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(54) **Title**: SYSTEM AND METHOD FOR SERUM BASED CANCER DETECTION

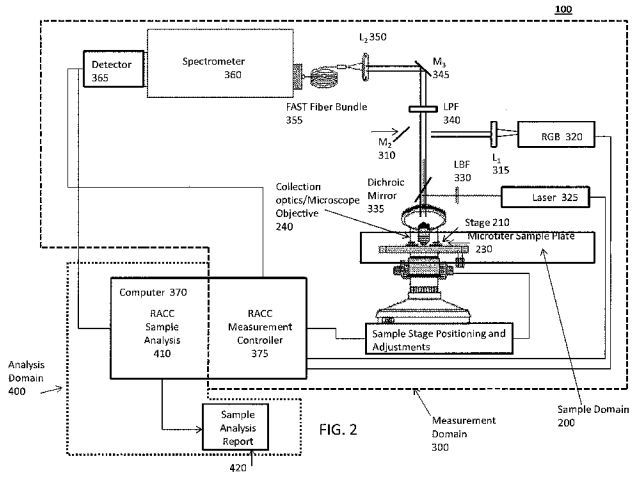


FIG. 2

(57) **Abstract**: A system and method for analyzing biological samples, such as dried human blood serum, to determine a disease state such as colorectal cancer (CRC). Using dried samples may hold potential for enhancing localized concentration and/or segmentation of sample components. The method may comprise illuminating at least one location of a biological sample to generate a plurality of interacted photons, collecting the interacted photons and generating at least one Raman data set representative of the biological sample. A system may comprise an illumination source to illuminate at least one location of a biological sample and generate at least one plurality of interacted photons, at least one mirror for directing the interacted photons to a detector. The detector may be configured to generate at least one Raman data set representative of the biological sample. The system and method may utilize a FAST device for multipoint analysis or may be configured to analyze a sample using a line scanning configuration.

WO 2014/074569 A1

SYSTEM AND METHOD FOR SERUM BASED CANCER DETECTION

RELATED APPLICATIONS

This Application claims priority under 35 U.S.C. 119(e) to the following pending provisional U.S. Patent applications: No. 61/796,268, filed on November 6, 2012, entitled "System and Method for Serum Based Cancer Detection," No. 61/797,686, filed on December 13, 2012, entitled "System and Method for Serum Based Cancer Detection, Staging, and Polyp Discrimination," No. 61/848,242, filed on December 28, 2012, entitled "Calibration Transfer Function for Biological Materials," and No. 61/765,524, filed on February 15, 2013, entitled "System and Method for Determining Disease Stage Using Raman Molecular Imaging." All of these applications are hereby incorporated by reference in their entireties.

BACKGROUND

Cancer is significant, not only in terms of mortality and morbidity, but also in terms of the cost of treating advanced cancers and the reduced productivity and quality of life of advanced cancer patients. Despite the common conception of cancers as incurable diseases, many cancers can be alleviated, slowed, or even cured if timely medical intervention can be administered. A widely recognized need exists for tools and methods for early detection of cancer.

Cancers arise by a variety of mechanisms, not all of which are well understood. Cancers, called tumors when they arise in the form of a solid mass, characteristically exhibit decontrolled growth and/or proliferation of cells. Cancer cells often exhibit other characteristic differences relative to the cell type from which they arise, including altered expression of cell surface,

secreted, nuclear, and/or cytoplasmic proteins, altered antigenicity, altered lipid envelope (i.e., cell membrane) composition, altered production of nucleic acids, altered morphology, and other differences. Typically, cancers are diagnosed either by observation of tumor formation or by observation of one or more of these characteristic differences. Because cancers arise from cells of normal tissues, cancer cells usually initially closely resemble the cells of the original normal tissue, often making detection of cancer cells difficult until the cancer has progressed to a stage at which the differences between cancer cells and the corresponding original normal cells are more pronounced. Depending on the type of cancer, the cancer can have advanced to a relatively difficult-to-treat stage before it is easily detectable.

Early definitive detection and classification of cancer is often crucial to successful treatment. Included in the diagnosis of many cancers is a determination of the type and grade of the cancer and the stage of its progression. This information can inform treatment selection, allowing use of milder treatments (i.e., having fewer undesirable side effects) for relatively early-stage, non- or slowly-spreading cancers and more aggressive treatment (i.e., having more undesirable side effects and/or a lower therapeutic index) of cancers that pose a greater risk to the patient's health.

When cancer is suspected, a physician will often have the tumor or a section of tissue having one or more abnormal characteristics removed or biopsied and sent for histopathological analyses. Typically, the time taken to prepare the specimen is on the order of one day or more. Communication of results from the pathologist to the physician and to the patient can further slow the diagnosis of the cancer and the onset of any indicated treatment. Patient anxiety can soar during the period between sample collection and diagnosis.

A recognized need exists to shorten the time required to analyze biological samples in

order to determine whether or not the sample is cancerous. Furthermore, it would be beneficial to use body fluids instead of traditional tissue/cellular samples, in order to minimize patient discomfort and improve patient acceptance of testing.

Spectroscopic techniques provide information about biological molecules and therefore hold potential for providing information about the biological sample's disease state. As the biological sample's state (e.g., the sample's metabolic state) changes from a normal state to a diseased state, spectroscopic techniques may provide information to indicate the change and serve to diagnose and predict the outcome of a disease.

Various types of spectroscopy and imaging may be explored for detection of various types of diseases in particular cancers. Because Raman spectroscopy is based on irradiation of a sample and detection of scattered radiation, it can be employed non-invasively and non-destructively, such that it is suitable for analysis of biological samples. Thus, little or no sample preparation is required. In addition, water exhibits very little Raman scattering, and Raman spectroscopy techniques can be readily performed in aqueous environments.

Raman spectroscopy provides information about the vibrational state of molecules. Many molecules have atomic bonds capable of existing in a number of vibrational states. Such molecules are able to scatter incident radiation that matches a transition between two of its allowed vibrational states and to subsequently emit the radiation. Most often, scattered radiation is re-radiated at the same wavelength, a process designated Rayleigh or elastic scattering. In some instances, the re-radiated radiation can contain slightly more or slightly less energy than the incident radiation (depending on the allowable vibrational states and the initial and final vibrational states of the molecule). The result of the energy difference between the incident and re-radiated radiation is manifested as a shift in the wavelength between the incident and re-

radiated radiation, and the degree of difference is designated the Raman shift (RS), measured in units of wavenumber (inverse length). If the incident light is substantially monochromatic (single wavelength) as it is when using a laser source, the scattered light which differs in wavelength can be more easily distinguished from the Rayleigh scattered light.

The Raman spectrum of a material can reveal the molecular composition of the material, including the specific functional groups present in organic and inorganic molecules. Raman spectroscopy is useful for detection of biological materials because most, if not all, of these agents exhibit characteristic "fingerprint" Raman spectra, subject to various selection rules, by which the agent can be identified. Raman peak position, peak width, peak shape, and adherence to selection rules can be used to determine molecular identity and to determine conformational information (e.g., crystalline phase, degree of order, protein secondary structure) for condensed phase materials.

In the past several years, a number of key technologies have been introduced into wide use that have enabled scientists to largely overcome the problems inherent to Raman spectroscopy. These technologies include high efficiency solid-state lasers, efficient laser rejection filters, and silicon (Si) charge coupled device (CCD) detectors. In general, the sample size determines the choice of image gathering optic. For example, a microscope is typically employed for the analysis of submicron to millimeter spatial dimension samples. For larger objects, in the range of millimeter to meter dimensions, macro lens optics are appropriate. For samples located within relatively inaccessible environments, flexible fiberscope or rigid borescopes can be employed. For very large scale objects, such as planetary objects, telescopes are appropriate image gathering optics.

For detection of images formed by the various optical systems, two-dimensional,

imaging focal plane array (FPA) detectors are typically employed. The choice of FPA detector is governed by the spectroscopic technique employed to characterize the sample of interest. For example, Si CCD detectors or complementary metal-oxide-semiconductor (CMOS) detectors are typically employed with visible (VIS) wavelength fluorescence and Raman spectroscopic imaging systems, while indium gallium arsenide (InGaAs) FPA detectors are typically employed with near-infrared (NIR) spectroscopic imaging systems.

In order to detect Raman scattered light and to accurately determine the Raman shift of that light, the sample should be irradiated with substantially monochromatic light, such as light having a bandwidth not greater than about 1.3 nanometers (nm), and preferably not greater than 1.0, 0.50, or 0.25 nm. Suitable sources include various lasers and polychromatic light source-monochromator combinations. It is recognized that the bandwidth of the irradiating light, the resolution of the wavelength resolving element(s), and the spectral range of the detector determine how well a spectral feature can be observed, detected, or distinguished from other spectral features. The combined properties of these elements (i.e., the light source, the filter, grating, or other mechanism used to distinguish Raman scattered light by wavelength) define the spectral resolution of the Raman signal detection system. The known relationships of these elements enable the skilled artisan to select appropriate components in readily calculable ways. Limitations in spectral resolution of the system (e.g., limitations relating to the bandwidth of irradiating light) can limit the ability to resolve, detect, or distinguish spectral features. The skilled artisan understands that and how the separation and shape of Raman scattering signals can determine the acceptable limits of spectral resolution for the system for any of the Raman spectral features described herein.

Spectroscopic imaging combines digital imaging and molecular spectroscopy techniques, which can include Raman scattering, fluorescence, photoluminescence, ultraviolet (UV), VIS and infrared (IR) absorption spectroscopies. When applied to the chemical analysis of materials, spectroscopic imaging is commonly referred to as chemical imaging. Instruments for performing spectroscopic (i.e. chemical) imaging typically comprise an illumination source, image gathering optics, focal plane array imaging detectors and imaging spectrometers.

For example, Raman chemical imaging (RCI) is a reagentless tissue imaging approach based on the scattering of laser light from tissue samples. The approach yields an image of a sample wherein pixels of the image is the Raman spectrum of the sample at the corresponding location. The Raman spectrum carries information about the local chemical environment of the sample at each location. RCI has a spatial resolving power of approximately 250 nm and can potentially provide qualitative and quantitative image information based on molecular composition, conformation and morphology.

Spectroscopic imaging of a sample can be implemented by one of several methods. First, a point-source illumination can be provided on the sample to measure the spectra at each point of the illuminated area. Line scanning may also be used where data is generated by illuminating a sample with a laser line. Spectra may also be collected over the entire area encompassing the sample simultaneously using an electronically tunable optical imaging filter such as an acousto-optic tunable filter (AOTF), a multi-conjugate tunable filter (MCF), or a liquid crystal tunable filter (LCTF). In an MCF, the organic material in such optical filters is actively aligned by applied voltages to produce the desired bandpass and transmission function. The spectra obtained for each pixel of such an image thereby forms a complex data set referred to as a hyperspectral image, which contains the intensity values at numerous wavelengths or the wavelength

dependence of each pixel element in this image. The method selected to generate spectroscopic data may depend on a variety of factors including the nature of the sample being analyzed, time required for analysis, and cost.

The ability to determine a disease state is critical to clinical diagnosis and cancer detection. Such testing often requires obtaining the spectrum of a sample at different wavelengths. Conventional spectroscopic devices operate over a limited range of wavelengths due to the operation ranges of the detectors, tunable filters, or other system components possible. This enables analysis in the UV, VIS, IR, NIR, short wave infrared (SWIR) mid-infrared (MIR), and long wave infrared (LWIR) wavelengths and to some overlapping ranges. These correspond to wavelengths of about 180-380 nm (UV), about 380-700 nm (VIS), about 700-2500 nm (NIR), about 850-1700 nm (SWIR) and about 2500-5000 nm (MIR), and about 5000-25000 nm (LWIR). Additional techniques include attenuated total reflectance (ATR) and fluorescence.

The most effective cure for cancer is early, pre-symptomatic detection. Once the presence of cancer is obvious, such as malignant and growing tumors combined with metastasis to other organs, the survival rate is very poor, especially in the cases of colorectal cancer (CRC). Early detection of colorectal cancer, the third most common cancer in the developed world, can result in a five plus year survival rate of 95%. However, late stage detection is reported to have disconcerting survival rates of only 5% combined with end of life medical costs skyrocketing up to hundreds of thousands of dollars. To date, early stage tumor markers have not been well receive by clinicians and insurers because of their poor reliability and inconsistent relevance to specific cancerous conditions. A need exists for an accurate and reliable system and method of detecting CRC, including early stage detection. Such a solution may hold potential for detecting

CRC in patients earlier than using traditional methods, monitor recurrence of CRC, and therefore allow a patient to seek treatment earlier, increasing survival rates.

SUMMARY

The present disclosure provides for a system and method for analyzing serum samples using spatially resolved Raman spectroscopy and/or Raman chemical imaging and supervised multivariate statistical analysis (i.e. chemometric) techniques to diagnose CRC and its precancerous lesions. In addition to detecting cancer, the system and method of the present disclosure may also hold potential for determining a cancer grade of a sample and to distinguish cancer from normal samples and/or the presence of polyps. Changes in the concentration or conformation of molecules in a sample may change as cancer progresses. These changes may be detected using the system and method disclosed herein and by analyzing changes in spectral bands between these stages. The disclosure provides for various embodiments comprising the use of spectroscopic, imaging, and sensor fusion techniques.

