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MINI-REVIEW

CANCER DIAGNOSIS BY FT-IR SPECTROPHOTOMETRY

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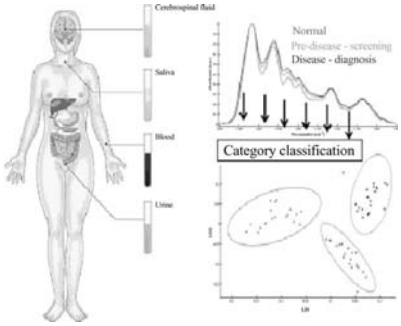
Fourier transform infrared spectroscopy (FTIR) is a fast and nondestructive analytical method. The infrared spectrum of a mixture serves as the basis to quantitate its constituents, and a number of common clinical chemistry tests have proven to be feasible using this approach. This mini-review provides some background to infrared spectroscopy including Fourier transform infrared spectroscopy in biological fluids. By this we provide the reader with sufficient background for selected applications in cancer diagnostic. This review focuses on biomedical FTIR applications, published in the period 2009-2014, used for early detection of cancer through qualitative and quantitative analysis.



INTRODUCTION*

Infrared (IR) spectroscopy is one of the most important analytical techniques available to scientists. One of the great advantages of IR spectroscopy is that any sample in virtually any state may be studied. As a consequence of improved instrumentation, a variety of new sensitive techniques have been developed in order to examine formerly intractable or difficult samples in biomedical research.[1-4]

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

Biostructure disorders (*e.g.*, uncontrolled cell division, invasive cell growth into adjacent tissue and metastatic implantation to other body sites) are called "cancer". Cancer is becoming the leading cause of death all around the world. Cancer-related diseases affect people in all age ranges, but the risk tends to increase with age. The highest death rates are recorded for lung, colon, breast and prostate cancers. Developments in cancer treatment are reported daily with serious attention paid to diagnostic methods. It is well known that a precise accurate diagnostic report is very helpful for drawing up strategies for treatment.

The risk of cancer in humans is increased by a wide spectrum of factors, which ranges from exposure to an identified agent, such as environmental chemicals or a virus, to a culturally determined behaviour, such as smoking, or to

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socio-economic conditions. We are today able to intervene on some of these factors. Only progress in the understanding of the mechanisms by which these factors act can lead to specific means of cancer prevention.

The main diagnostic method for cancer is histological confirmation that is provided by pathological examination of tissue samples that can be obtained from biopsy of surgery.[5,6] Usually in the first step, a piece of tissue is fixed by formaldehyde solution in order to preserve it physically. In the next step, a paraffin-embedding procedure is performed to make a hard block of sample, which can be sectioned by a microtome. The sliced sections are then glass mounted and stained by hematoxylin and eosin (H&E) to provide different colors for each biochemical structure of the tissue cells, prior to being investigated by pathological microscopic evaluations. The pathological inspections are based on some criteria, according to the cell size and visual morphology, which are different between normal and cancerous cells (*e.g.*, larger cell nucleus in cancer, multiple nuclei, irregular condition in cancer cases, or neoplastic invasion of malignant structures into normal ones). All the main diagnostic criteria in these cases are qualitative. Standard histopathological procedure involves tagging and visualizing the distribution and the structure of cellular components in tissue sections using light microscopy. Although used in clinical routine, this common approach is not free of pitfalls and is associated with environmental contamination by agents used for sample preparation.[7] The biopsy-based approach is time consuming and depends on the professional capabilities of the pathologist.

Innovative diagnostic methods that provide indications, complementary to the conventional histopathology, in particular the early biomolecular alterations under malignant conditions are under scrutiny. One such candidate method is the infrared (IR) spectral imaging which has the potential to provide, in a nondestructive and label-free manner, a biochemical fingerprint of cells and tissues.[8,9] As such, its potentials have been exploited in various IR spectroscopic studies applied to cells and tissues from different organs. In comparison to the conventional hematoxylin and eosin (HE)-stained reference histological images, the cluster images permitted to retrieve specific IR spectral signatures representative of the nontumoral and the tumoral epithelial components.

Cancer includes several different diseases originating from a defect in any cell in the body which generates progeny unrestricted by constraints imposed on their growth, division and differentiation by normal regulatory mechanisms of the body. The goal of early detection and screening is the diagnosis and treatment of cancer before it spreads beyond the organ of origin, perhaps even in its pre-invasive stage. This approach is preferable to trying to control cancer by systemic therapy. However, available early detection and screening techniques pick up many tumors at a relatively late stage in their natural history.[10] As a result, reduction in mortality with the currently available detection modalities is likely to be modest.

The optical techniques have been studied extensively as a proposal for the diagnosis of cancer, because instead of using an approach based on morphological changes, as currently occurs in histopathological studies, the analysis is automated and relies on the detection of biochemical changes that occur in tumor tissues.[11] One of the optical spectroscopy techniques that can effectively provide information concerning the structure and chemical composition of biological materials at the molecular level is Fourier transform infrared spectroscopy (FTIR).

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

Infrared (IR) spectroscopy has emerged in recent years as the analytical method of choice in an enormous variety of applications.[12,13] Molecular structure and function are strongly correlated. This aspect is particularly relevant in the case of proteins, which play important roles in cells biochemistry. Changes of structure may be easily detected in an IR spectrum and a cellular molecular marker may in fact be used to address a pathological status of tissue. Furthermore, comparison of spectra between healthy and cancerous tissue may improve understanding of pathogenesis of morbid process.

Infrared (IR) spectroscopy has emerged in recent years as the analytical method of choice in an enormous variety of applications. What has this revolution bring about? The clearest advantage is that no specific reagents are required. Automated,

repetitive analyses can therefore be carried out at very low cost. The appeal of these factors has spurred the development of a new generation of analytical IR spectrometers that combine high acquisition speed with superb spectral sensitivity. Powerful chemometric algorithms and software packages have emerged in parallel with the new hardware, and new applications emerge continually.

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

In a previous review we presented some important applications of FTIR microscopy in biomedical investigations.[14] The aim of the present review is to present some of the most important papers related to the usefulness of FTIR spectroscopy as an artifice for discriminating between normal and malignant cells, with varying degrees of dysplasia, published between 2009 and 2014.

