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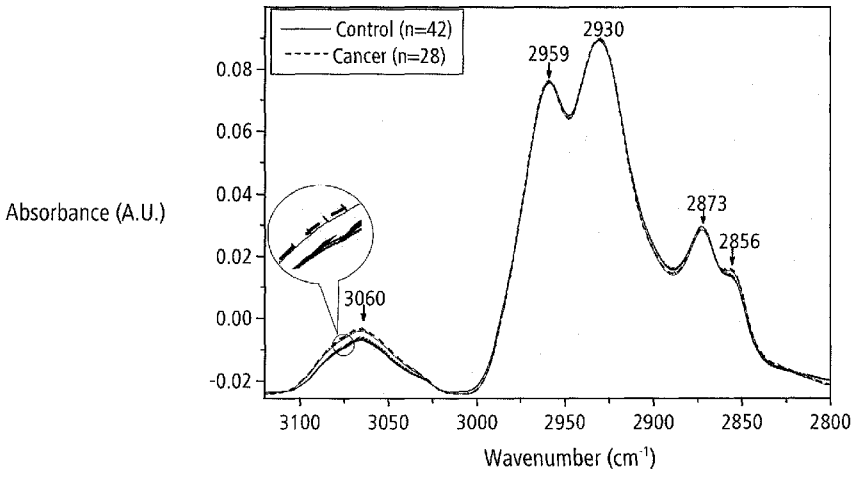
(54) **Title**: DIAGNOSIS OF CANCER

FIG. 1A



(57) **Abstract**: A method is provided including obtaining an infrared (IR) spectrum of a blood plasma sample by analyzing the blood plasma sample by infrared spectroscopy, and based on the infrared spectrum, generating an output indicative of the presence of a solid tumor or a pre-malignant condition. Other applications are also described.

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DIAGNOSIS OF CANCER

CROSS-REFERENCES TO RELATED APPLICATIONS

The present application claims the priority of US Provisional Patent Application 61/484,753 to Kapelushnik et al., entitled, "Diagnosis of cancer," filed May 11, 2011, which is incorporated herein by reference.

FIELD OF EMBODIMENTS OF THE INVENTION

Embodiments of the present invention relate generally to diagnosis and monitoring of a disease, and particularly to methods for diagnosis and monitoring of a malignant disease.

BACKGROUND

Analysis of certain markers (e.g., certain proteins, peptides, RNA molecules) in a patient's circulation may be useful in detection and/or monitoring of cancer. For example, studies have shown that analysis of a patient's blood plasma for certain oncofetal antigens, enzymes and/or miRNA molecules may assist in diagnosis and prognosis of certain types of cancer.

Fourier Transform Infrared (FTIR) spectroscopy is typically used to identify biochemical compounds and examine the biochemical composition of a biological sample. Typically, FTIR spectra are composed of several absorption bands, each corresponding to specific functional groups related to cellular components such as lipids, proteins, carbohydrates and nucleic acids.

FTIR spectroscopy is used for analysis of various compounds in blood plasma such as total proteins, creatinine, amino acids, fatty acids, albumin, glucose, fibrinogen, lactate, triglycerides, glycerol, urea, triglycerides, cholesterol, apolipoprotein and immunoglobulin.

Additionally, FTIR spectroscopy is commonly used to distinguish between normal and abnormal tissue by analyzing the changes in absorption bands of macromolecules such as lipids, proteins, carbohydrates and nucleic acids. Additionally, FTIR spectroscopy may be utilized for evaluation of cell death mode, cell cycle progression, and the degree of maturation of hematopoietic cells.

SUMMARY OF EMBODIMENTS OF THE INVENTION

In some applications of the present invention, a method and system are provided for the diagnosis and monitoring of multiple types of malignant neoplasms, particularly malignant solid tumors.

Additionally or alternatively, some applications of the present invention comprise diagnosis and monitoring of a pre-malignant condition.

Typically, the method comprises analysis of blood plasma samples from cancer patients by techniques of infrared (IR) spectroscopy, for example, FTIR spectroscopy and/or microspectroscopy. As provided by applications of the present invention, FTIR Optical Diagnosis Technology (FODT) allows analysis of biochemical changes in a blood plasma sample of a patient which can indicate the presence of a solid tumor. Processes such as carcinogenesis may trigger changes in a biochemical composition of a body fluid of a patient, e.g., blood plasma. These changes are typically represented by differences in the absorption and reflection spectra when analyzed by FTIR spectroscopy techniques and compared to plasma samples from control individuals who do not suffer from a malignant solid tumor, e.g., healthy controls.

Accordingly, biochemical analysis of blood plasma samples obtained from untreated cancer patients and control individuals who do not suffer from a malignant solid tumor is conducted by FTIR microspectroscopy (FTIR MSP) techniques. Subsequently, the FTIR spectra (absorption and/or reflection) of blood plasma samples of the cancer patients are compared to the FTIR spectra of blood plasma samples obtained from the controls.

The inventors have identified that the blood plasma samples obtained from cancer patients suffering from a malignant solid tumor produce FTIR spectra that differ from those of the control individuals who do not suffer from a malignant solid tumor, allowing distinguishing between the cancer patients and controls. Thus, some applications of the present invention can be used to diagnose cancer patients suffering from various types of malignancies, particularly solid tumors. The distinction by FTIR spectroscopy between controls and patients suffering from solid tumors is typically performed based on analysis of blood plasma samples and not of the actual tumor cells.

For some applications, a data processor is configured to analyze the IR spectrum, e.g., the FTIR spectrum, of the blood plasma sample of a subject and an output unit is configured to generate an output indicative of the presence of a solid tumor, based on the infrared (IR) spectrum. Additionally, the data processor is typically configured to calculate a second derivative of the infrared (IR) spectrum of the blood plasma sample and, based on the second derivative of the infrared (IR) spectrum, to generate an output indicative of the presence of a solid tumor.

Additionally, some applications of the present invention allow distinguishing between various types of solid tumors. For example, blood plasma samples obtained from a cancer patient suffering from a certain type of solid tumor produces an FTIR spectrum having a unique spectral pattern which is characteristic of the type of malignancy and distinct from spectra of other malignancy types.

For some applications, analysis by IR spectroscopy, e.g., FTIR spectroscopy of the biochemistry of blood plasma samples can be used for the screening of large populations, aiding in the early detection of cancer, including solid tumors. Infrared (IR) spectroscopy and microspectroscopy techniques (e.g., FTIR spectroscopy or FTIR Microspectroscopy) as described herein are typically simple, reagent-free and rapid methods, suitable for use as screening tests for large populations. Early detection of cancer generally enables early intervention and treatment, contributing to a reduced mortality rate.

There is therefore provided in accordance with some applications of the present invention a method including:

obtaining an infrared (IR) spectrum of a blood plasma sample by analyzing the blood plasma sample by infrared spectroscopy; and

based on the infrared spectrum, generating an output indicative of the presence of a solid tumor or a pre-malignant condition.

For some applications, generating the output includes generating the output indicative of the presence of the solid tumor.

For some applications, analyzing the blood plasma sample by infrared (IR) spectroscopy includes analyzing the blood plasma sample by Fourier Transformed Infrared (FTIR) spectroscopy, and obtaining the infrared (IR) spectrum includes obtaining a Fourier Transformed Infrared (FTIR) spectrum.

For some applications, analyzing the blood plasma sample by infrared (IR) spectroscopy includes analyzing the blood plasma sample by Fourier Transformed Infrared microspectroscopy (FTIR-MSP).

For some applications, analyzing includes assessing a characteristic of the blood plasma sample at at least one wavenumber selected from the group consisting of: 759 ± 4 cm-1, 987 ± 4 cm-1, 1172 ± 4 cm-1, 1270 ± 4 cm-1, 1283 ± 4 cm-1, and 1393 ± 4 cm-1.

For some applications, analyzing includes assessing the characteristic at at least two wavenumbers selected from the group.

For some applications, analyzing includes assessing the characteristic at at least three wavenumbers selected from the group.