The system and method disclosed herein provide for the use of multipoint Raman spectroscopy and/or imaging in conjunction with a fiber array spectral translator (FAST) device. The use of FAST enables full spectral acquisition for hundreds to thousands of spatially resolved spectra in a single image frame. Use of a FAST device overcomes the limitations of the prior art by dramatically increasing data acquisition rates compared to point scanning or current tunable filter based technologies. Software, hardware, and/or a combination of software and hardware may be used to extract the spatial/spectral information to reconstruct data. Furthermore, FAST is a rugged technology that operates over an extensive spectral range from UV to IR. Therefore, the system and method of the present disclosure hold potential for providing a simple, low-cost,

reagentless *in vitro* diagnostic test performed which may be performed on biological samples, such as dried blood serum samples. The analysis of dried blood serum samples also provides an advantage over other techniques for detecting CRC in that it is minimally invasive to a patient.

A system is provided for analyzing biological samples. The system may comprise an illumination source configured to illuminate at least one location of the biological sample and generate at least one plurality of interacted photons. The interacted photons may be directed to a spectrometer using at least one mirror. At least one detector may be configured to detect the interacted photons and generate at least one Raman data set representative of the biological sample. At least one processor may be configured to analyze the Raman data set and associate the biological sample with at least one disease state.

A method is provided that comprises illuminating at least one location of a biological sample to generate at least one plurality of interacted photons. The interacted photons may be collected and detected to generate at least one Raman data set representative of the biological sample. The Raman data set may be analyzed to associate the biological sample with at least one disease state.

The present disclosure also provides for a non-transitory storage medium containing machine readable program code, which, when executed by a processor, causes the processor to perform the following: illuminate at least one location of a biological sample to generate at least one plurality of interacted photons, collect the plurality of interacted photons, detect the plurality of interacted photos and generate at least one Raman data set representative of the biological sample, and analyze the Raman data set to associate the biological sample with at least one disease state.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are included to provide further understanding of the disclosure and are incorporated in and constitute a part of this specification illustrate embodiments of the disclosure, and together with the description, serve to explain the principles of the disclosure.

In the drawings:

FIG. 1 is illustrative of an exemplary housing configuration of a system of the present disclosure.

FIG. 2 is illustrative of a system of the present disclosure.

FIG. 3A is illustrative of a fiber array spectral translator (FAST) device of the present disclosure.

FIG. 3B is illustrative of exemplary sampling configurations of various embodiments of the present disclosure.

FIG. 4 is illustrative of a system of the present disclosure.

FIG. 5A is illustrative of a method of the present disclosure.

FIG. 5B is illustrative of a method of the present disclosure.

FIG. 6A is illustrative of one embodiment of a system of the present disclosure utilizing data fusion from multiple spectroscopic modalities.

FIG. 6B is illustrative of one embodiment of a system of the present disclosure utilizing data fusion from multiple spectroscopic modalities.

FIG. 7A is illustrative of a low throughput sampling configuration of one embodiment of the present disclosure.

FIG. 7B is illustrative of a high throughput sampling configuration of one embodiment of the present disclosure.

FIG. 8A is illustrative of the generation of a RACC (Raman Assay for Colorectal Cancer) Index for an exemplary set of sample data, illustrating the detection capabilities of the present disclosure for differentiating between normal, cancer, and polyp samples.

FIG. 8B is illustrative of a receiver operating characteristic (ROC) curve of an exemplary set of sample data.

FIG. 8C is illustrative of the generation of a RACC index for an exemplary set of sample data, illustrating the detection capabilities of the present disclosure for detecting a cancer grade.

FIG. 9A is illustrative of a ROC curve of an exemplary set of sample data.

FIG. 9B is illustrative of the generation of a RACC index for an exemplary set of sample data, illustrating the detection capabilities of the present disclosure for differentiating between CRC and normal samples.

FIG. 10 is illustrative of RCI data of CRC and normal samples.

FIG. 11A is illustrative of average class spectra for the CRC and normal samples illustrated in FIG. 10.

FIG. 11B is illustrative of the Variable Importance in Projection (VIP) Scores for the model differentiating CRC and normal samples illustrated in FIG. 10.

FIG. 12A is illustrative of the detection capabilities of the present disclosure to differentiate between CRC and normal samples using RCI data in a grid pattern sampling configuration.

FIG. 12B is illustrative of the detection capabilities of the present disclosure to differentiate between CRC and normal samples using RCI data in a ring pattern sampling configuration.

FIG. 12C is illustrative of statistical information relating to the sampling configurations illustrated in FIG. 12A and FIG. 12B.

FIG. 13A is illustrative of a fluorescence chemical image of a CRC sample.

FIG. 13B is illustrative of a fluorescence chemical image of a normal sample.

FIG. 14 is illustrative of the detection capabilities of the present discourse to differentiate between CRC and normal samples using data fusion.

FIG. 15 is illustrative of the ability of the present disclosure to analyze a variety of spectral features including those associated with protein conformation.

FIG. 16 is illustrative of exemplary spectral features of interest relating to assessing protein conformation.

FIG. 17 is illustrative of VIP scores for a model differentiating CRC and normal samples.

FIG. 18 A is illustrative of RCI data relating to amide 1 peak center of mass (COM).

FIG. 18B is illustrative of spectral data indicating a random coil conformation.

DETAILED DESCRIPTION

Reference will now be made in detail to the embodiments of the present disclosure, examples of which are illustrated in the accompanying drawings. Wherever possible, the same reference numbers will be used throughout the specification to refer to the same or like parts.

The present disclosure provides for a system and method for analyzing biological samples or components of biological samples. Examples of biological samples include, but are

not limited to, a bodily fluid such as urine, saliva, sputum, feces, blood, serum, plasma, mucus, pus, semen, fluid expressed from a wound, lavage, cerebrospinal fluid, vaginal fluid, and combinations thereof. Although this disclosure focuses on determining a disease state (detecting cancer or a normal sample) of a biological sample, the present disclosure also contemplates that the system and method disclosed herein may be used to determine other characteristics of a sample (e.g. a metabolic state, a hydration state, an inflammatory state, and combinations thereof) and precursor conditions such as the presence of polyps within its definition of disease state. Additionally, while the examples provided herein relate to the detection of CRC, the present disclosure is not limited to CRC and the system and method may be used to detect a wide variety of cancers. In addition to detecting whether or not a sample comprises cancer, the system and method may also be applied to determine a cancer grade (or disease grade).

The present disclosure provides for a system, further illustrated by FIGS. 1-4 for analyzing biological samples to determine a disease state. An exemplary housing of a system **100** is illustrated in FIG.1. As can be seen in FIG. 1, the system **100** may comprise a sample domain **200** for placing a sample under analysis, a measurement domain **300**, for generating at least one Raman data set representative of the sample placed in the sample domain **200**, and an analysis domain **400** for analyzing the data generated by the measurement domain **300**.

FIG. 2 is a more detailed representation of a system **100** of the present disclosure. As illustrated in FIG. 2, the sample domain **200** may further comprise a stage **210** for placing a sample. This stage **210** may be moved to analyze the various samples under analysis. In one embodiment, the sample may be affixed to a slide or placed in a well plate, such as a microtiter sample plate **230**. The sample may be placed under collection optics such as a microscope objective **240** for analysis.

The measurement domain **300** may comprise an RGB camera **320** configured to generate an RGB image representative of the sample. At least one mirror **310** may be configured to direct photons from the sample through at least one lens **315** to the RGB camera **320**. The RGB image generated may be used to help align the sample for analysis and/or be used to find morphological features or areas of interest in the sample. The RGB image may also be correlated with a Raman data set generated by the measurement domain **300**.

Still referring to FIG. 2, the measurement domain **200** may further comprise at least one laser illumination source **325** configured to emit illuminating photons that may be passed through a laser bandpass filter (LBF) **330** to filter out wavelengths of light that are not of interest and allow one or more wavelengths of light of interest to pass through. These filtered illuminating photons may be directed to the sample by at least one mechanism **335** such as a dichroic mirror or a dichroic beamsplitter.

The illuminating photons may illuminate the sample and generate at least one plurality of interacted photons. In one embodiment, these interacted photons may comprise at least one of: photons scattered by the sample, photons absorbed by the sample, photons reflected by the sample, photons emitted by the sample, and combinations thereof.

The plurality of interacted photons may be passed through a long pass filter (LPF) **340** to filter out photons having short wavelengths and directed by at least one mirror **345** through a lens **350** to a two-dimensional end of a FAST device **355**. A FAST device **355** is illustrated in more detail in FIG. 3A. In FIG. 3A, the FAST device **355** comprises a two-dimensional end **356** and a one-dimensional end **357**. In one embodiment, the two-dimensional end **356** may have an ordering such as serpentine ordering. The two-dimensional end **356** of the FAST device **355** may comprise a two-dimensional array of optical fibers drawn into a one-dimensional fiber stack

357. In one embodiment, the two-dimensional end **365** may be non-linear (which can be in any non-linear configuration, e.g., circular, square, rectangular, etc.) and the one-dimensional linear end **357** may be linear.

Interacted photons may be focused onto the input (two-dimensional end **365)** of a FAST device, which may consist of up to thousands of individual fibers, each fiber collecting the light scattered (or absorbed, reflected, and/or emitted) by a specific corresponding location in the excited area of a biological sample.

The one-dimensional fiber stack **357** (output end) may be orientated at the entrance slit of a spectrometer **360**, illustrated in both FIG. 2 and FIG. 3A. The spectrometer **360** can function to separate the plurality of photons into a plurality of wavelengths and provide a separate dispersive spectrum from each fiber. Multiple Raman spectra and therefore multiple interrogations of the sample area can be obtained in a single measurement cycle, in essentially the same time as in conventional Raman sensors.

Referring to FIG. 2, the photons may be detected at a detector **365** to generate a Raman data set representative of a biological sample. In one embodiment, a processor (and/or software) **370** may be used to extract spectral/spatial information that is embedded in a single frame generated by a detector **365**.

Referring to FIG. 3A, **361** is representative of an exemplary detector **365** output, **362** is representative of an exemplary spectral reconstruction, and **363** is representative of an exemplary image reconstruction.

In one embodiment, an area of interest can be optically matched by the FAST device to an area of a laser spot to maximize the collection Raman efficiency. In one embodiment, the present disclosure contemplates a configuration in which only the laser beam is moved for

scanning within a field of view (FOV). The present disclosure also contemplates a preferred embodiment, wherein the sample is moved and the laser beam is stationary.

It is possible to optically match the "scanning" FOV with the Raman collection FOV. The FOV is imaged onto a rectangular FAST device so that each FAST fiber is collecting light from one region of the FOV. The area per fiber which yields the maximum spatial resolution is easily calculated by dividing the area of the entire FOV by the number of fibers. Raman scattering is only generated when the laser excites a sample, so Raman spectra will only be obtained at those fibers whose collection area is being scanned by the laser beam. Scanning only the laser beam is a rapid process that may utilize off the shelf galvonmeter-driven mirror systems.

The construction of the FAST device **355** requires knowledge of the position of each fiber at both the two-dimensional end **356** and the distal end, one-dimensional end **357** of the array. Each fiber collects light from a fixed position in the two-dimensional array (imaging end) and transmits this light onto a fixed position on the detector **365** (through that fiber's distal end **357**).

Each fiber may span more than one detector row, allowing higher resolution than one pixel per fiber in the reconstructed image. In fact, this super-resolution, combined with interpolation between fiber pixels (i.e., pixels in the detector associated with the respective fiber), achieves much higher spatial resolution than is otherwise possible. Thus, spatial calibration may involve not only the knowledge of fiber geometry (i.e., fiber correspondence) at the imaging end and the distal end, but also the knowledge of which detector rows are associated with a given fiber.

One of the fundamental advantages of using a FAST device, over other spectroscopic methods, is speed of analysis. FAST technology can acquire a few to thousands of full spectral range, spatially resolved spectra simultaneously. A complete spectroscopic imaging data set can be acquired in the amount of time it takes to generate a single spectrum from a given material, especially for samples that are susceptible to laser induced photodamage. FAST devices can also be implemented with multiple detectors and color-coded FAST spectroscopic images can be superimposed on other high-spatial resolution gray-scale images to provide significant insight into the morphology and chemistry of the sample.

Utilizing a FAST device is one way of configuring a system **100** for what may be referred to as "multipoint" analysis. To perform multipoint analysis, the biological sample and field to be evaluated is illuminated in whole or in part, depending on the nature of the biological sample and the type of multipoint sampling desired. A field of illumination can be divided into multiple adjacent, non-adjacent, or overlapping points, and spectra can be generated at each of the points. In one embodiment, these spectra may be averaged. In another embodiment, an illumination spot size can be increased sufficiently to spatially sample/average over a large area of the sample. This may also include transect sampling.

By way of example, the entire sample can be illuminated and multipoint analysis performed by assessing interacted photons at selected points. Alternatively, multiple points of the sample can be illuminated, and interacted photons emanating from those points can be assessed. The points can be assessed serially (i.e., sequentially). To implement this strategy, there is an inherent trade off between acquisition time and the spatial resolution of the spectroscopic map. Each full spectrum takes a certain time to collect. The more spectra collected per unit area of a sample, the higher the apparent resolution of the spectroscopic map, but the

longer the data acquisition takes. In another embodiment, interacted photons can be assessed in parallel (i.e., simultaneously) for all selected points in an image field. This parallel processing of all points is designated chemical imaging, and can require significant data acquisition time, computing time and capacity when very large numbers of spatial points and spectral channels are selected, but require less data acquisition time, computing time and capacity when relatively small number of spectral channels are assessed.