INFRARED SPECTROSCOPY IN THE BIOLOGICAL FLUIDS

Water is the major molecular component within biological matrices, and strongly affects the utility of selected electromagnetic spectral regimes due to strong O–H absorptions, especially in the MIR region.[15] However, water has a relatively broad transmission window in the NIR, thereby enabling direct measurement of the biological specimen. However, despite the merits of operating in the NIR region, the information content and data interpretation of biological NIR spectra are frequently affected by relatively weak and highly convoluted absorption features.

In considering the use of IR spectroscopy for clinical analyses, we are confronted with the fact that the most abundant species found in all biological fluids is water, and the IR spectra reflect this fact. To illustrate the dominance of water in the IR spectra, Figs. 1 and 2 depict the absorption profiles for native serum in the MIR and NIR spectral regions.[16] Although some of the stronger solute absorptions do emerge in the MIR spectra, water clearly dominates the overall appearance. The NIR spectra are apparently devoid of any absorptions other than those of water. MIR and NIR spectroscopies in fact offer quite different, complementary, approaches to analysis. The richness of the MIR spectrum makes it instinctively appealing as the method of choice for analytical work, however NIR has practical benefits such as convenience in sample handling and the fact that the sample cells do not require specialized materials.

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

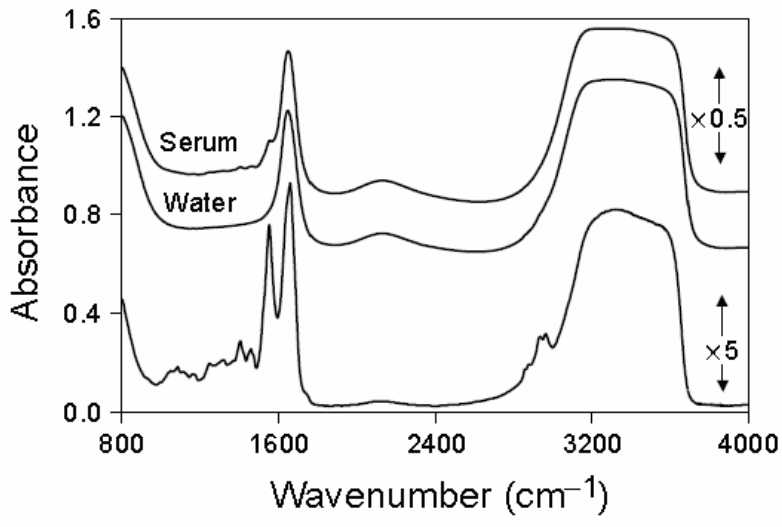

Fig. 1 – MIR absorption spectra of serum and water, collected with an optical path of 6 μm. The lower spectrum is the difference, water spectrum substracted from the serum one.

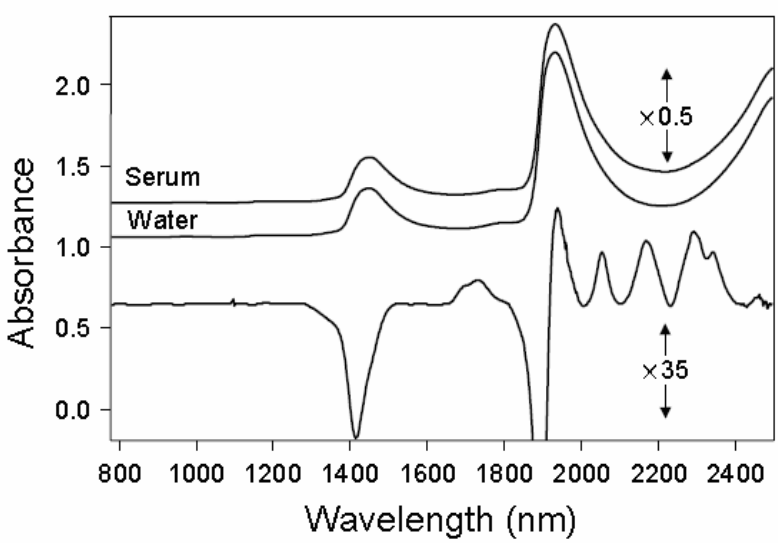

Fig. 2 – NIR absorption spectra of serum and water, collected with an optical path of 0.5 μm. The lower spectrum is the difference, water spectrum substracted from the serum one.

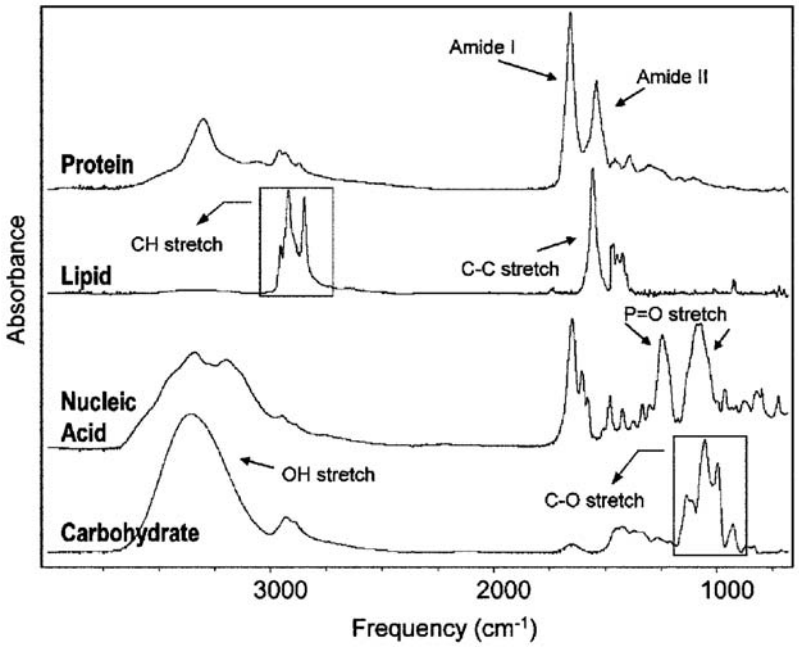

Fig. 3 – Various cellular components have dramatically different IR spectra as demonstrated by IR spectra of **(A)** a lipid (palmitic acid), **(B)** a protein (myoglobin), **(C)** a poly-nucleic acid, and **(D)** a carbohydrate (sucrose).

