Australian Journal of Basic and Applied Sciences, 5(9): 1734-1739, 2011
ISSN 1991-8178

# *In vivo* Spectral Analysis of Bladder Cancer Using Fourier Transform Infrared Spectroscopy, A comparative Study

[1]Nafie A. Al- Muslet, [2]Essam E. Ali

[1]Institute of laser – Sudan University of Science and Technology (www.sustech.edu), almogran - Khartoum / Republic of Sudan, P. O. Box (407).
[2]College of science and technology – Shendi University, Shendi / Republic of Sudan

**Abstract:** Recently, spectroscopic analysis of cancer tissues has received considerable attention to be a diagnosis technique due to its sensitivity to biochemical variations in the samples. In this study Fourier Transform Infrared Spectroscopy (FTIR) was used to analyze, spectroscopicaly, a number of bladder cancer tissues compared with normal bladder tissues. Twenty samples were collected from ten patients, diagnosed with bladder cancer, from different hospitals without any pretreatment. From each patient two samples were collected, one of them was normal and the second was cancerous tissue. FTIR spectrometer was used to differentiate between normal and cancerous bladder tissues via the change in the spectra of these samples. The investigations detected obvious spectroscopic changes in the proteins (1650, 1550 $cm^{-1}$), lipids (2925, 2850 $cm^{-1}$) and nucleic acids (1080, 1236 $cm^{-1}$). The results showed that FTIR spectroscopy holds much promising as a rapid, accurate, nondestructive method, and easy to use as an alternative method for identification and diagnosis of bladder cancerous tissues.

**Key words:** FTIR for malignancy diagnosis, spectroscopic changes in bladder cancer, spectroscopy in biology, nondestructive diagnosis.

## INTRODUCTION

A human body, on a simple level, is composed primarily of water, proteins, nucleic acids, lipids and carbohydrates. Changes in the body leading to diseases, such as cancer, are thought to be due to some biophysical and biochemical changes in one or all of these components. Vibrational spectra in the infrared (I.R) region are sensitive to the structure of these components, and then they too must change with the diseased state (Kazuo Nakamoto, 2009).

Fourier Transform Infrared Spectroscopy (FTIR) has in fact been shown to be very sensitive to the conformation of these biological changes (Gordon G. Hammes, 2005).

Although all biomolecules are important, the nucleic acids RNA and DNA are especially important because they carry within their structure the hereditary information that determines the identity and structure of proteins. The bands with the strongest intensity correspond to regions and assignments of the following vibrations: The 1750–1620 $cm^{-1}$ region corresponds to inplane double-bond vibrations of the bases. The spectra in this region are very sensitive to effects of hydrogen bond formation. The 1230 $cm^{-1}$ and 1090 $cm^{-1}$ band are assigned to antisymmetric and symmetric phosphate stretching vibrations, respectively. Ribose has a strong C–O band at 1120 $cm^{-1}$, which serves as a marker band for RNA in solution. In the solid, the most significant difference between the two nucleic acids is the ratio of intensity of the bands in the multiplet around 1055 $cm^{-1}$ (Gordon G. Hammes, 2005).

In the vibrational spectra, the amide bonds of proteins form so-called chromophores that give rise to nine strong characteristic bands that are named amide A, amide B and amides I–VII. Amide I band, which is due mostly to the C=O stretching vibrations of the peptide backbone, is by far the best characterized. It gives rise to an IR band in the 1600–1700 $cm^{-1}$ region and has been used for structural studies due to its high sensitivity to small changes in molecular geometry and hydrogen bonding of the peptide group. The amide II band, due largely to a coupling of CN stretching and inplane bending of the N–H group, is strong in the IR giving rise to a band in the 1480–1575 $cm^{-1}$ region. The amide II band is not often used for structural studies because it is less sensitive and is subject to interference from absorption bands of amino acid side-chain vibrations. The amide III band, arising from coupling of C–N stretching and N–H bending, and giving rise to bands in the 1230–1300 $cm^{-1}$ region, is fairly weak in the IR but quite strong in Raman but can also be mixed with vibrations of side-chains (Barbara Stuart, 2004).

