of these factors has spurred the development of a new generation of analytical IR spectrometers that combine high acquisition speed with superb spectral sensitivity. Powerful chemometric algorithms and software packages have arisen in parallel with the new hardware, and new applications emerge continually.

FT-IR spectroscopy results are able to show structural changes of cells at the molecular level in various human cancers. The structural changes are result of carcinogenesis caused by different modes of vibration in the molecules of the cells and tissues. The unique vibrational frequencies of major functional groups are characterized by the changes in the FT-IR spectra. Thus, the FT-IR spectra could show normal or malignant cells with their spectral characteristic appearance.

In a previous review we presented some important applications of FT-IR microscopy in biomedical investigations (Bunaciu et al., 2014). The aim of the present review is to present recent advancements in the potential use of FT-IR spectroscopy for discriminating between normal and malignant cells, with varying degrees of dysplasia.

Infrared Spectroscopy in Biological Fluids

Serum is composed of water, organic substances, and inorganic salts; it can reflect human beings' physiological and pathological conditions (Zhang et al., 2008) and is easier to collect and more suitable for rapid diagnosis.

Water is the major molecular component within biological matrices and strongly affects the utility of selected electromagnetic spectral regimes due to strong O—H absorptions, especially in the mid infrared region (MIR). However, water has a relatively broad transmission window in the near infrared region (NIR), thereby enabling direct measurement of the biological specimen. However, despite the merits of operating in the NIR region, the information content and data interpretation of biological NIR spectra are frequently affected by relatively weak and highly convoluted absorption features.

In considering the use of IR spectroscopy for clinical analyses, we are confronted with the fact that the most abundant species found in all biological fluids is water, and the IR spectra reflect this fact. To illustrate the dominance of water in the IR spectra, Figures 1 and 2 depict the absorption profiles for native serum in the MIR and NIR spectral regions (Shaw and Mantsch, 2010). Although some of the stronger solute absorptions do emerge in the MIR spectra, water clearly dominates the overall appearance. The NIR spectra are apparently devoid of any absorptions other than those of

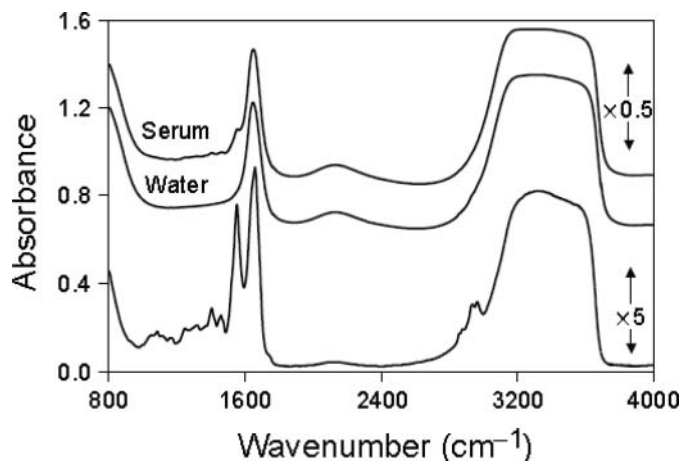


Fig. 1. MIR absorption spectra of serum and water, collected with an optical path of $6 \mu\text{m}$. The lower spectrum is a difference, with the spectrum of water subtracted from that of serum.

water. MIR and NIR spectroscopies in fact offer quite different, but complementary, approaches to analysis. The richness of the MIR spectrum makes it instinctively appealing as the method of choice for analytical work, however NIR has practical benefits such as convenience in sample handling and the fact that the sample cells do not require specialized materials. Whereas MIR spectroscopy of aqueous specimens typically requires optical path lengths of the order of microns, NIR transmission spectra are generally collected using path lengths of 0.5 mm or greater. The question of whether to use NIR or MIR spectroscopy for analytical purposes then translates to the question of whether the additional effort generally required to acquire MIR spectra is compensated for by other possible benefits such as greater analytical accuracy or smaller sample volume.