Human cells come in a variety of shapes and sizes: they may range from a configuration that is about 10 to 15 μm on edge, and nearly cubic in shape, to stratified (flattened) morphology, up to 60 μm in diameter and 5±10 μm thick, depending on the organ of origin. By dry weight, cells consist of about 60% protein and 25% nucleic acids, with the rest from other components (carbohydrates, phospholipids, and others).[17]

Vibrational spectroscopy offers a complete information on the chemical composition of samples regarding both, major and minor compounds, which present many characteristic bands in the infrared range (IR). Additionally, the presence of trace compounds can be modelized in some cases through the multivariate treatment of the whole IR spectra of well characterized samples based on the influence of molecules at low concentration levels on the size and shape of the bands of major compounds as can be seen in Fig. 3.[18]

The "bio-fingerprint region" (1800 cm^{-1} to 900 cm^{-1}) of the mid-infrared (mid-IR) spectrum contains the fundamental vibrational modes of key

chemical bonds that may be exploited to understand intracellular mechanisms. Therefore, IR spectroscopy produces a so-called "biochemical cell fingerprint" of the material under study, with direct association between peaks and chemical bonds[19-21] providing a non-destructive, screening approach to diagnosis [22] which can be done in a rapid manner. Biospectroscopy[21] is envisaged as an objective and robust tool to be used in cancer screening and diagnosis. Despite the increasing popularity of the field, there are several challenges to the developing application of biospectroscopy with regards to sample preparation, instrumentation and data handling; these need to be addressed before the technique can become a routine method in the clinical laboratory or the biological laboratory.

The chemical composition and structure of cells, tissues, and the composition of biofluids of biological entities are subject to variations at the molecular level, if affected by environmental factors, diseases, cancers, or other pathologies/ abnormalities. IR spectroscopy not only differentiates cells and tissues based on their characteristic spectral properties reflecting the chemical composition and structure, but also has the potential to serve as a diagnostic tool for detecting and discriminating different diseases or disease progression due to the induced changes of chemical composition and structure.

Three main sampling methods of FTIR spectroscopy exist, as can be seen in Fig. 4. Transmission mode experiments operate by transmitting IR radiation through the sample and substrate before the resulting radiation is detected. In using this technique, expensive IR transparent substrates are commonly used. Although transmission mode experiments are the most common, transmission spectra are subject to a variety of physical effects occurring when measuring the sample. Transflection mode experiments detect the absorbed IR radiation after it is transmitted through the sample, reflected off the substrate, and transmitted back through the sample. The final mode of FTIR spectroscopy is ATR-FTIR and operates on the principles of total internal reflection. By using this sampling mode of FTIR spectroscopy, the aforementioned unwanted contributions to spectra can be overcome. Past research in the field has shown that ATR-FTIR is well suited to the analysis of biofluids and dry films.[23] It has a wide applicability in quantifying various serum components of interest in clinical assays.[16] The sampling method also has the distinct advantage for analysing samples such as serum, because the sample can be placed and dried directly on the ATR crystal before spectra are measured.

Changes in the tissue biochemistry must precede any morphological or symptomatic manifestation associated with a diseased state,[24] so spectroscopic diagnosis is a logical alternative for the early detection of disease.

The clinical need for analytical methods that have the capability of performing intraoperative diagnosis is due to:

- limitations in preoperative diagnostic validity necessitating intraoperative diagnostics,
- long information delay in conventional intraoperative frozen section histology for single tissue pieces, and
- limitations of frozen section histology relying on tissue that has already been removed.[25]

The Fourier transform infrared (FTIR) spectroscopy, with the absorption of electromagnetic radiation from 400 to 4000 cm^{-1}, is sensitive to changes in molecular compositions and structures. It permits rapid collection of spectra obtained from millimeter-sized samples and could detect biochemical signatures of tissues that associated with generation and progression of disease.[26]

APPLICATIONS

Vibrational spectroscopy offers a unique opportunity to investigate the composition of unknown substances on a molecular basis. Although this fact was discovered long ago, it is the recent technical advances that have generated the strong and increasing interest in the application potential of molecular vibrational spectroscopy. Biological and medical applications, in particular, have progressed significantly in recent years.[27-32]