For some applications, analyzing includes assessing a characteristic of the blood plasma sample at at least one wavenumber selected from the group consisting of: 743 ± 4 cm-1, 793 ± 4 cm-1, 808 ± 4 cm-1, 847 ± 4 cm-1, 850 ± 4 cm-1, 895 ± 4 cm-1, 950 ± 4 cm-1, 961 ± 4 cm-1, 963 ± 4, 967 ± 4 cm-1, 975 ± 4 cm-1, 997 ± 4 cm-1, 1008 ± 4 cm-1, 1030 ± 4 cm-1, 1031 ± 4 cm-1, 1048 ± 4 cm-1, 1120 ± 4 cm-1, 1150 ± 4 cm-1, 1159 ± 4 cm-1, 1188 ± 4 cm-1, 1205 ± 4 cm-1, 1220 ± 4 cm-1, 1221 ± 4 cm-1, 1255 ± 4 cm-1, 1322 ± 4 cm-1, 1326 ± 4 cm-1, 1341 ± 4 cm-1, 1356 ± 4 cm-1 1370 ± 4 cm-1, 1372 ± 4 cm-1, 1402 ± 4 cm-1, 1415 ± 4 cm-1, and 1555 ± 4 cm-1, 1595 ± 4 cm-1, 1653 ± 4 cm-1, 1681 ± 4 cm-1.

For some applications, analyzing includes assessing the characteristic at at least two wavenumbers selected from the group.

For some applications, analyzing includes assessing the characteristic at at least three wavenumbers selected from the group.

For some applications, assessing the characteristic includes analyzing a band of the IR spectrum at at least one wavenumber selected from the group.

For some applications, the solid tumor includes a solid tumor in an organ selected from the group consisting of: lung, pancreas, prostate, bladder, and gastrointestinal tract, and generating the output includes generating an output indicative of the presence of a solid tumor in an organ selected from the group.

For some applications, the solid tumor includes breast cancer, and generating the output includes generating an output indicative of the presence of breast cancer.

For some applications, analyzing the blood plasma sample includes obtaining a second derivative of the infrared (IR) spectrum of the blood plasma sample.

For some applications, the blood plasma sample includes a dried blood plasma sample and analyzing the blood plasma sample includes analyzing the dried blood plasma sample.

For some applications, the infrared (IR) spectrum includes an absorption spectrum and obtaining the infrared (IR) spectrum includes obtaining the absorption spectrum.

For some applications, the infrared (IR) spectrum includes a reflection spectrum and obtaining the infrared (IR) spectrum includes obtaining the reflection spectrum.

There is further provided in accordance with some applications of the present invention a method for monitoring the effect of an anti-cancer treatment on a subject undergoing anti-cancer treatment for a solid tumor, for use with a first blood plasma sample separated from blood of the subject that was obtained prior to initiation of the treatment and a second blood plasma sample separated from blood of the subject that was obtained after initiation of the treatment, the method including:

obtaining IR spectra of the first and second blood plasma samples by analyzing the first and second blood plasma samples by IR spectroscopy; and

based on the IR spectra, generating an output indicative of the effect of the treatment.

For some applications, analyzing the first and second blood plasma samples by IR spectroscopy includes analyzing the samples by Fourier Transformed Infrared spectroscopy, and obtaining the IR spectra includes obtaining Fourier Transformed Infrared (FTIR) spectra.

For some applications, analyzing the first and second blood plasma samples by infrared (IR) spectroscopy includes analyzing the first and second blood plasma samples by Fourier Transformed Infrared microspectroscopy (FTIR-MSP).

For some applications the method includes, obtaining an IR spectrum of a third blood plasma sample separated from blood of the subject that was obtained following

termination of the treatment, by analyzing the third blood plasma sample by IR spectroscopy.

For some applications, analyzing includes assessing a characteristic of the blood plasma sample at at least one wavenumber selected from the group consisting of: 759 ± 4 cm-1, 987 ± 4 cm-1, 1172 ± 4 cm-1, 1270 ± 4 cm-1, 1283 ± 4 cm-1, and 1393 ± 4 cm-1.

For some applications, analyzing includes assessing the characteristic at at least two wavenumbers selected from the group.

For some applications, analyzing includes assessing the characteristic at at least three wavenumbers selected from the group.

For some applications, analyzing includes assessing a characteristic of the blood plasma sample at at least one wavenumber selected from the group consisting of: 743 ± 4 cm-1, 793 ± 4 cm-1, 808 ± 4 cm-1, 847 ± 4 cm-1, 850 ± 4 cm-1, 895 ± 4 cm-1, 950 ± 4 cm-1, 961 ± 4 cm-1, 963 ± 4, 967 ± 4 cm-1, 975 ± 4 cm-1, 997 ± 4 cm-1, 1008 ± 4 cm-1, 1030 ± 4 cm-1, 1031 ± 4 cm-1, 1048 ± 4 cm-1, 1120 ± 4 cm-1, 1150 ± 4 cm-1, 1159 ± 4 cm-1, 1188 ± 4 cm-1, 1205 ± 4 cm-1, 1220 ± 4 cm-1, 1221 ± 4 cm-1, 1255 ± 4 cm-1, 1322 ± 4 cm-1, 1326 ± 4 cm-1, 1341 ± 4 cm-1, 1356 ± 4 cm-1 1370 ± 4 cm-1, 1372 ± 4 cm-1, 1402 ± 4 cm-1, 1415 ± 4 cm-1, and 1555 ± 4 cm-1, 1595 ± 4 cm-1, 1653 ± 4 cm-1, 1681 ± 4 cm-1.

For some applications, analyzing includes assessing the characteristic at at least two wavenumbers selected from the group.

For some applications, analyzing includes assessing the characteristic at at least three wavenumbers selected from the group.

There is additionally provided in accordance with some applications of the present invention a method including:

obtaining an infrared (IR) spectrum of a blood plasma sample by analyzing the blood plasma sample; and

based on the infrared spectrum, generating an output indicative of the presence of a solid tumor or a pre-malignant condition.

For some applications, generating the output includes generating the output indicative of the presence of the solid tumor.

There is yet additionally provided in accordance with some applications of the present invention, a system for diagnosing a solid tumor, including:

a data processor, configured to analyze an infrared (IR) spectrum of a blood plasma sample of a subject; and

an output unit, configured to generate an output indicative of the presence of a solid tumor, based on the infrared (IR) spectrum.

For some applications, the data processor is configured to calculate a second derivative of the infrared (IR) spectrum of the blood plasma sample and, based on the second derivative of the infrared (IR) spectrum, to generate an output indicative of the presence of a solid tumor.

For some applications, the IR spectrum includes a Fourier Transformed Infrared (FTIR) spectrum, and the data processor is configured to calculate a second derivative of the FTIR spectrum.

For some applications, the data processor is configured to analyze the infrared (IR) spectrum by assessing a characteristic of the blood plasma sample at at least one wavenumber selected from the group consisting of: 759 ± 4 cm-1, 987 ± 4 cm-1, 1172 ± 4 cm-1, 1270 ± 4 cm-1, 1283 ± 4 cm-1, and 1393 ± 4 cm-1.

For some applications, the data processor is configured to analyze the infrared (IR) spectrum by assessing the characteristic at at least two wavenumbers selected from the group.

For some applications, the data processor is configured to analyze the infrared (IR) spectrum by assessing the characteristic at at least three wavenumbers selected from the group.

For some applications, the data processor is configured to analyze the infrared (IR) spectrum by assessing a characteristic of the blood plasma sample at at least one wavenumber selected from the group consisting of: 743 ± 4 cm-1, 793 ± 4 cm-1, 808 ± 4 cm-1, 847 ± 4 cm-1, 850 ± 4 cm-1, 895 ± 4 cm-1, 950 ± 4 cm-1, 961 ± 4 cm-1, 963 ± 4, 967 ± 4 cm-1, 975 ± 4 cm-1, 997 ± 4 cm-1, 1008 ± 4 cm-1, 1030 ± 4 cm-1, 1031 ± 4 cm-1, 1048 ± 4 cm-1, 1120 ± 4 cm-1, 1150 ± 4 cm-1, 1159 ± 4 cm-1, 1188 ± 4 cm-1, 1205 ± 4 cm-1, 1220 ± 4 cm-1, 1221 ± 4 cm-1, 1255 ± 4 cm-1, 1322 ± 4 cm-1, 1326 ± 4 cm-1,

1341 ± 4 cm-1, 1356 ± 4 cm-1 1370 ± 4 cm-1, 1372 ± 4 cm-1, 1402 ± 4 cm-1, 1415 ± 4 cm-1, and 1555 ± 4 cm-1, 1595 ± 4 cm-1, 1653 ± 4 cm-1, 1681 ± 4 cm-1.