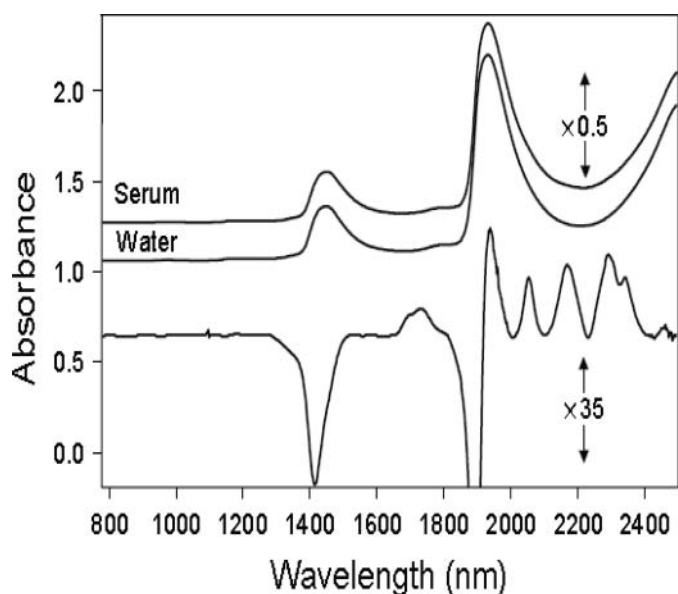


Fig. 2. NIR absorption spectra of serum and water, collected with an optical path of 0.5 mm . The lower spectrum is a difference, with the spectrum of water subtracted from that of serum.

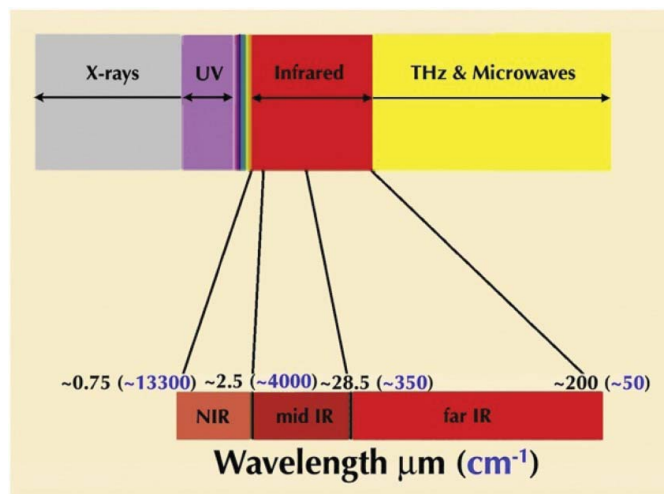


Fig. 3. The electromagnetic spectrum.

The IR spectral region ranges from the red end of the visible spectrum at 780 nm ($12,820 \text{ cm}^{-1}$) to the onset of the microwave region at a wavelength of 1 mm (10 cm^{-1}). Traditionally, this range is further subdivided into the near infrared (NIR), mid infrared (MIR), and far infrared (FIR), as can be seen in Figure 3.

The MIR region covers the range $400\text{--}4000 \text{ cm}^{-1}$ and is the region most familiar to the organic chemist as providing a “fingerprint” characteristic of molecular species. It is this region that includes the rich spectrum of absorptions corresponding to fundamental vibrations of the species being probed.

Human cells come in a variety of shapes and sizes: they may range from a configuration that is about 10 to $15 \mu\text{m}$ on edge, and nearly cubic in shape, to stratified (flattened) morphology, up to $60 \mu\text{m}$ in diameter and $5 \pm 10 \mu\text{m}$ thick, depending on the organ of origin. By dry weight, cells consist of about 60% protein and 25% nucleic acids, with the rest from other components (carbohydrates, phospholipids, and others) (Diem et al., 1999).

Vibrational spectroscopy offers complete information on the chemical composition of samples regarding both major and minor compounds, which present many characteristic bands in the infrared range (IR). Additionally, the presence of trace compounds can be modeled in some cases through the multivariate treatment of the whole IR spectra of well-characterized samples based on the influence of molecules at low concentration levels on the size and shape of the bands of major compounds (Miller et al., 2003), as can be seen in Figure 4.