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

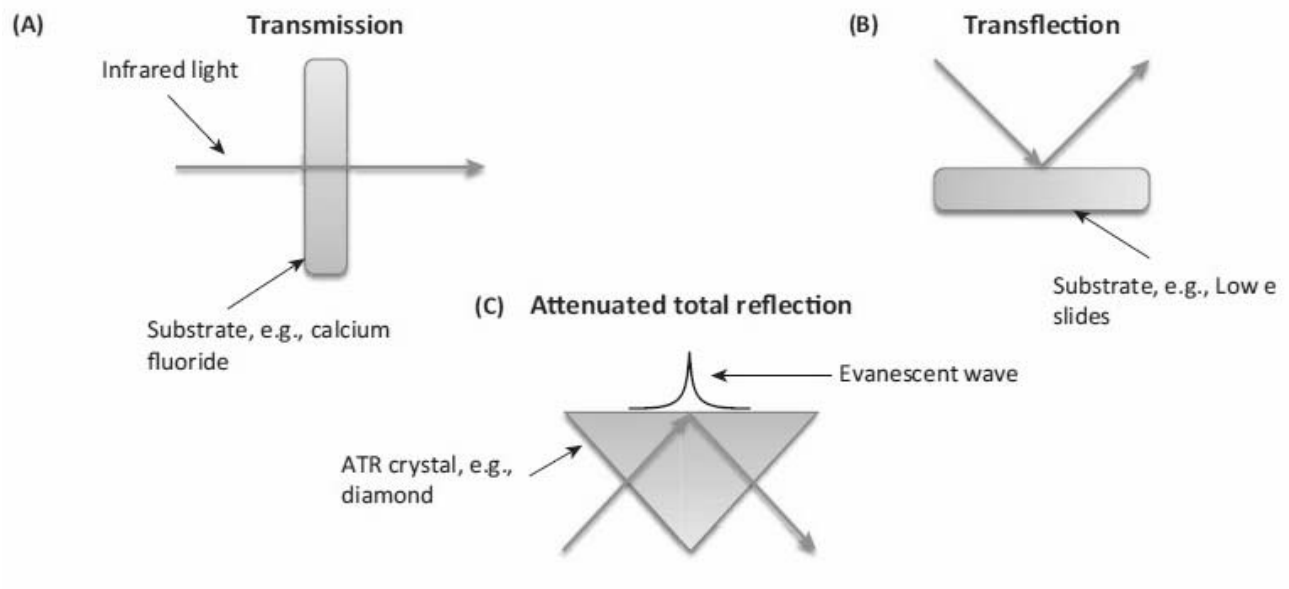

Fig. 4 – Examples of Fourier transform infrared sampling methods for: (A) transmission mode experiments; (B) transflection mode experiments; (C) attenuated total reflection (ATR) mode experiments.

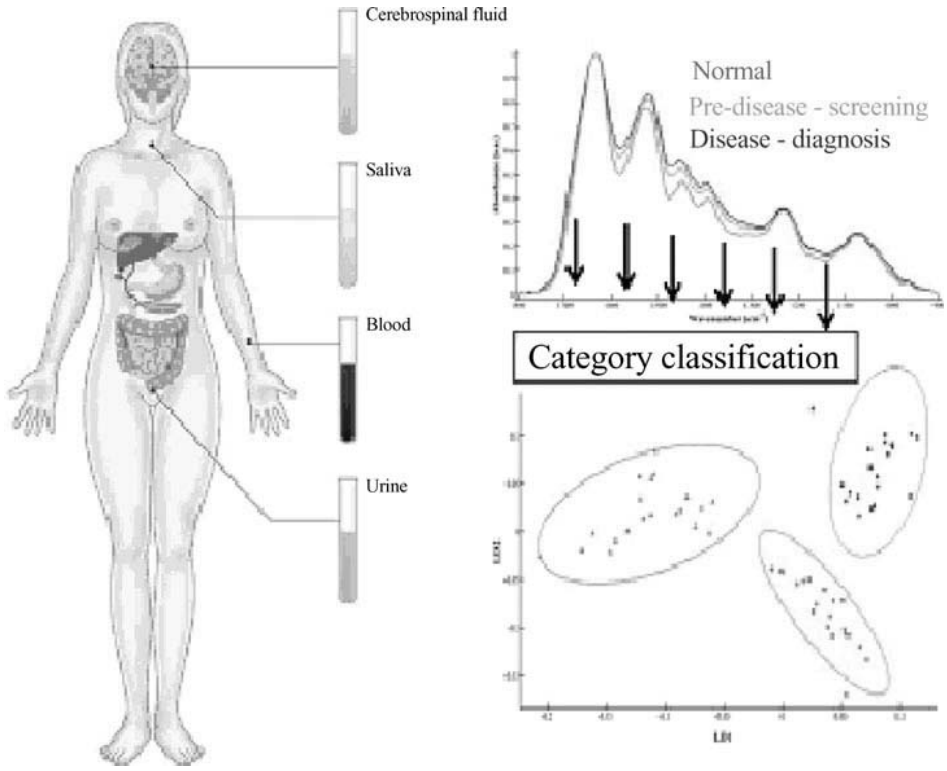

Fig. 5 – A variety of biofluids including blood, saliva, urine, and CSF are obtainable and applicable in a clinical setting. Other than CSF, these are relatively noninvasive. Secreted into these biofluids may be biomarkers of site-specific pathology reflecting either presymptomatic or emerging disease. Fingerprint spectra may diagnose the origin and grade of pathology based on a classification algorithm.

A wide range of biological studies have been covered by FTIR analysis. These studies include breast, cervix, lung, skin, gastrointestinal tissue, prostate, colon, cancer detection.

Using biofluids, specifically blood, urine, cerebrospinal fluid (CSF), or saliva (Fig. 5), is significantly less invasive as a screening tool.[33]

Breast cancer is the most frequent kind of cancer with women. This is the reason why a fast and early diagnosis is of enormous importance. The current diagnosis is more or less complicated and highly subjective. The most common methods for a screening are the mammography and ultrasound inspections. When there is a suspicion

of breast cancer, in most cases punching biopsies are taken from the patient. This is a time consuming method. The pathologist needs at least 0.5 h for a diagnosis. The findings are dependent on the subjective opinion of the corresponding doctor. This procedure features several limitations, including delays in providing the diagnostic results and being a subjective method, which has the potential for inter-observer disagreement.[6] To overcome these limitations new methods are needed, which allow a rapid, non-invasive and high-throughput diagnosis. Vibrational spectroscopic techniques, exhibit the potential to overcome these limitations and enable an additional way of diagnosing and staging of cancer.

Micro imaging Fourier transform infrared spectroscopy is able to monitor differentiation between normal and malignant tissues.[34] All the specimens, previously submitted to histological analysis, displayed abnormal spectra compared with the corresponding normal tissues with changes in many diagnostic bands like those arising from phosphate, C–O and CH stretching vibrational modes. These characteristic bands have been monitored as a function of the degree of cancer progression. Chemometric methods, such as principal component analysis (PCA) and hierarchical clustering analysis (HCA) have been used in order to distinguish spectra of neoplastic and normal zones.

A nondestructive method employing Fourier transform infrared (FTIR) microspectroscopy coupled with attenuated total reflectance (ATR) objective for the analysis of histopathological specimens was described.[35] Malignant breast tissue specimens have been analyzed to demonstrate the hypothesis that chemical changes taking place in biological tissue can be reliably and reproducibly identified. It was concluded that FTIR could objectively and reproducibly discriminate between DCIS (ductal carcinoma *in situ*) and IDC (invasive ductal carcinoma) grades without sample destruction. In the future, applications of FTIR approaches should become feasible in the nondestructive express classification of grades and diagnosis of breast carcinoma.