Lipids are critical to all biological media by forming the cell walls that keep biological media organized in their necessary compartments. The major absorption bands of a typical lipid are at 1738, 1465, 1255, 1168, 1095 and 1057, and 968 $cm^{-1}$ (Rina K. Dukor, 2002).

Sugars or saccharides are the most common carbohydrates present in biological media primarily as hexose sugars, such as glucose, where they are an immediate energy source. Pentose sugars are also present, mainly as

**Corresponding Author:** Nafie A. Al- Muslet, Institute of laser – Sudan University of Science and Technology (www.sustech.edu), almogran - Khartoum / Republic of Sudan, P.O. Box (407).
E-mail: mnmfa2008@yahoo.com

the ribose component of the nucleic acid backbone of DNA and RNA, both as the component monomers and the much longer polymers, as well as in the energy-transducing oligomeric pieces. Polysaccharides in the body are found either in a free state or combined with proteins, in a complex known as glycoproteins. The only polysaccharide in the body that is not bound to a protein is glycogen. The major absorption bands for glycogen solution are observed at 1153, 1105, 1082, 1043, 1025, 996 (weak shoulder) and 931 $cm^{-1}$. The corresponding bands for the solid spectrum are 1149, 1078, 1043 (weak shoulder), 1016, 996 and 931 $cm^{-1}$. The spectra are highly dependent on the degree of hydration (Rina K. Dukor, 2002).

***2 - Materials and Methods:***

This section describes the used FTIR spectrometer, tissue preparation, and sampling for FTIR.

***FTIR Spectrometer:***

The Fourier transform infrared spectrometer used in this study is type (FTIR-430, Jascow, Japan).This instrument has a ceramic IR source, KBr beam splitter, and DLATGS IR detector. FTIR spectra of the samples were obtained in the spectral range 4000 to 400 $cm^{-1}$ with a scanning speed of 2 mm/sec and resolution of 4 $cm^{-1}$.

***Tissue preparation:***

Twenty fresh tissues of bladder cancer and normal bladder tissue were obtained from 10 patients in many hospitals. The tissues were preserved in 10% formaldehyde solution and sampled immediately after removing during the operations. All the patients were diagnosed by physicians as Squamous and Transitional cell carcinoma with endoscopic biopsy. There were 6 males and 4 females, aged from 48 to 79 years old. None of the patients had radiotherapy or chemotherapy before surgery.

Normal and cancer tissues were cut into small sizes and put in vacuum container beside silica jell to absorb $H_2O$ in order to make the tissues dry. A very small amount of the solid tissue, approximately (2 mg), was finely grounded and intimately mixed with approximately 200 mg of dry potassium bromide (KBr) powder. The samples were placed between two disks and put under mechanical pressure of 20 tons fixed for several minutes. Re-crystallization of the KBr results in a clear glassy disk of about 1 mm thick. This disk is now ready to be analyzed by FTIR spectrometer.

A total of twenty FTIR spectra were obtained from the normal and cancer samples. All measurements were carried out under room light and at room temperature.

## RESULTS AND DISCUSSION

***FTIR Spectra for Bladder Normal and Cancer Tissues:***

Representative spectra recorded by the FTIR spectrometer of normal and cancer bladder tissues are shown in figures (1) to (10). The comparison can be done easily for each patient's samples.