The present disclosure provides for assessing interacted photons at multiple points in a FOV (e.g., the field of magnification for a microscope) that together represent only a portion of the area of the FOV (multipoint). It has been discovered that sampling the FOV at points representing a minority of the total area of the field (e.g., at two, three, four, six, ten, fifty, one hundred, or more) points representing, in sum, 25%, 5%, 1%, or less of the field). The points can be single pixels of an image of the FOV or areas of the field represented in an image by multiple adjacent or grouped pixels. The shape of areas or pixels assessed as individual points is not critical. For example, circular, annular, square, or rectangular areas or pixels can be assessed as individual points. Lines of pixels may also be assessed in a line scanning configuration. FIG. 3B is illustrative of exemplary sampling configurations of the various embodiments of the present disclosure.

The area corresponding to each point of a multipoint analysis can be selected or generated in a variety of known ways. In one embodiment, structured illumination may be used. By way of example, a confocal mask or diffracting optical element placed in the illumination or collection optical path can limit illumination or collection to certain portions of the sample having a defined geometric relationship.

Spectroscopic analysis of multiple points in a FOV (multipoint analysis) allows high

quality spectral sensing and analysis without the need to perform spectral imaging at every picture element (pixel) of an image. Optical imaging (e.g. RGB imaging) can be performed on the sample (e.g., simultaneously or separately) and the optical image can be combined with selected spectral information to define and locate regions of interest. Rapidly obtaining spectra from sufficient different locations of this region of interest at one time allows highly efficient and accurate spectral analysis and the identification of components in samples. Furthermore, identification of a region of interest in a sample or in a FOV can be used as a signal that more detailed Raman scattering (or other) analysis of that portion of the sample or FOV should be performed.

The high numbers of optical fibers required for FAST spectroscopic and/or imaging applications place extraordinary demands on the imaging spectrograph which the multipoint method addresses. Instead of having millions of pixels, multipoint analysis can utilize larger diameter fibers in bundles containing two to thousands of fibers. In the multipoint method of spectral sensing and analysis, complete spectral imaging (which would require at least thousands of adjacent pixels to create a physical image) is not required. Instead, spectral sensing performed at two to thousands of points simultaneously can rapidly (on the order of seconds) provide high quality spatially resolved spectra from a wide variety of points on the sample needed for analysis and identification. Thus, even if the precise geometric arrangement of the points analyzed in the FOV is not known, the points nonetheless have a defined geometrical arrangement which can span a sample or a FOV. The analyzed points may be informative regarding the disease state of a biological sample.

Referring again to FIG. 2, photons may be delivered to a spectrometer **360** wherein the spectrometer is configured to filter the interacted photons into a plurality of wavelengths. A

detector **365** may be configured to generate at least one Raman data set representative of the sample. In one embodiment, the Raman data set may comprise at least one of: at least one Raman spectrum and at least one Raman chemical image. In one embodiment, the detector **365** may further comprise at least one of: a CCD detector, an intensified charge coupled device (ICCD) detector, an InGaAs detector, an indium antimonide (InSb) detector, and a mercury cadmium telluride (MCT) detector.

The system **100** may further comprise at least one processor **370**. The processor **370** may function to carry out various functions in both the measurement domain **300** and the analysis domain **400**. In the measurement domain **300**, the processor **370** may comprise a measurement controller **375** that may comprise software to control various features of the system **100** such as data acquisition and calibration of the system **100**.

The system **100** may also comprise an analysis domain **400**, configured to analyze the data generated by the measurement domain **300**. The processor **370** may function in the analysis domain **400** to analyze the Raman data set. An analysis report **420** may be generated based on this analysis. This analysis report **420** may comprise a determination of disease state of a biological sample under analysis.

In one embodiment, the system **100** may further comprise at least one reference database comprising at least one reference data set, wherein each reference data set is associated with a known disease state. This reference data may be stored in the processor **370** and accessed to analyze the Raman data set generated from the biological sample.

FIG. 4 is provided to illustrate another embodiment of a system **100** of the present disclosure. In the embodiment of FIG. 4, the system **100** does not comprise a FAST device **355**, but rather operates using a line scanning configuration. Here, interacted photons are directed

directly to a spectrometer **360**. Other aspects of the system **100** may be the same as those in the embodiment of FIG. 2.

The present disclosure also provides for a method for analyzing biological samples to determine a disease state. In one embodiment, the biological sample may comprise at least one tissue. The present disclosure contemplates that this tissue may comprise a body fluid, such as blood, or a component of a tissue such as serum or plasma. When analyzing a tissue component, a method of the present disclosure may comprise processing a biological sample prior to analysis to remove any cellular or other debris from the sample. Analysis of body fluids holds potential for providing a less invasive mechanism of detecting disease than traditional biopsy methods.

One embodiment of a method of the present disclosure is illustrated in FIG. 5. In such an embodiment, the method **500** may comprise illuminating at least one location of a biological sample to generate at least one plurality of interacted photons in step **510**. These interacted photons may comprise at least one of: photons scattered by the biological sample, photons absorbed by the biological sample, photons reflected by the biological sample, and photons emitted by the biological sample.

In step **520**, the plurality of interacted photons may be collected. In one embodiment, the plurality of interacted photons may be passed through a FAST device to a spectrometer. In another embodiment, wherein a line scanning approach is used, the plurality of interacted photons may be passed directly to a spectrometer without the use of a FAST device. In either embodiment, the spectrometer may be configured to separate the plurality of interacted photons into a plurality of wavelengths.

In step **530** the plurality of interacted photons may be detected to generate at least one Raman data set representative of the biological sample. The present disclosure contemplates this

Raman data set may comprise at least one of: at least one Raman spectrum and at least one Raman chemical image. In step **540**, the Raman data set may be analyzed to associate the biological sample with at least one disease state. In one embodiment, the disease state may comprise at least one of: cancer, normal, and the presence of polyp. Where the disease state comprises cancer, analyzing the biological sample may further comprise determining at least once cancer grade. Where the disease state comprises normal, the method may further comprise determining at least one non-cancerous condition associated with the biological sample.
In one embodiment, the present disclosure contemplates generating multiple data sets for each patient over time. In such an embodiment, the system and method disclosed herein may be utilized to analyze biological samples for not only screening patients for cancer but also to monitor patients for recurrence, disease progression, or remission.

The present disclosure contemplates the determination of a disease state may be achieved by assessing one more component of a biological sample. Examples of components that may be measured include, but are not limited to: a chemical agent, a biological toxin, a microorganism, a bacterium, a protozoan, a virus, a protein, a flavonoid, a keratinoid, a metabolite, an enzyme, an electrolyte, a nucleic acid, and combinations thereof. The conformation of proteins in a biological sample (ordered or disordered) may also be analyzed.

Examples of metabolites that may be measured include, but are not limited to: those associated with the TCA cycle (succinate, isocitrate, citrate), tryptophan metabolism, (5-hydrozytryptophan, 5-hydroxyindolecetate, tryptophan), gut flora metablosim (2-hydroxyhippurate, phenlylacetatem phenylacetylglutamine, p-hydroxyphenyacetate, p-cresol), and others (5-oxoproline, N-acetyl-aspatem 3-methyl-histidine, histidine, myristate, putrescine, kynurenate). Examples of nucleic acids that may be analyzed include, but are not limited to:

SEPT9 methylated DNA, non-specific RNA SERS, secreted and cell surface gene. Other analytes that may be measured include but are not limited to CEA, CA-19, E-selectin, nucleosomes, and combinations thereof. In one embodiment, the present disclosure provides for analyzing trace level analytes modulating the blood serum proteins present in the biological sample.

In one embodiment, analyzing the biological sample **540** may further comprise the steps represented in FIG. 5B. In such an embodiment, analyzing **540** may comprise applying an instrument response correction in step **540a**. In one embodiment, an instrument response correction may further comprise at least one calibration transfer function to align misaligned spectra.

A calibration transfer function may comprise generating two or more spectral data sets representative of at least one biological sample. Reference points on the spectra may be selected where the points are common to both sets of spectra to determine a calibration transfer. As disclosed herein, a nonlinear spectral shift may exist between different data populations due to instrument and/or sample differences. In one embodiment, four spectral peaks corresponding to 1002 cm^{-1}, 1035 cm^{-1}, 1450 cm^{-1}, and 1672 cm^{-1} may be selected. However, the present disclosure is not limited to these wavelengths and others may be applied. A piecewise linear correction is then applied to the data using these known peaks as reference points to shift and stretch the spectra. In one embodiment, the spectra may then be combined into a single data set for analysis.

Instrument factors cause interference to low-intensity spectra. Removal of these factors may reveal subtle Raman signals. These factors may be removed by comparing the collected and empirical spectra of a standard reference material. Other processing steps may be applied such

as cosmic correction and flatfielding. Cosmic events occur randomly and may be seen as bright pixels in an image. For example, cosmic events may be removed by using a median filter that compares nearby neighboring pixels. Flatfielding is a process that may be used to improve uniformity of signal across the illuminated FOV. This may be performed by determining the illuminating pattern over a standard uniform material and then extracting this pattern from the sample images.

Referring again to FIG. 5B, spectra may be processed, which may include spectral truncation **540b**, baseline correction **540c**, and vector normalization **540d,** which are known in the art. Baseline correction removes variability in the data due to fluctuating baseline, which may be affected by several factors including tissue fluorescence and background interference. For example, the first two spectral data points and the last two spectral data points may be offset to the zero baseline. Normalization places spectra on the same intensity scale so that they can be directly compared. One method of normalization renders integrated area under the spectra that are equal for all data.

The analysis **540** may further comprise applying one or more steps to remove outlier data or data that is not suitable for analysis (sampling error, etc.). In step **540e**, intra-patient outlier rejection may be applied to the data to remove from analysis outlier spectra from the patient data. In step **540f**, whole-patient outlier rejection may be applied to remove all data associated with a patient if it is not suitable for analysis.

In step **540g**, at least one algorithm may be applied to perform supervised classification of the data. This algorithm may comprise support vector machines (SVM) and/or relevance vector machines (RVM). In another embodiment, the algorithm may comprise at least one chemometric technique. Examples of chemometric techniques that may be applied include, but

are not limited to: multivariate curve resolution, principle component analysis (PCA), k means clustering, band target entropy minimization (BTEM) method, adaptive subspace detector, cosine correlation analysis, Euclidian distance analysis, partial least squares regression, spectral mixture resolution, a spectral angle mapper metric, a spectral information divergence metric, a Mahalanobis distance metric, and spectral unmixing.

In one embodiment, the cheometric technique may comprise partial least squares discriminant analysis (PLSDA). A prediction from PLSDA is usually a value between zero and one, where one indicates membership within a class and zero indicates non-membership within a class.

In one embodiment, a model may be built repeatedly using a "leave one patient out" (LOPO) cross validation until all samples have been tested. To further analyze the results, ROC curves may be generated. A ROC curve is a plot of sensitivity and specificity and may be used as a test to select a threshold score that maximizes sensitivity and specificity.

Partial Least Squares (PLS) factor selection is an important step in PLSDA model building/evaluation process. The retention of too many PLS factors leads to overfitting of the class/spectra data which may include systematic noise sources. The retention of too few PLS factors leads to underfitting of the class/spectra data. A confusion matrix is typically employed as a Figure or Merit (FOM) for the optimal selection of PLS factors. A misclassification rate for the PLSDA model is evaluated as a function of PLS factors retained. The misclassification rate, although an important parameter, is not very descriptive of the final ROC curve which is the basis for model performance. This method uses an alternative FOM for the optimal selection of PLS factors based upon parameters from the ROC curve such as the Area Under the ROC (AUROC) as well as the minimum distance to an ideal sensor. This approach overcomes the

limitations of the prior art because ROC curves are not currently used for selecting factors. The ROC curve is traditionally created at the end of an evaluation process to determine the performance of the model, not to select parameters for building the model.

Referring again to FIG. 5B, a sample analysis report may be generated in step **540h**. This analysis report may be generated by the RACC sample analysis **410** functionality of a processor **370**, while operating in an analysis domain **400**. The analysis report may comprise a determination of a disease state, cancer grade, or other conclusion drawn from the analysis of the biological sample.

The analysis report generated in step **540h** may also comprise a RACC index representative of the biological sample under analysis. Here, analyzing the biological sample **540** may further comprise computing a RACC index for each biological sample. This RACC index represents a score for cancer and may be generated by applying at least one algorithm. In order to predict the class membership of a sample (e.g. cancer or normal), a threshold needs to be determined from the training data. Any sample with a RACC index above the threshold will be classified as cancer, and any sample with a RACC index below the threshold will be classified as normal. The threshold corresponds to the optimal operating point on the ROC curve that is generated by processing the training data. It is selected such that the performance of the classifier is as close to an ideal sensor as possible. An ideal sensor has a sensitivity of 100%, a specificity equal to 100%, an AUROC of 1.0, and is represented by the upper left corner of the ROC plot. To select the optimal operating point, a threshold is swept across the observed RACC indices. The true positive, true negative, false positive, and false negative classifications are calculated at each threshold value to yield the sensitivity and specificity results. The optimal operating point is the point on the ROC curve that is the minimum distance from the ideal

sensor. The threshold that corresponds to this sensitivity and specificity is selected as the threshold for the model. Alternatively, the threshold can be calculated by using a cluster method, such as Otsu's method. A histogram may be calculated using the RACC indices from the training data, and Otsu's method splits the histogram into two parts or classes.

