Sys Rev Pharm 2020;11(12):1932-1937
A multifaceted review journal in the field of pharmacy

Detection Of Urinary Bladder Cancer By (ATR-FTIR) Spectroscopy

Enas Sulaiman Yousif [1], Duraid Taha Abdulkareem[2], Nafea sami Enad alboaisa [3] and Ehab Jasim Mohammad*[4]

[1] Physiology and Medical Physics Department, College of Medicine – University of Anbar, Ramadi, Iraq.
[2] Surgery Department, College of Medicine – University of Anbar, Ramadi, Iraq.
[3] Pathology Department, College of Medicine – University of Anbar, Ramadi, Iraq.
[4] FEBU, FABMS,FICMS, MBChB, Consultant Urologist, College of Medicine, Ibn Sina University of Medical and Pharmaceutical Sciences, Baghdad, Iraq
ehabgmh@yahoo.comCorresponding Author:

ABSTRACT

Due to the spectroscopic analysis sensitivity to the molecular structure and biochemical variations in the cells, it has received considerable attention recently to be a diagnosis technique of cancer tissues. In the study reported here, a spectra of Attenuated Total Reflection (ATR) Fourier Transform Infrared (FTIR) Spectroscopy, ware taken for 46 samples of urinary bladder tissues that were previously histopathologically specified by pathologist experienced as: 23 normal (N) samples and 23 transitional cell carcinoma (TCC) samples. Several spectral variations were detected in the frequency range between 400 cm-1 and 4000 cm-1.The ratio of bands intensities of A2925/A2853, A1650/A1540 and A1343/A1450 supplied conformational changes of lipids, protein and collagen respectively in the urinary bladder tissues. Between normal and cancerous tissues, there are noticeable variance in the spectral features because during carcinogenesis, the transformation from a normal to a cancerous state accompany variation in molecules structure, where the concentration of lipid is lower in cancerous urinary bladder tissues, unlike normal tissue, while the content of protein and collagen have been increased in cancerous urinary bladder tissues. These differences in the spectral information serve for the diagnosis of urinary bladder cancer and the results displayed that ATR-FTIR spectroscopy is a promising as a rapid, accurate, and simple method utilized for identification and diagnosis of cancerous urinary bladder tissues.

Keywords: Bladder cancer, ATR-FTIR spectroscopy, FTIR spectroscopy; Automatic detection, Vibrational Spectroscopy.

Correspondence:
Ehab Jasim Mohammad
FEBU, FABMS, FICMS, MBChB, Consultant Urologist, College of Medicine, Ibn Sina University of Medical and Pharmaceutical Sciences, Baghdad, Iraq

*Corresponding author: Ehab Jasim Mohammad
ehabgmh@yahoo.com email-address:

INTRODUCTION

The UBC (Urinary bladder cancer) is one of the main current health burdens in the world [1]. It stands fifth cancer in terms of incidence rates when both genders are considered together [2]. The latest World Health Organization (WHO) statistics rankings data in 2018 published that, bladder cancer in Iraq reached 7.39 of total death. The age adjusted Death Rate is 8.45 per 100.000 of population ranks Iraq #1 worldwide.

UBC has a very high recurrence rate and more than half will recur within 5 years, which is why early detection of Urinary bladder cancer is essential to prevent recurrence and early progression [2].