A simple and rapid method for the detection of breast cancer with IR-spectroscopy was developed.[36] The method needs only 1 μL of a serum sample. The serum sample is dried on a suitable sample carrier such as a Si-plate. Every disease leaves a typical fingerprint in the IR-spectrum of serum. To be sure that there is no any interference with other diseases the breast cancer patients tested against 11 other diseases separately. Breast cancer was assigned to 79% to the correct group. These results suggest that IR-spectroscopy in combination with intelligent mathematical evaluation tools such as ANN or cluster analysis is a good tool for the diagnosis of breast cancer.

In order to apply FTIR spectroscopy as a routine tool for biomedical diagnostics of tissue samples, strong and reliable classifiers are needed.[37] Frequently, the number of available tissue samples is restricted and due to that data sets consist of a small number of samples, often less than 100. This can result in unstable classifiers, which perform poorly on unseen data. To overcome this limitation several support vector machines (SVM) were used. As these results show, the application of SVM ensembles in biomedical diagnostics using FTIR spectroscopy can be highly beneficial.

Histopathology forms the gold standard for the diagnosis of breast cancer and FT-IR spectroscopic imaging has been proposed to be a potentially powerful adjunct to current histopathological techniques. Most studies using FT-IR imaging for breast tissue analysis have been in the transmission or transmission-reflection mode, in which the wavelength and optics limit the data to a relatively coarse spatial resolution (typically, coarser than 5 μm × 5 μm per pixel). This resolution is insufficient to examine many histologic structures. So attenuated total reflectance (ATR) FT-IR imaging incorporating a Germanium optic can allow for a four-fold increase in spatial resolution due to the material's high refractive index in the mid-IR.[38] The authors emploied ATR FT-IR imaging toward examining cellular and tissue structures that constitute an important component of breast cancer diagnosis. They reported the extraction of intact chromosomes from breast cancer cells and their spatially localized analysis as a novel approach to understand changes associated with the molecular structure of DNA in breast cancer.

Cervical cancer is a leading cause of mortality and morbidity after breast cancer; representing approximately 12% of all cancers in women worldwide. FTIR spectroscopy has been utilized as an emerging method applied to the study of the structural changes of cells at the molecular level in various human cancers. Over the past decades, there have been a number of studies done to investigate the possibility of the FTIR technique as a screening tool for cervical cancer.

Inspired from the great potential of the Fourier-transform infrared (FTIR) spectroscopy as a screening tool for cervical cancer, a paper proposed an intelligent classification of cervical pre-cancerous cells based on the FTIR spectra.[39] It consists of two parts: the extraction of FTIR characteristics and the intelligent classification of the pre-cancerous cells. Correlation test proves the capability of the proposed PCABFE (Peak-corrected area-based features' extraction) to be as effective as the manual extraction by human experts, while the HMLP (Hybrid Multilayered Perceptron) network produces a good classification performance with 97.4% of accuracy.

Cervical cancer screening programmes have greatly reduced the burden associated with this disease. However, conventional cervical cytology screening still lacks sensitivity and specificity. There is an urgent need for the development of a low-cost robust screening technique.[40] By generating a spectral "biochemical-cell fingerprint", Fourier-transform infrared (FTIR) spectroscopy has been touted as a tool capable of segregating grades of dysplasia. Following FTIR spectroscopy, derived spectra were examined for segregation between classes in scores plots generated with subsequent multivariate analysis. Deeper understanding of the underlying changes in the transition between cervical cytology classes (normal vs. low-grade vs. high-grade) is required in order to develop biospectroscopy tools as a screening approach. This will then allow for the development of blind classification algorithms.

Ovarian cancer is the sixth most common cancer among women worldwide, and mortality rates from this cancer are higher than for other gynecological cancers. The detection of neoplastic changes by optical spectroscopy techniques such as FTIR, Raman, and fluorescence spectroscopy, has been one of the most active areas of recent research into the discrimination of oral, cervical, breast and other cancers. These methods are more objective, less time-consuming, and have the ability to be applied in vivo. A review of the literature shows that there are very few reports of studies on ovarian cancer diagnosis by optical spectroscopy.[41, 42]

Infrared (IR) spectroscopy of blood plasma or serum is a rapid, versatile, and relatively non-invasive approach which could characterize biomolecular alterations due to cancer and has potential to be utilized as a screening or diagnostic tool.[43] Classification results for ovarian cancer were remarkable (up to 96.7%), This pilot study suggests that ATR-FTIR spectroscopy of blood is a robust tool for accurate diagnosis, and carries the potential to be utilized as a screening test for ovarian cancer in primary care settings. The proposed classification machine is a powerful tool which could be applied to classify the vibrational spectroscopy data of different biological systems (*e.g.*, tissue, urine, saliva), with their potential application in clinical practice.

In recent years, infrared (IR) spectroscopy has gained attention as a simple and inexpensive method for the biomedical study of several diseases. In their study, Mehrotra et co-workers[44] infrared spectra of normal and malignant ovarian tissues were recorded in the 650 cm^{-1} to 4000 cm^{-1} region. Post surgical tissue samples were taken from the normal and tumor sections of the tissue. Significant spectral differences between the normal and the ovarian cancerous tissues were observed. In particular changes in frequency and intensity in the spectral region of protein, nucleic acid and lipid vibrational modes were observed.

Gastric cancer is rampant in many countries around the world, it is the fourth most common cancer worldwide.[45] Almost two-thirds of the cases occur in developing countries and 42% in China alone. Survival for gastric cancer is moderately good only in Japan (52%), where mass screening by photofluoroscopy has been practiced since the 1960s. NIR spectroscopy, as a sensitive analytical technique with practical advantages, can record the response of chemical bonds in functional groups (*e.g.*, O–H, C–H, and N–H bands) to the NIR spectrum, which is related to the primary structural components of organic molecules. Therefore, any alteration in the composition of the tissues can be detected and used for diagnostic purposes.[46] In the present study, the authors investigated the qualitative NIR spectral differences between gastric cancer and normal tissues in surgically resected gastric specimens using FT-NIR equipped with a fiber-optic probe to mimic in situ clinical measurements. The spectra from cancerous and normal tissues were collected using Fourier transform near-infrared spectroscopy (FT-NIR) equipped with a fiber-optic probe. These present results indicate that CH-stretching first, combination band and second overtone regions can serve as diagnostic markers for gastric cancer.