In one embodiment, the method **500** may further comprise generating at least one additional spectroscopic and/or imaging data set representative of the sample using a modality other than Raman. For example, the method **500** may further comprise generating at least one RGB image representative of the biological sample. This RGB image may be used to assess locations and/or features of interest within the sample. The RGB image may also be correlated with a Raman data set.

In addition to augmenting Raman data sets with RGB images, the present disclosure also contemplates that the method **500** may further comprise applying data fusion. In such an embodiment, other spectroscopic and/or imaging techniques may be combined with Raman data to augment the data and analyze biological samples to determine a disease state.

For example, one option for implementing data fusion is to use both Raman and fluorescence modalities and fuse the scores from each sensor using a method such as Image Weighted Bayesian Fusion (IWBF). In one embodiment, Monte Carlo methods may be used to find a set of weights which minimized the number of false positive pixels in the fused detection image when the detection threshold was set to find all the true positive pixels. The terms can also be combined using other methods such as linear regression, neural networks, fuzzy logic, etc.

Fusion often provides better discrimination performance and allows for improvements on the score distribution. Fusion can create distributions with a smaller range and variance than

results from individual sensors. This can be beneficial because the threshold that is selected to discriminate the two classes relies heavily on the distribution of scores within a class. The tighter the distribution of scores is within a class and the larger difference between the classes, the better the performance of the model will be.

In embodiments utilizing sensor fusion, the system embodiments illustrated in FIGS. 2 and 4 may be altered to provide for additional components to enable generation of data using different spectroscopic and/or imaging modalities. For example, in an embodiment where fluorescence data is fused to Raman data, additional components may comprise a fluorescence light source and one or more dichroic mirrors and/or beamsplitters to direct illuminating photons to a biological sample and to direct interacted photons to the appropriate detectors. In one embodiment, a Rayleigh rejection filter may be used to filter interacted photons before being directed to a FAST device and/or to a spectrometer. The present disclosure also contemplates that other filters may be used.

FIGS. 6A and 6B are provided to further illustrate potential system configurations for data fusion. FIGS. 6A and 6B are intended to further enhance the system in FIGS. 2 and 4, and the same reference characters are used to refer to same or like parts. In FIG. 6A, one spectrometer **360** and one detector **365** may be used. Here, an additional illumination source, a fluorescence light source, **326** is provided to illuminate at least one location of a sample, for example in a well plate **230**. Interacted photons generated may be passed through collection optic **240** and be directed via at least one dichroic mirror/beamsplitter **336** through a Rayleigh rejection filter **351** and to the two-dimensional end **356** of a FAST device **355**. In this embodiment, the spectrometer **360** may comprise a split grating spectrometer. A split grating spectrometer **360** is illustrated in more detail by **367**. The photons may be separated into a

plurality of wavelengths by the spectrometer **360** and detected by a detector **365** to generate both a Raman data set and a fluorescence data set, wherein the fluorescence data set may comprise at least one of: at least one fluorescence spectrum and at least one fluorescence chemical image. An exemplary detector image is illustrated by **380** and exemplary Raman and fluorescence spectra are illustrated by **390** and **391**.

Another embodiment utilizing Raman/fluorescence data fusion is illustrated in FIG. 6B. Here, two separate spectrometers, **360** and **361** are configured to receive interacted photons from the one-dimensional end **357** of a FAST device **355**. Each spectrometer may filter the interacted photons into a plurality of wavelengths and two detectors, **365** and **366,** may be configured to detect these photons. One detector **365** may be configured to generate a Raman data set and the other detector **366** may be configured to generate a fluorescence data set. Exemplary detector images are illustrated by **380** and **381**. Exemplary Raman spectra are illustrated by **390** and exemplary fluorescence spectra are illustrated by **391**.

In addition to the embodiments of the system and method already discussed herein, the present disclosure also provides for a non-transitory storage medium containing machine readable program code. In one embodiment, this non-transitory storage medium containing machine readable program code which, when executed by a processor, causes the processor to perform the following: illuminate at least one location of a biological sample to generate at least one plurality of interacted photons, collect the plurality of interacted photons, detect the plurality of interacted photons, generate at least one Raman data set representative of the biological sample, and analyze the Raman data set to associate the biological sample with at least one disease state. In one embodiment, the storage medium, when executed by a processor, further causes the processor to pass the interacted photons through a FAST device.

EXAMPLES

FIGS. 7-17 are provided to illustrate the detection capabilities of the present disclosure for determining a disease state of a biological sample. Human blood samples collected from patients were removed from freezer storage and thawed at room temperature for approximately 1 hour. The samples were vortexed for approximately 15 seconds. 2.5 microlitres of human blood serum were dropped onto an aluminum-coated microscope slide via a micropipetter and allowed to dry for approximately 18 – 20 hours.

FIG. 7A is illustrative of an exemplary sample preparation utilizing a microscope slide. However, as illustrated in FIG. 7B, the present disclosure also contemplates a 96 well plate may also be used to hold samples. It is noted that duplicates of each sample (patient) were used along with both positive and negative controls.

FIG. 8A is illustrative of the detection capabilities of the present disclosure. A RACC index score was generated for each sample and plotted on a RACC discrimination plot. A threshold was applied based on a corresponding ROC curve (FIG. 8B) to determining an optimal operating point. As can be seen from the plot, samples could be associated with disease stages based on their location on the plot. Samples falling below the threshold were classified as normal. Samples falling above the threshold were classified as either CRC or the presence of polyps (a potential precursor condition). For samples determined to be CRC, cancer grades can be assigned based on the RACC index. Cancer staging of the samples is illustrated in more detail in FIG. 8C, with each plot representing the mean and standard deviation for the samples belonging to each stage.

FIGS. 9A and 9B are provided to further illustrate the detection capabilities of the present disclosure and represent the results of a second study. Here, 11 CRC samples and 21 normal samples were analyzed using SVM. The ROC curve (FIG. 9A) was used to select a threshold to apply to the data as illustrated in the plot of FIG. 9B. As can be seen from FIG. 9B, CRC samples were distinguished from normal samples.

FIG. 10 illustrates high definition Raman images of samples represented by the data of FIGS. 9A and 9B, using an SVM analysis. FIG. 10 illustrates two samples from the population analyzed, one representative of a normal sample and one representative of a CRC sample. The hypercube data for each patient (sample) was analyzed against two sets of data, one corresponding to CRC and one corresponding to normal. The images illustrate a RACC index at each pixel for each sample comprising either a CRC or a normal sample. As can be seen from differences in the images, CRC and normal score images hold potential for analyzing biological samples to screen patients for cancer.

FIGS. 11A and 11B illustrate spectral data representative of the droplets of FIG. 10. FIG. 11A illustrates average class spectra for both CRC and normal samples. The differences in the spectra are clear and are indicative of the potential of Raman spectroscopy to aid in cancer screening. FIG. 11B illustrates the VIP scores for CRC samples. VIP estimates the importance of each variable in the projection used in a model and is often used for variable selection. A variable with a VIP Score close to or greater than 1 (one) can be considered important in given model. In one embodiment, spectral features that dominate the discriminating power in supervised classification models may be used to reduce the number of wavenumbers evaluated (only input the ones of importance into the chemometric/supervised learning model). Examples of spectral features may include, but are not limited to: about 502 cm^{-1}, about 524 cm^{-1}, about

540 cm^{-1}, about, 559 cm^{-1}, about 850 cm^{-1}, about 992 cm^{-1}, about 999 cm^{-1}, about 1010 cm^{-1}, about 1213 cm^{-1}, about 1274 cm^{-1}.

FIGS. 12A and 12B illustrate the potential benefits of implementing a multipoint sampling approach as contemplated by the present disclosure. FIG. 12A illustrates sampling in a grid pattern. As can be seen from the RACC index plot, CRC samples and normal samples were easily differentiated when data was generated using this sampling approach. Similarly, in FIG. 12B, CRC samples were easily differentiated from normal samples when the data was generated using a ring sampling approach. The method of the present disclosure may overcome the limitations of the prior art by enabling sampling of an outer ring of a sample (between the center of the spot and the periphery). The present embodiment can be differentiated from other techniques, such as Drop Coating Deposition Raman (DCDR). DCDR is a method that can be used to improve Raman detections in samples with low concentrations of proteins. The method comprises deposition of a potein in a solution onto a hydrophobic surface, which is prepared using a thin layer of a hydrophobic material (such as a Tienta substrate). When the solvent is removed (via drying), dried proteins in a sample may be locally enriched in an outer edge of the sample (the periphery of the sample). In contrast, the present disclosure provides for the use of samples that contain high concentrations of proteins. The method is reagentless and, unlike DCDR, does not require treatment of the samples with a solution. Also, as illustrated by FIGS. 3B and 12A, the present disclosure is not limited to sampling the periphery of a sample and holds potential for discriminating between CRC and normal samples using data obtained from the center portion of a sample.

FIG. 12C is provided to illustrate statistical data regarding the sampling approaches of FIGS. 12A and 12B. A histogram is calculated using the RACC indices from the training data,

and Otsu's method splits the histogram into two parts or classes (difference between the means). The ring sampling approach improved the statistics of the model by providing a greater difference between class means and by reducing the class standard deviation.

FIGS. 13A-13B and FIG. 14 are provided to illustrate the capabilities of the present disclosure to fuse data from multiple modalities. FIGS. 13A and 13B represent fluorescence images of a CRC patient and a normal patient, respectively. In one embodiment, RACC indices resulting from SVM applied to Raman spectra for a patient were fused with RACC indices calculated from SVM applied to fluorescence spectra for the same patient. Fusion was done using IWBF. The fused results improved the RACC index distribution. In this example, fusion took advantage of the small distribution of the RACC indices for CRC samples in the fluorescence data and improved the distribution of the RACC index for CRC in the Raman data. Similarly for the normal samples, fusion improved the RACC index distribution of the fluorescence samples and capitalized on the tight distribution of RACC indices in the Raman data. The results of data fusion are illustrated in FIG. 14. As can be seen from the FIGS, data from multiple spectroscopic modes may be used to provide a more robust data set than either modality alone.

As discussed herein, the present disclosure contemplates that in one embodiment, a manifold of spectral features may be evaluated to determine a disease state of a biological sample. FIGS. 15-18 are provided to further illustrate an embodiment of the present disclosure wherein protein conformation is assessed as at least a primary factor in determining whether a sample comprises CRC. For example, FIG. 15 illustrates the average Raman spectra associated with CRC and Normal blood serum samples for exemplary data. The Raman spectra exhibit scattering from blood serum proteins as the dominant molecular moieties. Raman spectroscopy

has demonstrated capability for the detection of protein conformation, and the basis of discrimination between CRC and normal serum samples arises chiefly from changes in the conformation of one or more high abundance serum proteins. FIG. 16 summarizes several Raman spectral features observable in blood serum Raman spectra that indicate blood serum protein conformation. Analysis of these spectral features, where the identified wavenumber (cm^{-1}) position corresponds to the approximate centroid of the spectral feature, suggests that CRC blood serum samples contain increased Random Coil protein conformation relative to Normal blood serum samples. Specifically, the CRC Raman spectra evidence an increase in the shoulder band centered at 1660.6 cm^{-1}, which can be measured as an increase in the center of mass (COM) of the Amide I peak and is an indication of increased Random Coil protein conformation.

In comparison, the Normal Raman spectra evidence a reduced COM to 1660.3 cm^{-1}, which indicates more ordered, α-helix, protein conformation. Other observable changes that indicate the general trend of higher degree of Random Coil protein conformation in CRC spectra and higher degree of α -helix protein conformation in Normal spectra include: (1) increase at 1263 cm^{-1} (Amide III spectral feature) in Normal spectra; (2) increase at 941 cm^{-1} (C-C Stretch of Polypeptide Backbone spectral feature) in Normal spectra; and (3) increase in 857/827 cm^{-1} doublet ratio (Tyrosine Fermi Resonance Doublet) in CRC spectra. FIG. 17 illustrates the VIP Scores generated for these samples.

FIG. 18A is illustrative of RCI data relating to amide 1 peak COM. Amide 1 vibration is a result of primarily (about 80%) C=O stretching mode, with minor contributions from C-N stretching and Cα-CN deformation. It is also sensitive to protein secondary structure. FIG. 18B is illustrative of spectral data from these samples that illustrate differences between the CRC

spectrum and the normal spectrum. This difference may indicate a random coil conformation and be used to distinguish between CRC samples and normal samples.

While the disclosure has been described in detail in reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope of the embodiments. Thus, it is intended that the present disclosure cover the modifications and variations of this disclosure provided they come within the scope of the appended claims and their equivalents.