The “bio-fingerprint region” (1800 to 900 cm^{-1}) of the MIR spectrum contains the fundamental vibrational modes of key chemical bonds that may be exploited to understand intracellular mechanisms. Therefore, IR spectroscopy produces a so-called “biochemical cell fingerprint” of the material under study, with direct association between peaks and chemical bonds (Kelly et al., 2011; Martin and Pollock, 2010; Naumann, 2001), providing a nondestructive, screening approach to diagnosis (Shin and Markey, 2006) that can be

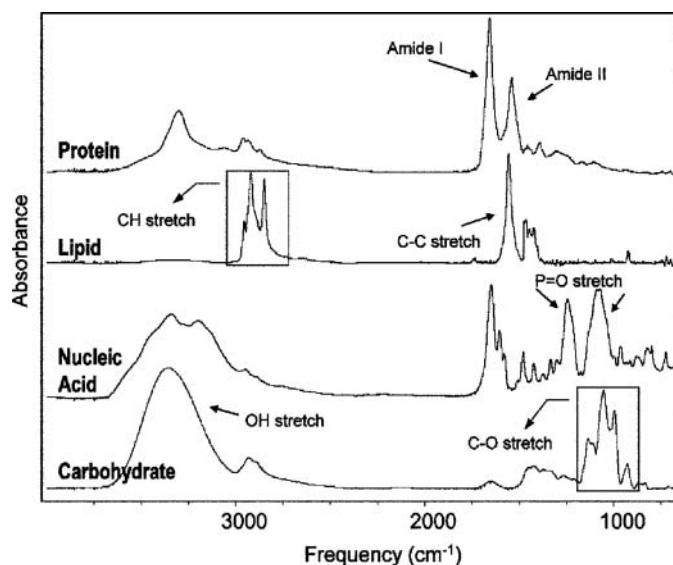


Fig. 4. Various cellular components have dramatically different IR spectra as demonstrated by IR spectra of a protein (myoglobin), a lipid (palmitic acid), a polynucleic acid, and a carbohydrate (sucrose).

done rapidly. Biospectroscopy (Kelly et al., 2011) is envisaged as an objective and robust tool to be used in cancer screening and diagnosis. Despite the increasing popularity of the field, there are several challenges to the developing application of biospectroscopy with regards to sample preparation, instrumentation, and data handling; these need to be addressed before the technique can become a routine method in the clinical or biological laboratory.

The chemical composition and structure of cells and tissues and the composition of biofluids of biological entities are subject to variations at the molecular level if affected by environmental factors, diseases, cancers, or other pathologies/abnormalities. IR spectroscopy not only differentiates

cells and tissues based on their characteristic spectral properties reflecting the chemical composition and structure, but also has the potential to serve as a diagnostic tool for detecting and discriminating different diseases or disease progression due to induced changes of chemical composition and structure.

Three main sampling methods of FT-IR spectroscopy exist, as can be seen in Figure 5. Transmission mode experiments operate by transmitting IR radiation through the sample and substrate before the resulting radiation is detected. In using this technique, expensive IR transparent substrates are commonly used. Although transmission mode experiments are the most common, transmission spectra are subject to a variety of physical effects occurring when measuring the sample. Transflection mode experiments detect the absorbed IR radiation after it is transmitted through the sample, reflected off the substrate, and transmitted back through the sample. The final mode of FT-IR spectroscopy is attenuated total reflection (ATR)-FT-IR; it operates on the principles of total internal reflection. By using this sampling mode of FT-IR spectroscopy, the aforementioned unwanted contributions to spectra can be overcome. Previous research in the field has shown that ATR-FT-IR is well suited to the analysis of biofluids and dry films. It has a wide applicability in quantifying various serum components of interest in clinical assays (Shaw and Mantsch, 2010). The sampling method also has the distinct advantage in analyzing samples such as serum, because the sample can be placed and dried directly on the ATR crystal before spectra are measured.

