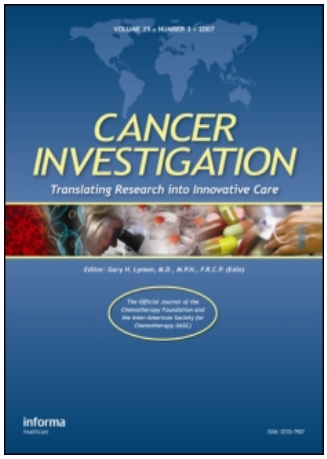

Cancer Investigation
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597231

Cancer Diagnosis by Discrimination between Normal and Malignant Human Blood Samples Using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy

M. Khanmohammadi [a]; M. A. Ansari [a]; A. Bagheri Garmarudi [a]; G. Hassanzadeh [b]; G. Garoosi [c]
[a] Department of Chemistry, Faculty of Science, Imam Khomeini International University, Qazvin, Iran
[b] Tehran University of Medical Science, Tehran, Iran
[c] Department of Agricultural Biotechnology Engineering, Imam Khomeini International University, Qazvin, Iran

Online Publication Date: 01 September 2007
To cite this Article: Khanmohammadi, M., Ansari, M. A., Garmarudi, A. Bagheri, Hassanzadeh, G. and Garoosi, G. (2007) 'Cancer Diagnosis by Discrimination between Normal and Malignant Human Blood Samples Using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy', Cancer Investigation, 25:6, 397 - 404
To link to this article: DOI: 10.1080/02770900701512555
URL: http://dx.doi.org/10.1080/02770900701512555

Cancer Investigation, 25:397–404, 2007
ISSN: 0735-7907 print / 1532-4192 online

DOI: 10.1080/02770900701512555

informa
healthcare

ORIGINAL ARTICLE
Imaging, Diagnosis, Prognosis

Cancer Diagnosis by Discrimination between Normal and Malignant Human Blood Samples Using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy

M. Khanmohammadi, Ph.D.,[1] M.A. Ansari, M.Sc.,[1] A. Bagheri Garmarudi, M.Sc.,[1] G. Hassanzadeh, Ph.D.,[2] and **G. Garoosi, Ph.D.[3]**

Department of Chemistry, Faculty of Science, Imam Khomeini International University, Qazvin, Iran.[1]
Tehran University of Medical Science, Tehran, Iran.[2]
Department of Agricultural Biotechnology Engineering, Imam Khomeini International University, Qazvin, Iran.[3]

ABSTRACT

FTIR spectroscopy is a common technique for cancer diagnosis. Applied tissue samples are heterogeneous and may be damaged in preparation procedures. Easier sampling, more available samples and also easier process with assured results would be interesting. Whole blood samples include all of these qualifications and our hypothesis was the bio-molecular changes in blood which manifest themselves in different optical signatures, detectable by FTIR spectroscopy. Noncancerous blood samples were differentiated from cancerous ones using ATR-FTIR spectroscopy and LDA classification method. Procedure was 100 percent and 90 percent accurate in prediction of cancerous or noncancerous situation for 33 known and 10 unknown samples, respectively.

INTRODUCTION

Cancer is one of the leading causes of death in the world. Many efforts have been made to improve the diagnostic methods in order to reduce the amount of death by cancer disease. Nowadays, the pathologic methods are most common procedures for cancer diagnostic studies. Although spectroscopy has been also applied as an efficient tool for these studies.

Keywords: Linear discriminant analysis, Cancer, Chemometrics, ATR-FTIR, Blood.
Correspondence to:
M. Khanmohammadi
Department of Chemistry
Faculty of Science
Imam Khomeini International University
P.O. Box 288
Qazvin, Iran
email: mkhanmohammadi@yahoo.com or mkhanmohammadi@ikiu.ac.ir

Recently Fourier-transform infrared (FTIR) spectroscopy has been employed to detect cancer tissues from noncancerous ones in different types of cancer such as colon cancer, cervical cancer, gastric cancer and bowel disease (1–7). In the most of these researches the infrared spectral characteristic has been studied. Specific regions of the spectra have been analyzed by statistical tools to study variations in metabolites that signified changes between the 2 pathological conditions.

In a research work, normal and cancerous breast tissue samples have been detected by FTIR spectroscopy with an attenuated total reflection (ATR) probe, comparing the band variations by using standard statistic methods. The results demonstrate that bands of protein, lipid, carbohydrate, and nucleic acid from cancerous samples are significantly different from those normal ones (8).

However, the preparing of biopsy samples involves 4 steps: fixation, embedding, sectioning and mounting on a glass slide, and finally staining, in which fixation and embedding process are dangerous, as the treatment may distort the cell structure and immersion of tissues in lipid solvents, such as xylene, dissolves the tissue lipids (9). On the other hand, any tissue samples are

normally very heterogeneous, comprising several components and different cell types.