What is claimed is:

1. A method comprising:

illuminating at least one location of a biological sample to generate a plurality of interacted photons;

collecting the plurality of interacted photons;

detecting the plurality of interacted photons and generating at least one Raman data set representative of the biological sample;

analyzing the Raman data set to associate the biological sample with at least one disease state.

2. The method of claim 1 wherein the biological sample further comprises at least one body fluid.

3. The method of claim1 wherein the biological sample further comprises at least one tissue.

4. The method of clam 3 wherein the tissue further comprises blood.

5. The method of claim 1 wherein the biological sample further comprises at least one component of a tissue.

6. The method of claim 4 wherein the component further comprises at least one of: serum and plasma.

7. The method of claim 1 further comprising passing the plurality of interacted photons through a fiber array spectral translator device.

8. The method of claim 1 wherein the illuminating further comprises illuminating a plurality of locations of the biological sample.

9. The method of claim 8 wherein the plurality of points is further illuminated by structured illumination.

10. The method of claim 8 wherein the plurality of locations further have a defined geometric relationship.

11. The method of claim 8 wherein the plurality of locations further comprise a line.

12. The method of claim 1 wherein the illuminating is further achieved using wide-field illumination.

13. The method of claim 1 wherein the disease state further comprises at least one of: cancer, normal, and the presence of polyps.

14. The method of claim 13 wherein the disease state comprises cancer, further comprising determining at least one cancer stage associated with the biological sample.

15. The method of claim 1 wherein the cancer further comprises colorectal cancer.

16 The method of claim 13 wherein the disease state comprises normal, further comprising determining at least one non-cancerous condition associated with the biological sample.

17. The method of claim 16 wherein the non-cancerous condition further comprises at least one of: an immune response and an inflammatory response.

18. The method of claim 1 wherein the illuminating is further achieved by using an infrared laser.

19. The method of claim 18 wherein the infrared laser further comprise a 785 nm laser.

20. The method of claim 1 wherein the Raman data set further comprises at least one Raman spectrum.

21. The method of claim 1 wherein the Raman data set further comprises at least one Raman chemical image.

22. The method of claim 1 wherein analyzing further comprises applying at least one algorithmic technique.

23. The method of claim 22 wherein the algorithmic technique further comprises at least one chemometric technique.

24. The method of claim 23 wherein the chemometric technique further comprises at least one of: multivariate curve resolution, principal component analysis, k means clustering, band t. entropy method, adaptive subspace detector, cosine correlation analysis, Euclidian distance analysis, partial least squares regression, spectral mixture resolution, a spectral angle mapper metric, a spectral information divergence metric, a Mahalanobis distance metric, and spectral unmixing.

25. The method of claim 23 wherein the chemometric technique further comprises partial least squares discriminant analysis.

26. The method of claim 22 wherein the algorithmic technique further comprises support vector machines.

27. The method of claim 1 further comprising generating at least one RGB image representative of the biological sample.

28. The method of claim 27 further comprising fusing the RGB image and the Raman data set.

29. The method of claim 1 wherein analyzing the Raman data set further comprises applying at least one of: whole patient outlier rejection and intra-patient outlier rejection.

30. The method of claim 1 wherein analyzing the Raman data set further comprises applying at least one calibration transfer function, wherein the calibration transfer function further comprises a piece-wise linear function.

31. The method of claim 1 wherein analyzing the Raman data set further comprises assessing a protein conformation of the biological sample.

32. The method of claim 31 further comprising associating a disordered protein conformation with a disease state comprising cancer.

33. The method of claim 31 further comprising assessing at least one spectral feature of interest to thereby assess the protein conformation, wherein the spectral feature further comprises at least one of: a wavelength of approximately 1660cm^{-1}, a wavelength of approximately 941cm^{-1}, a wavelength range of approximately 1230cm^{-1}-1300cm^{-1}, and a wavelength ratio of approximately 857cm^{-1}/827cm^{-1}.

34. A system comprising:

an illumination source configured to illuminate at least one location of a biological sample to generate at least one plurality of interacted photons;

at least one mirror configured to direct the interacted photons to a spectrometer, wherein the spectrometer is configured to filter the interacted photons into a plurality of wavelengths;

at least one detector configured to detect the interacted photons and generate at least one Raman data set representative of the biological sample; and

at least one processor, configured to analyze the Raman data set and associated the biological sample with at least one disease state.

35. The system of claim 34 wherein the illumination source further comprises an infrared laser.

36. The system of claim 35 wherein the infrared laser further comprises a 785 nm laser.

37. The system of claim 34 further comprising a fiber array spectral translator device configured to receive the plurality of interacted photons, and wherein the fiber array spectral translator device further comprises a two dimensional array of optical fibers drawn into a one dimensional fiber stack so as to effectively convert a two-dimensional field of view into a curvilinear field of view, and wherein the one-dimensional fiber stack is optically coupled to the spectrometer.

38. The system of claim 34 wherein the detector further comprises at least one of: a CCD detector, an ICCD detector, an InGaAs Detector, an IbSb detector, and a MCT detector.

39. The system of claim 34 further comprising a RGB detector configured to generate a at least one RGB image representative of the biological sample.

40. The system of claim 39 further comprising at least one mirror configured to direct the interacted photons to the RGB detector.

41. The system of claim 34 further comprising a stage configured for holding a biological sample.

42. The system of claim 34 further comprising at least one reference data set, wherein the reference data set is associated with at least one known disease state.

43. The system of claim 42 wherein known disease state further comprises at least one of: cancer, normal, and polyp.

44. The system of claim 42 wherein the known disease state further comprises at least one non-cancerous condition.

45. The system of claim 44 wherein the non-cancerous disease state further comprises at least one of: an immune response and an inflammatory response.

46. The system of claim 34 wherein the Raman data set further comprises at least one Raman spectrum.

47. The system of claim 34 wherein the Raman data set further comprises at least one Raman chemical image.

48. The system of claim 34 wherein the illumination source is further configured to illuminate the sample using wide-field illumination.

49. The system of claim 34 wherein the illumination source is further configured to illuminate the biological sample at a plurality of points.

50. The system of claim 49 wherein the plurality of points further comprises a line.

51. The system of claim 34 wherein the illumination source is further configured for illuminating the sample using structured illumination.

52. The system of claim 34 wherein the processor is further configured to analyze the Raman data set by applying at least one algorithmic technique.

53. The system of claim 52 wherein the analyzing further comprises comparing the Raman data set to at least one reference data set.

54. A non-transitory storage medium containing machine readable program code, which, when executed by a processor, causes the processor to perform the following:

illuminate at least one location of a biological sample to generate a plurality of interacted photons;

collect the plurality of interacted photons;

detect the plurality of interacted photons and generating at least one Raman data set representative of the biological sample;

analyze the Raman data set to associate the biological sample with at least one disease state.

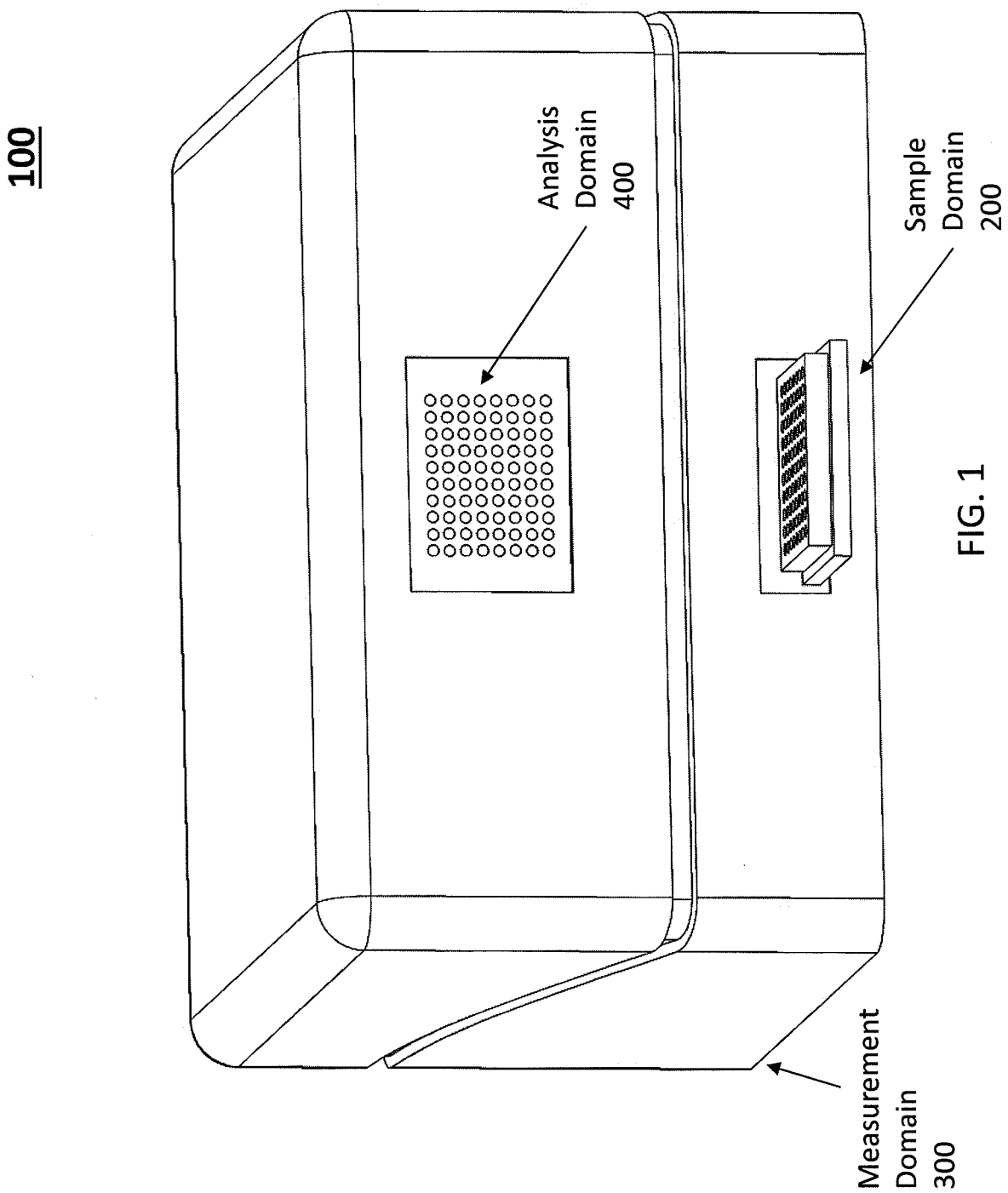

100
Analysis Domain 400
Sample Domain 200
Measurement Domain 300

FIG. 1

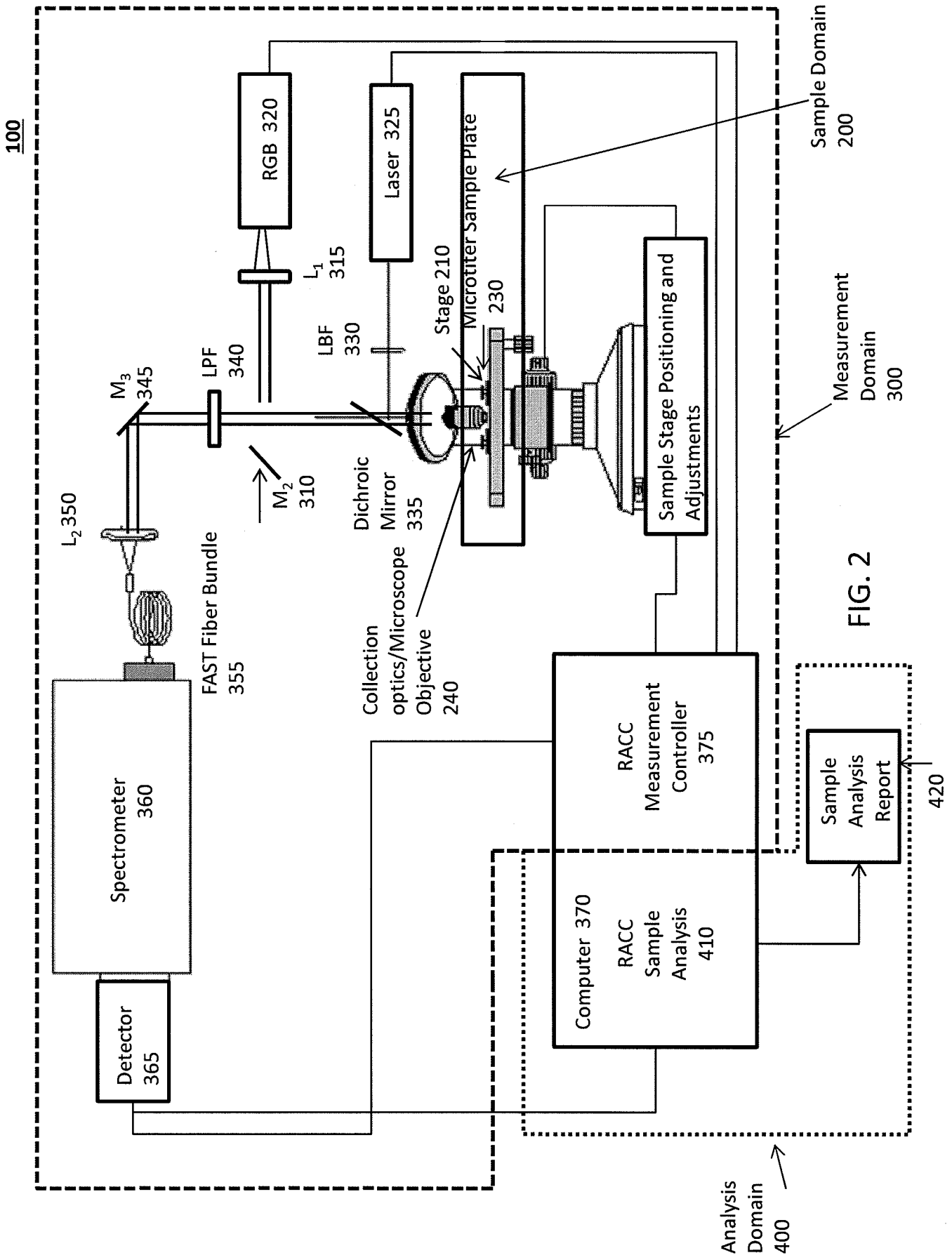

100
RGB 320
Laser 325
Sample Domain 200
Microtiter Sample Plate 230
Stage 210
L_1 315
LBF 330
LPF 340
M_3 345
M_2 310
Dichroic Mirror 335
Sample Stage Positioning and Adjustments
Measurement Domain 300
L_2 350
FAST Fiber Bundle 355
Collection optics/Microscope Objective 240
Spectrometer 360
Detector 365
RACC Measurement Controller 375
Computer 370
RACC Sample Analysis 410
Sample Analysis Report
420
Analysis Domain 400