According to the increase incidence of UB cancer patients all over the world in general, and in Iraq specially**,** rapid, precise, minimally invasive, and inexpensive screening methods must be found. The gold standard to detect urinary bladder cancer is cystoscopy, which is an examination of the urinary bladder by insert a rigid or flexible endoscope through the urethra [3], the biopsies are sent for histopathological examination. by an expert pathologist. In this investigation, we concentrate on the detection of bladder cancer (BC) by Attenuated Total Reflection (ATR) Fourier Transform Infrared (FTIR) Spectroscopy. The issue that cause a problem is that the IR absorption of water is very extreme, this can be solved in one of many ways like dehydration of specimen, the water signal electronic subtraction or application of attenuated total reflectance (ATR) spectroscopy [4] and that was the option that we chose**.** Attenuated Total Reflection (ATR) Fourier Transform Infrared (FTIR) spectroscopy is an analytical technique that can be utilized broadly to examine a wide range of various molecules in a various state. ATR FTIR spectroscopy includes directing the IR light at an interface between an infrared transparent material with a big index of refraction named the internal reflection element (IRE, for example a prism made of germanium or ZnSe) and a specimen on the IRE surface [5]. The incidence angle of IR beam is bigger than the critical angle because of that, total internal reflection happens. A radiation standing wave named (an evanescent wave) is instituted at the reflecting surface and reacts with the specimen attenuating the IR light beam exiting the IRE. In FTIR spectroscopy, any specimen can be examined by electromagnetic radiation facing to the region of mid-IR to produce an absorbance spectrum. This spectrum can be considered a "fingerprint" characteristic of any biological specimen or chemical matter [4]. In biological cells and tissues, Lipids, proteins, collagen, are the important compositional, structural, and functional biomolecules. The alteration of cells from normal to malignant condition, induces not only alterations in the relative amounts of biomolecules but also in the structures of biomolecules [6]. FTIR spectroscopy has been utilized for a long time to characterize chemical compounds and used to examine the modes of vibration of molecules functional groups, this technique is sensitive to

molecular structure; thus, FT-IR measures the alterations in the contents and structure of biomolecules in cells by identifying the positions and relative intensities of various spectral bands of large biological molecules. The fingerprint region is approximately 1800–900 cm^{-1} in spectral of biological samples, this fingerprint region have a series of determined bands that can be used to differentiate the structure of complex biological specimens and to recognize a particular molecule [7]. Several studies have indicated the efficacy of FTIR spectroscopic approach in classifying urinary bladder pathologies [2,3,8,9]. The FT-IR spectra features are very useful tools in probing biomolecular composition of normal and cancer urinary bladder tissues, that mean we are able to supply a new medical device with great promise in terms of bladder cancer automatic detection [3]. Because of that, evaluation of these technique is requisite before considering for routine clinical use.

EXPERIMENTAL WORK

In this study, the human normal and malignant urinary bladder tissues samples of patients aged 29–65 years old (median age is 47 years old) were collected from Al- Ramadi hospitals and some privet hospitals in AL-Ramadi city. After the transurethral resection, the tissues removed were stored in 10% formaldehyde solution. Two pieces of tissue were removed, each approximately 2 cm in diameter. One was cut from the center of the lesion (abnormal) and the other from the far (normal) edge of the removed tissue. Each urinary bladder tissue is divided into two parts, the first part goes to the pathological examination and undergo to the standard procedure. The second part of the samples goes to our lab to conduct spectral analysis. Normal and cancerous tissues were cut into approximately 2 mm sizes and dried for 3 days for ATR-FTIR investigation. These urinary bladder tissues investigated spectroscopically using Fourier Transform Infrared Spectrometer (FT-IR) by (ATR) method. The ATR-FTIR that is used in our laboratory is (FTIR-670,Thermo Nicolet, NEXUS). The samples FT-IR spectra were gained in the spectral range of 4000 to 400 cm^{-1}. Each spectrum was acquired with 32 scans and 4cm^{-1} resolution. For each patient sample, we have measured FT-IR spectra for normal tissue and abnormal tissue as shown in Table (1). By using ATR-FTIR spectroscopy, qualitative analysis of normal and abnormal urinary bladder tissues have been carried out by characterizing the position (wave number) of the peaks and the assignment of infrared absorption bands of the urinary bladder tissues samples spectra. Also quantitative analysis of normal and abnormal urinary bladder tissue have been carried out by obtaining the relative intensity of the major absorption bands to estimate the contents of functional biomolecules like lipids, proteins and collagen.

Table (1): Number of cases studied with ATR-FTIR.

No.	Histopathological examination results	Total number of cases
1	Normal (N)	23
2	Transitional cell carcinoma (TCC)	23
3	Total samples number	46

RESULTS AND DISCUSSION

Histopathological Examination Results

In this investigation, normal and malignant urinary bladder tissue specimens were obtained after transurethral resection from urinary bladders of patients. Histological image of the cross section of the formalin-fixed human bladder tissue stained with H&E (hematoxylin-eosin) as observed under a microscope is shown in Figure (1). The histopathological analysis included 23 normal specimens (N) and 23 transitional cell carcinoma specimens (TCC).