It has been tried to distinct the premalignant and malignant cells and tissues from their normal state using specific parameters obtained from FTIR spectra, making it a rapid and reagent-free method. It could be called as a substantial progress in computational methods to increase the sensitivity, making it an objective and sensitive technique suitable for medical demands (10). FTIR spectroscopy with ATR also has been utilized to estimate the secondary structure of amide I in normal and cancer tissue of human colon results, in which it was shown that conformational changes of the secondary structure of protein in normal and cancerous human colon tissue are: increment in beta-turns and composition of alpha-helixes and also decrement in beta-sheets due to cancer while the composition of random coils would not be significantly different between normal and cancer tissues (11). Glycogen levels in normal and cancerous tissues have also been measured by the FTIR spectroscopic method. Its reliability has been confirmed by chemical analysis of the same tissues used for the FTIR spectroscopic measurements, suggesting that this spectroscopic method has a high specificity and sensitivity in discriminating human cancerous tissues from noncancerous tissues (12). ATR-FTIR technique also has been used for analysis of healthy and breast cancer affect people's hair, which was shown to increase the -sheet/disorder structures (relative to -helix structures) and C–H lipid content of hair from breast cancerous patients. Thus, it was supposed that the presence of breast cancer appears to alter the hair growth process, resulting from changes in the composition and conformation of cell membrane and matrix materials of hair fiber. The results were tested for other types of cancer such as prostate and lung but results were not completely reliable (13).

Nowadays chemometrics has become a powerful tool for many analytical purposes. Gazi et al. applied chemometric treatment of FTIR spectra, using the linear discriminant algorithm, to demonstrate a promising method for the classification of benign and malignant tissues. Using the principle component analysis, they achieved the separation of FTIR spectra of prostate cancer cell lines derived from different metastatic sites for the first time. Of course all merits of sample preparation were yet remained (14). Some other chemometric methods such as "Soft Independent Modeling of Class Analogy (SIMCA)" (15) and "Noise Band Factor Cluster Analysis (NBFCA)" (16) also have been researched for classification of spectral data obtained from tissue sample studies.

As mentioned before, studying the tissue samples has drawbacks such as difficult sampling, low speed sample preparation and very sensitive keeping conditions. Thus, it seems necessary to look after a substitute for tissue samples which would be easier in providing and analysis, while its results are reliable. According to our review, there is no report in all around the world to indicate the analysis of whole blood by ATR-FTIR spectroscopy using discriminant analysis for cancer diagnosis. In this work we replaced whole blood sample instead of the tissue, because blood is a homogeneous sample and the sample preparation procedure is very simple.

As we know, ATR has added a quantitative analysis power to the FTIR spectroscopy. Also, both the quality and the quantity of the samples are possible to be analyzed by using ATR-FTIR spectroscopy. It is mentioned that "quality" stands for studying the presence of a proposed functional group and "quantity" stands for measurements indicating the concentration of foresaid functional group in the sample. So it may be possible to compare 2 groups of bloods: cancerous and noncancerous.

The Linear Discriminant Analysis (LDA), one of the chemometrics classification methods, was employed to classify the spectra of both cancerous and noncancerous samples.

MATERIALS AND METHODS

Sample preparation

Sample preparation in this work is very simple and it is one of the advantages of proposed method which involves providing 2 ml blood of both noncancerous and cancer affected people. Of course, all keeping conditions for blood samples must be the same. All used malignant samples were from different forms of cancer and were obtained from 40 to 65-year-old randomly selected peoples.

As mentioned, all blood samples were kept in same condition, 25°C in EDTA vials, being analyzed till 20 hours after bleeding. EDTA was used as anticoagulant agent.

ATR-FTIR spectroscopy

About 2 ml of blood was placed on a zinc-selenide ATR cell. Measurements on blood samples were performed using the FTIR spectrometer ABB-BOMEM, MB-100, active in mid-IR region with DTGS detector. Thirty-six scans were performed in the wavenumber region of 2000–900 cm^{-1} in each measurement.

The main problem in this region is the absorbance of water which consists about 90 percent of blood. To avoid of this problem, we used water as background spectrum. As can be seen in Figures 1 and 2, in this way the absorbance of water does not affect the absorbance of the other blood components. The wave number region of 2000–900 cm^{-1} was chosen for the analysis because the previous studies have demonstrated many spectral differences between cancer status and noncancerous status in this region (1–7, 17–20). In the other words, there are some functional groups, existing in this region for which their infrared signal in noncancerous and cancerous samples would be different with each other. Amides are an example of these functional groups and Figures 2-1 and 2-2 show the absorbance related to amide I and amide II (1650 cm^{-1} and 1550 cm^{-1}) are the sharpest peaks in this region.