Since serum can reflect human beings' physiological and pathological conditions, FTIR spectroscopy was used to compare gastric cancer

patients' serum with healthy persons' serum.[47] The H2959/H2931 ratio might be a standard for distinguishing gastric cancer patients from healthy persons. the result showed that the RNA/DNA ratios of gastric cancer patients' serum were obviously lower than those of healthy persons' serum. The results suggest that FTIR spectroscopy may be a potentially useful tool for diagnosis of gastric cancer.

Colorectal cancer is a major public health problem, being the third most common cancer and the fourth leading cause of cancer deaths worldwide. IR spectroscopy has demonstrated several advantages such as being a rapid procedure, requiring minimal sample preparation, working by automated algorithms, collecting spectral data within minutes, being compatible with interpretation techniques. Molecular vibrations in IR spectroscopy would probe the chemical composition and structural properties of tissue samples without external markers. Fourier transform infrared spectroscopy (FTIR) is known to be a powerful tool that provides the ability to study secondary conformation of intact protein in biological tissues for the diagnosis of disease states.

It is important to explore a noninvasive and rapid method for detection of colon cancer biopsies. Initially, principal component analysis was applied to examine the degree of separation between tissue samples.[48] This study tries to demonstrate that attenuated total reflectance-fourier transform infrared (ATR-FTIR) microspectroscopy in combination with chemometric methods can reliably distinguish malignant colon tissues from healthy ones. There were significant differences in the FTIR spectra of normal and cancerous colon biopsies in the 1800-900 cm^{-1} spectral region. The SIMCA results demonstrated that the accuracy, specificity, and sensitivity of the proposed diagnostic method were 93.3, 100, and 88.2%, respectively, which could help satisfy clinical diagnostic requirements.

The process of carcinogenesis in the colon progresses through several overlapping, stages, making the evaluation process challenging, as well as subjective.[49] Fourier-transform infrared spectroscopy, being quantitative and objective, has the capacity for automation and relevance to cancer diagnosis. These results highlight investigations on the application of Fourier-transform infrared spectroscopy (particularly microscopy) in colon cancer diagnosis and parallel developments in data analysis techniques for the characterization of spectral signatures of malignant tissues in the colon.

Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) microspectroscopy was applied for detection of colon cancer according to the spectral features of colon tissues.[50] Several chemometric methods such as analysis of variance (ANOVA), cluster analysis (CA) and linear discriminate analysis (LDA) were applied for classification of IR spectra. Utilizing the chemometric techniques, clear and reproducible differences were observed between the spectra of normal and cancer cases, suggesting that infrared microspectroscopy in conjunction with spectral data processing would be useful for diagnostic classification.

Lung cancer is one of the most prevalent malignant tumors worldwide and the leading cause of cancer death globally. It accounts for 13% (1.6 million) of the total cancer cases and 18% (1.4 million) of the deaths in 2008. Due to the increase of smokers and deterioration of environment, the incidence and mortality of lung cancer have increased gradually in recent years. At present three methods such as chest X-ray, CT and bronchoscope are widely used for clinical diagnosis of lung cancer. Although these methods can improve the ability to diagnose lung cancer, they are still less effective for detecting lung cancer at early stage.[51] So it is very urgent to develop an effective method for early detection of lung cancer. From the late 1980s till now, FTIR spectroscopy has been used to explore a number of diseases, including lung cancer research.

Survival time for lung cancer is poor with over 90% of patients dying within five years of diagnosis primarily due to detection at late stage. The main objective of some research projects was to evaluate Fourier transform infrared spectroscopy (FTIR) as a high throughput and cost effective method for identifying biochemical changes in sputum as biomarkers for detection of lung cancer.[52] A panel of 92 infrared wavenumbers had absorbances significantly different between cancer and normal sputum spectra and were associated with putative changes in protein, nucleic acid and glycogen levels in tumours. The results suggest that FTIR applied to sputum might have high sensitivity and specificity in diagnosing lung cancer with potential as a non-invasive, cost-effective and high-throughput method for screening.

Optical observation of lung cancer tissues using attenuated total reflectance-Fourier transform infrared microscope (ATR-FTIR) and confocal Raman microscope were also tried for cancer

diagnostic.[53] A total of six malignant tissues, seven tissues adjacent to cancer, and nine normal tissues from nine patients with known lung cancer were studied. High-quality spectra from human tissues were obtained only in a few seconds. The results revealed that some of the spectral characteristics varied significantly between normal and malignant tissues, that is, IR peak positions, Raman shift, and the spectral intensities.

Attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy could be used as a diagnostic tool for detecting and discriminating lung cancer.[54] A pilot study on 60 samples was performed to distinguish malignant and nonmalignant lung tissues with ATR-FTIR spectroscopy. Peak positions, intensities, and full width at half maximum of each absorbent band were measured, and the relative intensity ratios were calculated. The sensitivity and specificity of the discriminants were all 96.7% so ATR-FTIR spectroscopy can be considered a promising method for the detection of malignant lung tissue and could be proved useful in lung tumor surgery.

Prostate cancer is the most common cancer and second most common cause of cancer-related deaths of men; however, the diagnosis in individual patients can be problematic, which has led to inappropriate treatment. An interesting study was performed in order to establish a link between FTIR data and the local biopotential of prostate cancers using fixed and stained tissues.[55] The importance of the staging disease for directing therapy has been well documented; and this study suggests FTIR could be used to categorize tissues into those that are aggressive and have the potential to move beyond the prostate, and those that remain localized to the prostate. The authors found that biochemical changes associated with prostate cancer could be discriminated by FTIR to classify confined and locally invasive prostate cancers. These findings could enable the development of improved diagnostic and prognostic methods for the detection and treatment of prostate cancers.

Cancer is certainly one of the major civilization diseases since the last century and nowadays among the different cancer pathologies, the prostate cancer is one of the most common neoplasm man pathologies.[56] FTIR spectra of tissues samples in different conditions: healthy, hyperplastic and cancerous stages, reveal differences that address the occurrence of chemical compositions changes in the examined samples. In the case of prostate tissue sections the results show the possibility to determine the intensity ratio of the CH_2 and CH_3 bands set at 2930 cm^{-1} and 2960 cm^{-1}, respectively.