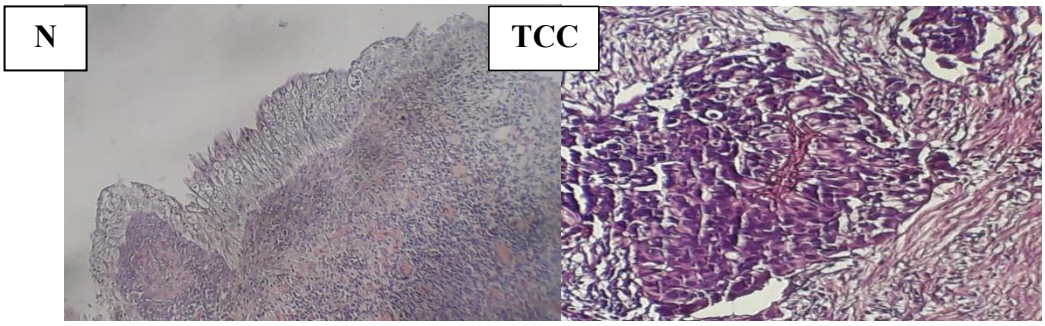

Figure (1): Histological image cross section of formalin-fixed urinary bladder tissue with H&E (hematoxylin-eosin). (N): normal urinary bladder tissue and (TCC): Transitional cell carcinoma**.**

Qualitative & Quantitative Analysis of FT-IR spectroscopy

Before we discuss in detail the qualitative and quantitative analysis of FT-IR spectrum of normal and cancer urinary bladder tissues, we first characterize the position of the peaks and the IR absorption bands assignment of the bladder tissues. The FT-IR spectra of normal bladder tissues (N) and transitional cell carcinoma bladder tissues (TCC) that shown in Figure (2) and listed in Table (2) are distinguished by seven notable peaks at 1343, 1450, 1540, 1650, 1743, 2853, and 2925 cm^{-1}. The spectra display spectacular changes in peak heights, while no considerable shift in the frequency was observed. The spectral feature of cancerous tissues convert because of the changes in the structure of molecules that combine the mutation from a normal to abnormal condition [10].

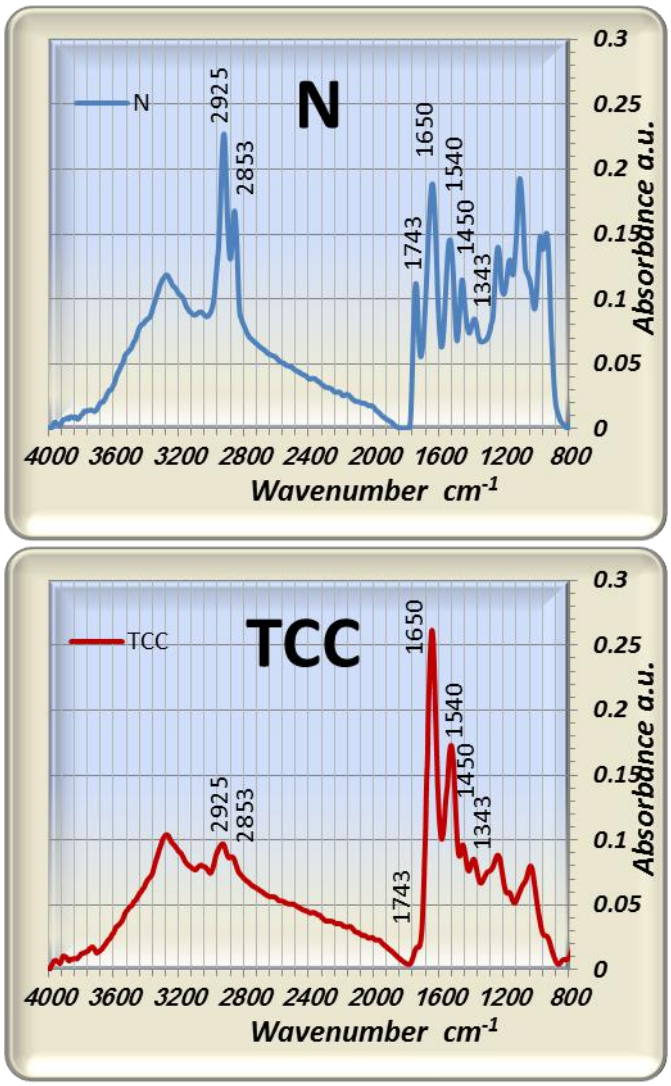

Figure (2) : FT-IR spectra for (N):normal and (TCC): Transitional cell carcinoma of urinary bladder tissues.