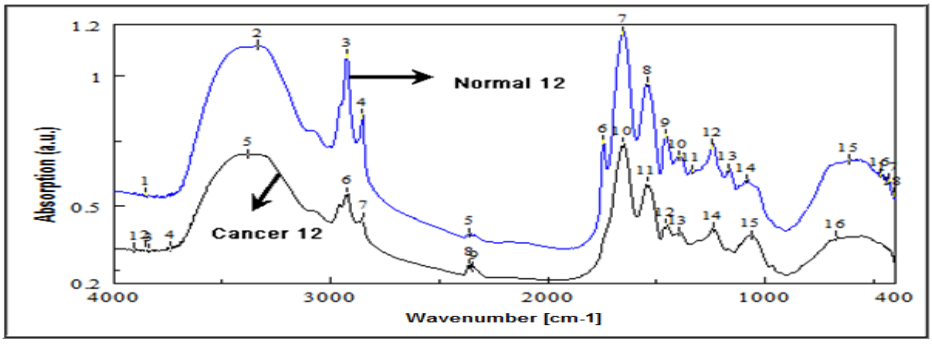


**Fig. 1:** FTIR spectra of normal and cancer bladder (patient 1)

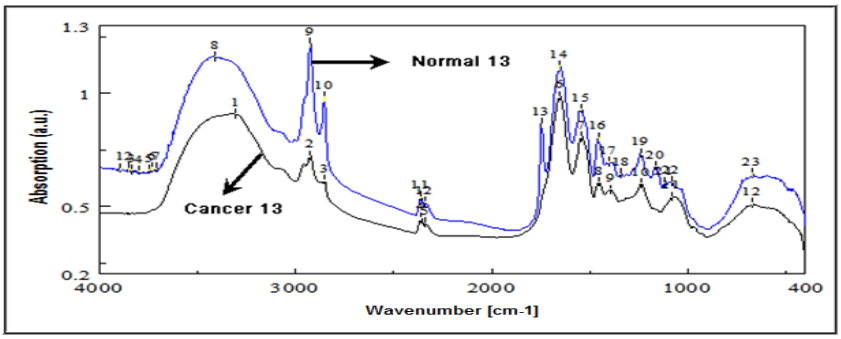


**Fig. 2:** FTIR spectra of normal and cancer bladder (patient 2)

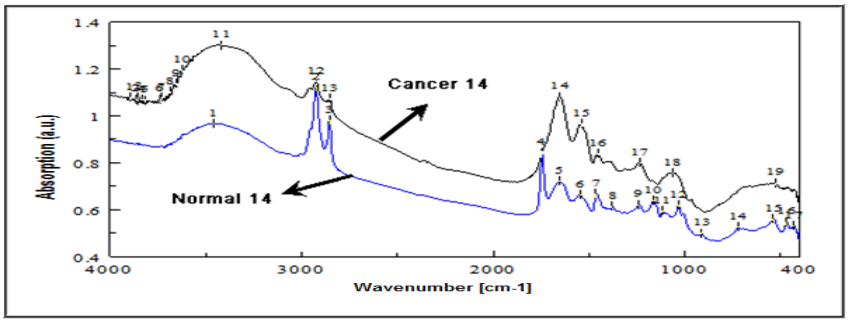


**Fig. 3:** FTIR spectra of normal and cancer bladder (patient 3)

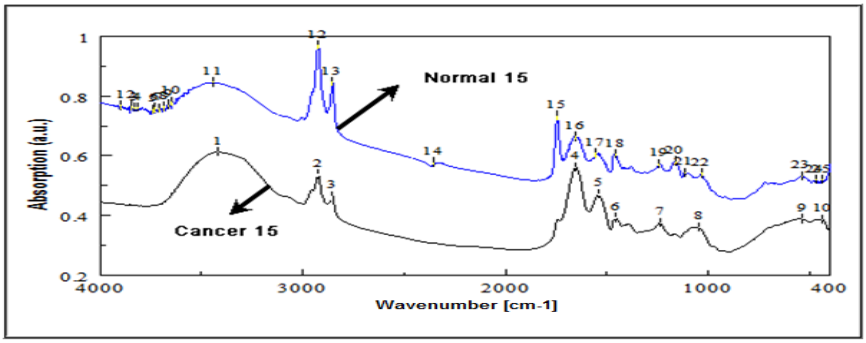


**Fig. 4:** FTIR spectra of normal and cancer bladder (patient 4)

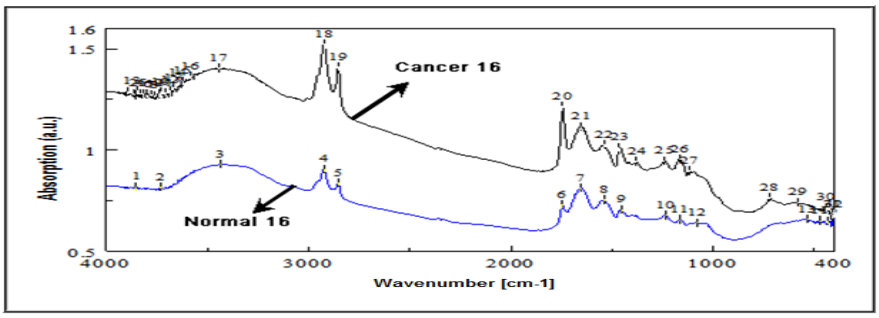


**Fig. 5:** FTIR spectra of normal and cancer bladder (patient 5)

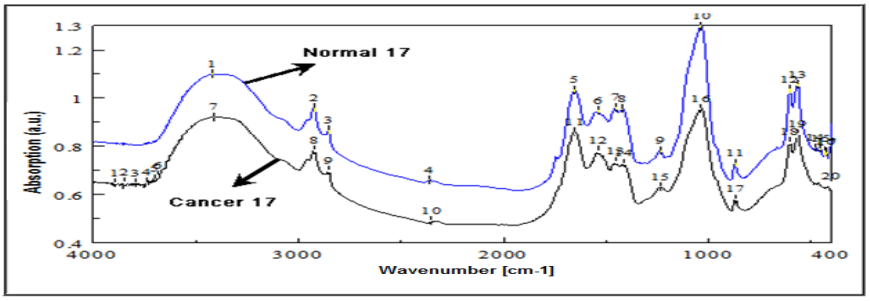


**Fig. 6:** FTIR spectra of normal and cancer bladder (patient 6)

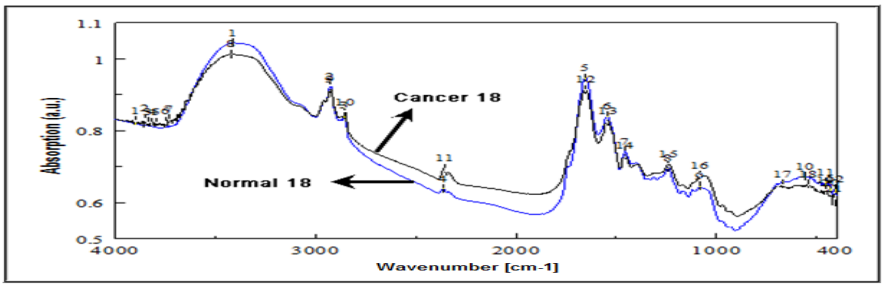


**Fig. 7:** FTIR spectra of normal and cancer bladder (patient 7)

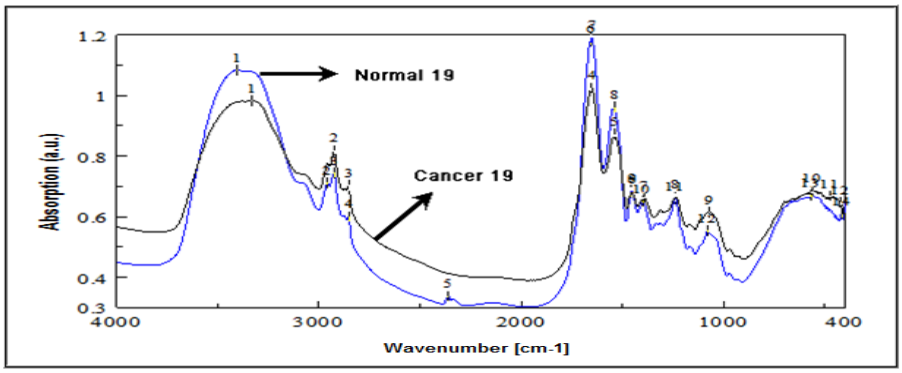