Statistical analysis of FTIR spectra

"Chemometrics" is a chemical discipline that uses mathematical and statistical methods to design or select optimal measurement procedures and experiments, and to provide maximum chemical information by analyzing chemical data. One of the first and the most publicized success stories in chemometrics

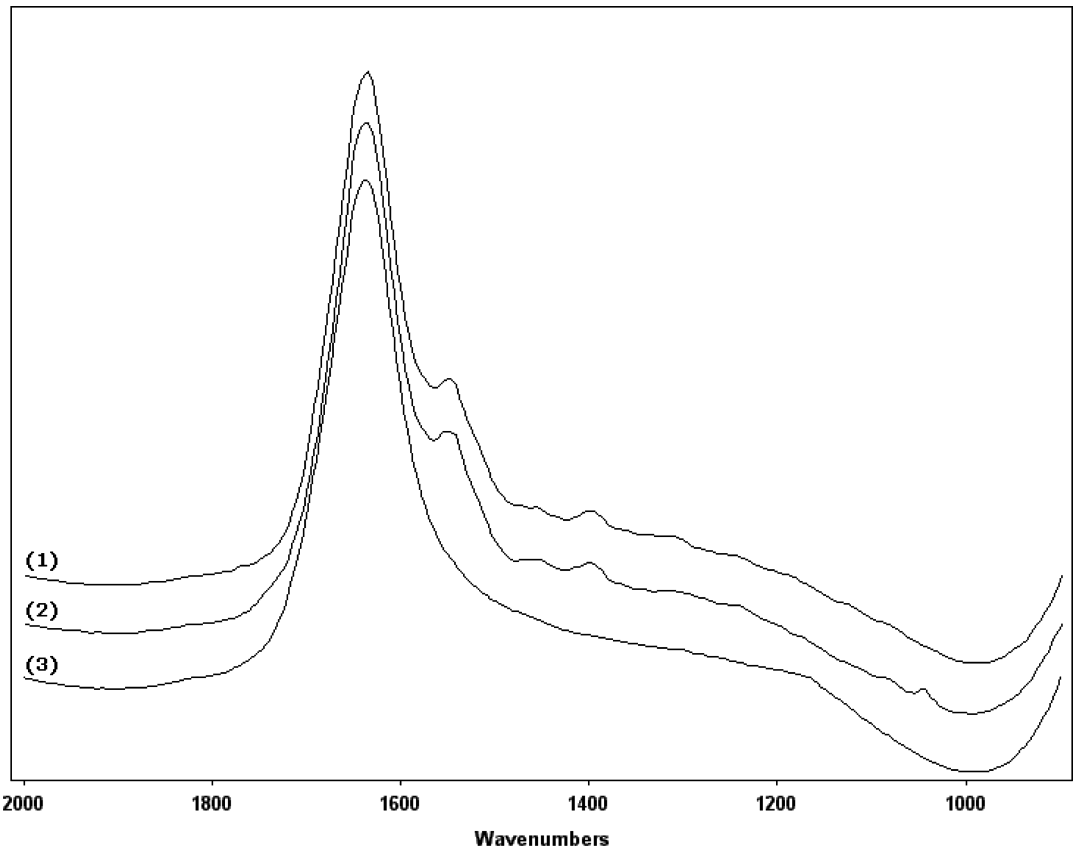

Figure 1. The ATR-FTIR absorption spectra of (1) cancerous blood, (2) noncancerous blood, and (3) water sample, using air as a reference.

is pattern recognition. Much chemistry involves using data to determine patterns. There are a large number of methods for supervised pattern recognition, mostly aimed at classification. Multivariate statisticians have developed many discriminant functions, some of direct relevance to chemists. Consider using a chemical method such as infrared spectroscopy to determine whether a sample of brain tissue is cancerous or not. A method can be set up in which the spectra of 2 groups, cancerous and noncancerous samples, are recorded. Then some form of mathematical model is set up. As a standard model, finally by using the proposed model, the diagnosis of an unknown sample can be predicted.

Supervised pattern recognition

Supervised pattern recognition–also called classification—requires a training set of known groupings to be available in advance, and tries to answer a precise question as to the class of an unknown sample is used to assign samples to a number of groups (or classes). It differs from cluster analysis where, although the relationship between samples is important, there are no predefined groups. Although there are numerous algorithms in the literature, chemists and statisticians often use a common strategy for classification with no care to what algorithm is employed:

a) Modeling the training set: The first step is normally to produce a mathematical model between some measurements (e.g., spectra) on a series of objects and their known groups. These objects are called a training set. Then a parameter which representing the percentage correctly classified (%CC) can be calculated. After application of the algorithm, the origin (or class) of each spectrum is predicted. A difficulty in many real situations is that it can be expensive to perform experiments that result in large training sets. Spectroscopy is a common method for screening and chemometrics combined with spectroscopy acts like a "sniffer dog" in a customs checkpoint trying to detect drugs. The dog may miss some cases, and may even get excited when there are no drugs, but there will be a good chance the dog is correct.

b) Test sets and cross-validation: However, normally training sets give fairly good predictions, because the model itself has been formed using these datasets, but this does not mean that the method is yet safe to use in practical situations. A recommended next step is to test the quality of predictions using an independent *test* set. This is a series of samples that has been left out of the original calculations, and is like a "blind test." These samples are assumed at first step to be of unknown membership class, and then the model from the training set is applied to these samples. Using a test set to determine the quality of predictions is indeed a form of "*validation.*"

c) Improving the data: If the model is not very satisfactory, there are some ways to improve it. Using a different computational algorithm could be the first one and the second one could

