Fourier transform infrared spectroscopy in cancer detection

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The rapid developments in the field of infrared spectroscopy in the past decade have demonstrated a potential for disease diagnosis using noninvasive technologies. Several earlier studies have highlighted the advantage of using infrared spectroscopy both in the near- and mid-infrared regions for diagnostic purposes at clinical levels. The areas of focus have been the distinction of premalignant and malignant cells and tissues from their normal state using specific parameters obtained from Fourier transform infrared spectra, making it a rapid and reagent-free method. While it still requires pilot studies and designed clinical trials to ensure the applicability of such systems for cancer diagnosis, substantial progress has been made in incorporating advances in computational methods into the system to increase the sensitivity of the entire setup, making it an objective and sensitive technique suitable for automation to suit the demands of the medical community. The development of fiber-optics systems for infrared spectroscopy have further opened up new and modern avenues in medical diagnosis at various levels of cells, tissues and organs under laboratory and clinical conditions.

Among the different spectroscopic methods that have been evaluated for distinction between normal and neoplastic tissues, Fourier transform infrared (FTIR) spectroscopy has shown huge potential as the technique of the future. Infrared (IR)-based techniques in biomedicine have become a reality with a large amount of information accumulated from clinical studies, trials and the developments in instrumentation for IR spectroscopy. This technique, being reagent free, can rapidly and noninvasively detect changes in the biochemical composition of cells and tissues (at the molecular level), especially during carcinogenesis. In the present article however, the significant progress made during the last 5–6 years shall be discussed and its implications for cancer diagnosis highlighted, leaving the more basic aspects to the interested readers by citing relevant references. The prominent areas where FTIR-spectroscopy may be used in cancer diagnosis in the future are:

- Differentiation of normal and diseased tissues in organs – breast [1], colon [2], liver [3] and cervix [4]; detection of early stages of malignancy (e.g., polyp that is considered premalignant), which are not yet evident using standard methods [2]
- Monitoring abnormal cell growth and proliferation in tissue sections – colonic crypts [5], cervical epithelium [6] (both possess well-established pattern [zones] of cell growth and exfoliation)
- Distinguishing between normal and abnormal cell–cell scrapings (cervix), biopsies from smaller organs like the prostate [7] and thyroid [8] and body fluids [9]
- Differentiation between cancer and other pathologic conditions with similar clinical manifestation – IBD and colon cancer [10]
- Monitor the effect of anticancer therapy–chemotherapy in leukemia [11], and tumor grading – lymphoid tumors [12]

The examples mentioned above clearly suggest that FTIR has the potential to develop into a powerful tool in cancer diagnosis. The common methodology for FTIR spectroscopy is outlined and finally, a brief summary of the impact and implications of FTIR spectroscopy on future oncology is provided. A brief discussion of the progress made in the diagnosis of cancer through FTIR spectroscopy will focus on cervical cancer as a case study.

History of FTIR spectroscopy for cancer diagnosis

Cervical cancer as a case study

Cervical cancer (especially invasive squamous cell carcinoma) is the second most prevalent cancer among women after skin cancer, constituting between 80–90% of cervical cancers, and is a major public health problem and leading cause of death in developing countries [13]. Screening for cervical malignancies is therefore important and currently undertaken through Papanicolaou

Keywords: biomarkers, cancer grading, diagnosis, Fourier transform infrared spectroscopy

future medicine

(PAP) smears or biopsy examinations. An initial report by Wong and colleagues examined the possibility of FTIR spectroscopy for cervical cancer diagnosis, although later the differences were found to be due to erroneous sampling at different layers of cervical epithelium and not due to actual differences due to malignancy [14]. Morris and colleagues studied samples that contained cells collected from cervical canals using FTIR and observed changes in spectra at various stages of cervical intraepithelial neoplasia (CIN) that were of diagnostic potential [15]. Another report, dealt with the distinction of normal and dysplastic cells in cervical samples using FTIR and reported the similarity of the dysplastic cell spectra with HeLa cells' spectra [16]. The important observation was that the glycogen absorbance was a prominent diagnostic feature and this combined with prinicple-component analysis ushered in a new era of FTIR diagnosis of cervical malignancies using microscopic FTIR facilities. This was followed by systemic studies of exfoliated cells for tracing diagnostic changes [17,18]. Cases of histologically normal cervical samples with abnormalities in chemical composition were observed using FTIR [19], bringing the new concept of detecting chemical changes that are undetectable by conventional methods. At the same time, other groups compared the sensitivity of FTIR with standard diagnostic procedures [20]. The development of advanced computational methods to analyze the spectra was also in progress, with several groups identifying important digital methods for the distinction of normal and cancerous tissues [18,21]. The presence of contamination in samples is a major obstacle in FTIR measurements as no staining is undertaken. Samples from the cervix are usually contaminated with materials such as blood, microorganisms and cellular debris. These impurities affect the actual absorption pattern of IR by cervical cells/tissues and can greatly alter the results and interpretations of the spectral data. Thus, there were also studies concerning this issue [22,23] and attempts made to overcome these hindrances. Meanwhile, the biopsies from the cervix were studied by FTIR spectroscopy for the detection of cancer and to understand the spectral variations during the differentiation of cervical epithelium [24,25]. Comparison of FTIR spectra was made between biopsies and exfoliated cell samples to see if similar trends were observed in the distinction of normal from cancerous cervical cells/tissues [26,27]. A detailed description of FTIR spectroscopy and cervical cancer was reviewed by Diem and colleagues [28] and methodology for chemical mapping at the cellular level were described [29]. Recently, chemical mapping and changes occurring in FTIR spectra during biopsy processing have been reported by Gazi and colleagues [30], which will help to identify biochemicals that are common to fresh and formalin-fixed tissues for *in situ* diagnosis in future. During the past few years, progress has been made by such approaches in eliminating factors leading to the removal of confounding variables [31,32]. More recently, the application of linear discriminate analysis (LDA) and quadratic discriminate analysis (QDA) for spectral analysis and utilization of probabilistic neural networks (PNN) for grading of premalignancy CIN and identification of cancer were studied [33,34]. This has further boosted the prospect of having automated methods based on FTIR spectroscopy and advanced computational methods. Similarly, much progress has been made in image analysis of cervical tissues [35], which will enhance the screening capacities in the hands of the pathologist in the future. Thus, FTIR spectroscopy would be an important tool in cancer diagnosis in future. The following sections describe how FTIR accessories work in tissue and cell analysis and future trends in the development and application of this method.