For some applications, the data processor is configured to analyze the infrared (IR) spectrum by assessing the characteristic at at least two wavenumbers selected from the group.

For some applications, the data processor is configured to analyze the infrared (IR) spectrum by assessing the characteristic at at least three wavenumbers selected from the group.

There is still additionally provided in accordance with some applications of the present invention, a computer program product for administering processing of a body of data, the product including a computer-readable medium having program instructions embodied therein, which instructions, when read by a computer, cause the computer to:

obtain an infrared (IR) spectrum of a blood plasma sample by analyzing the blood plasma sample by infrared spectroscopy; and

based on the infrared spectrum, generate an output indicative of the presence of a solid tumor.

The present invention will be more fully understood from the following detailed description of embodiments thereof, taken together with the drawings, in which:

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A-F are graphs representing FTIR absorption spectra and the second derivative of absorption spectra and analysis thereof, based on blood plasma from several cancer patients and controls, derived in accordance with some applications of the present invention; and

Fig. 2 is a graph representing values of the second derivative of absorption spectra of blood plasma samples from cancer patients compared to blood plasma samples from healthy controls, derived in accordance with some applications of the present invention.

DETAILED DESCRIPTION OF THE EMBODIMENTS

Some applications of the present invention comprise diagnosis of a solid tumor by FTIR microspectroscopy (MSP) techniques. For some applications, FTIR Optical Diagnosis Technology (FODT) is used to diagnose a solid tumor based on biochemical

properties of a blood plasma sample of a subject. Some applications of the present invention comprise obtaining a blood sample from a subject and analyzing plasma from the sample by FTIR-MSP techniques for the detection of a malignancy, specifically a solid tumor. Typically, blood plasma of a patient suffering from a solid tumor is identified as exhibiting FTIR spectra that are different from FTIR spectra produced by blood plasma from a subject who does not suffer from a solid tumor (for some applications the control group may include subjects suffering from a pathology that is not a solid tumor). Accordingly, some applications of the present invention provide a useful method for the detection of cancer, specifically solid tumors. FTIR spectra of a blood plasma sample obtained from a cancer patient with a solid tumor generally reflect biochemical changes which occur in the blood plasma of the patient in response to the tumor.

For some applications, methods of the present invention are used to diagnose a type of solid tumor and/or a stage of the cancer.

For some applications, methods of the present invention can be used to provide monitoring and follow up of cancer patients during and after treatment, e.g., chemotherapy treatment. Typically, changes in FTIR spectra of blood plasma samples of solid-tumor patients who are undergoing treatment can indicate biochemical changes in response to the treatment. This biochemical information can provide insight into the effect of treatment on the patient and/or the tumor.

METHODS USED IN SOME EMBODIMENTS OF THE PRESENT INVENTION

A series of protocols are described hereinbelow which may be used separately or in combination, as appropriate, in accordance with applications of the present invention. It is to be appreciated that numerical values are provided by way of illustration and not limitation. Typically, but not necessarily, each value shown is an example selected from a range of values that is within 20% of the value shown. Similarly, although certain steps are described with a high level of specificity, a person of ordinary skill in the art will appreciate that other steps may be performed, *mutatis mutandis*.

In accordance with some applications of the present invention, the following methods were applied:

Obtaining patient and control populations

All studies were approved by the local Ethics Committee of the Soroka University Medical Center and conducted in accordance with the Declaration of Helsinki. Qualified personnel obtained informed consent from each individual participating in this study.

The patient population included 28 cancer patients diagnosed with the following primary tumors:

Breast (n=13), lung (n=4), pancreas (n=1), prostate (n=1) bladder (n=1), and gastrointestinal (n=7) and unknown origin (n=1) cancers.

The diagnosis of cancer was determined by clinical, surgical, histological, and pathologic diagnosis. The pathologic stage of the tumor was determined according to tumor–node–metastasis (TNM) classification, as described in "TNM Classification of Malignant Tumours", by Sobin LH. et al., 7th Edition, New York: John Wiley, 2009. A control group (n=42) included healthy volunteers who underwent detailed clinical questioning, at the Soroka University Medical Center and Ben-Gurion University.

Collection of blood samples

1-2 ml of peripheral blood was collected in 5 ml EDTA blood collection tubes (BD Vacutainer® Tubes, BD Vacutainer, Toronto) from patients and healthy controls using standard phlebotomy procedures. Samples were processed within 2 hours of collection. It is to be noted that any other suitable anticoagulant may be used in collection and processing of the blood samples.

Isolation of plasma from peripheral blood samples

Blood from cancer patients and healthy controls was diluted 1:1 in isotonic saline (0.9% NaCl solution). The diluted blood was applied carefully to Histopaque 1077 gradients (Sigma Chemical Co., St. Louis, Missouri, USA) in 15 ml collection tubes, and centrifuged at 400 g for 30 min.

To discard platelets and cell debris, the plasma was transferred to 1.5 ml eppendorf tubes and centrifuged at 3,200 g for 10 min. The supernatant was transferred to a new eppendorf tube, and 0.5 µl of plasma was deposited on a zinc selenide (ZnSe) slide. It is noted that any other suitable slide may be used, e.g., reflection measurements may be

carried out using a gold slide. The slide was air dried for 1 hour under laminar flow to remove water. The dried plasma was then subjected to FTIR microscopy.

FTIR-Microspectroscopy

Fourier Transform Infrared Microspectroscopy (FTIR-MSP) and Data Acquisition Measurements were performed using the FTIR microscope Nicolet Centaurus with a liquid-nitrogen-cooled mercury-cadmium-telluride (MCT) detector, coupled to the FTIR spectrometer Nicolet iS10, OMNIC software (Nicolet, Madison, WI) using OPUS software (Bruker Optik GmbH, Ettlingen, Germany). To achieve high signal-to-noise ratio (SNR), 128 coadded scans were collected in each measurement in the wavenumber region 700 to 4000 cm−1. The measurement site was circular, with a diameter of 100 μm and spectral resolution of 4 cm−1 (0.482 cm−1 data spacing). To reduce plasma sample thickness variation and achieve proper comparison between different samples, the following procedures were adopted:

1. Each sample was measured at least five times at different spots.

2. Analog to Digital Converter (ADC) rates were empirically chosen between 2000 to 3000 counts / sec (providing measurement areas with similar material density).

3. The obtained spectra were baseline corrected using the rubber band method, with 64 consecutive points, and normalized using vector normalization in OPUS software as described in an article entitled "Early spectral changes of cellular malignant transformation using Fourier transformation infrared microspectroscopy", by Bogomolny et al., 2007. J Biomed Opt.12:024003.

In order to obtain precise absorption values at a given wavenumber with minimal background interference, the second derivative spectra were used to determine concentrations of bio-molecules of interest. This method is susceptible to changes in FWHM (full width at half maximum) of the IR bands. However, in the case of biological samples, all samples (plasma) from the same type are composed of similar basic components, which give relatively broad bands. Thus, it is possible to generally neglect the changes in band FWHM, as described in an article entitled "Selenium alters the lipid content and protein profile of rat heart: An FTIR microspectroscopy study", by Toyran et al., Arch.Biochem.Biophys. 2007 458:184-193.

Statistical analysis:

Statistical analysis was performed using the student T-test. P-values < 0.05 were considered significant. Statistical analysis was performed using STATISTICA software (STATISTICA, StatSoft, Inc., Tulsa, OK).

Artificial Neural Network analysis:

Alyuda NeuroIntelligence 2.2 (Alyuda Research Inc.) is a Neural Network Software for Classifying Data.

EXPERIMENTAL DATA

The experiments described hereinbelow were performed by the inventors in accordance with applications of the present invention, and using the techniques described hereinabove.

In a set of experiments, blood plasma from 42 healthy controls was analyzed by FTIR-MSP, and a typical FTIR-MSP spectral pattern was established for control blood plasma. Additionally, blood plasma samples from 28 cancer patients suffering from multiple types of solid tumors were subjected to FTIR-MSP analysis and compared to the control FTIR-MSP spectral pattern. The blood plasma was obtained by preliminary processing of the peripheral blood in accordance with the protocols described hereinabove with reference to isolation of plasma from peripheral blood samples. The blood plasma samples were then analyzed by FTIR-MSP in accordance with the protocols described hereinabove with reference to FTIR-MSP.