In this investigation, the main spectral contribution assigned to lipid peaks were 2925 and 2853 cm^{-1} caused by antisymmetric stretching of CH_2 and symmetric stretching of CH_2, respectively [11]. The methylene (-CH_2-) group band in the 2925 and 2853 cm^{-1} peaks is enhanced in the spectra of noncancerous tissue. Figure (2) reveal that they are markedly reduced in the spectra of cancerous tissues compared with the spectra of normal tissues. Also the other vibrational modes of lipid appear at 1743 cm^{-1} band, which is assigned to the vibration of the C=O (ester group) stretching vibration mode [12,13], this band is existent in normal tissues, whereas it is weak and absent in (TCC) tissues as shown in Figure (2). The reduction of fat cells in cancerous tissues may be accounted to two reasons, during tumor development, normal tissues, including fat cells, are eliminated by the proliferating malignant tissue, explaining the lack of fat cells in the tissue, the second reason is the fat in the region of the cancerous tissue is consumed because of the increased nutritional and energy necessity of the growthing carcinoma [14]. Proteins peaks due to the protein amide I peak caused by carbonyl (C=O) stretching was at 1650 cm^{-1}; and the protein amide II due to (N-H) bending was at 1540 cm^{-1} [15,16]. The variation in the spectral features of the cancerous and noncancerous tissues in the intensity of the peaks at 1650 and 1540cm^{-1} are dramatic. From Figure (2) It is clear that the spectrum of protein band from the cancerous tissue were very increased compared with those of normal tissues. This result can explain the protein amount increases in cancerous tissues during malignancy [17,18]. Collagen vibrational modes appear at 1343 peak due to CH_3CH_2 wagging and 1450 cm^{-1} peak caused by CH_3CH_2 deformation modes, respectively [19,20].

Table (2): Peak positions and assignments of urinary bladder tissues spectra.

Peak pos (cm^{-1})	Major assignment
2925	CH_2 antisymmetric stretching (lipid)
2853	CH_2 symmetric stretching (lipid)
1743	C=O ester stretching mode (lipid)
1650	(C=O) carbonyl stretching, Amide I (protein)
1540	N-H bending mode, amide II (protein)
1450	CH_3CH_2 deformation mode
1343	CH_3CH_2 wagging mode

The other important spectral parameter is the relative intensity of the major absorption bands for obtaining the quantitative information about the biomolecules contents in these tissues [21]. A variation in peak intensity and shape indicates considerable biochemical changes [15]. To quantitatively recognize the N and TCC samples, we calculated three absorbance ratios by using the measured peak heights. Variation in these ratios between N and TCC of urinary bladder tissue samples were calculated from the

spectra in Figure (2) and listed in Table (3). In the present investigation, the absorbance ratio A_{2925}/A_{2853} is used to measure the ratio of lipid content and reflected the total lipid content in the N and TCC tissues [6,22]. The mean values of this absorbance ratio are 1.46, and 1.13 for the N and TCC samples respectively. The percentage rates of change in lipid content of TCC from N sample type is −22.6%, where (−) means decrease and (+) means increase. From these results we can recognize that this ratio decreased in cancerous tissues as shown in Figure (3). This decrease in intensity or the vanishing of the lipid bands reveal a lack in the relative number of methyl groups in the cancerous cells during carcinogenesis [22].