Conventional FT-IR spectroscopical techniques have been already applied to biomedical samples, and it is now well established that typical absorption bands observed in these types of samples are (Wong et al., 1991):

- i. Amide I (NH₂)
- ii. Amide II (NH)
- iii. C—H alkyl bending in proteins

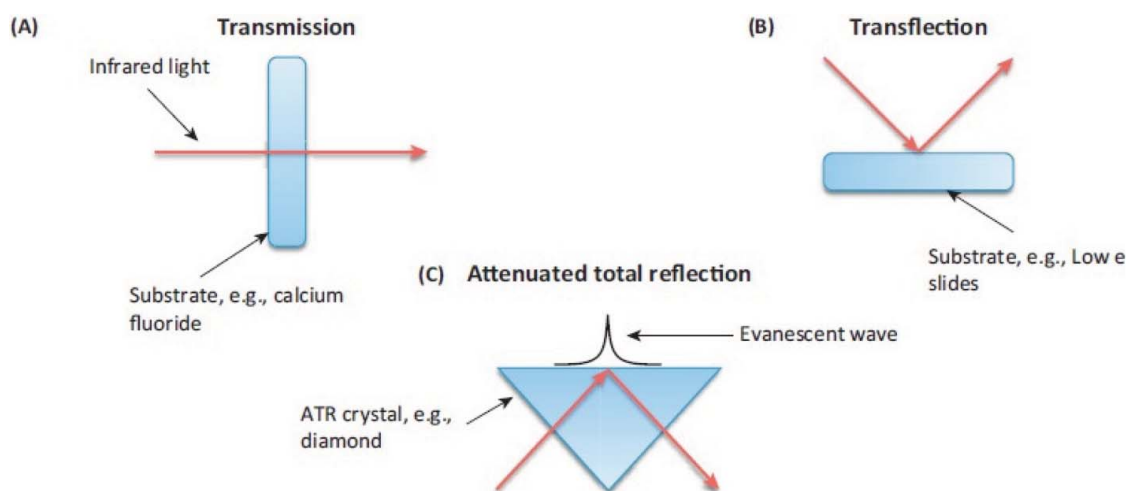


Fig. 5. Examples of Fourier transform-infrared (FT-IR) sampling methods for: (A) transmission mode experiments, (B) transflection mode experiments, and (C) attenuated total reflection (ATR) mode experiments.

- iv. Asymmetric phosphate (PO^{-3}_4) stretching in nucleic acids (NA II)
- v. C—O stretching in carbohydrates
- vi. Symmetric phosphate (PO^{-3}_4) stretching in nucleic acids (NA I)
- vii. Glycogen

FT-IR spectroscopy, with the absorption of electromagnetic radiation from 400 to 4000 cm^{-1} , is sensitive to changes in molecular compositions and structures in tissue biochemistry (Fernandez et al., 2005). It permits rapid collection of spectra obtained from millimeter-sized samples and could detect biochemical signatures of tissues that are associated with generation and progression of disease (Das et al., 2008).

Applications

Vibrational spectroscopy offers a unique opportunity to investigate the composition of unknown substances on a molecular basis. Although this fact was discovered long ago, it is recent technical advances that have generated strong and increasing interest in the application potential of molecular vibrational spectroscopy. Biological and medical applications, in particular, have progressed significantly in recent years (Bellisola and Sorio, 2012; Büttner Mostaço-Guidolin et al., 2009; Fass, 2008; Madsen et al., 2010; Mfoumou et al., 2012; Movasaghi et al., 2008).

The recent “renaissance” of applied vibrational spectroscopy is reflected by the interest of the medical community in this technology. Possibly, the number of related publications in the medical database MEDLINE may be considered indicative of this interest. Indeed, a variety of human body fluids, tissues, and calcified minerals have been investigated up to now. The quantitative analysis of metabolites in serum, the classification of diseased tissue, and the spectral identification of bacteria are only a few examples within the present repertoire of the medical application potential.

A wide range of biological studies has been covered by FT-IR analysis. These studies include breast, cervix, lung, skin, gastrointestinal tissue, prostate, and colon cancer detection.

Breast Cancer

Breast cancer is the most frequent kind of cancer in women, thus fast and early diagnosis is of enormous importance. Currently, diagnosis is more or less complicated and highly subjective. The most common methods for a screening are the mammography and ultrasound inspections. When there is a suspicion of breast cancer, in most cases punching biopsies are taken from the patient. This is a time-consuming method; the pathologist needs at least 30 minutes for a diagnosis. The findings are dependent on the subjective opinion of the corresponding doctor. This procedure features several limitations, including delays in providing the diagnostic results and being a subjective method, which has the potential for inter-observer disagreement (Kendall et al., 2009). To overcome these limitations new methods are needed that allow rapid,

noninvasive, and high-throughput diagnosis. Vibrational spectroscopic techniques exhibit the potential to overcome these limitations and enable an additional way of diagnosing and staging of cancer.