**Fig. 8:** FTIR spectra of normal and cancer bladder (patient 8)

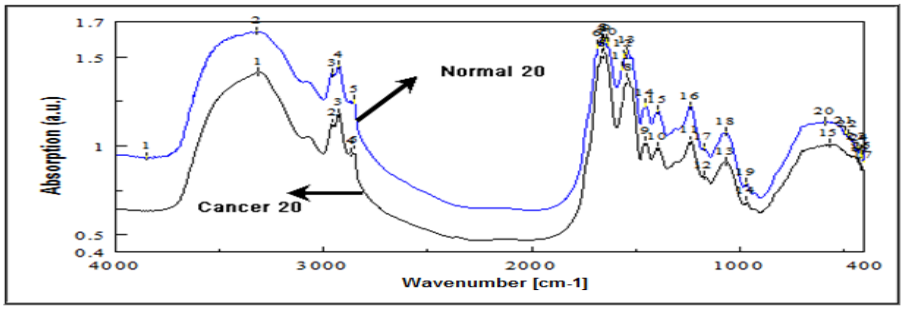


**Fig. 9:** FTIR spectra of normal and cancer bladder (patient 9)

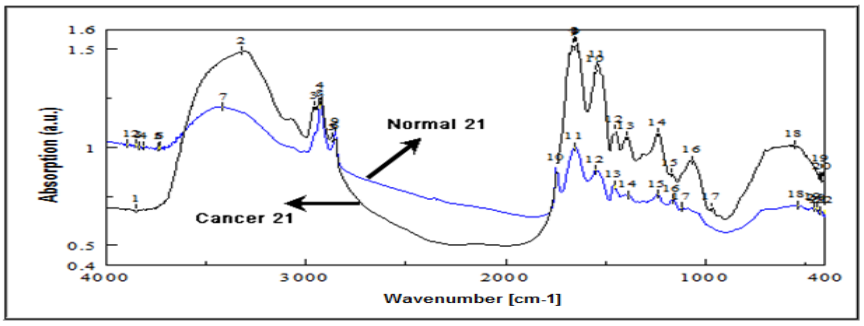


**Fig. 10:** FTIR spectra of normal and cancer bladder (patient 10)

In figures (1) to (10), the main spectral contributions are assigned to proteins around 3300, 1650, 1550, 1450, 1400 and 1240 $cm^{-1}$ and lipids around 3000, 2930, 2925, 2850, 1740, and 1450 $cm^{-1}$. The bands around 1236 $cm^{-1}$ and 1080 $cm^{-1}$ are due mainly to the asymmetric and symmetric phosphate stretching mode of nucleic acids. The bands around 1200 $cm^{-1}$ and 1030 $cm^{-1}$ are arising from C-OH stretching vibrations of collagen carbohydrate moieties, C-N stretching vibrations of the collagen backbone and $CH_2$ wagging vibrations of collagen side chains. In addition to lipids, proteins and nucleic acids, carbohydrates are also presented in all cells. The major absorption by carbohydrates is found in the 1000-1200 $cm^{-1}$ region of the spectra. Typical infrared absorption spectra of normal and malignant tissues are dominated by two absorption bands at 1650 and 1540 $cm^{-1}$ known as amide I and II, respectively. Amide I band arises from the C = O hydrogen bonded stretching vibrations, and amide II from the C-N stretching and a CNH bending vibrations. The weaker protein bands at 1450 and 1400 $cm^{-1}$ are associated with the asymmetric and symmetric $CH_3$ bending vibrations. The peaks at 1080 and 1236 $cm^{-1}$ are attributed to $PO_2$ (Nucleic acids), and the bands at 2925 and 2850 $cm^{-1}$ are $CH_2$, $CH_3$ stretching vibrations of phospholipids.