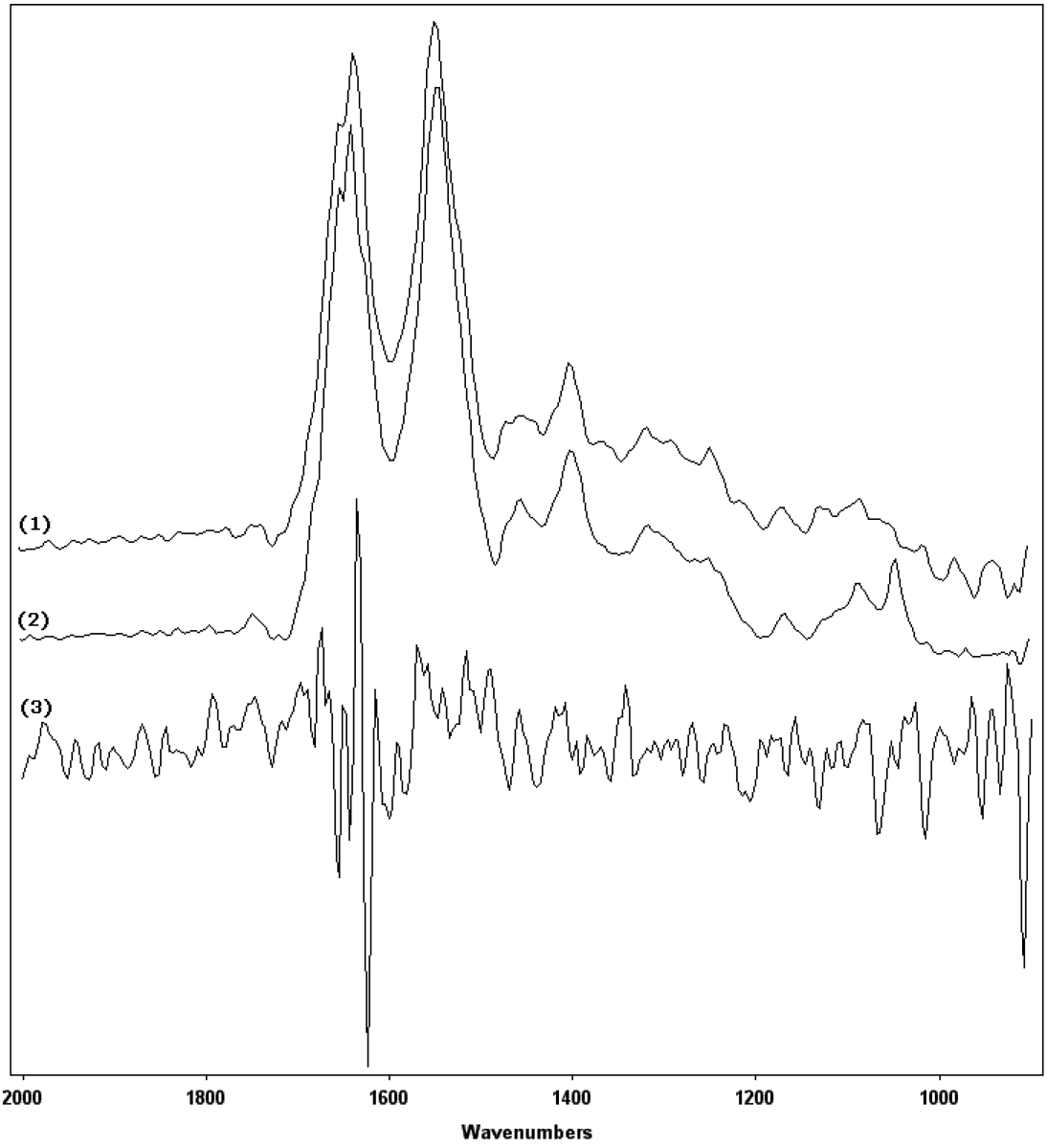

Figure 2. The ATR- FTIR absorption spectra of (1) cancerous blood, (2) noncancerous blood, and (3) water sample, using water as a reference.

be modifying the existing method. But a common approach might be involving wavelength selection in spectroscopy.

d) Applying the model: Once a satisfactory model is available, it can then be applied to unknown samples, using analytical data such as spectra or chromatograms, to make predictions. Usually by this stage, special software is required that is tailor-made for a specific application and measurement technique. The software also will have to determine whether a new sample really fits into the training set or not.

Discriminant analysis

Classification methods are based on statistical procedures while objects (or sometimes variables) are analyzed with the focus on samples belonging to categorical classes, groups or clusters. Most traditional approaches for classification in science are called *discriminant analysis* and are often also called forms of "hard modeling." The majority of statistically principal software packages contain substantial numbers of procedures, referred to various names such as linear (or Fisher) discriminant analysis and canonical variates analysis. One of the most powerful classification methods is Linear Discriminant Analysis (LDA).

The LDA method may be applied with 2 different objectives, first as a predictive method, with the goal of formulating a discrimination rule used to predict or allocate unknown samples in predefined classes, and second as an exploratory tool to increase understanding of the differences between classes (9).