Micro-imaging FT-IR spectroscopy is able to monitor differentiation between normal and malignant tissues (Anastasopoulou et al., 2009). All the specimens previously submitted to histological analysis displayed abnormal spectra compared with the corresponding normal tissues, with changes in many diagnostic bands like those arising from phosphate, C—OH, and CH stretching vibrational modes. These characteristic bands have been monitored as a function of the degree of cancer progression. Chemometric methods, such as principal component analysis (PCA) and hierarchical clustering analysis (HCA), have been used in order to distinguish spectra of neoplastic and normal zones.

A nondestructive method employing FT-IR microspectroscopy coupled with attenuated total reflectance (ATR) objective for the analysis of histopathological specimens was described (Rehman et al., 2010). Malignant breast tissue specimens have been analyzed to demonstrate the hypothesis that chemical changes taking place in biological tissue can be reliably and reproducibly identified. It was concluded that FT-IR could objectively and reproducibly discriminate between DCIS (ductal carcinoma in situ) and IDC (invasive ductal carcinoma) grades without sample destruction. In the future, applications of FT-IR approaches should become feasible in the nondestructive express classification of grades and diagnosis of breast carcinoma.

A simple and rapid method for the detection of breast cancer with IR spectroscopy was developed (Backhaus et al., 2010). The method needs only 1 μL of a serum sample. The serum sample is dried on a suitable sample carrier such as an Si-plate. Every disease leaves a typical fingerprint in the IR spectrum of serum. To be sure that there is no any interference with other diseases the breast cancer patients were tested against 11 other diseases separately. Breast cancer was assigned to 79% to the correct group. These results suggest that IR spectroscopy in combination with intelligent mathematical evaluation tools such as artificial neural network (ANN) or cluster analysis is a good tool for the diagnosis of breast cancer.

In order to apply FT-IR spectroscopy as a routine tool for biomedical diagnostics of tissue samples, strong and reliable classifiers are needed (Sattlecker et al., 2011). Frequently, the number of available tissue samples is restricted and due to that data sets consist of a small number of samples, often fewer than 100. This can result in unstable classifiers that perform poorly on unseen data. To overcome this limitation several support vector machines (SVM) were used. As these results show, the application of SVM ensembles in biomedical diagnostics using FT-IR spectroscopy can be highly beneficial.

Histopathology forms the gold standard for the diagnosis of breast cancer, and FT-IR spectroscopic imaging has been proposed to be a potentially powerful adjunct to current histopathological techniques. Most studies using FT-IR imaging for breast tissue analysis have been in the transmission or transmission-reflection mode, in which the wavelength and

optics limit the data to a relatively coarse spatial resolution (typically, coarser than $5\ \mu\text{m} \times 5\ \mu\text{m}$ per pixel). This resolution is insufficient to examine many histologic structures. So ATR-FT-IR imaging incorporating a Germanium optic can allow a four-fold increase in spatial resolution due to the material's high refractive index in the mid-IR (Walsh et al., 2012). The authors employed ATR-FT-IR imaging for examining cellular and tissue structures that constitute an important component of breast cancer diagnosis. They reported the extraction of intact chromosomes from breast cancer cells and their spatially localized analysis as a novel approach to understand changes associated with the molecular structure of DNA in breast cancer.

Cervical Cancer

After breast cancer, cervical cancer is a leading cause of morbidity and mortality in women, representing approximately 12% of all cancers in women worldwide. Inspired by the great potential of the FT-IR spectroscopy as a screening tool for cervical cancer, an intelligent classification of cervical precancerous cells has been proposed based on the FT-IR spectra (Jusman et al., 2012). It consists of two parts: the extraction of FT-IR characteristics and the intelligent classification of the precancerous cells. A correlation test proved the ability of the proposed PCABFE (peak-corrected area-based features' extraction) to be as effective as manual extraction by human experts, while the HMLP (hybrid multilayered perceptron) network produces good classification performance with 97.4% of accuracy.