Reference is made to Figs. 1A-F, which are graphs representing FTIR absorption spectra and the second derivative of absorption spectra and analysis thereof, for blood plasma from 28 cancer patients and 42 healthy controls, derived in accordance with some applications of the present invention.

FTIR-MSP analysis of blood plasma typically generated spectra in the region of 4000-700 cm-1. The spectra are composed of several absorption bands, each corresponding to specific functional groups of specific macromolecules. Figs. 1A-B show average FTIR-MSP spectra of blood plasma of healthy controls and cancer patients in the regions of 3150-2830 cm-1 (Fig. 1A) and 1800-700 cm-1 (Fig. 1B), after baseline correction and vector normalization. The main absorption bands are marked and the mean

± SEM is represented by the gray region around the average solid (control) and dotted (cancer) lines The absorption bands observed in the FTIR spectra shown in Figs. 1A-B generally correspond to vibrations of functional groups of molecules which are present in blood plasma. For example, proteins (e.g., albumin and globulins), nutrients (e.g., glucose, amino acids, fatty acids, and monoglycerides) fibrinogen, electrolytes, solutes, hormones, enzymes, vitamins and other cellular components; each has its own spectral fingerprint that together compose the entire spectra of the plasma sample. Each spectrum of a single plasma sample represents the average of five measurements at different sites for each sample.

As shown in Figs. 1A-B, the FTIR-MSP spectra derived from analysis of blood plasma from the cancer patients exhibited a different spectral pattern when compared to the FTIR-MSP spectra of blood plasma of healthy controls.

Reference is made to Fig. 1A. Typically, the spectral region 3150-2830 cm-1 contains absorption bands due to symmetric and asymmetric CH3 (at 2959 cm-1, 2873 cm-1) and CH3 (at 2930 cm-1, 2856 cm-1) stretching vibrations corresponding mainly to proteins and lipids respectively. Another absorption band located at ~3060 cm-1 in this region typically is due to N-H stretching, and corresponds to Amide B. The absorption at the band corresponding to Amide B was found to be significantly higher ($p < 2*10^{-4}$) in the blood plasma samples of cancer patients when compared to blood plasma from the healthy controls, as revealed by calculation of the area under the Amide B band (at 3014 cm-1 to 3110 cm-1) following cut, baseline correction and Min-Max normalization at 2800 cm-1 to 3150 cm-1.

Reference is made to Fig. 1B which shows a spectral region of 1800-700 cm-1. Additionally, the insert in Fig. 1B shows a detailed view of the 1400-900 cm-1 spectral region which has several overlapping bands which correspond to multiple functional groups of plasma components. The detailed view of the 1400-900 cm-1 spectral region shows several differences between blood plasma samples of cancer patients compared to healthy controls, e.g., a reduction at 1400 cm-1 (containing COO^- symmetric stretch) typically corresponding to protein.

Reference is made to Figs. 1C and 1E. In order to increase accuracy and achieve effective comparison between the blood plasma samples of the cancer patients and the healthy controls, the second derivative of the baseline-corrected, vector-normalized FTIR

spectra was used. Results are presented in Figs. 1C and 1E. As shown, the second derivative spectral pattern of blood plasma samples from the cancer patients differed significantly from the FTIR-MSP spectral pattern of blood plasma of the healthy controls. The main absorption bands are marked and the mean ± SEM is represented by the gray area around the solid (controls) and dotted (cancer) lines.

Reference is made to Figs. 1D and 1F, which are graphs showing an analysis of the second derivative data shown in Figs. 1C and 1E. The graphs in Figs. 1D and 1F represent the variation between the second derivative of the spectral pattern of blood plasma samples from the cancer patients and healthy controls, obtained in accordance with applications of the present invention.

Fig. 2 shows a graph representing values of the second derivative of absorption spectra at wavenumbers A1-A21 of blood plasma samples from cancer patients compared to blood plasma samples from healthy controls, derived in accordance with some applications of the present invention. Statistical analysis was performed and P-values are provided. As shown, the second derivative of blood plasma from the cancer patients differed significantly from the second derivative analysis of FTIR-MSP spectral pattern from blood plasma of healthy controls.

Table I lists the wavenumbers shown in Fig 2. Typically, blood plasma samples were analyzed by FTIR-MSP techniques using these wavenumbers to distinguish between healthy controls and cancer patients.

Table I:

	Wavenumber (cm-1) ± 4
A1	743
A2	759
A3	847
A4	963
A5	967
A6	987
A7	1030
A8	1150
A9	1172
A10	1205
A11	1221
A12	1270
A13	1283
A14	1326
A15	1356
A16	1372
A17	1393
A18	1555
A19	1595
A20	1653
A21	1681

The data obtain by analysis of the blood plasma samples may be further analyzed by any suitable method known in the art, e.g., Artificial Neural Network and/or Cluster

Analysis, and/or Principal Component Analysis, and/or Linear Discriminant Analysis (LDA) and/or Non Linear Discriminant Analysis.

For example, data obtained in accordance with applications of the present invention was analyzed by artificial neural network (ANN). Several biomarkers shown in Table I which were statistically significant ($p < 0.05$) were served as an input vector for the ANN analysis. According to the ANN, 2 spectra out of 42 controls were rejected from the analysis. These two spectra were suspected for improper sample preparation. Twenty eight spectra were randomly selected for training, 17 for validation and 23 for test. This procedure was repeated at least ten times (each time with different sets for training, test and validation) to confirm repeatability of the results. All of the ANN analysis results presented high sensitivity and specificity of about 85% and 90%, respectively.

Reference is made to Figs. 1 and 2.

It is further noted that the scope of the present invention includes the use of only one wavenumber biomarker for detection and/or monitoring of a solid tumor, as well as the use of two, three, four, or more wavenumbers.

Additionally, the scope of the present invention includes using any IR spectral feature or any feature derived from analysis of an IR spectral feature (e.g., any type of peak analysis), to indicate the presence of a solid tumor.

It is also noted that the scope of the present invention is not limited to any particular form or analysis of IR spectroscopy. For example, IR spectroscopy may include Attenuated Total Reflectance (ATR) spectroscopy techniques.

Although applications of the present invention are described hereinabove with respect to spectroscopy, microspectroscopy, and particularly FTIR spectroscopy, the scope of the present invention includes the use of analysis techniques with data obtained by other means as well (for example, using a monochromator or an LED, at specific single wavenumbers, and/or FTIR imaging).

It is additionally noted that the scope of the present invention is not limited to blood plasma and may apply to any treated or untreated blood component. For example, techniques and methods described herein may alternatively be applied to blood serum.

It will be understood by one skilled in the art that aspects of the present invention described hereinabove can be embodied in a computer running software, and that the software can be supplied and stored in tangible media, e.g., hard disks, floppy disks, a USB flash drive, or compact disks, or in intangible media, e.g., in an electronic memory, or on a network such as the Internet.

It will be appreciated by persons skilled in the art that the present invention is not limited to what has been particularly shown and described hereinabove. Rather, the scope of the present invention includes both combinations and subcombinations of the various features described hereinabove, as well as variations and modifications thereof that are not in the prior art, which would occur to persons skilled in the art upon reading the foregoing description.

CLAIMS

1. A method comprising:

obtaining an infrared (IR) spectrum of a blood plasma sample by analyzing the blood plasma sample by infrared spectroscopy; and

based on the infrared spectrum, generating an output indicative of the presence of a solid tumor or a pre-malignant condition.

2. The method according to claim 1, wherein generating the output comprises generating the output indicative of the presence of the solid tumor.

3. The method according to claim 1 or claim 2, wherein analyzing the blood plasma sample by infrared (IR) spectroscopy comprises analyzing the blood plasma sample by Fourier Transformed Infrared (FTIR) spectroscopy, and wherein obtaining the infrared (IR) spectrum comprises obtaining a Fourier Transformed Infrared (FTIR) spectrum.

4. The method according to claim 3, wherein analyzing the blood plasma sample by infrared (IR) spectroscopy comprises analyzing the blood plasma sample by Fourier Transformed Infrared microspectroscopy (FTIR-MSP).