Morphological and histomorphological evaluation of this disease is a well established technique for the cancer classification and has remained relatively unchanged since several decades, although it remains a time consuming and subjective technique, with unsatisfactory levels of inter-and intra-observer discrepancy. Novel approaches for histological recognition are necessary to identify and to investigate cancer in detail.[57] Fourier transform infrared (FTIR) spectroscopic imaging has become an essential tool for the detection, identification and characterization of the molecular components of biological processes, such as those responsible for the dynamic properties of cancer progression. Major advantage of this new technique is the acquisition of local molecular expression profiles while maintaining the topographic integrity of the tissue and avoiding time-consuming extraction, purification and separation steps. By using this method it is possible to investigate the spatial distribution of proteins, lipids, carbohydrates, cholesterols, nucleic acids, phospholipids and small molecules within biological systems by in situ analysis of tissue sections. With this method it is possible to distinguish between cancer and noncancer areas within a prostate cancer tissue with a resolution of 6.25 μm x 6.25 μm on frozen sections.

CONCLUSIONS

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

expertise on sample preparation, as well as on cytology, histology, and pathology. In contrast with these traditional techniques, IR spectroscopy holds promise as a rapid, label-free analytical route, which is potentially labor and time-saving, and requires a minimum amount of training, in particular if data processing and mining is an integrated component of a diagnostic system. As a conclusion we believe that noninvasive, rapid, accurate, and convenient analysis of tissues can be performed with Fourier-transform infrared spectroscopy if the infrared fiber optics and endoscopy technologies can be combined successfully. IR-spectroscopy opens the chance for a rapid and simple diagnostics which is nearly independent of the operator. Serum, plasma or urine served as sample matrix. Body fluids are routinely analysed by clinical chemists to obtain information on pathological processes.

REFERENCES

1. D. I. Ellis and R. Goodacre, *Analyst*, **2006**, *131*, 875-885.
2. C. Petibois and G. Deleris, *Trends in Biotechnol.*, **2006**, *24*, 455-462.
3. J. Wang, M. Sowa, H. H. Mantsch, A. Bittner and H. M. Heise, *TrAC Trends Anal. Chem.*, **1996**, *15*, 286-296.
4. R. N. A. H. Lewis and R. N. Mcelhaney, in H. H. Mantsch and D. Chapman (Eds.), "Infrared Spectroscopy of Biomolecules", Wiley-Liss, NewYork, USA, 1996, p. 1597.
5. L. G. Luna, "Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology", New York, McGraw-Hill, 1968.
6. C. Kendall, M. Isabelle, F. Bazant-Hegemark, J. Hutchings, L. Orr, J. Babrah, R. Baker and N. Stone, *Analyst*, **2009**, *134*, 1029-1045.
7. S. Mordechai, R. K. Sahu, Z. Hammody, S. Mark, K. Kantarovich, H. Guterman, A. Podshyvalov, J. Goldstein and S. Argov, *J. Microsc.*, **2004**, *215*, 86-91.
8. F. L. Martin, J. G. Kelly, V. Llabjani, P. L. Martin-Hirsch, I. I. Patel, J. Trevisan, N. J. Fullwood, and M. J. Walsh, *Nat. Protoc.*, **2010**, *5*, 1748-1760.
9. M. Khanmohammadi and A. B. Garmarudi, *TrAC Trends in Anal. Chem.*, **2011**, *30*, 864-874.
10. J. M. Bishop , *Science*, **1987**, *235*, 305-311.
11. D. Sidransky, *Int. J. Cancer*, **1995**, *64*, 1-2.
12. A. A. Bunaciu, H. Y. Aboul-Enein and S. Fleschin, *Applied Spectroscopy Review*, **2010**, *45*, 206-219.
13. R. A. Shaw and H. H. Mantsch, *J. Molec. Structure*, **1999**, *480-481*, 1-13.
14. A. A. Bunaciu, S. Fleschin and H. Y. Aboul-Enein, *Critical Reviews in Analytical Chemistry*, **2014**, *44*, 270-276.
15. X. D. Zhang, J. F. Li , Q. Q. Zhao, L. Zhang and C. Pan, *Laser Infrared*, **2008**, *38*, 267-269.
16. R. A. Shaw and H. H. Mantsch , "Infrared Spectroscopy in Clinical and Diagnostic Analysis" in "Encyclopedia of Analytical Chemistry", Robert A. Meyers (Ed.) Ó John Wiley & Sons Ltd, Chichester, 2010, p. 1-20.
17. M. Diem, S. Boydston-White and L. Chiriboga, *Applied Spectroscopy*, **1999**, *53*, 148A-161A.
18. L. M. Miller, G. D.Smith and G. L. Carr, *J. Biol. Phy.*, **2003**, *29*, 219-230.
19. D. Naumann, *Appl. Spectrosc. Rev.*, **2001**, *36*, 239-298.
20. F. L. Martin and H. M. Pollock, "Microspectroscopy as a tool to discriminate nano-molecular cellular alterations in biomedical research", in "Oxford Handbook of Nanoscience and Technology", A. V. Narlikar and Y. Y. Fu (Eds.), Oxford University Press, Oxford, UK, 2010, vol. 2, p. 285-336.
21. J. G. Kelly, J. Trevisan, A. D. Scott, P. L. Carmichael, H. M. Pollock, P. L. Martin-Hirsch and F. L. Martin, *J. Proteome Res.*, **2011**, *10*, 1437-1448.
22. H. Shin and M. K. Markey, *J. Biomed. Inf.*, **2006**, *39*, 227-248.
23. E. Diessel, S. Willmann, P. Kamphaus, R. Kurte, U. Damm and H. M. Heise, *Appl. Spectrosc*. **2004**, *58*, 442-450.
24. D. Fernandez, R. Bhargava, S. M. Hewitt and I. W. Levin, *Nat. Biotechnol.*, **2005**, *23*, 469-474.
25. L. Chin, J. N. Andersen and P. A. Futreal, *Nat. Med.*, **2011**, *17*, 297-303.
26. K. Das, C. Kendall, I. Martin, C. Fowler, J. Christie-Brown and N. Stone, *J. Photochem Photobiol B*, **2008**, *92*, 160-164.
27. L. Fass , *Molecular Oncology*, **2008**, *2*, 115-152.
28. Z. Movasaghi, R. Shazza and I. Rehman, *Applied Spectroscopy Reviews*, **2008**, *43*, 134-179.
29. L. Büttner Mostaço-Guidolin, L. Sayuri Murakami, A. Nomizo and L. Bachmann, *Applied Spectroscopy Reviews*, **2009**, *44*, 438-455.
30. R. Madsen, T. Lundstedt and J. Trygg, *Anal. Chim. Acta*, **2010**, *659*, 23-33.
31. G. Bellisola and C. Sorio, *Am. J. Cancer Res.*, **2012**, *2*, 1-21.
32. A. L. Mitchell, K. B. Gajjar, G. Theophilou, F. L. Martin, and P. L. Martin-Hirsch, *J. Biophoton.*, **2014**, *7*, 153-165.
33. E. Mfoumou, N. Sivakumar, A. Yasmeen, A. Al Moustafa and I. Stiharu, *Medical Hypotheses*, **2012**, *79*, 171-173.
34. J. Anastassopoulou, E. Boukaki, C. Conti, P. Ferraris, P. E. Giorgini, C. Rubini, S. Sabbatini, T. Theophanides and G. Tosi, *Vibrational Spectroscopy*, **2009**, *51*, 270-275.
35. S. Rehman, Z. Movasaghi, J. A. Darr and I. U. Rehman, *Applied Spectroscopy Reviews*, **2010**, *45*, 355-368.
36. J. Backhaus, R. Mueller, N. Formanski, N. Szlama, H. G. Meerpohl, M. Eidt and P. Bugert, *Vibrational Spectroscopy*, **2010**, *52*, 173-177.
37. M. Sattlecker, R. Baker, N. Stone and C. Bessant, *Chemometrics and Intelligent Laboratory Systems*, **2011**, *107*, 363-370.
38. M. J. Walsh, S. E. Holton, A. Kajdacsy-Balla and R. Bhargava, *Vibrational Spectroscopy*, **2012**, *60*, 23-28.
39. Y. Jusman, N. A. Mat Isa, R. Adnan and N. H. Othman, *Ain Shams Engineering Journal*, **2012**, *3*, 61-70.
40. N. C. Purandare, I. I. Patel, J. Trevisan, N. Bolger, R. Kelehan, G. von Buenau, P. L. Martin-Hirsch, W. J. Prendiville and F. L. Martin, *Analyst*, **2013**, *138*, 3909-3916.
41. M. Brewer, U. Utzinger, E. Silva, D. Gershenson, R. C. Bast Jr., M. Follen and R. Richards-Kortum, *Laser Surg. Med.*, **2001**, 29, 128-135.
42. E. F. Petricoin, A. M. Ardekani, B. A. Hitt, P. J. Levine, V. A. Fusaro, S. M. Steinberg, G. B. Mills, C. Simone, D.