Cervical cancer screening programs have greatly reduced the burden associated with this disease. However, conventional cervical cytology screening still lacks sensitivity and specificity. There is an urgent need for the development of a low-cost robust screening technique (Purandare et al., 2013). By generating a spectral "biochemical-cell fingerprint," FT-IR spectroscopy has been touted as a tool capable of segregating grades of dysplasia. Following FT-IR spectroscopy, derived spectra were examined for segregation between classes in score plots generated with subsequent multivariate analysis. Deeper understanding of the underlying changes in the transition between cervical cytology classes (normal to low-grade to high-grade) is required in order to develop biospectroscopy tools as a screening approach. This will then allow for the development of blind classification algorithms.

Ovarian Cancer

Ovarian cancer is the sixth most common cancer among women worldwide, and mortality rates from this cancer are higher than for other gynecological cancers. The detection of neoplastic changes by optical spectroscopy techniques such as FT-IR, Raman, and fluorescence spectroscopy has been one of the most active areas of recent research into the discrimination of oral, cervical, breast, and other cancers. These methods are more objective and less time-consuming and have the ability to be applied *in vivo*. A review of the literature shows that there are very few reports of studies on

ovarian cancer diagnosis by optical spectroscopy (Brewer et al., 2001; Petricoin et al., 2002).

IR spectroscopy of blood plasma or serum is a rapid, versatile, and relatively noninvasive approach that could characterize biomolecular alterations due to cancer and has the potential to be utilized as a screening or diagnostic tool (Gajjar et al., 2013). Classification results for ovarian cancer were remarkable (up to 96.7%). This pilot study suggests that ATR-FT-IR spectroscopy of blood is a robust tool for accurate diagnosis and carries the potential to be utilized as a screening test for ovarian cancer in primary care settings. The proposed classification machine is a powerful tool that could be applied to classify the vibrational spectroscopy data of different biological systems (e.g., tissue, urine, saliva), with potential application in clinical practice.

Mehrotra et al. (2010) recorded the infrared spectra of normal and malignant ovarian tissues in the 650 to $4000\ \text{cm}^{-1}$ region. Post-surgical tissue samples were taken from the normal and tumor sections of the tissue. Significant spectral differences between the normal and the ovarian cancerous tissues were observed. In particular, changes in frequency and intensity in the spectral region of protein, nucleic acid, and lipid vibrational modes were observed.

Gastric Cancer

Gastric cancer is rampant in many countries around the world. By estimate, it is the fourth most common cancer worldwide (Kamangar et al., 2006). Almost two-thirds of the cases occur in developing countries and 42% in China alone. Survival for gastric cancer is moderately good only in Japan (52%), where mass screening by photofluoroscopy has been practiced since the 1960s. NIR spectroscopy, as a sensitive analytical technique with practical advantages, can record the response of chemical bonds in functional groups (e.g., O—H, C—H, and N—H bands) to the NIR spectrum, which is related to the primary structural components of organic molecules. Therefore, any alteration in the composition of the tissues can be detected and used for diagnostic purposes (Yi et al., 2013). In the Yi et al. study, the authors investigated the qualitative NIR spectral differences between gastric cancer and normal tissues in surgically resected gastric specimens using FT-NIR equipped with a fiber-optic probe to mimic *in situ* clinical measurements. The spectra from cancerous and normal tissues were collected using Fourier transform-near infrared spectroscopy (FT-NIR) equipped with a fiber-optic probe. These results indicate that CH stretching first, combination band, and second overtone regions can serve as diagnostic markers for gastric cancer.

Since serum can reflect human beings' physiological and pathological conditions, FT-IR spectroscopy was used to compare gastric cancer patients' serum with healthy persons' serum (Sheng et al., 2013). The H2959/H2931 peak height ratio might be a standard for distinguishing gastric cancer patients from healthy persons; the result showed that the RNA/DNA ratios of gastric cancer patients' serum were obviously lower than those of healthy persons' serum. The results suggest that FT-IR spectroscopy may be a potentially useful tool for diagnosis of gastric cancer.