Principles & advantages of FTIR spectroscopy

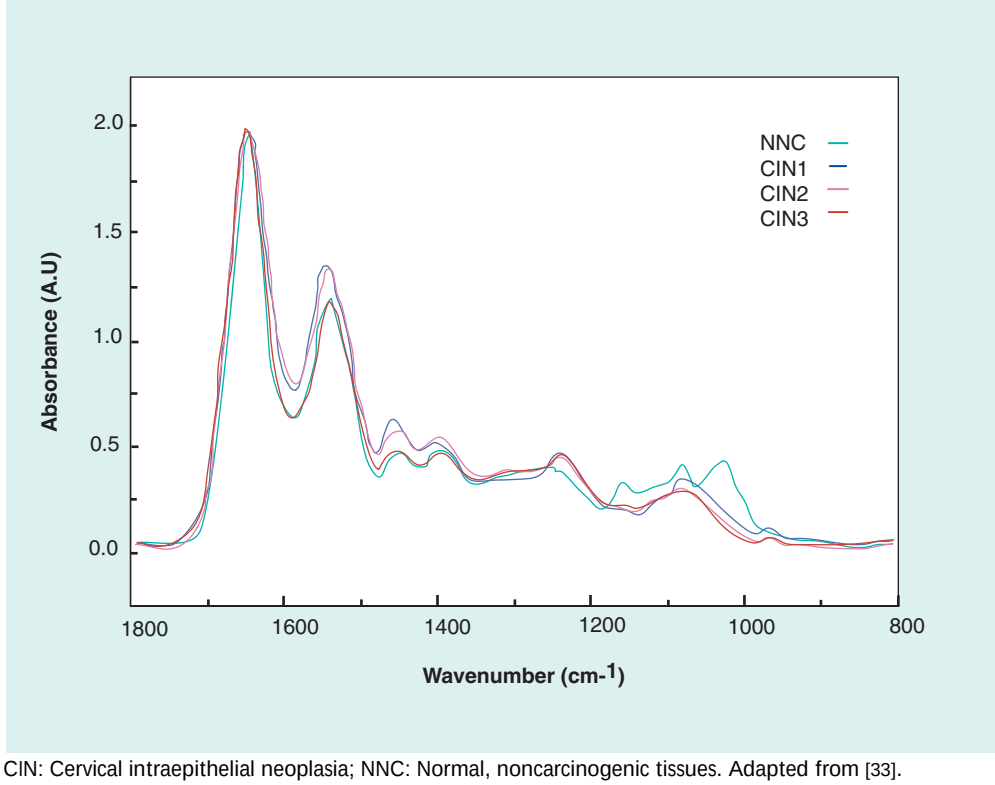
IR spectroscopy is the study of the interaction of IR radiation with matter. Earlier instruments were dispersive and thus intensity at each wave number was measured separately. This method, although popular, was time consuming. On the other hand, FTIR spectroscopy contains no monochromators but an optical element that is composed of an interferometer, allowing simultaneous measurements of a complete region of wave numbers in a short time span. The Fourier transform computerized methodology transfers the spectrum from optical path difference (intereferogram) into the frequency domain and thus decreases noise levels and makes data collection more rapid and easier to interpret.

In contrast to UV, x-rays and gamma rays, which are currently used in medicine, IR rays are non-destructive to biological samples. Compared with magnetic resonance imaging (MRI) and positron emission tomography (PET) it requires smaller amounts of samples (biopsies) and is also faster than Raman spectroscopy for *ex vivo* analysis. Thus, FTIR spectroscopy has

an advantage in analysis of tissues, fluids and cells. IR radiation promotes vibration of the covalent bonds of molecules within the sample that absorbs it. The wavelength of IR radiation that is absorbed depends upon the nature of the covalent bond (i.e., atoms involved and the type of bond) and the strength of any intermolecular interactions (van der Waal's interactions, H-bonding). Various biomolecular components give a characteristic IR spectrum, [36–38] that is akin to a biochemical fingerprint of that tissue, allowing measurements of complex molecular vibrational modes that contain valuable information on changes occurring due to diseases such as cancer. For example, simultaneous variation in several metabolites can be monitored in case of CIN or cancer, and these parameters singularly or in combination can provide a very useful diagnostic evaluation (Figures 1–3). It is cost effective and simple to operate when infrastructure is established. Since the sample preparation does not require exclusive and special treatments, the method would ensure a rapid diagnosis. Portions of samples obtained for histochemical studies or other analysis [39] can be used for FTIR spectroscopy and thus, it would enable valuable supplementary data. For example, it would help distinguish polyps in an objective method compared with histology [40].