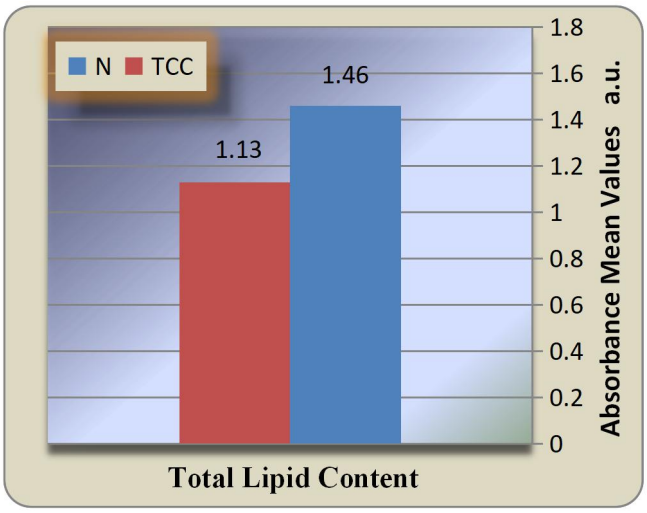

Figure (3): Variation of standard absorbance's ratios of lipid content between (N): normal and (TCC): Transitional cell carcinoma of urinary bladder tissues**.**

One of the fundamental components of living systems are proteins. Proteins with lipids and collagen constitute the molecules that play important roles in biology.

The absorbance ratio A_{1650}/A_{1540} is used to measure the ratio of protein content [6,21,22]. The mean values of this absorbance ratio are 1.31 and 1.52 for the N and TCC samples respectively as shown in Figure (4). The percentage rates of change in proteins content of TCC from N sample type is +16%. In this investigation, the ratio between the bands at 1650 and 1540 cm^{-1} increased in malignant tissues of the samples compared with that in normal tissues. The intensity of the amide I and amid II bands at 1650 and 1540 cm^{-1} increased in the spectra of carcinoma samples compared with those of the normal tissue maybe because of the tumor cells proliferation increased [22,23].

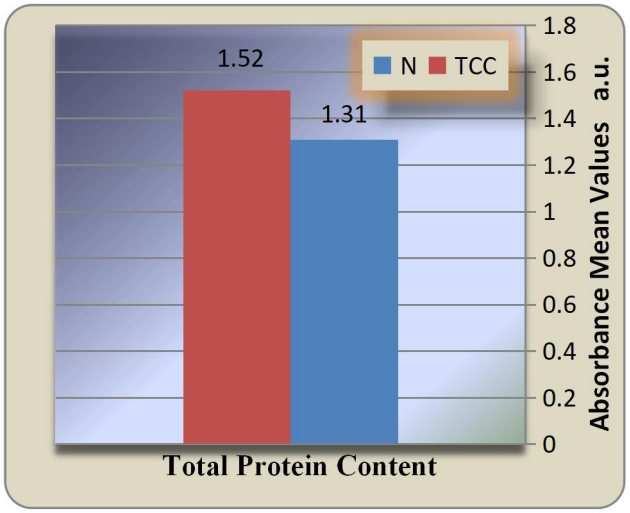

Figure (4): Variation of standard absorbance's ratios of Protein content between (N): normal and (TCC): Transitional cell carcinoma of urinary bladder tissues

Fibrosis occurs in malignant bladder lesions and includes proliferation of stroma. Fibrotic tissue is mainly composed of collagen, the amount of which increased in the proteins presence, like fibrinogen [7]. The absorbance ratio of A_{1343}/A_{1450} provides a measure of the ratio of collagen content [24,25]. The mean values of this absorbance ratio are 0.79, and 0.91 for the N, and TCC samples, respectively as shown in Figure (5). The percentage rates of change in content of TCC from N sample type is +19.7%. In the present investigation, the ratio of the band intensities at 1343 and 1450 cm^{-1} that represents the content of collagen in the normal and malignant tissues, this ratio increased in the cancerous tissues compared with that in normal tissue.