Colorectal Cancer

Colorectal cancer is a major public health problem, being the third most common cancer and the fourth leading cause of cancer deaths worldwide. It is important to explore a noninvasive and rapid method for detection of colon cancer biopsies. Initially, principal component analysis was applied to examine the degree of separation between tissue samples (Khanmohammadi et al., 2009). This study tried to demonstrate that ATR-FT-IR microspectroscopy in combination with chemometric methods can reliably distinguish malignant colon tissues from healthy ones. There were significant differences in the FT-IR spectra of normal and cancerous colon biopsies in the 1800–900 cm^{-1} spectral region. The soft independent modeling of class analogy (SIMCA) results demonstrated that the accuracy, specificity, and sensitivity of the proposed diagnostic method were 93.3, 100, and 88.2%, respectively, which could help satisfy clinical diagnostic requirements.

The process of carcinogenesis in the colon progresses through several overlapping, stages, making the evaluation process challenging, as well as subjective (Sahu and Mordechai, 2010). FT-IR spectroscopy, being quantitative and objective, has the capacity for automation and relevance to cancer diagnosis. These results highlight investigations on the application of FT-IR spectroscopy (particularly microscopy) in colon cancer diagnosis and parallel developments in data analysis techniques for the characterization of spectral signatures of malignant tissues in the colon.

ATR-FT-IR microspectroscopy was applied for detection of colon cancer according to the spectral features of colon tissues (Khanmohammadi et al., 2011). Several chemometric methods such as analysis of variance (ANOVA), cluster analysis (CA), and linear discriminate analysis (LDA) were applied for classification of IR spectra. Utilizing the chemometric techniques, clear and reproducible differences were observed between the spectra of normal and cancer cases, suggesting that infrared microspectroscopy in conjunction with spectral data processing would be useful for diagnostic classification.

Nonlinear feature extraction methods, neighborhood preserving embedding (NPE), and supervised NPE (SNPE) were employed to effectively represent the IR spectral features of stomach and colon biopsy tissues for classification and improve the classification accuracy for diagnosis of malignancy (Lee et al., 2013). The aim was to utilize the NPE and SNPE's capability of capturing nonlinear spectral behaviors by simultaneously preserving local relationships in order to effectively recognize minute spectral differences among classes. Overall results demonstrate that NPE and SNPE could be potential feature-representation strategies useful in biomedical diagnosis based on vibrational spectroscopy where effective recognition of minute spectral differences is critical.

Lung Cancer

Lung cancer is one of the most prevalent malignant tumors worldwide and the leading cause of cancer death globally. It accounts for 13% (1.6 million) of the total cancer cases and

18% (1.4 million) of the deaths in 2008. Due to the increase of smokers and deterioration of the environment, the incidence and mortality of lung cancer have increased gradually in recent years. At present three methods, chest X-ray, CT, and bronchoscope, are widely used for clinical diagnosis of lung cancer. Although these methods can improve the ability to diagnose lung cancer, they are still less effective for detecting lung cancer at early stages (Sudetja, 2003). Therefore, it is very urgent to develop an effective method for early detection of lung cancer. From the late 1980s up to now, FT-IR spectroscopy has been used to explore a number of diseases, including lung cancer.

Survival time for lung cancer is poor, with over 90% of patients dying within five years of diagnosis, primarily due to detection at a late stage. The main objective of some research projects was to evaluate FT-IR spectroscopy as a high-throughput and cost-effective method for identifying biochemical changes in sputum as biomarkers for detection of lung cancer (Lewis et al., 2010). A panel of 92 infrared wavenumbers had absorbances significantly different between cancer and normal sputum spectra and were associated with putative changes in protein, nucleic acid, and glycogen levels in tumors. The results suggest that FT-IR applied to sputum might have high sensitivity and specificity in diagnosing lung cancer with potential as a noninvasive, cost-effective, and high-throughput method for screening.

Optical observation of lung cancer tissues using ATR-FT-IR and confocal Raman microscope was performed (Lv et al., 2011). A total of six malignant tissues, seven tissues adjacent to cancer, and nine normal tissues from nine patients with known lung cancer were studied. High-quality spectra from human tissues were obtained only in a few seconds. The results revealed that some of the spectral characteristics varied significantly between normal and malignant tissues, that is, IR peak positions, Raman shift, and spectral intensities.