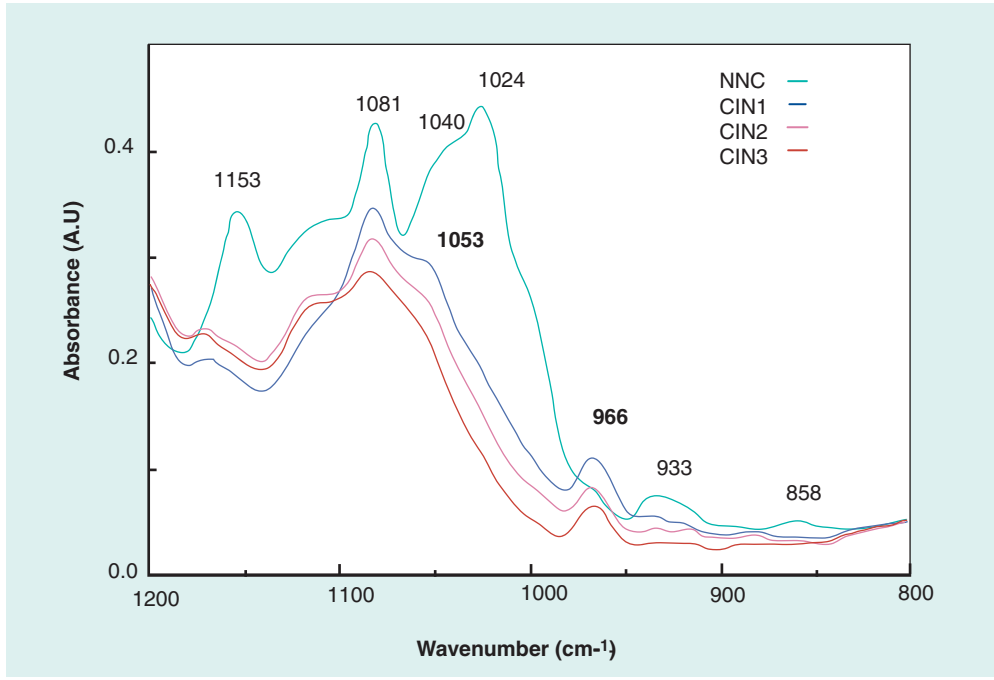
Current trends indicate that in the near future it would be possible, to develop instruments based on FTIR spectroscopy for diagnosis of cancer at various levels in tissues from biopsies to in situ diagnosis in the patient in the operating theater (with further technologic developments). FTIR spectroscopy can be used in oncology, not only for diagnosis of normal from cancer, but also to understand basic processes such as changes in metabolite concentration [41,42] and changes in secondary structure of biomolecules (DNA) prior to histologic manifestation [43–45]. One future utilization of FTIR spectroscopy that would have an advantage over existing methods of diagnosis (molecular and immuno-reaction based) is the identification of suitable common biomarkers for different types of malignancies, irrespective of their nature and origin. Some important insights into such common origins of cancer in different organs using FTIR spectroscopy was reported earlier [46]. Availability of

Figure 1. Mid-infrared spectra of cervical biopsies representing the absorbance pattern of different premalignant stages in the wavenumber region 800–1800 cm^{-1}.



CIN: Cervical intraepithelial neoplasia; NNC: Normal, noncarcinogenic tissues. Adapted from [33].

Figure 2. Expanded region between 800–1200 cm^{-1} of the absorbance due to carbohydrates (glycogen), phosphates and nucleic acids.



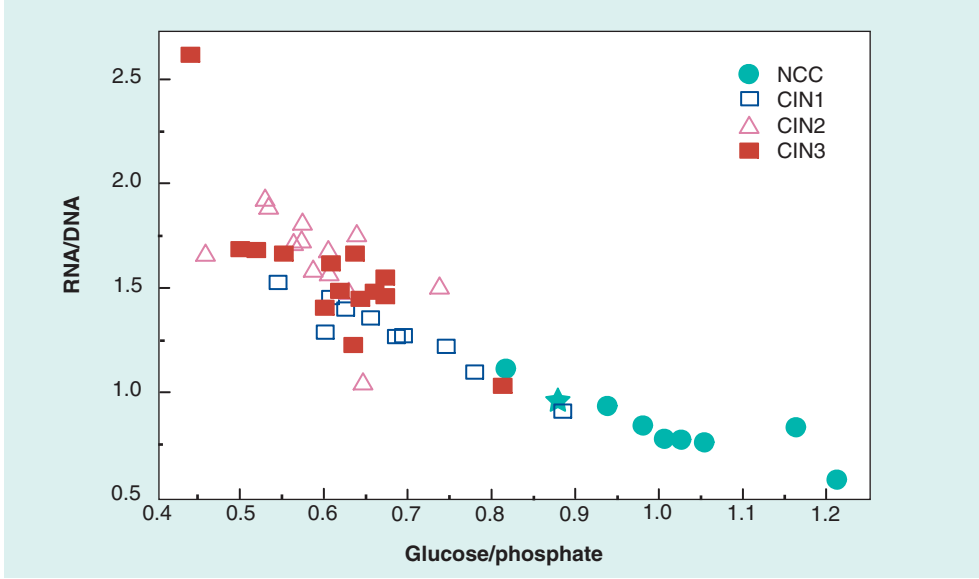
CIN: Cervical intraepithelial neoplasia; NNC: Normal, noncarcinogenic tissues. Adapted from [33].

a large amount of spectral data and multiple diagnostic wave numbers do not rule out such a possibility in the future. Some initial results in this aspect are encouraging [47–49]. Moreover, the availability of computational methods and neural networks could help to focus on some of the common features (wavelets) in all cancers.