5. The method according to claim 1 or claim 2, wherein analyzing comprises assessing a characteristic of the blood plasma sample at at least one wavenumber selected from the group consisting of: 759 ± 4 cm-1, 987 ± 4 cm-1, 1172 ± 4 cm-1, 1270 ± 4 cm-1, 1283 ± 4 cm-1, and 1393 ± 4 cm-1.

6. The method according to claim 5, wherein analyzing comprises assessing the characteristic at at least two wavenumbers selected from the group.

7. The method according to claim 5, wherein analyzing comprises assessing the characteristic at at least three wavenumbers selected from the group.

8. The method according to claim 1 or claim 2, wherein analyzing comprises assessing a characteristic of the blood plasma sample at at least one wavenumber selected from the group consisting of: 743 ± 4 cm-1, 793 ± 4 cm-1, 808 ± 4 cm-1, 847 ± 4 cm-1, 850 ± 4 cm-1, 895 ± 4 cm-1, 950 ± 4 cm-1, 961 ± 4 cm-1, 963 ± 4, 967 ± 4 cm-1, 975 ± 4 cm-1, 997 ± 4 cm-1, 1008 ± 4 cm-1, 1030 ± 4 cm-1, 1031 ± 4 cm-1, 1048 ± 4 cm-1, 1120 ± 4 cm-1, 1150 ± 4 cm-1, 1159 ± 4 cm-1, 1188 ± 4 cm-1, 1205 ± 4 cm-1, 1220 ± 4 cm-1, 1221 ± 4 cm-1, 1255 ± 4 cm-1, 1322 ± 4 cm-1, 1326 ± 4 cm-1, 1341 ± 4 cm-1, 1356 ± 4

cm-1 1370 ± 4 cm-1, 1372 ± 4 cm-1, 1402 ± 4 cm-1, 1415 ± 4 cm-1, and 1555 ± 4 cm-1, 1595 ± 4 cm-1, 1653 ± 4 cm-1, 1681 ± 4 cm-1.

9. The method according to claim 8, wherein analyzing comprises assessing the characteristic at at least two wavenumbers selected from the group.

10. The method according to claim 8, wherein analyzing comprises assessing the characteristic at at least three wavenumbers selected from the group.

11. The method according to claim 8, wherein assessing the characteristic comprises analyzing a band of the IR spectrum at at least one wavenumber selected from the group.

12. The method according to claim 1 or claim 2, wherein the solid tumor includes a solid tumor in an organ selected from the group consisting of: lung, pancreas, prostate, bladder, and gastrointestinal tract, and wherein generating the output comprises generating an output indicative of the presence of a solid tumor in an organ selected from the group.

13. The method according to claim 1 or claim 2, wherein the solid tumor includes breast cancer, and wherein generating the output comprises generating an output indicative of the presence of breast cancer.

14. The method according to claim 1 or claim 2, wherein analyzing the blood plasma sample comprises obtaining a second derivative of the infrared (IR) spectrum of the blood plasma sample.

15. The method according to claim 1 or claim 2, wherein the blood plasma sample includes a dried blood plasma sample and analyzing the blood plasma sample comprises analyzing the dried blood plasma sample.

16. The method according to claim 1 or claim 2, wherein the infrared (IR) spectrum includes an absorption spectrum and obtaining the infrared (IR) spectrum comprises obtaining the absorption spectrum.

17. The method according to claim 1 or claim 2, wherein the infrared (IR) spectrum includes a reflection spectrum and obtaining the infrared (IR) spectrum comprises obtaining the reflection spectrum.

18. A method for monitoring the effect of an anti-cancer treatment on a subject undergoing anti-cancer treatment for a solid tumor, for use with a first blood plasma

sample separated from blood of the subject that was obtained prior to initiation of the treatment and a second blood plasma sample separated from blood of the subject that was obtained after initiation of the treatment, the method comprising:

obtaining IR spectra of the first and second blood plasma samples by analyzing the first and second blood plasma samples by IR spectroscopy; and

based on the IR spectra, generating an output indicative of the effect of the treatment.

19. The method according to claim 18, wherein analyzing the first and second blood plasma samples by IR spectroscopy comprises analyzing the samples by Fourier Transformed Infrared spectroscopy, and wherein obtaining the IR spectra comprises obtaining Fourier Transformed Infrared (FTIR) spectra.

20. The method according to claim 19, wherein analyzing the first and second blood plasma samples by infrared (IR) spectroscopy comprises analyzing the first and second blood plasma samples by Fourier Transformed Infrared microspectroscopy (FTIR-MSP).

21. The method according to claim 18, further comprising obtaining an IR spectrum of a third blood plasma sample separated from blood of the subject that was obtained following termination of the treatment, by analyzing the third blood plasma sample by IR spectroscopy.

22. The method according to claim 18, wherein analyzing comprises assessing a characteristic of the blood plasma sample at at least one wavenumber selected from the group consisting of: 759 ± 4 cm-1, 987 ± 4 cm-1, 1172 ± 4 cm-1, 1270 ± 4 cm-1, 1283 ± 4 cm-1, and 1393 ± 4 cm-1.

23. The method according to claim 22, wherein analyzing comprises assessing the characteristic at at least two wavenumbers selected from the group.

24. The method according to claim 22, wherein analyzing comprises assessing the characteristic at at least three wavenumbers selected from the group.

25. The method according to claim 18, wherein analyzing comprises assessing a characteristic of the blood plasma sample at at least one wavenumber selected from the group consisting of: 743 ± 4 cm-1, 793 ± 4 cm-1, 808 ± 4 cm-1, 847 ± 4 cm-1, 850 ± 4 cm-1, 895 ± 4 cm-1, 950 ± 4 cm-1, 961 ± 4 cm-1, 963 ± 4, 967 ± 4 cm-1, 975 ± 4 cm-1, 997 ± 4 cm-1, 1008 ± 4 cm-1, 1030 ± 4 cm-1, 1031 ± 4 cm-1, 1048 ± 4 cm-1, 1120 ± 4

cm-1, 1150 ± 4 cm-1, 1159 ± 4 cm-1, 1188 ± 4 cm-1, 1205 ± 4 cm-1, 1220 ± 4 cm-1, 1221 ± 4 cm-1, 1255 ± 4 cm-1, 1322 ± 4 cm-1, 1326 ± 4 cm-1, 1341 ± 4 cm-1, 1356 ± 4 cm-1 1370 ± 4 cm-1, 1372 ± 4 cm-1, 1402 ± 4 cm-1, 1415 ± 4 cm-1, and 1555 ± 4 cm-1, 1595 ± 4 cm-1, 1653 ± 4 cm-1, 1681 ± 4 cm-1.

26. The method according to claim 25, wherein analyzing comprises assessing the characteristic at at least two wavenumbers selected from the group.

27. The method according to claim 25, wherein analyzing comprises assessing the characteristic at at least three wavenumbers selected from the group.

28. A method comprising:

obtaining an infrared (IR) spectrum of a blood plasma sample by analyzing the blood plasma sample; and

based on the infrared spectrum, generating an output indicative of the presence of a solid tumor or a pre-malignant condition.

29. The method according to claim 28, wherein generating the output comprises generating the output indicative of the presence of the solid tumor.

30. A system for diagnosing a solid tumor, comprising:

a data processor, configured to analyze an infrared (IR) spectrum of a blood plasma sample of a subject; and

an output unit, configured to generate an output indicative of the presence of a solid tumor, based on the infrared (IR) spectrum.

31. The system according to claim 30, wherein the data processor is configured to calculate a second derivative of the infrared (IR) spectrum of the blood plasma sample and, based on the second derivative of the infrared (IR) spectrum, to generate an output indicative of the presence of a solid tumor.

32. The system according to claim 31, wherein the IR spectrum includes a Fourier Transformed Infrared (FTIR) spectrum, and wherein the data processor is configured to calculate a second derivative of the FTIR spectrum.

33. The system according to claim 30, wherein the data processor is configured to analyze the infrared (IR) spectrum by assessing a characteristic of the blood plasma

sample at at least one wavenumber selected from the group consisting of: 759 ± 4 cm-1, 987 ± 4 cm-1, 1172 ± 4 cm-1, 1270 ± 4 cm-1, 1283 ± 4 cm-1, and 1393 ± 4 cm-1.

34. The system according to claim 33, wherein the data processor is configured to analyze the infrared (IR) spectrum by assessing the characteristic at at least two wavenumbers selected from the group.