A. Fishaman, E. C. Kohn and L. A. Loitta, *Lancet*, **2002**, *59*, 572-577.
43. K. Gajjar, J. Trevisan, G. Owens, P. J. Keating, N. J. Wood, H. F. Stringfellow, P. L. Martin-Hirsch and F. L. Martin, *Analyst*, **2013**, *138*, 3917-3926.
44. R. Mehrotra, G. Tyagi, D. K. Jangir, R. Dawar R. and N. Gupta N., *J.Ovarian Research*, **2010**, 3, 27.
45. F. Kamangar, G. Dores and W. J. Anderson, *Clin. Oncol.*, **2006**, *24*, 2137-2150.
46. W. S. Yi, D. S. Cui, Z. Li, L. L. Wud, A. G. Shen and J. M. Hu, *Spectrochim. Acta Part A: Molecular and Biomolecular Spectroscopy*, **2013**, *101*, 127-131.
47. D. Sheng, Y. Wu, X. Wang, D. Huang, X. Chen and X. Liu, *Spectrochim. Acta Part A: Molecular & Biomolecular Spectroscopy*, **2013**, *116*, 365-369.
48. M. Khanmohammadi, A. B. Garmarudi, K. Ghasemi, H. K. Jaliseh and A. Kaviani, *Medical Oncology*, **2009**, *26*, 292-297.
49. R. K. Sahu and S. Mordechai, *Future Oncology*, **2010**, *6*, 1653-1667.
50. M. Khanmohammadi, A. B. Garmarudi, S. Samani, K. Ghasemi and A. Ashuri, *Pathology & Oncology Research*, **2011**, *17*, 435-441.
51. G. Sutedja, *Eur. Respir. J.*, **2003**, *21: Suppl.*, 57s-66s.
52. P. D. Lewis, K. E. Lewis., R. Ghosal, S. Bayliss, A. J. Lloyd, J. Wills, R. Godfrey, P. Kloer and L. A. J. Mur, *BMC Cancer*, **2010**, *10*, Article Number: 640.
53. J. Lv, L. Zhang, J. Feng, Y. Liu, Z. Wang, M. Zhao and R. Shi R., *Spectroscopy Letters*, **2011**, *44*, 312-317.
54. X. Sun , Y. Xu, J. Wu, Y. Zhang and K. Sun, *J. Surgical Research*, **2013**, *179*, 33-38.
55. M. A. Mackanos and C. H. Contag, *Trends in Biotechnology*, **2009**, *27*, 661-663.
56. W. M. Kwiatek, C. Paluszkiewicz, A. Banas, A. Kisiel, M. Podgorczyk, A. Marcelli, M. C. Guidi and M. Piccinini, *Acta Physica Polonica A*, **2009**, *115*, 602-605.
57. C. Pezzei, J. D. Pallua, G. Schaefer, C. Seifarth, V. Huck-Pezzei, L. K. Bittner, H. Klocker, G. Bartsch, G. K. Bonn and C. W. Huck, *Molecular Biosystems*, **2010**, *6*, 2287-2295.