Both univariate and multivariate models are used in discrimination but more often, several measurements are required to

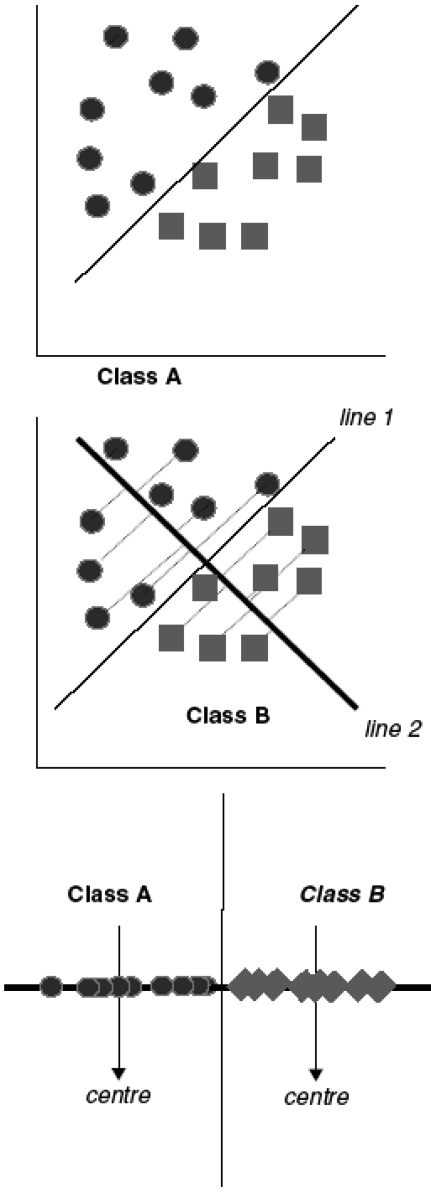

Figure 3. Discrimination between 2 classes, and projection.

determine the group to which a sample belongs. So multivariate would be much more applied.

Figure 3 is a proposal example of multivariate procedure. The objects represented by circles are clearly distinct from those represented by squares, but neither of the 2 measurements alone can discriminate between these groups and, therefore, both are essential for classification. It is possible, however, to draw a line between the two groups. If so, an object above the line, belongs to class A, otherwise belongs to class B. Graphically, this can be represented by *projecting* the objects on to a line at right angles to the discriminating line, as demonstrated in Figure 3. The projection can now be converted to a position along line 2 of the figure. The distance can be converted to a number, analogous to a "score." Objects with lower values belong to class A, whereas those with higher values belong to class B.

Many chemometricians use the Mahalanobis distance, sometimes called the "statistical" distance, between objects. In areas such as spectroscopy it is normal that some wavelengths or regions of the spectra are more useful than others for discriminant analysis.

It must be mentioned that the LDA method finds a decision boundary between different classes by Fisher and Mahalanobis distances. The boundary or hyper plane is calculated such that the variance between the classes is maximized and the variance within the individual classes is minimized. The linear discriminant functions' weights (w) are found as the eigenvectors of the following matrix:

$$G^{-1}Hw = \lambda w, \qquad [1]$$

while λ is the eigenvalue and the matrix G is derived from the covariance matrix (c) of different classes or groups (g).

Singular value decomposition (SVD) is performed on to obtain the w. In the case that a matrix titled "loading" is concluded from SVD, which is w, and is applied to obtain the discriminant score (S):

$$G = (n-g)C = (n-g)\frac{1}{n-g}\sum_{j=1}^{g}(n_j - 1)C_j \qquad [2]$$

So that:

$$C_j = \frac{1}{n_j - 1}\sum_{Legj}(x_{1i} - \bar{x}_{ji})(\bar{x}_{ik} - \bar{x}_{jk}) \qquad [3]$$

while n is the number of total samples, n_j is the number of samples in group j, and Leg_j indicates that 1 is a member of group j.

Matrix H describes the spread of the group means over the grand average.

$$H = \sum_{j=1}^{g} n_j(\bar{x}_j - \bar{x})(\bar{x}_j - \bar{x})^T \qquad [4]$$

$$\bar{x} = \frac{1}{n}\sum_{j=1}^{1} n_j\bar{x}_j \qquad [5]$$

The linear combination for a discriminant analysis is derived from following equation:

$$S = w_{11}X_1 + w_{12}X_2 + \cdots + w_{1p}X_p, \qquad [6]$$

where S is the discriminant score, w_i is the discriminant weight for independent variable i and X_i is the independent variable i. In order to predict the unknown object, it is assigned to that class for which its centroid has the smallest Euclidian distance (21):

$$\text{Min}\|W^T(Xu - Xj)\| \text{ with } j = 1, \ldots, g$$

The obtained IR absorption spectra were analyzed by discriminant analysis software which was written by MATLAB7 software.

RESULTS

Typical IR absorption spectra of noncancerous and cancerous blood samples in the range of 2000–900 cm^{-1} (Figure 1). Water has been used as background for these spectra. The spectra are dominated by 2 absorbance bands at 1643 and 1544 cm^{-1} known as amide I and II, respectively. Amide I arises from the C═O hydrogen bonded stretching vibrations, and amide II from the C─N stretching and a CNH bending vibrations. The weaker aminoacid side chain from peptides and proteins at 1456 and 1401 cm^{-1} are associated with the asymmetric and symmetric CH_3 bending vibrations (22). The absorption peaks at 1243 and 1075 cm^{-1} are related to the PO2 ionized asymmetric and symmetric stretching, respectively (1, 23).