35. The system according to claim 33, wherein the data processor is configured to analyze the infrared (IR) spectrum by assessing the characteristic at at least three wavenumbers selected from the group.

36. The system according to claim 30, wherein the data processor is configured to analyze the infrared (IR) spectrum by assessing a characteristic of the blood plasma sample at at least one wavenumber selected from the group consisting of: 743 ± 4 cm-1, 793 ± 4 cm-1, 808 ± 4 cm-1, 847 ± 4 cm-1, 850 ± 4 cm-1, 895 ± 4 cm-1, 950 ± 4 cm-1, 961 ± 4 cm-1, 963 ± 4, 967 ± 4 cm-1, 975 ± 4 cm-1, 997 ± 4 cm-1, 1008 ± 4 cm-1, 1030 ± 4 cm-1, 1031 ± 4 cm-1, 1048 ± 4 cm-1, 1120 ± 4 cm-1, 1150 ± 4 cm-1, 1159 ± 4 cm-1, 1188 ± 4 cm-1, 1205 ± 4 cm-1, 1220 ± 4 cm-1, 1221 ± 4 cm-1, 1255 ± 4 cm-1, 1322 ± 4 cm-1, 1326 ± 4 cm-1, 1341 ± 4 cm-1, 1356 ± 4 cm-1 1370 ± 4 cm-1, 1372 ± 4 cm-1, 1402 ± 4 cm-1, 1415 ± 4 cm-1, and 1555 ± 4 cm-1, 1595 ± 4 cm-1, 1653 ± 4 cm-1, 1681 ± 4 cm-1.

37. The system according to claim 36, wherein the data processor is configured to analyze the infrared (IR) spectrum by assessing the characteristic at at least two wavenumbers selected from the group.

38. The system according to claim 36, wherein the data processor is configured to analyze the infrared (IR) spectrum by assessing the characteristic at at least three wavenumbers selected from the group.

39. A computer program product for administering processing of a body of data, the product comprising a computer-readable medium having program instructions embodied therein, which instructions, when read by a computer, cause the computer to:

obtain an infrared (IR) spectrum of a blood plasma sample by analyzing the blood plasma sample by infrared spectroscopy; and

based on the infrared spectrum, generate an output indicative of the presence of a solid tumor.

FIG. 1A

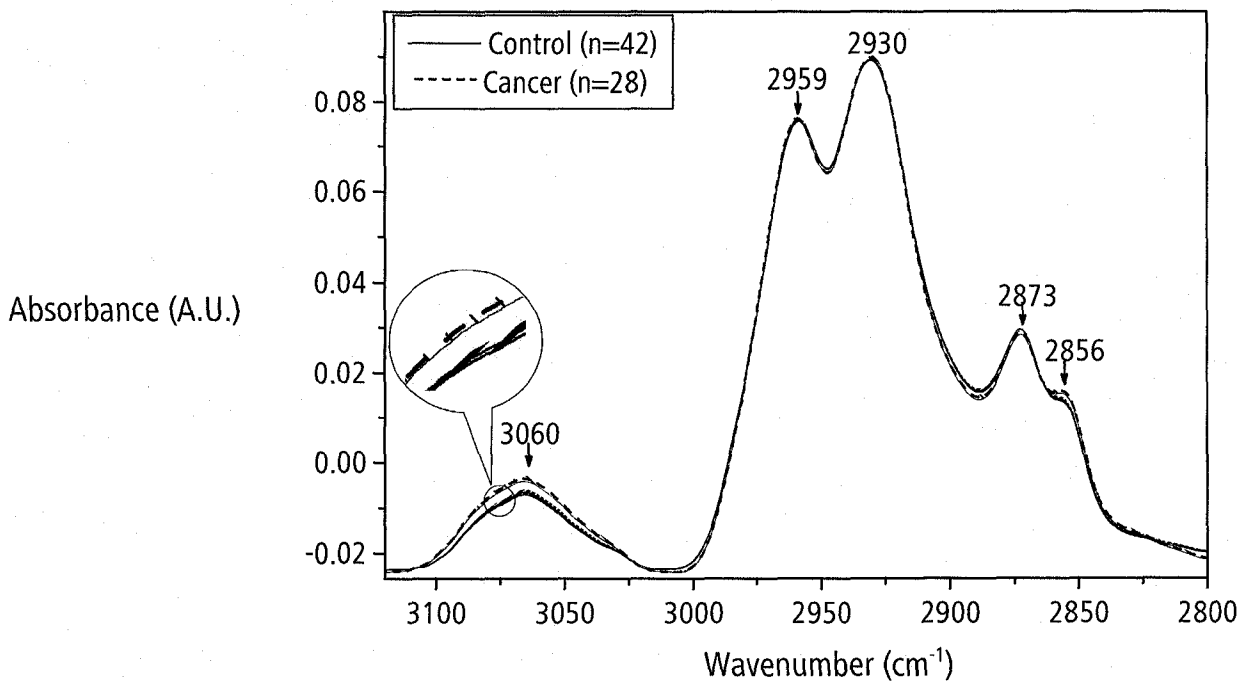

Control (n=42)
Cancer (n=28)
2959
2930
2873
2856
3060
0.08
0.06
0.04
0.02
0.00
-0.02
3100
3050
3000
2950
2900
2850
2800
Absorbance (A.U.)
Wavenumber (cm-1)

FIG. 1B

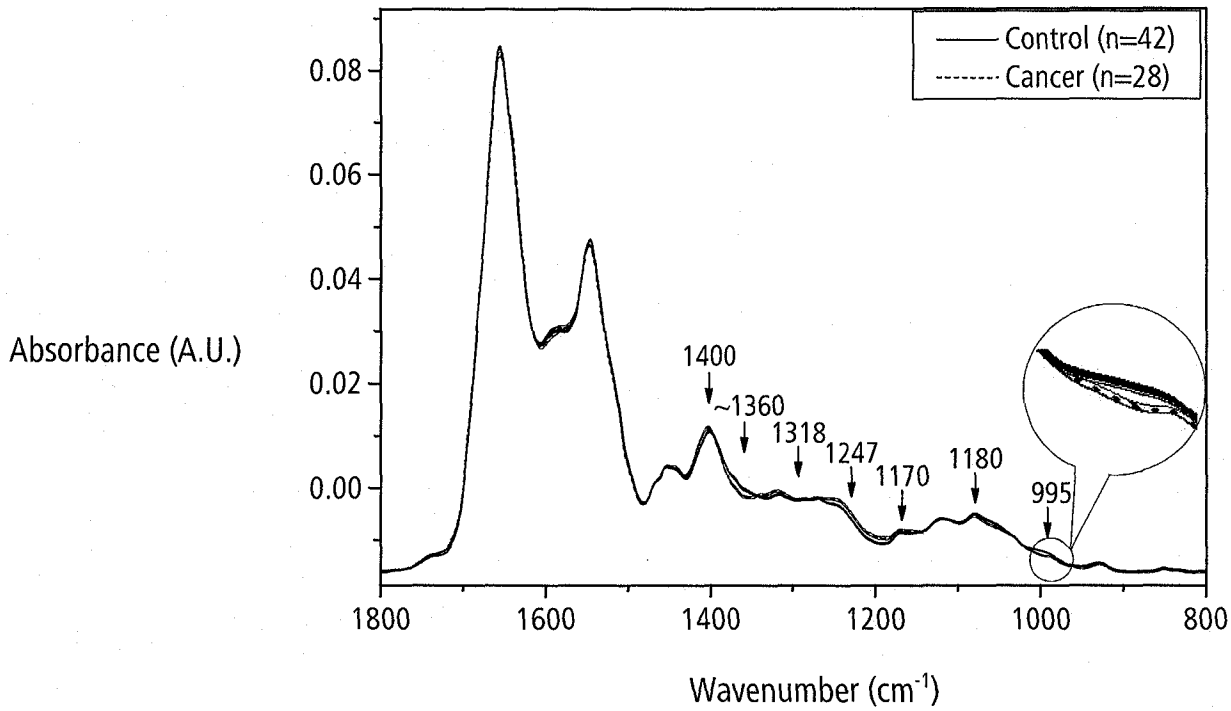

Control (n=42)
Cancer (n=28)
1400
~1360
1318
1247
1170
1180
995
0.08
0.06
0.04
0.02
0.00
1800
1600
1400
1200
1000
800
Absorbance (A.U.)
Wavenumber (cm-1)