Utilities of FTIR spectroscopy

The high prevalence of colonic and cervical cancers [13] requires rapid techniques for early and foolproof diagnosis. While a FTIR-based method suitable for *in situ* diagnosis is a significant achievement [50], progress has been made in identifying, grading and also predicting cancer in biopsies (samples) from these two organs that are amenable to endoscopic studies. Other than these, FTIR spectroscopy has been used for cancer detection in other organs/tissues such as the stomach [51], esophagus [52] and oral mucosa [53], which are part of the digestive tract. The requirement of very small amounts of samples (a few microliters or cells) has an added advantage that a separate sample procurement is not required and minute portions of samples taken for other conventional studies are used. This is especially useful for cancers of organs such as the thyroid, prostate, and bone marrow, where needle biopsies can be performed or only small amounts of samples are available for analysis. Micro quantities of lymphocytes have been examined by FTIR spectroscopy to identify and grade the type of leukemia [12]. Cervical smears or exfoliated cells are also subjected to such analyses [17,54]. The accurate staging/grading of the premalignancy/cancer is an important determinant of a successful treatment, as false grading could lead to a wrong prognosis. Various types of polps have been successfully graded utilizing FTIR-microspectroscopy (MSP) and neural networks [2]. Similarly, the various stages of CIN have been classified using FTIR-MSP [33]. Differentiation of melanoma and nevi by FTIR-MSP has also been successful [55]. Since FTIR spectroscopy can distinguish between normal and cancerous tissues and also between different grades of malignancies, in principle it would be possible to calculate the extent of spread of cancer/premalignancy using biopsies at a regular distance from the foci of cancer and reassess resection margins for individual patients. For example, in the case of colon, where abnormal crypts can be identified in a very early stage (Figures 4 & 5) [5,56]. Use of imaging systems can also help to re-evaluate the shoulder regions and identify any abnormality present in tissues. While most cancers have a distinct clinical manifestation, at times they show

Figure 3. Parameters derived from spectral intensities to differentiate stages of premalignancy (RNA/DNA: I(1121 cm^{-1})/I(1020 cm^{-1}) and glucose/phosphate: I(1030 cm^{-1})/I (1080 cm^{-1})).



The color codes are the same as for Figure 1. Each point represents a biopsy. The star symbol represents a biopsy that was initially diagnosed as normal by the pathologist but from Fourier transform infrared-microspectorscopy, tallied with cervical intraepithelial neoplasia (CIN)1. It was later confirmed from re-examination by the pathologist as CIN1.
NNC: Normal, noncarcinogenic tissues. Adapted from [33].

similarity with other diseases. For example, acute gastritis and inflammatory bowel diseases are known to progress to cancer. Thus, it is important to distinguish these diseases from cancer using sensitive techniques such as FTIR spectroscopy [10]. In the future, analysis of shoulder regions as well as accurate grading and identification of premalignancy may be used to predict relapse of cancer or onset of premalignancy. Such studies will depend on the high accuracy and predictability of FTIR spectroscopy in tandem with advanced computational methods such as neural networks.

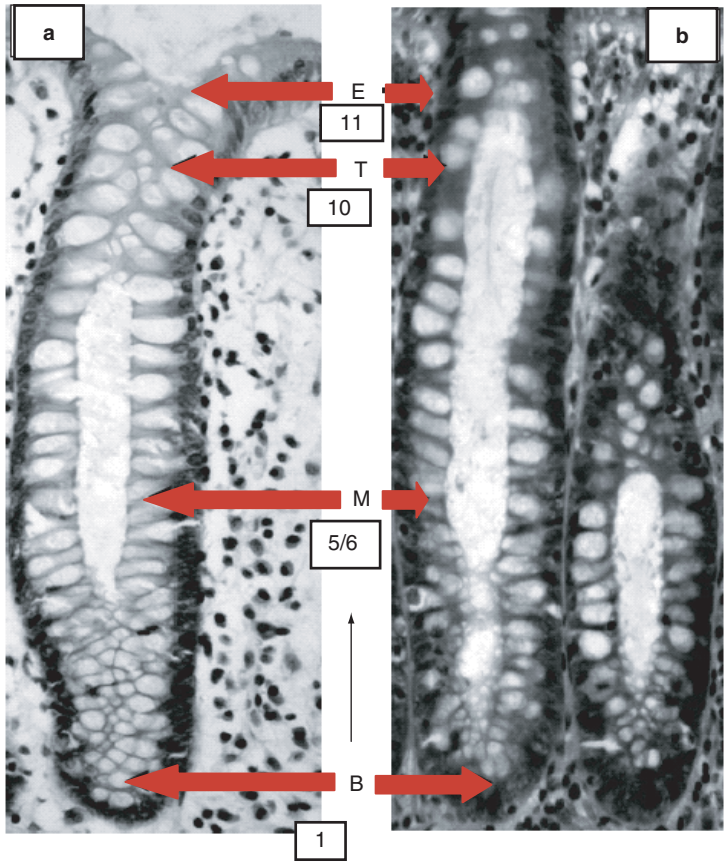
Computational & technologic accessories for cancer diagnosis by FTIR

FTIR spectroscopy requires accessories for increasing its diagnostic potential (sensitivity and specificity) such as advanced computational methods, to efficiently handle and classify large amounts of spectral data. Such methods can range from simple cluster analysis of spectra based on algorithms (such as Ward's algorithm) in specific regions of the spectra that contain prominent features and markers, to utilization of complex probabilistic neural networks and artificial neural networks softwares (that are based on wavelet features) to predict/diagnose cancer.