The bands at 1025 and 1045 cm^{-1} in IR spectra are responsible for the vibrational modes of ─CH2OH groups and the C─O stretching coupled with C–O bending of the carbohydrates, C─OH groups (includes glucose, fructose, glycogen, etc.).

In this work, 14 cancerous and 19 noncancerous blood samples were classified as calibration members. The following types of cancer exist in the cancerous class: 4 breast cancer, 4 skin cancer, 3 gastric cancer, 1 bladder cancer, 1 esophagus, and 1 colon cancer. Ten unknown blood samples were used for prediction: 6 noncancerous blood, 2 skin cancer, 1 breast cancer, and 1 colon cancer.

Patients must be selected from those who are not treated by chemotherapy, because the blood samples must be pure, otherwise the comparison between noncancerous and cancerous samples are failed.

It was important to find biomolecular changes between 2 classes: noncancerous and cancerous. For, this reason LDA was employed using MATLAB (version 7: Maths Works, Inc., Natick, MA, USA). In order to apply the MALAB software according to our aim, an algorithm was designed and its m.file was introduced in this environment which was employed to perform the linear discriminant analysis.

The first step was to classify the spectra of both classes. Figure 4 shows the s-value of 33 blood samples, classified by LDA (14 cancerous and 19 noncancerous). The s-value of each sample in the calibration are shown in Table 1. Second step was to predict the unknown samples by calibration. In this step, 4 cancerous samples and 6 noncancerous samples were employed supposing that numbers 1–4 and 5–10 related to cancerous and noncancerous samples, respectively.

The s-values of unknown samples are shown in the Table 2; the s-values related to the cancerous samples are numbers 1–4 and the others are related to the normal samples.

The s-values less than −0.0313 are classified in the cancerous group and the s-values bigger than −0.0313 are classified in the normal group. For unknown samples 3 of the 4 cancer samples and 6 of the 6 normal samples were correctly classified (90%CC). The LDA program could predict these 10 samples with 90 percent accuracy. So LDA can classify the spectra of blood samples with high accuracy.

DISCUSSION

In the present study using ATR-FTIR spectroscopy, at the first, we classified the 33 spectra of human blood samples in two groups of noncancerous and cancerous by LDA. In the second

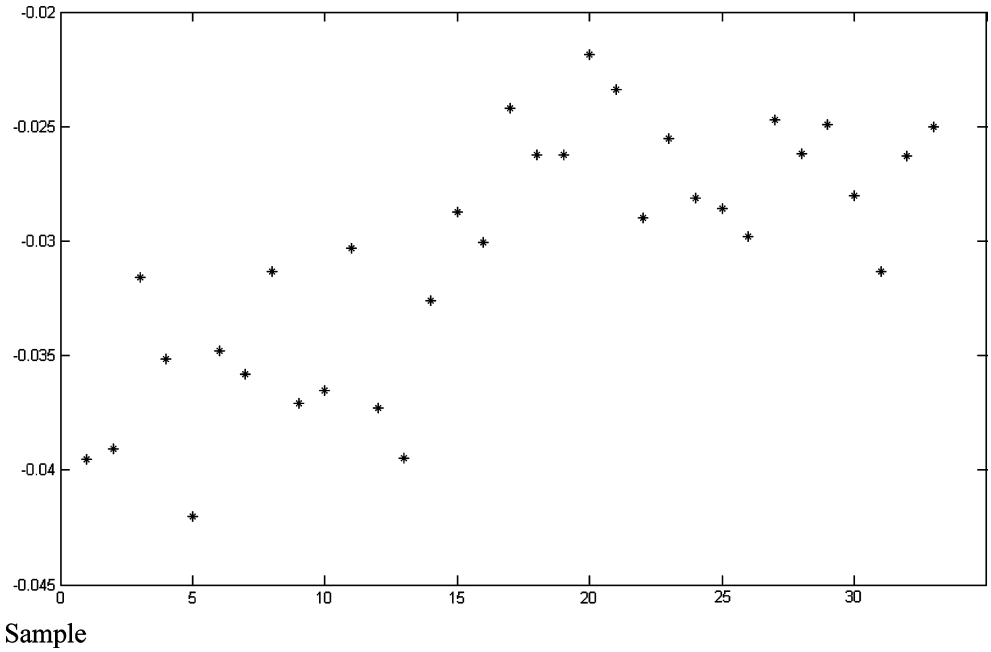

Figure 4. The graph of s-values for 33 blood samples. (cancerous class: samples 1–14 and noncancerous class: samples 15–33).

Table 1. The s-values related to the 14 cancerous and 19 noncancerous samples. The s-values less than -0.0313 are classified in the cancerous group and the s-values bigger than −0.0313 are classified in the normal group

	s-values				
Cancerous	−0.0395	−0.0391	−0.0316	−0.0351	−0.0420
	−0.0348	−0.0358	−0.0313	−0.0371	−0.0365
	−0.0303	−0.0373	−0.0395	−0.0326	
Noncancerous	−0.0287	−0.0300	−0.0242	−0.0262	−0.0262
	−0.0219	−0.0234	−0.0290	−0.0255	−0.0281
	−0.0286	−0.0298	−0.0247	−0.0262	−0.0249
	−0.0280	−0.0313	−0.0263	−0.0250	

step 10 unknown samples were predicted as noncancerous or cancerous.