FIG. 2

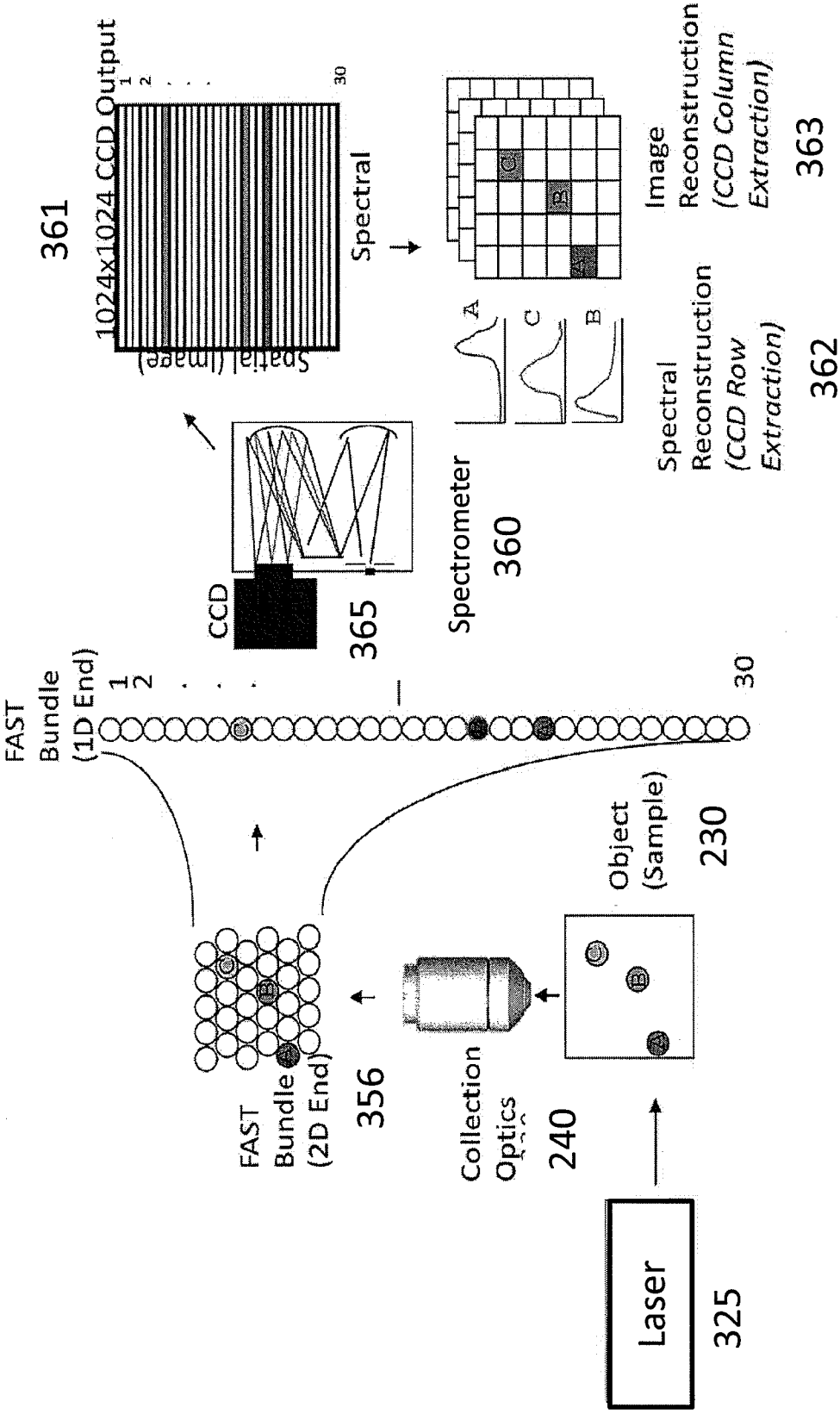

Laser
325
Object (Sample)
230
Collection Optics
240
FAST Bundle (2D End)
356
FAST Bundle (1D End)
357
CCD
365
Spectrometer
360
Spectral Reconstruction (CCD Row Extraction)
362
Image Reconstruction (CCD Column Extraction)
363
1024x1024 CCD Output
361
Spatial (Image)
Spectral

FIG. 3A

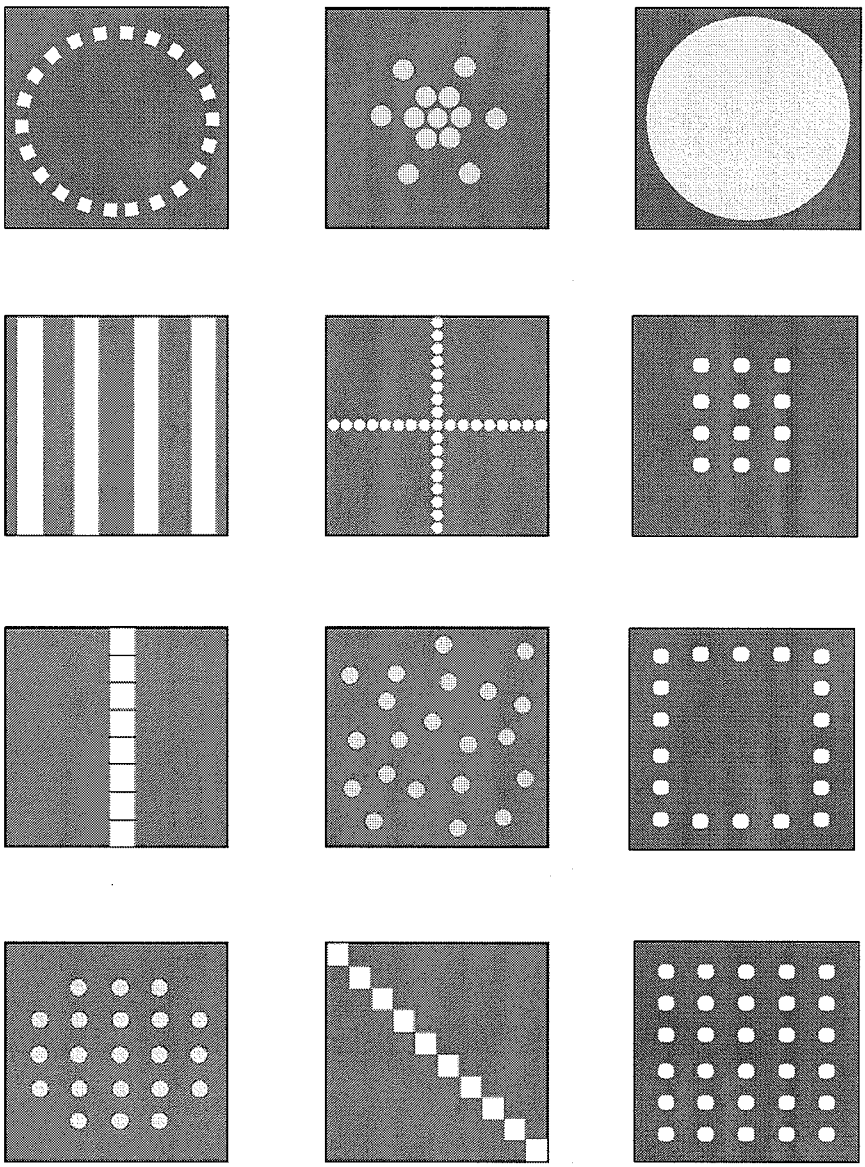


FIG. 3B

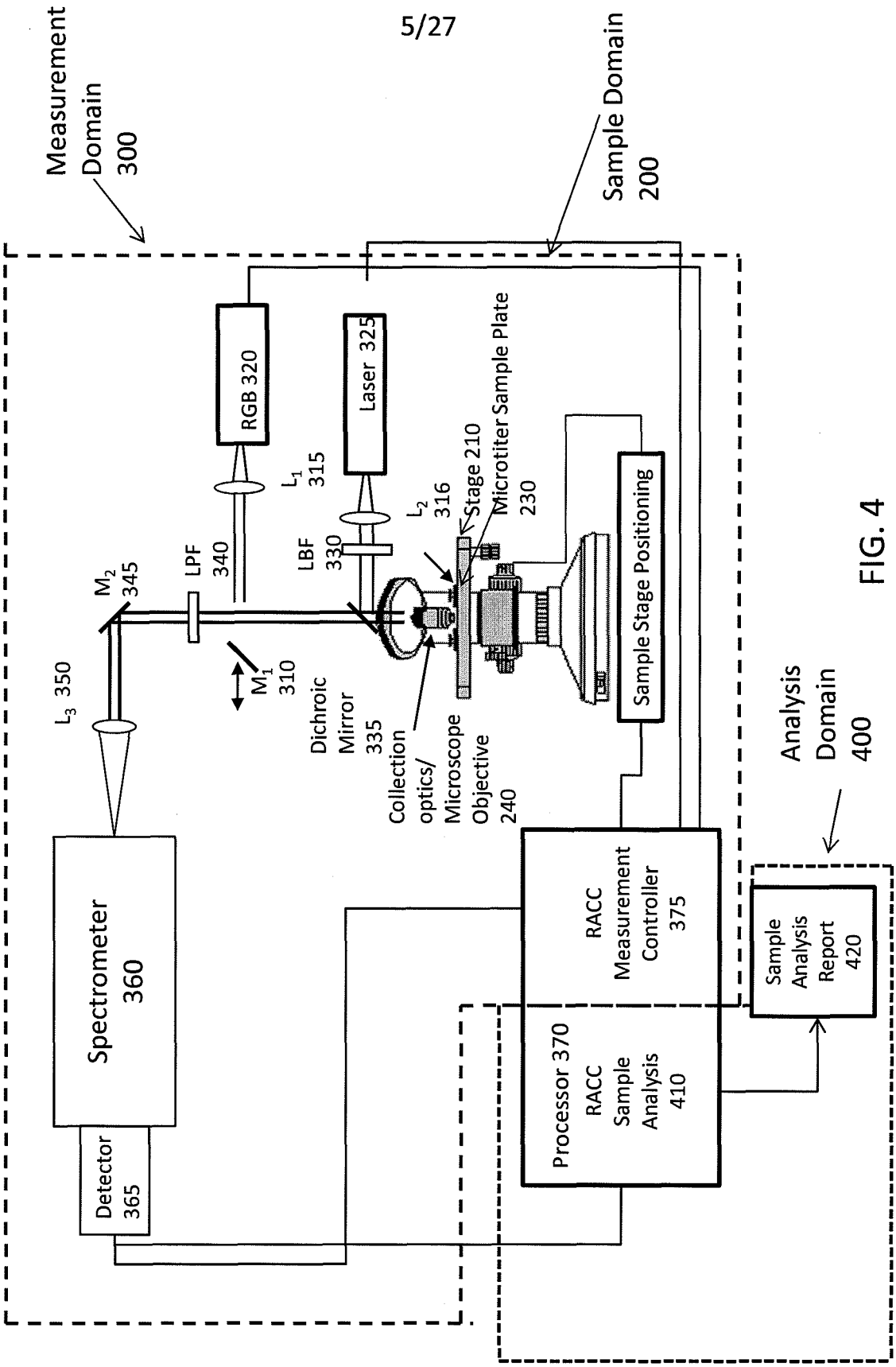

Measurement Domain 300
Sample Domain 200
Analysis Domain 400
RGB 320
Laser 325
L_1 315
L_2 316
Stage 210
Microtiter Sample Plate 230
LPF 340
LBF 330
M_2 345
M_1 310
L_3 350
Dichroic Mirror 335
Collection optics/ Microscope Objective 240
Sample Stage Positioning
Spectrometer 360
Detector 365
RACC Measurement Controller 375
Processor 370
RACC Sample Analysis 410
Sample Analysis Report 420