ATR-FT-IR spectroscopy could serve as a diagnostic tool for detecting and discriminating lung cancer (Sun et al., 2013). A pilot study on 60 samples was performed to distinguish malignant and nonmalignant lung tissues with ATR-FT-IR spectroscopy. Peak positions, intensities, and full width at half maximum of each absorbent band were measured, and the relative intensity ratios were calculated. The sensitivity and specificity of the discriminants were all 96.7%, so ATR-FT-IR spectroscopy can be considered a promising method for the detection of malignant lung tissue and could be proved useful in lung tumor surgery.

Prostate Cancer

Prostate cancer is the most common cancer and second most common cause of cancer-related deaths of men; however, the diagnosis in individual patients can be problematic, which has led to inappropriate treatment (Kwiatk et al., 2009).

A interesting study was performed in order to establish a link between FT-IR data and the local biopotential of prostate cancers using fixed and stained tissues (Mackanos and Contag, 2009). The importance of staging the disease for directing therapy has been well documented; and this study

suggests FT-IR could be used to categorize tissues into those that are aggressive and have the potential to move beyond the prostate and those that remain localized to the prostate. The authors found that biochemical changes associated with prostate cancer could be discriminated by FT-IR to classify confined and locally invasive prostate cancers. These findings could enable the development of improved diagnostic and prognostic methods for the detection and treatment of prostate cancers.

FT-IR spectra of tissue samples in different conditions, healthy, hyperplastic, and cancerous stages, reveal differences that address the occurrence of chemical composition changes in the examined samples. In the case of prostate tissue sections the results show the possibility of determining the intensity ratio of the CH₂ and CH₃ bands set at 2930 and 2960 cm⁻¹, respectively.

Morphological and histomorphological evaluation of this disease is a well-established technique for cancer classification and has remained relatively unchanged for several decades, although it remains a time-consuming and subjective technique, with unsatisfactory levels of inter- and intra-observer discrepancy. Novel approaches for histological recognition are necessary to identify and investigate cancer in detail (Pezzei et al., 2010). FT-IR spectroscopic imaging has become an essential tool for the detection, identification, and characterization of the molecular components of biological processes, such as those responsible for the dynamic properties of cancer progression. A major advantage of this new technique is the acquisition of local molecular expression profiles while maintaining the topographic integrity of the tissue and avoiding time-consuming extraction, purification, and separation steps. By using this method it is possible to investigate the spatial distribution of proteins, lipids, carbohydrates, cholesterol, nucleic acids, phospholipids, and small molecules within biological systems by in situ analysis of tissue sections. By applying this method, it is possible to distinguish between cancerous and noncancerous areas within prostate cancer tissue with a resolution of 6.25 μm × 6.25 μm on frozen sections.

Conclusions

Fourier transform-infrared (FT-IR) spectroscopic methods for the detection of cancerous and precancerous cells and tissues offer several advantages over the conventional histocytological methods, which rely on the intrinsically subjective visual examination of cells by trained pathologists. These advantages include: minimal sample preparation, rapid measurement time, automated diagnosis, sample recovery, and instrumentation that is inexpensive and easy to operate. Furthermore, FT-IR objectively detects the biochemical composition of a cell or cell population and has the sensitivity and selectivity to differentiate between samples based on changes localized to any one of many cellular constituents. Conventional biomedical diagnostics such as cancerous cell or tissue determination is usually time consuming and labor intensive, and requires expertise in sample preparation as well as cytology, histology, and pathology. In contrast to

these traditional techniques, IR spectroscopy holds promise as a rapid, label-free analytical route, which is potentially labor- and time-saving and requires a minimum amount of training, in particular if data processing and mining are integrated components of a diagnostic system. Finally, we believe that noninvasive, rapid, accurate, and convenient analysis of tissues can be performed with FT-IR spectroscopy if the infrared fiber optics and endoscopy technologies can be combined successfully. IR spectroscopy opens the chance for a rapid and simple diagnostics that is nearly independent of the operator. However, more technical instrumentations need to be introduced to overcome some of its practical limitations.

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