The wave number region of 2000–900 cm^{-1} involves the bands related to asymmetric and symmetric phosphate stretching modes, asymmetric, and symmetric CH_3 bending modes, C─O stretching mode of C─OH groups and collagen. So it is possible that the biomolecular changes made by cancer disease manifest themselves in this wavenumber region.

Blood was selected as sample instead of tissue sample because of its homogeneity and simple preparation. To classify the blood samples into cancerous and noncancerous classes, the LDA method was correctly employed.

LDA is a strong classification method that finds a decision boundary between classes so as the variance between the classes is maximized and the variance within the individual classes is minimized. Applying this method, surprisingly, revealed some advance benefits in addition to accurate and reliable results. Modeling test was not expensive, just a simple bleeding is enough for sample preparation, no data improvement was needed as the MATLAB software results a reliable accuracy, no change in the wavenumber region was done as our selected region—due to our previous studies and research on wavenumber region selection—but with satisfactory results, and at last but not at least, sampling time till 20 hours after bleeding and condition has no effect on prediction results and it is just enough to define a standard condition for sample preservation.

Different types of cancer were used in this study because of discrimination between noncancerous condition and all of the cancerous conditions. It made this method a powerful one for cancer diagnosis, because it is not dependent on cancer types. First, 33 blood samples were classified in 2 noncancerous and cancerous groups, and then 10 unknown samples were predicted based on this classification. Over the last decade, studies using vibrational spectroscopy have been conducted on samples from a variety of organs.

Table 2. The s-values related to unknown samples. For unknown samples 3 of the 4 cancer samples and 6 of the 6 normal samples were correctly classified (90 percent correct classification)

	s-values				
Unknown samples	−0.0352	−0.0329	−0.0346	−0.0247	−0.0162
	−0.0129	−0.0268	−0.0267	−0.0265	−0.0288

The results of all these studies, on a gross level, can be summarized as follows: "normal and malignant tissues can be differentiated on the order of 80–100 percent accuracy with the use of some statistical analysis" (24). Our initial results showed that the normal and malignant blood samples could be identified with about 90 percent accuracy.

The present study showed the cancerous blood samples were correctly segregated from the noncancerous samples using LDA. The 90 percent sensitivity in cancer detection suggests that this ATR-FTIR method is a promising method for cancer diagnosis. FTIR spectroscopy is a promising tool for cancer diagnosis because of the rapid processing time and easy operation.

The simple method for sample preparation, quick diagnosis and high accuracy are other advantages of this method. Our results suggest that ATR-FTIR spectroscopy is a useful technique for cancer diagnosis. It is noticeable that all of samples either in calibration series or prediction were from people who had been tested by pathologic cancer diagnosis methods, earlier and spectroscopy-chemometrics results were completely compatible with pathologic results. Therefore, normal can be differentiated from abnormal with good accuracy.

REFERENCES

1. Lasch, P.; Wasche, W.; McCarthy, W.J.; Muller, G.; Naumann, D. Imaging of human colon carcinoma thin sections by FTIR microspectrometry. Cell Mol. Biol. **1998**, *44*, 189–201.
2. Argov, S.; Ramesh, J.; Salman, A.; Sinelnikov, I.; Goldstein, J.; Guterman, H.; Mordechai, S. Diagnostic potential of Fourier-transform infrared microspectroscopy and advanced computational methods in colon cancer patients. J. Biom. Optics **2002**, *7*, 1–5.
3. Ramesh, J.; Salman, A.; Argov, S.; Goldstein, J.; Sinelnikov, I.; Walfisch, S.; Guterman, H.; Mordechai, S. FTIR microscopic studies on normal, polyp, and malignant human colonic tissues. Subsurf. Sens. Technol. Appl. **2001**, *2*, 99–112.
4. Salman, A.; Argov, S.; Shau, R.K.; Bernshtain, E.; Walfisch, S.; Mordechai, S. Probing cell proliferation in the human colon using vibrational spectroscopy: a novel use of FTIR-microspectroscopy. Vibrational Spectroscopy **2004,** *34*, 301–308.
5. Cohenford, M.A.; Rigas, B. Cytologically normal cells from neoplastic cervical samples display extensive structural abnormalities on IR spectroscopy: Implications for tumor biology. Proc. Natl. Acad. Sci. USA **1998,** *95*, 15327–15332.
6. Argov, S.; Sahu, R.K.; Bernshtain, E.; Salman, A.; Shohat, G.; Zelig, U.; Mordechai, S. Inflamatory bowel diseases as an intermediate stage between normal and cancer: A FTIR-microspectroscopy approach. Biopolymers **2004**, *75*, (5), 384–392.
7. Fujioka, N.; Morimoto, Y.; Arai, T.; Kikuchi, M. Discrimination between normal and malignant human gastric tissues by Fourier-transform infrared spectroscopy. Cancer Det. Prev. **2004**, *28*, 32–36.
8. Ling, X.F.; Xu, Z.; Xu, Y.Z.; Li, Q.B.; Zhou, S.; Zhang, L.; Zhao, H.M.; Hou, C.S.; Wang, L.X.; Hou, K.Y.; Zhou, X.S.; Wu, J.G. FTIR spectroscopic explorations of clinical practice of breast cancer. Guang Pu Xue Yu Guang Pu Fen Xi **2005**, *25* (2), 198–200, (Article in Chinease - http://www.ncbi.nlm.nih.gov)
9. Chalmers, J.M.; Griffiths, P.R. *Handbook of vibrational spectroscopy*. John Wiley & Sons, Chichestir; 2002, vol. 3.