Statistical methods are useful to identify suitable parameters obtained from the spectral data (e.g. ratio of intensities at specific wavenumbers) that can be used as biomarkers for cancer. Although there can be many diagnostic wave numbers, intensities based on known sources of absorbance are more useful as they directly correlate with the biologic sources [37]. The availability of a large number of such intensities makes it necessary to screen them using statistical tests such as 't' tests to identify the most sensitive (Table 1) [47]. The utilization of artificial and probabilistic neural network softwares (Table 2), linear discriminate analysis, principal component analysis [18], and Ward's algorithm or Euclidean distances for cluster analysis have opened up new avenues for classifying and grading of tissues/cells. These methods being suitable for automation would lead to rapid and objective diagnosis of samples, reducing false negatives and false positives. The necessity of such complex computational methods has been well discussed in earlier works [2,8].

With the development of optical fibers that are highly transparent in the mid-IR, it was realized that IR fibers could be used as attenuated total reflection (ATR) elements.

Figure 4. Crypts from human colon stained with hematoxylin-eosin showing the measurement sites along the crypt using Fourier transform infrared (FTIR)-microspectroscopy.



Symbols represent: (B) measurements at the base of the crypt, (M) middle of the crypt, (T) top of the crypt and (E) zone of exfoliation. The arrow shows the direction of the FTIR-microspectorscopy measurements.
Both the normal **(a)** and abnormal **(b)** crypts as designated by FTIR spectra show similar (normal) morphology in the histochemical review. Number 1 refers to the first measurement site at the base and 11 represents the top of the crypt.
The other numbers indicate the position with respect to these points, with an estimated equal spacing along the crypt height. Adapted from [5].

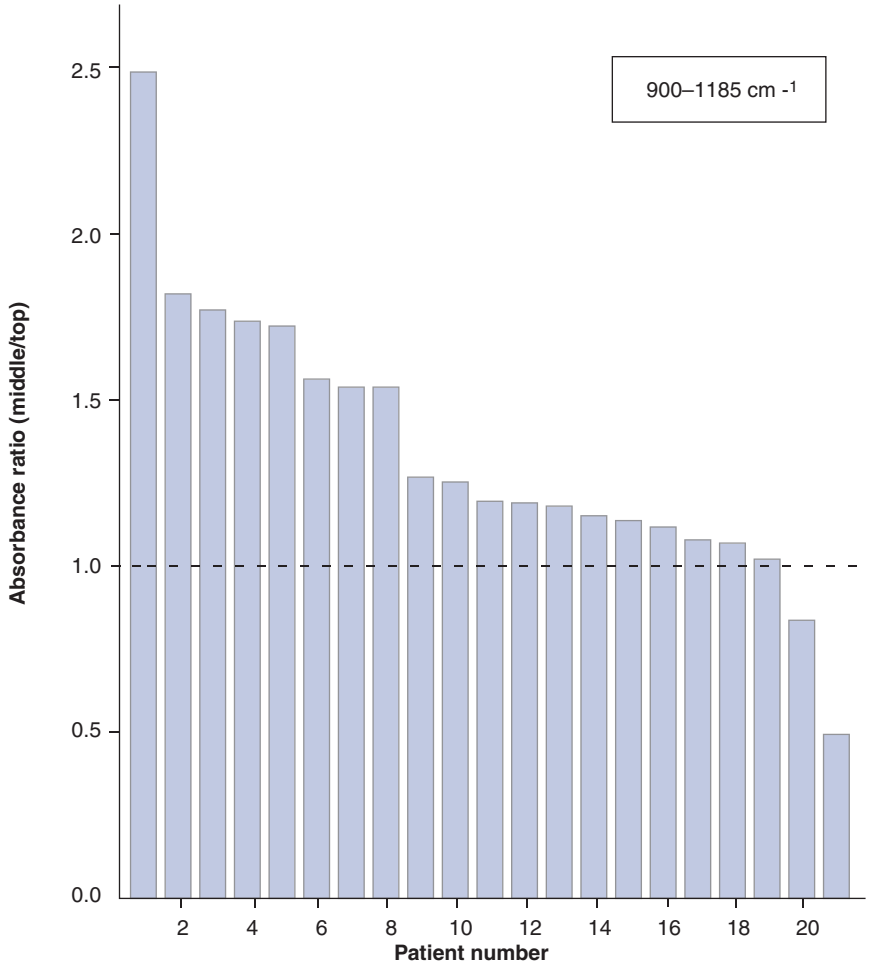
These fibers offer great advantages over the traditional ATR elements. The IR fibers are flexible, inexpensive, and can be quite long (many meters), thus enabling IR spectroscopy of samples remote from the instrument. This is called fiberoptic evanescent wave spectroscopy (FEWS) (Figure 6). FTIR-FEWS would prove useful for organs such as the skin [57], where the probe can be used to scan the surfaces (for diagnosis of skin cancer and melanoma). They can also be incorporated into instruments such as endoscopes, colonoscopes and colposcopes to enable diagnoses of cancer *in situ* [50,56], although currently, the water content in tissues is a major hindrance. Such studies are still in the preliminary stage [34].