Significant differences were found between normal and malignant bladder tissues in the FTIR spectra. Through the spectral analysis, the spectral characteristics of malignancy were as follows: the cancerous tissue had higher absorption in the N-H region (3500-3000 $cm^{-1}$), amide I (1650-1660 $cm^{-1}$), amide II (1550-1540 $cm^{-1}$) which are indicated by higher intensity of these bands when compared to normal tissues. The bands for the C-H stretching vibration in the region around 2850 $cm^{-1}$ and C = O band near 1745 $cm^{-1}$ become weaker and even disappeared. The peak of amide I band is shifted to a lower wavenumber. The intensity of amides II bands becomes weaker. Also we found slight shifts in the positions between peaks of normal and cancer tissues which are in agreement with Qing-Bo Li *et al* and P.Venkatachalam et al (2005; 2008).

It can be noticed also that some cancerous bladder tissues had high absorption bands at (3417 $cm^{-1}$) and (1650 $cm^{-1}$), compared to normal bladder tissue, this is attributed to the increment of lipids and proteins in cancerous tissue when compared with normal bladder tissue. As for the Nucleic acids, apparently the cancerous bladder tissues had less absorbance at (1081 $cm^{-1}$), compared with the normal bladder tissue.

For further comparison between the spectra of normal and malignant tissues, the ratio between the intensities of proteins bands (amide $I_{1650}/I_{1550}$), Lipids bands ($I_{2925}/I_{2850}$) and Nucleic acids bands ($I_{1080}/I_{1236}$) for all spectra, are listed in table (1).

**Table 1:** The ratio of intensities for proteins, lipids and nucleic acids bands:

| Sample | Proteins $I_{1650}/I_{1550}$ | | Lipids $I_{2925}/I_{2850}$ | | Nucleic acids $I_{1080}/I_{1236}$ | |
|---|---|---|---|---|---|---|
| No. | Normal | Cancer | Normal | Cancer | Normal | Cancer |
| 1 | 1.2026 | 1.2600 | 1.2703 | 1.2146 | 0.8143 | 0.9338 |
| 2 | 1.2048 | 1.2211 | 1.2422 | 1.1712 | 0.8301 | 0.9067 |
| 3 | 1.0911 | 1.1198 | 1.1444 | 1.0678 | 0.9940 | 0.9449 |
| 4 | 1.0942 | 1.1899 | 1.1450 | 1.1527 | 0.9449 | 0.9617 |
| 5 | 1.0757 | 1.0886 | 1.0817 | 1.0789 | 0.9452 | 0.9413 |
| 6 | 1.0894 | 1.0996 | 1.1074 | 1.1194 | 1.6584 | 1.5140 |
| 7 | 1.1266 | 1.1063 | 1.0952 | 1.0751 | 0.9254 | 0.9560 |
| 8 | 1.2396 | 1.1773 | 1.2402 | 1.1640 | 0.8390 | 0.9234 |
| 9 | 1.0592 | 1.1622 | 1.1550 | 1.2270 | 0.8802 | 0.8848 |
| 10 | 1.1356 | 1.1039 | 1.1296 | 1.1931 | 0.9033 | 0.8685 |

***3-2 Intensity ratio of proteins (amide I/II):***

The intensity ratio between amide I and amide II ($I_{1650}/I_{1550}$) bands is increased in the cancerous tissues for samples (1, 2, 3, 4, 5, 6 and 9) when compared to normal tissues. The increase of the protein absorption in the cancerous tissues indicated that a number of C - O bonds from proteins and hydrogen bonds in the C - OH groups of amino acids were destroyed after cells became malignant. An increase in the intensity of the amide I

can be observed in the spectra of cancerous tissues compared to the normal tissues and this is may be a result of the increased proliferation of the malignant tissues. The results indicated that the amide I/II ratios are much higher for the malignant tissues than that for normal tissues; the same observation was noticed by P.Venkatachalam *et al.,* (2008).