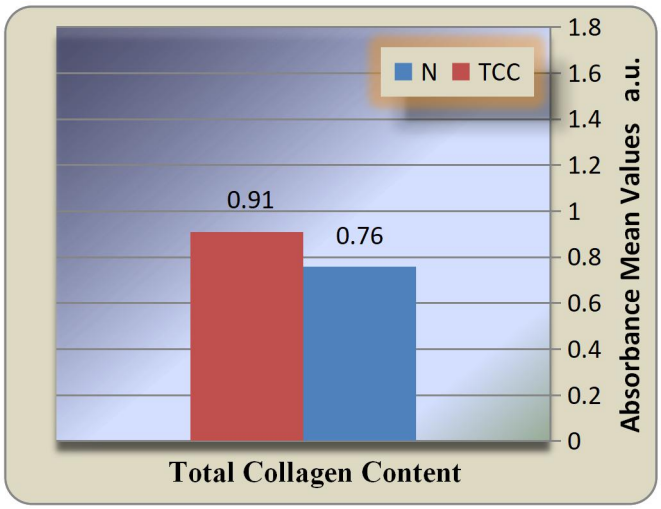

Figure (5): Variation of standard absorbance's ratios of collagen content between (N): normal and (TCC): Transitional cell carcinoma of urinary bladder tissues**.**

The results in Table (3) show a clear variance in the ATR-FTIR spectra between normal and malignant tissues in the spectral range of 3000–1000 cm^{-1} by the ratios of the peak intensity of spectral peaks characteristic of the cellular components, and they are consistent with previous findings [22,24,26].

Table(3): Comparison of standard ratio between normal and cance urinary bladder tissue.

Standard Ratio	**N**	**TCC**	**Rate of Change From N**
A_{2925}/A_{2853} **Lipid**	1.46	1.13	−22.6%
A_{1650}/A_{1540} **Protein**	1.31	1.52	+16%
A_{1343}/A_{1450} **Collagen**	0.76	0.91	+19.7%

(+) means increase, (−) means decrease

CONCLUSIONS

The spectrum of transitional cell carcinoma (TCC) of urinary bladder tissue is remarkably different from the normal (N) tissue, whereas the spectral changes indicate alterations in content of lipids, proteins and collagen. The concentration of lipid cells is lower in cancerous than in normal urinary bladder tissues, although changes in content of protein, and collagen have been implicated in tumorigenesis, where a high level of protein and collagen were observed in transitional cell carcinoma (TCC) of urinary bladder tissues may show that carcinogenesis depend on the alterations in the composition of connective tissues. These results are consistent with the histopathological results. Therefore the present study shows that ATR-FTIR spectroscopy can be used to recognize cancerous from noncancerous of urinary bladder tissues and this new technique promises practicality, rapid, accurate, inexpensive and spectrum acquisition with high-quality.

REFERENCES

1. Julian Ollesch, Margot Heinze, H. Michael Heise, Thomas Behrens, Thomas Bruning and Klaus Gerwert, It's in your blood: spectral biomarker candidates for urinary bladder cancer from automated FTIR spectroscopy, J. Biophotonics 7(2014) 1–12
2. Shaoxin Li, Linfang Li, Qiuyao Zeng, Yanjiao Zhang3, Zhouyi Guo, Zhiming Liu, Mei Jin, Chengkang Su, Lin Lin, Junfa Xu & Songhao Liu, Characterization and noninvasive diagnosis of bladder cancer with serum surface enhanced Raman spectroscopy and genetic algorithms, Scientific Reports, 5(2015)1-7
3. Siouar Bensaida, Amar Kachenouraa, Nathalie Costeta, Karim Bensalaha, Hugues Tarield, Lotfi Senhadji, Noninvasive detection of bladder cancer using mid-infrared spectra classification, Expert Systems with Applications, 89(2017) 333–342.
4. David I. Ellis, George G. Harrigan and Royston Goodacre, Metabolic Fingerprinting With Fourier Transform Infrared Spectroscopy, U.S.A.,(2003).
5. Stefanie E. Glassford, Bernadette Byrne, Sergei G. Kazarian, Recent applications of ATR FTIR spectroscopy and imaging to proteins,U.K.,(2013).
6. Tiyu Gao, Yunxiang Ci , Fourier Transform Infrared Spectroscopic features of human breast benign and malignant tissues, Vib. Spec. J., 3(2004) 35-42.
7. Renata A. Bitar Cartera, Airton A. Martina, Mario M. Nettob, FT-Raman Spectroscopy Study of Human Breast Tissue, Biomedical Vibrational Spectroscopy, 5321(2004)190-198.
8. Gok, S.; Aydin, O.Z.; Sural, Y.S.; Zorlu, F.; Bayol, U.; Severcan, F. Bladder cancer diagnosis from bladder wash by Fourier transform infrared spectroscopy as a novel test for tumor recurrence. J. Biophotonics, 9(2016) 967–975.
9. 9-Kar-Yan Su and Wai-Leng Lee, Fourier Transform Infrared Spectroscopy as a Cancer Screening and Diagnostic Tool: A Review and Prospects, Cancers 12(2020)1-19
10. Enas S. Yousif, Nabeil I. Fawaz, Bilal J. Mohamed, Human Breast Tissue Cancer Diagnosis by FT-IR Spectroscopy,9(2015)19-26
11. Abasalt Hosseinzadeh, Mohammad Javad and Taerreh Khadjvand, Fourier transform infrared