The utilization of histopathologic studies on consecutive sections in parallel helps to further complement the observations. This is useful when databases are built for use as a reference to classify unknown samples into normal/neoplastic/premalignant/cancer. Similarly in the case of leukemia, fluorescence assorted cell sorters, based on immunotyping, polymerase chain reaction for identification of gene mutations and cytology for translocations, is used to diagnose the type of leukemia in tandem with FTIR spectroscopy to examine the validity of separating one type of leukemia from another [11]. Likewise, data obtained through other methods, such as colonoscopy and colposcopy, can give information on the condition of the organ that is being studied and draw a correlation between the FTIR results and *in situ* conditions.

Diagnosis using FTIR spectroscopy

Simple IR spectrometers can collect biochemical information from dried homogeneous samples (plasma, whole blood, blood components or body fluids) mounted on suitable transparent window materials. FTIR microscopy can be utilized for fresh, frozen or fixed tissues where microtomed tissue sections are available. Tissues such as colon or cervix are easier to measure, as they have defined zones of proliferation. Usually the consecutive tissue section (which is identical) is stained histochemically so that a direct comparison can be made between the two methods to confirm the diagnosis. Imaging techniques facilitate data output, allowing large arrays of spectral measurements using focal plane area detectors. These systems are also very useful when an entire tissue is mapped at pixel level using the spectra obtained at each point using focal plane area technology. The spectral data obtained for each point is later subjected to various mathematical analyses (such as cluster analysis) and the data used to generate a color code for each point. The color intensities are then reassembled to obtain a pseudo-colored image. In these cases, the malignant tissues are colored differently from the normal tissue. Although this type of analysis takes longer, it maps the whole area simultaneously, identifying malignancies at any point. In a microscopic study there is a possibility of missing certain areas during random sampling for cancer

Figure 5. Changes in the ratio of carbohydrate content of normal and abnormal crypts as determined by the integrated absorbance in the region 900–1185 cm^{-1}.



The ratio represents the variation in the carbohydrate content at the middle of the crypt relative to the top of the crypt. The dotted line represents the ratio of 1.0 when levels of the metabolites at the middle and at the top are equal. Adapted from [5].

detection. The imaging system has been used for various organs such as colon [58], cervix [35], breast [59], head and neck tumors [60].

FEWS systems are based on ATR elements inside or outside an FTIR system (Figure 6). In this case, samples are placed in contact with the IR transmitting prisms or flat wave-guides that serve as ATR elements. The evanescent waves that 'escape' from the ATR elements are absorbed in the samples, at some wavelengths that are characteristic to the sample. Therefore, by measuring the transmission of an ATR element in contact with a sample, one actually measures the IR absorption of that sample. The advantage is that one does not have to prepare a thin sample. In the case of biologic samples this is a very convenient way of measuring IR absorption of tissues *in vivo* or *in situ* [50,56].

Steps in FIR spectroscopy of biologic samples

Samples that contain little or no water are suitable for FTIR spectroscopy in the mid-IR region. The conventional formalin-fixed biopsies sliced to thin sections (about 10 µm) are suitable, after proper deparaffinization, for evaluation with both the imaging systems as well as microscopic systems [2]. The processing itself reduces the moisture present in the tissues. Additionally, both fresh and frozen sections of biopsies have shown encouraging results for diagnosis of cancer. Similarly in the case of leukemia, air-dried lymphocytes formed a good sample [11], although drying has been shown to affect some spectral features. Cells from PAP smears can be analyzed by mounting on CaF_2, BaF or ZnSe

Table 1. Effectiveness of different RNA/DNA ratios on distinction of normal, premalignant (polyp) and malignant colonic tissues.

Source		996/966	1121/1020	1244/1230
Normal and cancer	t-value	8.849	7.569	0.776
	p-value	3.57×10^{-13}	9.05×10^{-11}	0.440
Normal and polyp	t-value	7.493	8.596	0.819
	p-value	3.469×10^{-11}	1.65×10^{-13}	0.415
Polyp and cancer	t-value	2.256	0.500	0.186
	p-value	0.027	0.618	0.853

The values denote the ratios of absorbance at the given wavenumber after spectral processing for baseline and normalization to the amide I peak. It can be seen that the I (996 cm^{-1})/I (966 cm^{-1}) is the best parameter yielding the highest 't' value. In addition, this parameter is also a better indicator of the grading of colonic premalignancy and not only cancer. Adapted from [47].