10. Sahu, R.K.; Mordechai, S. Fourier transform infrared spectroscopy in cancer detection. Future Oncology **2005**, *1*, (5), 635–647.

11. Chen, Y.J.; Hsieh, Y.W.; Cheng, Y.D.; Liao, C.C. Study on the secondary structure of protein in amide I band from human colon cancer tissue by Fourier-transform infrared spectroscopy. Chang Gung Med. J. **2001**, *24* (9), 541–546.

12. Yano, K.; Sakamoto, Y.; Hirosawa, N.; Tonooka, S.; Katayama, H.; Kumaido K.; Satomi, A. Applications of Fourier transform infrared spectroscopy, Fourier transform infrared microscopy and near-infrared spectroscopy to cancer research. Spectroscopy **2003,** *17* (2–3), 315–321.

13. Lyman, D.J.; Murray-Wijelath J. Fourier Transform Infrared Attenuated Total Reflection Analysis of Human Hair: Comparison of Hair from Breast Cancer Patients with Hair from Healthy Subjects. Applied Spectroscopy **2005**, *59* (1), 26–32.

14. Gazi, E.; Dwyer, J.; Gardner, P.; Ghanbari-Siahkali, A.; Wade, A.P.; Miyan, J.; Lockyer, N.P.; Vickerman, J.C.; Clarke, N.W.; Shanks, J.H.; Scott, L.J.; Hart C.A.; Brown, M. Applications of Fourier transform infrared microspectroscopy in studies of benign prostate and prostate cancer. A pilot study. J. Pathology **2003,** *201* (1), 99–108.

15. Li, Q.B.; Yang, L.M.; Ling, X.F.; Wang, J.S.; Zhou, X.S.; Shi, J.S.; Wu, J.G. Application of the SIMCA method to cancer diagnosis with Fourier-transform infrared spectroscopy]. Guang Pu Xue Yu Guang Pu Fen Xi. **2004**, *24* (4), 414–417. [in Chinese: http://www.ncbi.nlm.nih.gov

16. Sukuta, S.; Bruch, R. F. Noise-band factor analysis of cancer Fourier transform infrared evanescent-wave fiber optical (FTIR-FEW) spectra. Optical Biopsy IV, Robert R. Alfano; Ed., Proc. SPIE, 2002; Vol. 4613, 198–207.

17. Ramesh, J.; Argov, S.; Salman, A.; Yuzhelevski, M.; Sinelnikov, I.; Goldstein, J.; Erukhimovitch, V.; Mordechai, S. Spectroscopic evidence for site-specific cellular activity in the tubular gland in human intestine. Eur. Biophysics J. **2002**, *30* (8), 612–616.

18. Huleihel, M.; Salman, A.; Erukhimovitch, V.; Ramesh, J.; Hammody, Z.; Mordechai, S. Novel spectral method for the study of viral carcinogenesis in vitro. J. Biochem. Biophys. Methods **2002**, *50*, 111–121.

19. Gazi, E.; Dwyer, J.; Lockyer, N.P.; Gardner, P.; Vickerman, J.C.; Miyan, J.; Claire, A.H.; Brown, M.; Shanks, J.H.; Clarke, N.W. The combined application of FTIR microspectroscopy and TOF-SIMS imaging in the study of prostate cancer. Faraday Discussions **2004**, *126*, 41–59.

20. Bruni, P.; Conti, C.; Giorgini, E.; Pisani, M.; Rubini, C. ; Tosi, G. Histological and microscopy FT-IR imaging study on the proliferative activity and angiogenesis in head and neck tumours. Faraday Discussions **2004**, *126*, 19–26. [discussion 77–92].

21. Otto, M. *Chemometrics, statistics and computer application in analytical chemistry.* Wiley, VCH, Weinheim, Germany; 1998.

22. Ramesh, J.; Salman, A.; Hammody, Z.; Cohen, B.; Gopas, J.; Grossman, N.; Mordechai, S. FTIR microscopic studies on normal and H-Ras oncogene transfected cultured mouse fibroblasts. Eur. Biophys. J. **2001,** *30*, 250–255.

23. Krupnik, E.; Jackson, M.; Bird, R.P.; Smith, I.C.P.; Mantsch, H.H. Infrared spectroscopic characteristics of normal and malignant colonic epithelium. Proc. SPIE Int. Soc. Opt. Eng. **1998**, *3257*, 307–310.

24. Dukor R.K. *Vibrational Spectroscopy in the Detection of Cancer in: Handbook of vibrational spectroscopy, vol. 5*, John Wiley & Sons Ltd, New York; 2002.