microspectroscopy as a diagnostic tool for distinguishing between normal and malignant human gastric tissue, J. Biosci., 36 (2011) 669–677
12. Abigail S. Haka,1 Zoya Volynskaya,1 Joseph A. Gardecki, In vivo Margin Assessment during Partial Mastectomy Breast Surgery Using Raman Spectroscopy, Cancer Res, 66(2006) 3317-3330.
13. Tiyu Gao, Yunxiang Ci , Fourier Transform Infrared Spectroscopic features of human breast benign and malignant tissues, Vib. Spec. J., 3(2004) 35-42.
14. Jin Guang WU, YI Zhuang XU, Cuan Wen, Distinguishing Malignant from Normal Oral Tissues Using FT-IR Fiber Optic Techniques, FTIR Fiber Optics, 23(2001)186-197.
15. C.-H. Liu, Y. Zhou, Y. Sun, J. Y. Li, L. X. Zhou, Resonance Raman and Raman Spectroscopy for Breast Cancer Detection, Technology in Cancer Research and Treatment, 12 (2013) 371-377.
16. 16- E.B. Hanlon, R Manoharan, T-W Koo, K E Shafer, Prospects for in vivo Raman spectroscopy, Phys. Med. Biol., 45 (2000) 6-59.
17. Jakub Surmacki, Jacek Musial, Radzislaw Kordek and Halina Abramczyk, Raman imaging at biological interfaces: applications in breast cancer diagnosis, Molecular Cancer, 12(2013)48
18. Ceren Aksoy and Feride Severcan, Role of Vibrational Spectroscopy in Stem Cell Research, 27 (2012) 167–184.
19. Barbara Stuart, Infrared Spectroscopy: Fundamentals and Applications, U.S.A., (2011).
20. Heinz Fabian, Ngoc Anh Ngo Thi , Michael Eiden , Diagnosing benign and malignant lesions in breast tissue sections by using IR-microspectroscopy, Biochimica et Biophysica Acta 1758 (2006) 874–882.
21. Safaa K. H. Khalil, Mostafa M. Khodeir, Rasha Abd El-Hakam, Rezq Abd El-Monem Rezq, Spectroscopic Study for Detection and Grading of Breast Carcinoma In vitro, Australian Journal of Basic and Applied Sciences, 3(2009) 2419-2428.
22. Venkatachalam, L. Lakshmana Rao, N. Kiishna Kumar, Anupama Jose and Shaiju S.Nazeer, Diagnosis of Breast Cancer Based on FT-IR
23. Spectroscopy, American Institute of Physic, 8(2010)144-149.
24. E B Hanlon, R Manoharan, T-W Koo, K E Shafer**,** Prospects for *in vivo* Raman spectroscopy, Phys. Med. Biol., 45 (2000) 6-59.
25. M. V. P. Chowdary, K. Kalyan Kumar, Stanley Mathew, C.Murali and Jacob Kurien, Biochemical Correlation of Raman Spectra of Normal, Benign and Malignant Breast Tissues: A Spectral Deconvolution Study ,Biopolymers, 91(2009) 540.
26. Shazza Rehman ,Vibrational Spectroscopy for Tissue Analysis, U.K., (2013).
27. Tiyu Gao, Jun Feng and Yunxiang Ci, Human breast carcinomal tissues display distinctive FTIR spectra: implication for the histological characterization of carcinomas, Analytical Cellular Pathology, 18 (1999) 87–93.