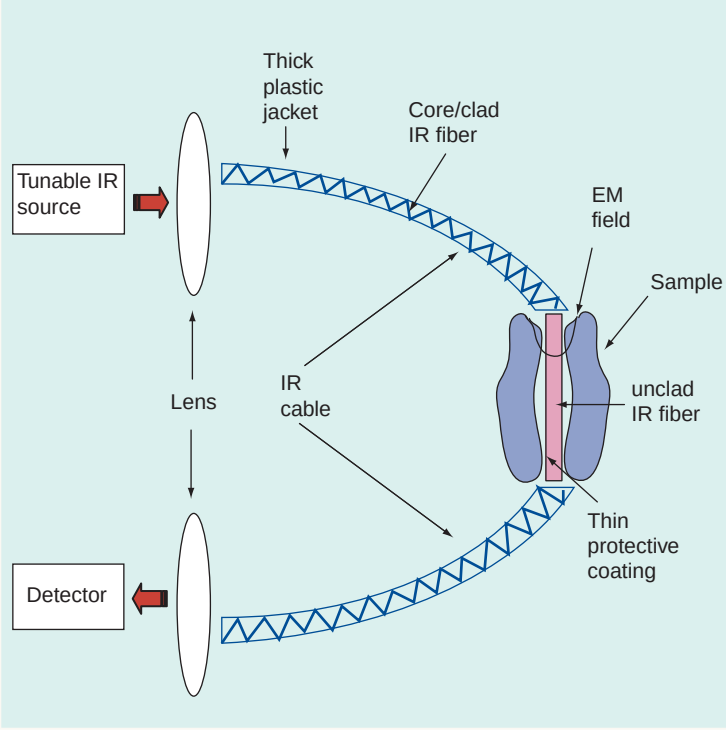
discs. Since the contaminations can affect spectral data, procedures to reduce these confounding variables are required [31,32]. The spectral data are usually collected over the range of 600–4000 cm^{-1} with several co-added scans (128/256) to improve the signal to noise ratio. The measurement time varies from several seconds (microscopy/spectroscopy) to a few hours in the case of complete imaging of a section by focal plane area detectors (single point detector systems). The spectra are measured either in transmission mode (FTIR spectroscopy, microscopy) or in reflectance mode (FTIR spectroscopy, FEWS-ATR-based systems) depending on the type of tissue being studied. Intensities at many wave numbers are simultaneously measured, but only a few of these wave numbers or spectral regions show diagnostic potentials (depending on the type of tissue being studied). Spectral analysis includes basic analysis such as baseline corrections, normalization (min–max or area normalization) before the particular absorbance is used. Several workers have provided important diagnostic wave numbers, among which the phosphate band intensity at approximately 1080 cm^{-1} has been shown to be a valuable one [2,12]. Specific wave numbers or regions of absorbance in the IR spectra of cells/tissues are most affected due to carcinogenesis or diseases. In the case of cervical tissues, for example, the glycogen levels are depleted in cancer cells compared with the levels in corresponding normal cells. Therefore, the region between 900–1185 cm^{-1} has a lower absorbance, and intensities at specific wave numbers are used to formulate ratios for distinction of normal and cancer cells/tissues [33,34]. These diagnostic bands with a corresponding biochemical origin are considered as biomarkers. Similarly, the nucleic acid levels are altered during cancer, and absorbance bands of these molecules are also used as markers [47,48]. Occasionally portions of the spectra can be subjected to analyses by supervised or unsupervised methods such as artificial neural networks, cluster analysis, linear discriminate analysis and principal component analysis to classify the samples. The utilization of artificial neural networks has especially helped to increase the sensitivity of the technique and accurately classify tissues, reducing false positive and false negative results [33]. Similarly, cluster analysis has been

Table 2. Fourier transform infrared assessment as a confusion matrix: the percentage of correct and incorrect test diagnoses for normal and three grades of cervical intraepithelial neoplasia patients.

Estimated	Normal	CIN I	CIN II	CIN III
Source				
Normal	91	9	0	0
CIN I	6	84	1	9
CIN II	0	4	90	6
CIN III	0	7	10	83

CIN: Cervical intraepithelial neoplasia. Adapted from [33]

Figure 6. Schematic representation of a fiberoptic evanescent wave spectroscopy (FEWS) based on attentuated total reflection (ATR) system using IR transmitting fibers, suitable for *in situ*/*in vivo* diagnosis of diseases like cancer that show remarkable spectral differences in the mid-IR region.



The unclad portion (magnified for clarity) is in contact with the tissue surface whose spectra is measured.
EM: Electromagnetic; IR: Infrared.

helpful in predicting the possibility of some samples having a tendency to progress to malignancy [5,33].

Sample preparation for cancer diagnosis

A minute part of the sample being used for conventional diagnosis can always be diverted for FTIR studies providing a supplementary tool for the pathologist to confirm results from other parallel studies. Additional processing with reagents other than deparaffinization or separation of pure cell components (in the case of cells) is not required for FTIR studies. Similarly, the method is suitable for the evaluation of tissues from animal models. Often, both for research purposes as well as for clinical evaluation of tissues, *in vitro* cell cultures are used and FTIR spectra can be obtained to monitor transformation of cell cultures. Thus, the methodology can be extended to *in vitro* studies of carcinogenesis [61]. *In situ* detection using ATR-FEWS systems would be suitable in organs approachable by surface sensing technologies and endoscopic methods. Probes have been developed for scanning tissue surfaces (such as skin) using these systems. Further studies would help to modify the existing instruments for different organs, in parallel with suitable computational methods for diagnosis of cancer *in situ*. These systems would be directly useful in the operating theater by allowing the surgeon to remove any malignancy on the spot, without waiting for results from pathology laboratories. Detection of cancer in tissues *in vivo*, especially of melanoma and cancers on other body surfaces, would be a reality with the rapid advances being made in developing IR-based equipments and optical guides for surface sensing.