FIG. 4

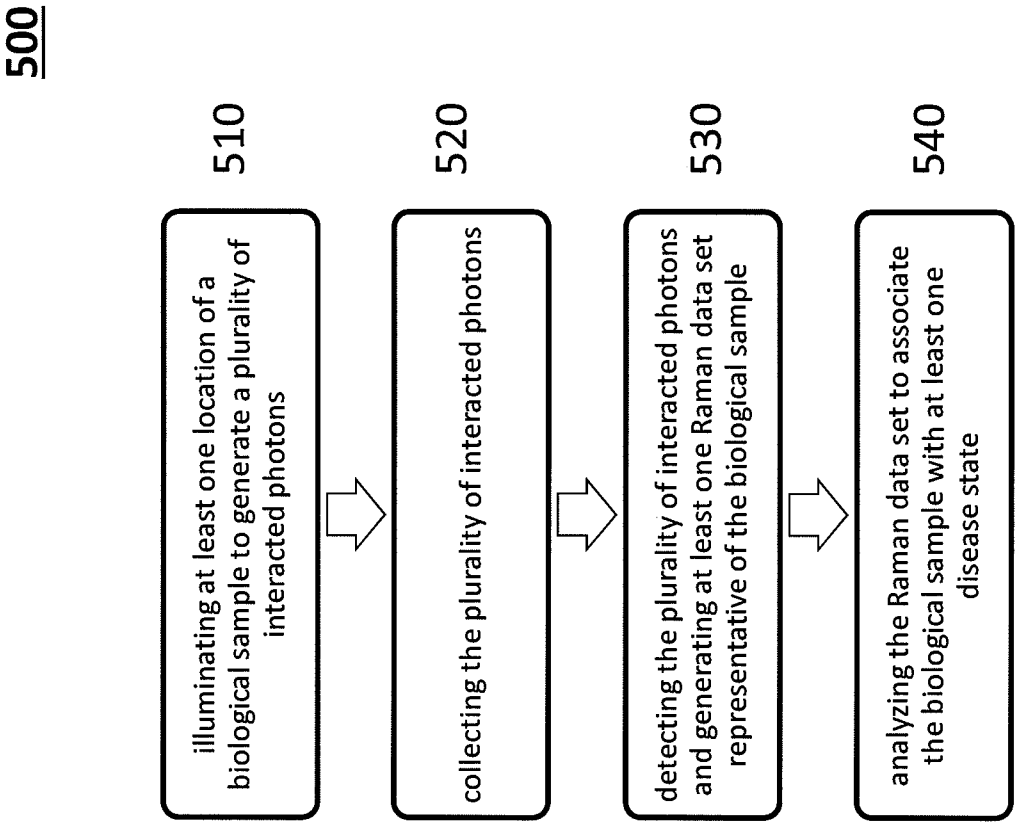

500
510
illuminating at least one location of a biological sample to generate a plurality of interacted photons
520
collecting the plurality of interacted photons
530
detecting the plurality of interacted photons and generating at least one Raman data set representative of the biological sample
540
analyzing the Raman data set to associate the biological sample with at least one disease state

FIG. 5A

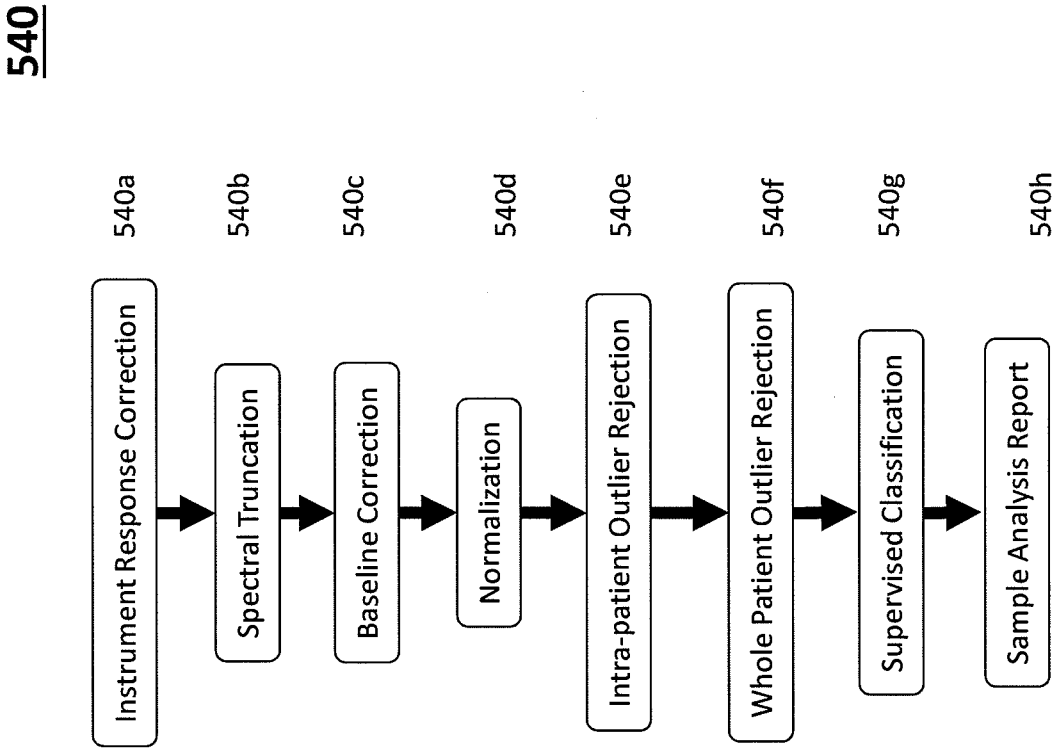

540
540a
Instrument Response Correction
540b
Spectral Truncation
540c
Baseline Correction
540d
Normalization
540e
Intra-patient Outlier Rejection
540f
Whole Patient Outlier Rejection
540g
Supervised Classification
540h
Sample Analysis Report

FIG. 5B

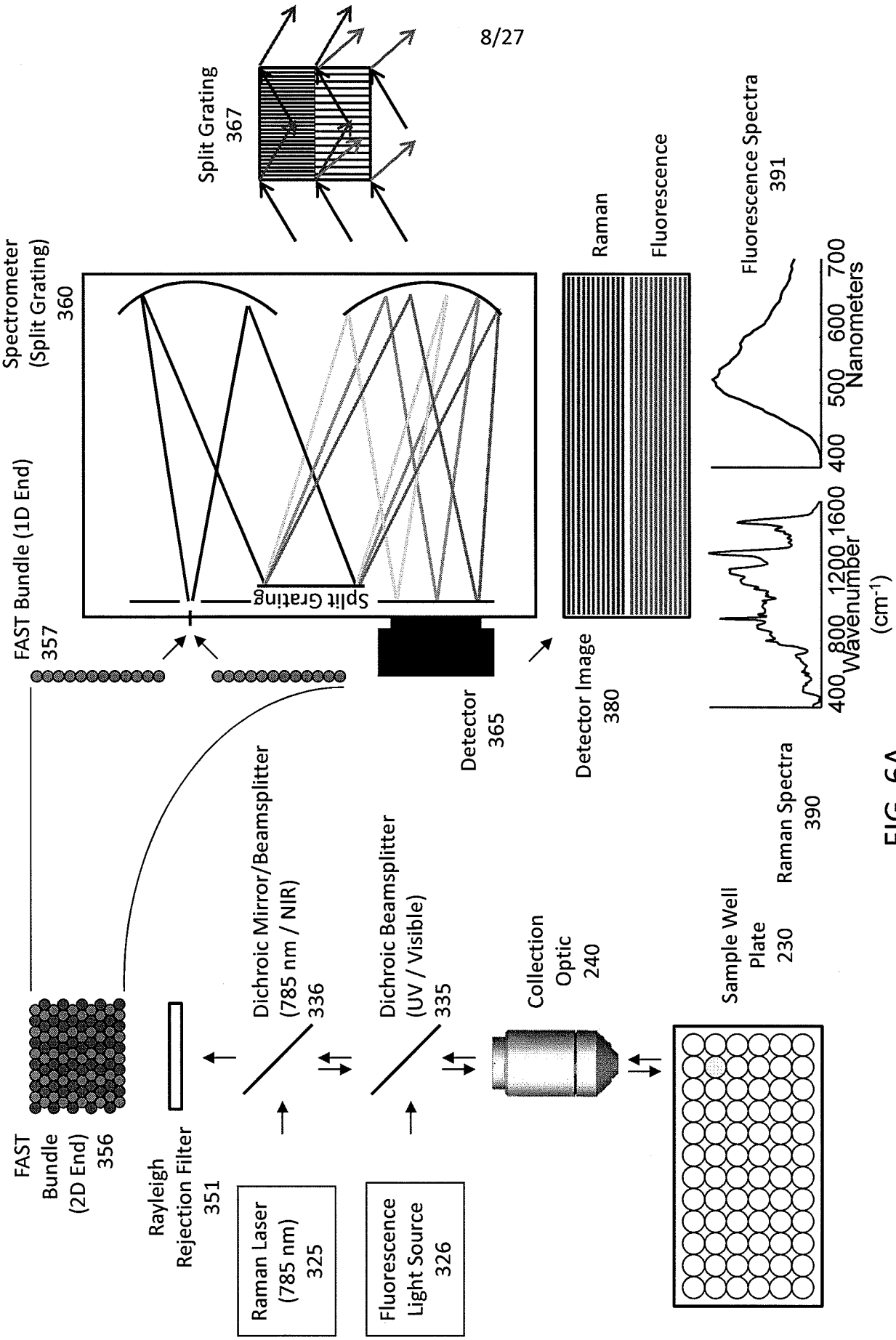

Split Grating
367
Spectrometer
(Split Grating)
360
FAST Bundle (1D End)
357
Split Grating
Raman
Fluorescence
Fluorescence Spectra
391
400 500 600 700
Nanometers
400 800 1200 1600
Wavenumber
(cm-1)
Detector
365
Detector Image
380
Raman Spectra
390
Sample Well
Plate
230
Collection
Optic
240
Dichroic Mirror/Beamsplitter
(785 nm / NIR)
336
Dichroic Beamsplitter
(UV / Visible)
335
FAST
Bundle
(2D End)
356
Rayleigh
Rejection Filter
351
Raman Laser
(785 nm)
325
Fluorescence
Light Source
326

FIG. 6A

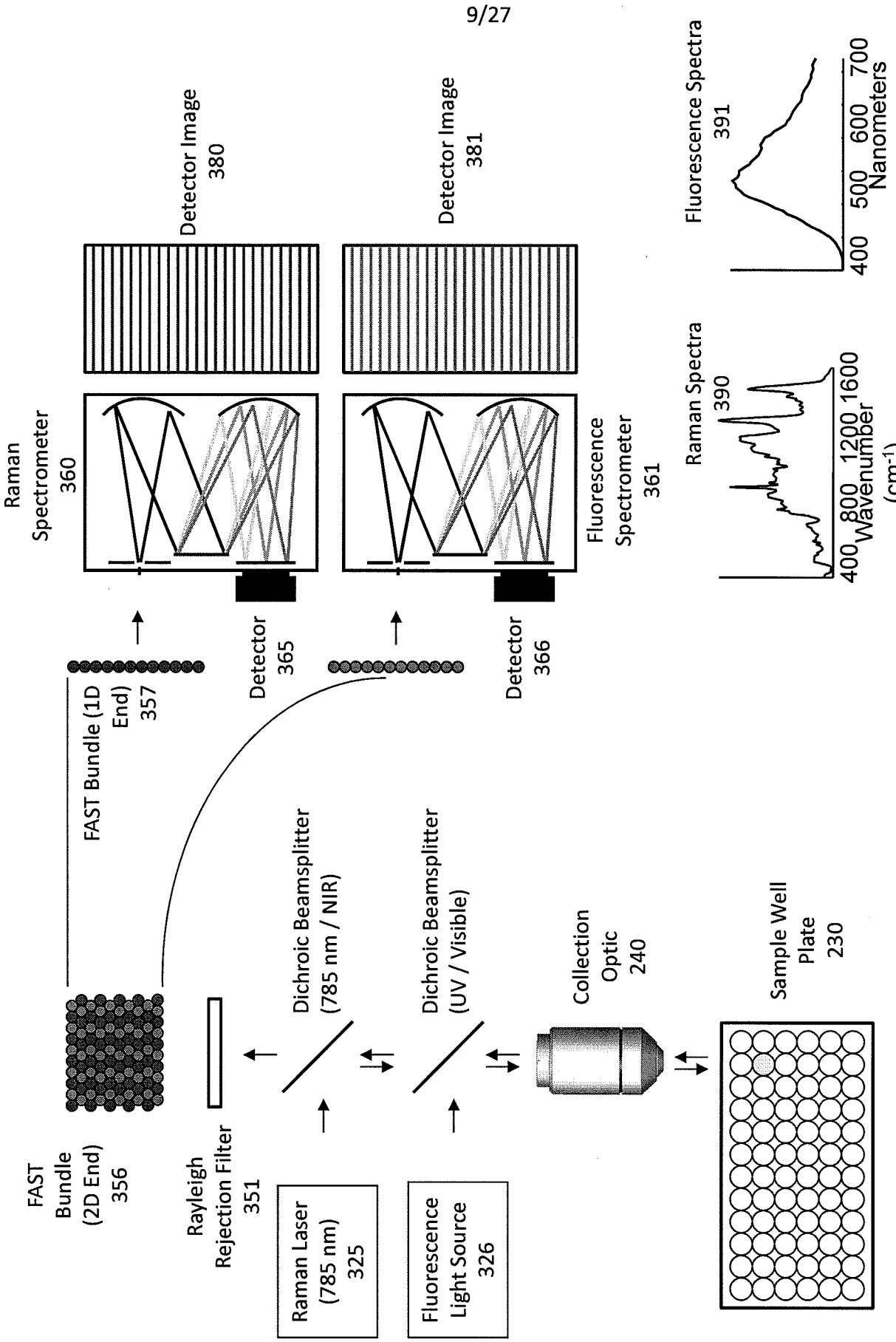

FAST Bundle (2D End) 356
Rayleigh Rejection Filter 351
Raman Laser (785 nm) 325
Fluorescence Light Source 326
Dichroic Beamsplitter (785 nm / NIR)
Dichroic Beamsplitter (UV / Visible)
Collection Optic 240
Sample Well Plate 230
FAST Bundle (1D End) 357
Detector 365
Detector 366
Raman Spectrometer 360
Fluorescence Spectrometer 361
Detector Image 380
Detector Image 381
Raman Spectra 390
400 800 1200 1600
Wavenumber (cm-1)
Fluorescence Spectra 391
400 500 600 700
Nanometers