FIG. 1C

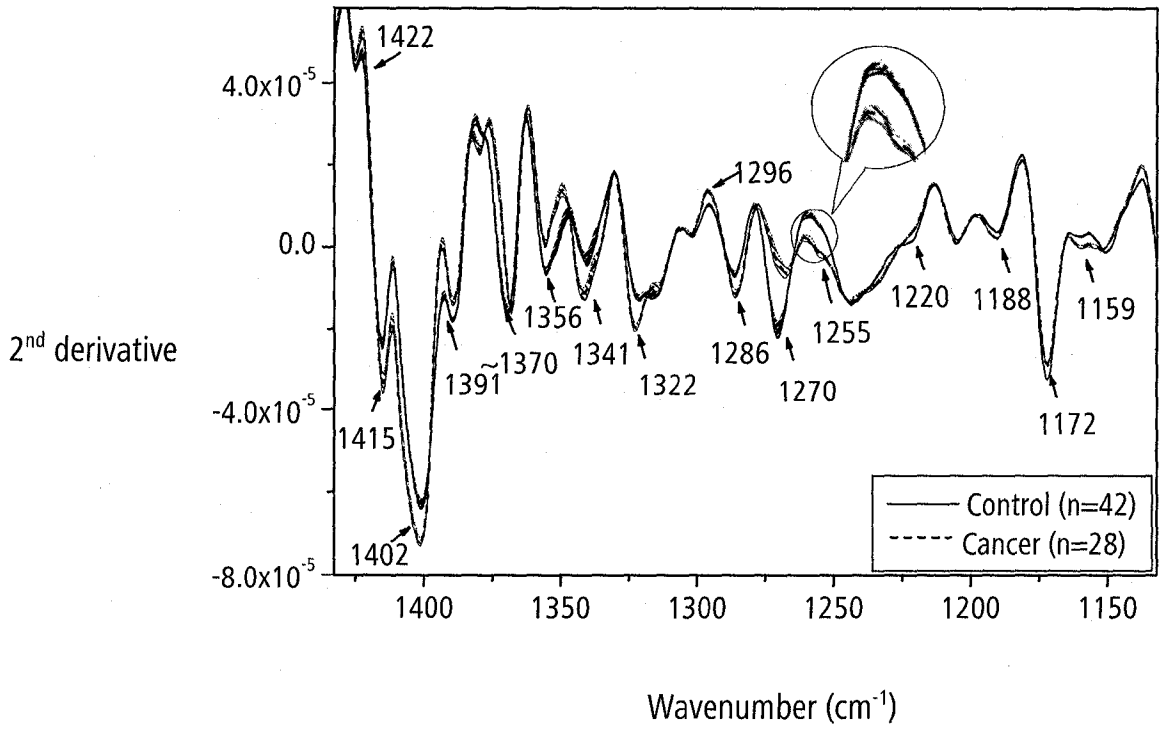

2nd derivative
4.0x10-5
0.0
-4.0x10-5
-8.0x10-5
1422
1415
1402
1391
~1370
1356
1341
1322
1296
1286
1270
1255
1220
1188
1172
1159
Control (n=42)
Cancer (n=28)
1400
1350
1300
1250
1200
1150
Wavenumber (cm-1)

FIG. 1D

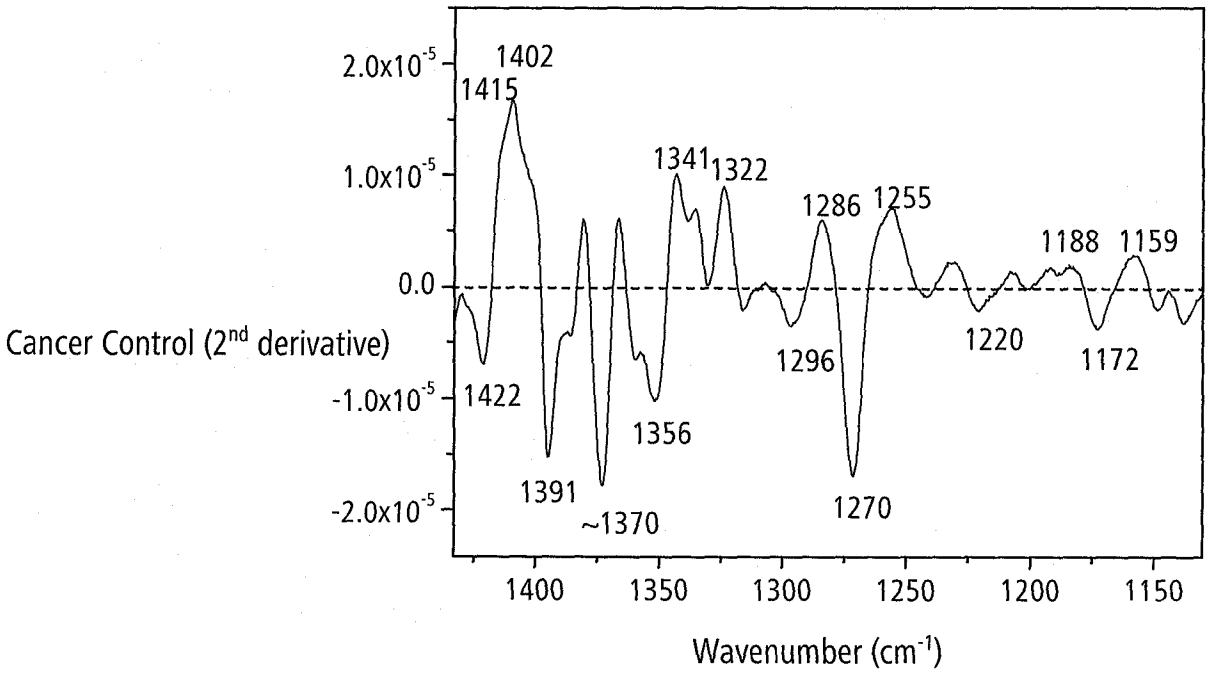

Cancer Control (2nd derivative)
2.0x10-5
1.0x10-5
0.0
-1.0x10-5
-2.0x10-5
1402
1415
1422
1391
~1370
1356
1341
1322
1296
1286
1270
1255
1220
1188
1172
1159
1400
1350
1300
1250
1200
1150
Wavenumber (cm-1)

FIG. 1E

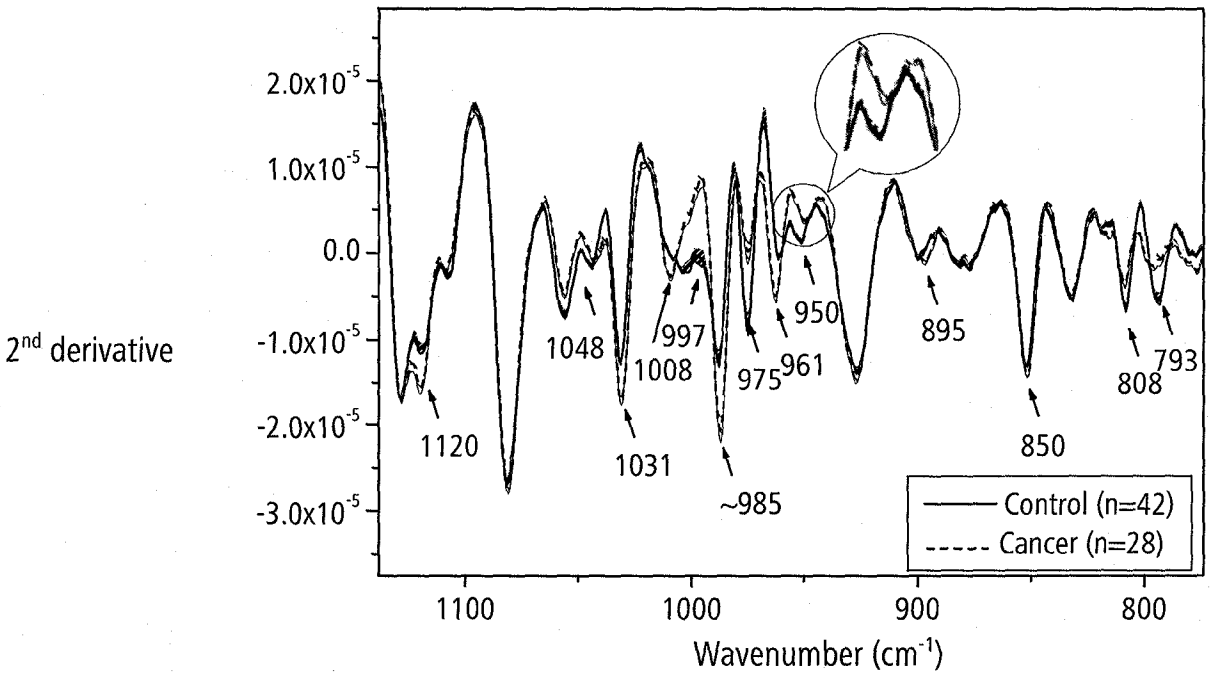

2nd derivative
2.0x10-5
1.0x10-5
0.0
-1.0x10-5
-2.0x10-5
-3.0x10-5
1120
1048
1031
1008
997
~985
975
961
950
895
850
808
793
Control (n=42)
Cancer (n=28)
1100
1000
900
800
Wavenumber (cm-1)