Future perspective

FTIR spectroscopy has been shown to provide important clues regarding the changes in the biochemical composition of cells and tissues, especially during carcinogenesis. It may be used to test its efficiency compared with current methods [62,63], so that in future it can replace existing methods after its sensitivity is established. Thus, current diagnosis methods that inculcate constraints such as time and money can be substituted by FTIR spectroscopy. This method can be used as an intermediate step between light microscopy and more complicated (molecular biology-based) studies to decrease the number of samples to be analyzed in detail. In particular, the method would find utility in identification of subtle biochemical changes, which are not apparent by morphology in the histologic review, during diagnosis of premalignancy in resection margins [5]. Similarly, mapping of sections away from the foci of cancer would help to define the exact margins of resection without relying solely on convention and helping the surgeons to make decisions for each individual on an objective basis.

The availability of intense IR beams from synchrotron sources have helped to elucidate biochemical changes within single cells and understand the cell cycle and growth at a molecular level using IR spectroscopy [64]. Such studies would further help to understand growth and/or differentiation within organs or tissues to define normal spectral changes [65]. Similarly, the changes in spectra due to microbial infections [66] or induction of malignancy by genetic [67] or environmental factors may lead to the definition of spectral parameters to trace the origin of malignancy to different causes. However, such studies would require in-depth analysis of the

spectra from the same organ with different origins of cancer and also of different organs with a similar cause, such as a genetic basis or viral infections. Thus, the versatility of FTIR spectroscopy could be used to elucidate multiple facets of the cancers in a short time using variation at different wave numbers. Other techniques that are now being studied for applications in oncology can also use FTIR to supplement the information obtained [68]. Comparisons could be made that would help to identify the advantages and disadvantages of other methods and modify systems based on FTIR to overcome their disadvantage or improve their advantages.

The advances made in FEWS systems and alleviation of interference from water in tissues would usher in an entire new chapter in the utilization of IR-based endoscopic instruments capable of real time, *in vivo*/*in situ* diagnosis of diseases, especially of organs like the digestive and reproductive tracts. Thus, routine check up for progression or regression of malignancy would become more convenient for patients and surgeons, avoiding traumatic surgical interventions at defined intervals. This would be helpful for organs that are easily accessible to fiber-optic systems, such as the oral cavity [69].

The imaging of tissues using focal plane area detectors and multipixel spectral data by clustering shows promise in obtaining pseudocolored images where the cancer or diseased tissue would be colored in a different manner compared with normal tissue, making it easy for a pathologist to screen large number of biopsies or even store the digital images for follow-up and use other software programs to diagnose the samples and give a digital report.

Utilization of methods such as surface enhanced IR absorption may become helpful to identify abnormal RNA and DNA levels from smaller quantities of samples [70]. Synchrotron radiation-FTIR based [71] studies can be carried out at the cellular level to understand more basic aspects of the biochemical changes during normal and abnormal cell divisions and their characterization from spectral data.

The advances in the development of software and computational methods would enable the utilization of FTIR spectroscopy on a regular basis in diagnosis, follow-up and prediction of cancer. Further improvement and research in other areas, and collaboration between physicists, computer scientists, pathologists, oncologists and surgeons would pave the way for making FTIR-based diagnosis a powerful new tool in oncology.

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Executive summary

Advantages of Fourier transform infrared (FTIR) spectroscopy
• Harmless, rapid, noninvasive and simultaneous monitoring of several biomolecules.
• Cost effective and simple to operate when infrastructure is established.
• Provides supplementary information on data obtained by immunochemical methods.
• Suitable for *ex vivo*, *in vitro* and *in vivo* (with future developments) applications.
• Possible common biomarkers for different cancers (a possibility).
Utilities of FTIR spectroscopy
• Diagnosis of cancer in biopsies of organs such as the cervix and colon.
• Evaluation of carcinogenesis from Papanicolaou (PAP) smears, needle biopsies and blood samples.
• Distinction and grading of neoplasia, premalignancy and cancer.
• Extent of spread of cancer.
• Distinction between cancer and diseases with a similar clinical manifestation.
• Prediction of recurrence or early transformation into malignancy.
Computational & technologic accessories for cancer diagnosis by FTIR
• Suitable statistical methods, such as 't' tests.
• Advanced computational methods such as probabilistic neural networks and artificial neural networks softwares, linear discriminate analysis, prinicipal component analysis and cluster analysis algorithms.

Executive summary

- Fiber optic evanescent wave spectroscopy systems for *in vivo*/*in vitro* applications.
- Complementary methods such as histopathology and immunochemistry/molecular biology.

Currently available methods using FTIR spectroscopy

- Conventional FTIR spectrometers to analyze dried films of blood, cells and body fluids.
- FTIR microscopy to view and measure microscopic sites of fresh, frozen or fixed tissues.
- Utilization of imaging techniques to obtain a pseudo-colored image of sections being observed simultaneously under a microscope (chemical mapping).
- Fiberoptic evanescent wave spectroscopy systems based on attenuated total reflection probes/sensors.

Steps in cancer diagnosis using FTIR spectroscopy

- Sample preparation.
- Data collection.
- Data analysis.
- Computational methods.
- Diagnosis/predictions.

Approaches for FTIR spectroscopy

- *Ex vivo* analysis of biopsies and cells/tissues.
- *In vitro* studies in cell cultures.
- *In situ* detection using attenuated total reflection systems in organs approachable by surface sensing technologies/ endoscopic methods.

Future perspective

- *In vivo* detection of cancer.
- Utilization of techniques such as surface enhanced infrared absorption and synchrotron radiation-FTIR to understanding biochemical processes.

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