***3-3 Intensity ratio of Lipids ($I_{2925}/I_{2850}$):***

The intensity ratio of lipids $I_{2925}/I_{2850}$ reflected the total lipids content in the tissues. In the present investigation, this ratio is decreased in the cancerous tissues for samples (1, 2, 3, 5, 7 and 8) compared with the normal tissues. The decrease in the intensity, or disappearance of the lipid bands, implies a decrease in the relative number of methyl groups in the malignant tissues. This decrease in the number of methyl groups is consistent with the hypomethylation seen during carcinogenesis. The results indicated that the ratio of Lipids $I_{2925}/I_{2850}$ is decreased for the malignant tissues compared with that of normal tissues; the same observation was noticed by Qing-Bo Li *et al.,* (2005).

***3-4 Intensity ratios of Nucleic acids ($I_{1080}/I_{1236}$):***

The ratio of bands intensities at 1236 and 1080 $cm^{-1}$ provides a measure of Nucleic acids and phosphate changes in the tissues of samples (1, 2, 4, 7, 8 and 9). In the present study, this ratio is increased in the cancerous tissues compared with normal tissue which reflects the higher activity of the malignant cells. The increased concentration of phospholipids in tumors may be due to the increase in phospholipids degradation, which can result in the modification of composition, structure and stability of the membranes resulting in membrane dyes function. These results indicated that the Nucleic acids and phospholipids intensity ratios are much higher in the malignant tissues than in normal tissues, this is in good agreement with the results of Jun-Kai Du et al and Zhiwei Huang *et al.,* (2009; 2004).

***Conclusions:***

In this study significant spectroscopic differences were found between normal and malignant bladder tissues, investigated by FTIR, where the spectra changes indicate alterations in the proteins, lipids and nucleic acids.

FTIR may be considered as a promising diagnostic tool for malignancy, beside that it is a fast technique and has high resolution.

## REFERENCES

Barbara Stuart, 2004. Infrared Spectroscopy Fundamentals and Applications, Copyright by John Wiley & Sons, Canada.

Gordon G. Hammes, 2005. Spectroscopy for the Biological Sciences, Copyright by John Wiley and Sons, Inc, New Jersey.

Jun-Kai Du, Jing-Sen Shi, Xue-Jun Sun, Jian-Sheng Wang, Yi-Zhuang Xu, Jin-Guang Wu, Yuan-Fu Zhang and Shi-Fu Weng, 2009. Fourier transform infrared spectroscopy of gallbladder carcinoma cell line, Copyright by Hepatobiliary Pancreat Disn Inc.

Kazuo Nakamoto, 2009. Infrared and Raman Spectra of Inorganic and Coordination Compounds, Part A: Theory and Applications in Inorganic Chemistry, Sixth Edition, Copyright by John Wiley & Sons, Inc, New Jersey.

Qing-Bo Li, Zhi Xu, Neng-Wei Zhang, Li Zhang, Fan Wang, Li-Min Yang, Jian-Sheng Wang, Su Zhou, Yuan-Fu Zhang, Xiao-Si Zhou, Jing-Sen Shi and Jin-Guang Wu, 2005. In vivo and in situ detection of colorectal cancer using Fourier transform infrared spectroscopy, Copyright by The WJG Press and Elsevier Inc. Amsterdam.

Rina, K. Dukor, 2002. Vibrational Spectroscopy in the Detection of Cancer, Copyright by John Wiley & Sons Ltd, USA.

Venkatachalam, P., L. Lakshmana Rao, N. Kiishna Kumar, Anupama Jose and Shaiju S. Nazee, 2008. Diagnosis of Breast Cancer Based on FT-IR Spectroscopy, Perspectives in Vibrational Spectroscopy, Copyright by American Institute Of Physics, USA.

Zhiwei Huang, Annette Mcwilliams1, Harvey Lui, David . Mclean, Stephen Lam and Haishan Zeng1, 2004. Near-Infrared Raman Spectroscopy for Optical Diagnosis of Lung Cancer, Copyright By Wiley Liss, Inc Canada.