FIG. 1F

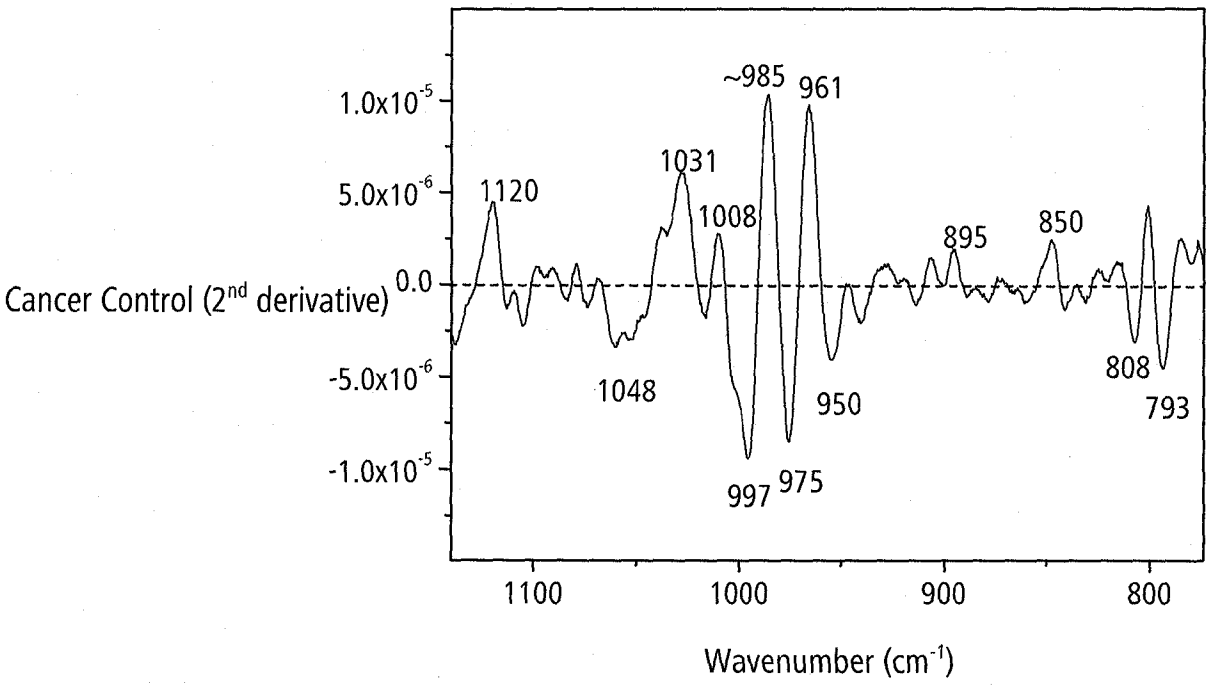
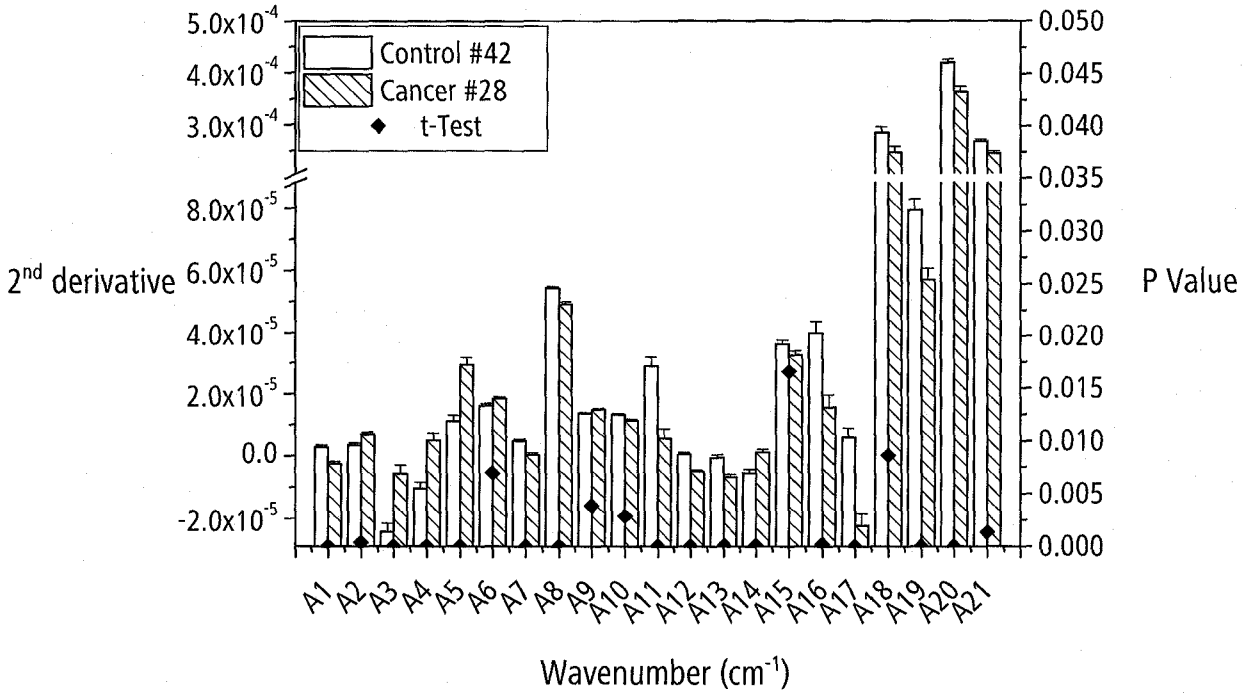

Cancer Control (2nd derivative)
1.0x10-5
5.0x10-6
0.0
-5.0x10-6
-1.0x10-5
1120
1048
1031
1008
~985
997
975
961
950
895
850
808
793
1100
1000
900
800
Wavenumber (cm-1)

FIG. 2


Control #42
Cancer #28
t-Test
2nd derivative
P Value
5.0x10-4
4.0x10-4
3.0x10-4
8.0x10-5
6.0x10-5
4.0x10-5
2.0x10-5
0.0
-2.0x10-5
0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
A1 A2 A3 A4 A5 A6 A7 A8 A9 A10 A11 A12 A13 A14 A15 A16 A17 A18 A19 A20 A21
Wavenumber (cm-1)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2012/000187

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/48 (2012.01)
USPC - 436/64
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - G01J 3/42, 3/457; G01N 33/48, 33/494 (2012.01)
USPC - 250/339.07, 339.08; 436/64

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	US 6,841,388 B2 (DUKOR et al) 11 January 2005 (11.01.2005) entire document	1-13, 16, 17, 28-30, 33-39 --- 14, 15, 31, 32
Y	US 2009/0004682 A1 (KITAMURA et al) 01 January 2009 (01.01.2009) entire document	14, 15, 31, 32
A	US 2007/0003921 A1 (ANDRUS) 04 January 2007 (04.01.2007) Abstract, para 0017, 0026-0028	1-39
A	US 5,261,410 A (ALFANO et al) 16 November 1993 (16.11.1993) Abstract, col 3, lines 19-54, col 4, lines 41-56	1-39

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
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"P" document published prior to the international filing date but later than the priority date claimed
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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 August 2012	31 AUG 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774