FIG. 6B

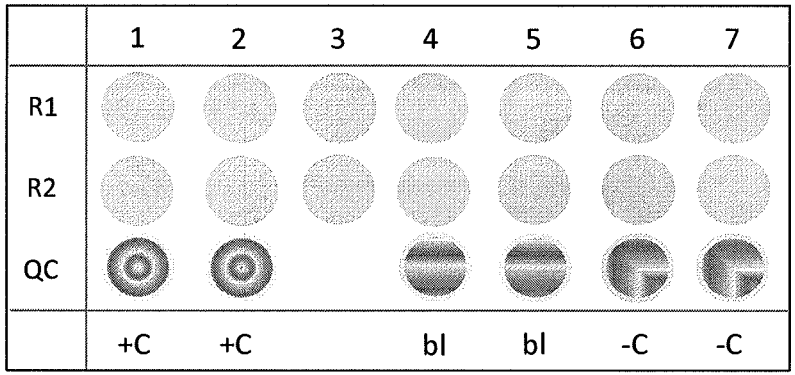


FIG. 7A – Aluminum-coated glass slide (Low Throughput Configuration); 7 patients; 2 duplicates

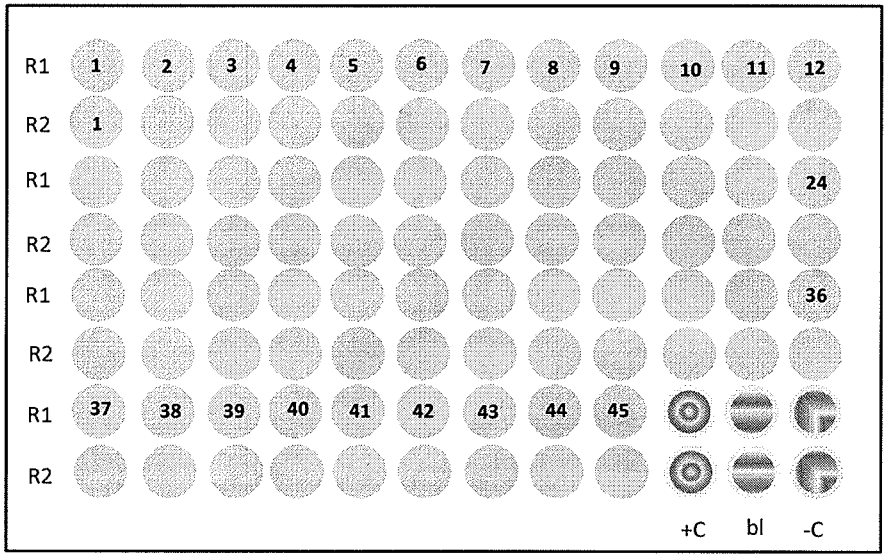


FIG. 7B – 96-Well Plate (High Throughput Configuration) 45 patients; 2 duplicates

Legend

- bl = blank
- +C = positive control
- - C = negative control

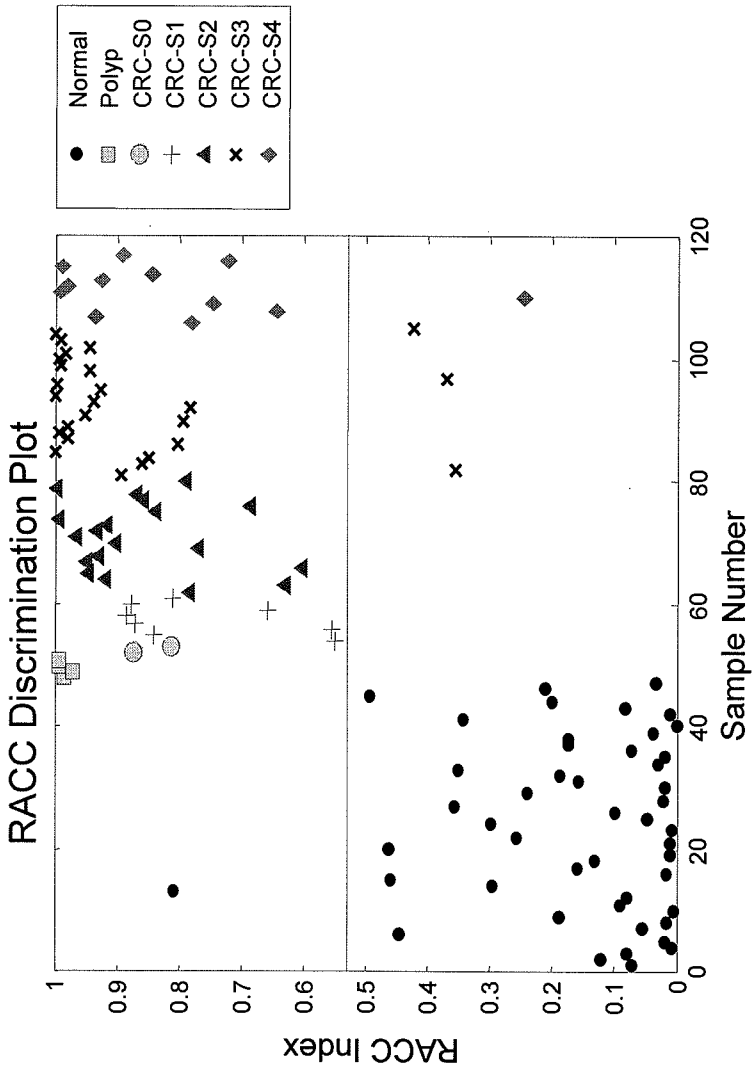

RACC Discrimination Plot
Normal
Polyp
CRC-S0
CRC-S1
CRC-S2
CRC-S3
CRC-S4
RACC Index
Sample Number
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
100
120

FIG. 8A

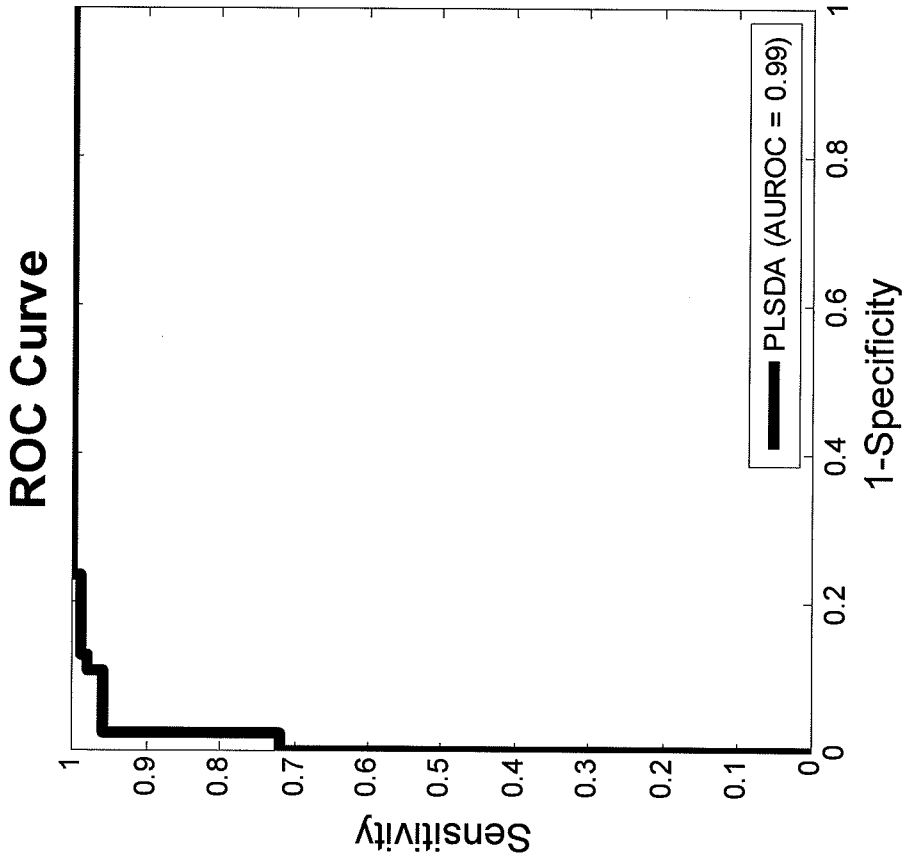

ROC Curve
Sensitivity
1-Specificity
PLSDA (AUROC = 0.99)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0.2
0.4
0.6
0.8

FIG. 8B

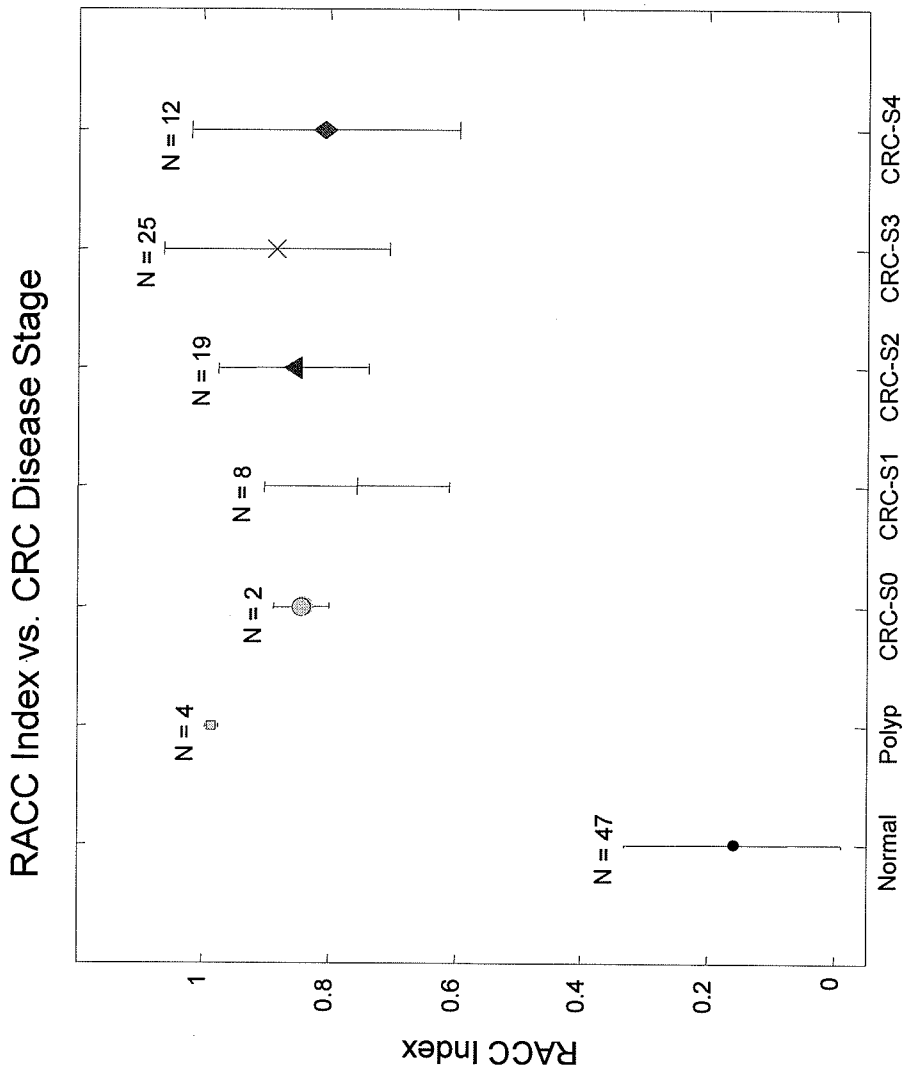

RACC Index vs. CRC Disease Stage
RACC Index
0
0.2
0.4
0.6
0.8
1
N = 4
N = 2
N = 8
N = 19
N = 25
N = 12
N = 47
Normal
Polyp
CRC-S0
CRC-S1
CRC-S2
CRC-S3
CRC-S4
CRC Disease Stage

FIG. 8C

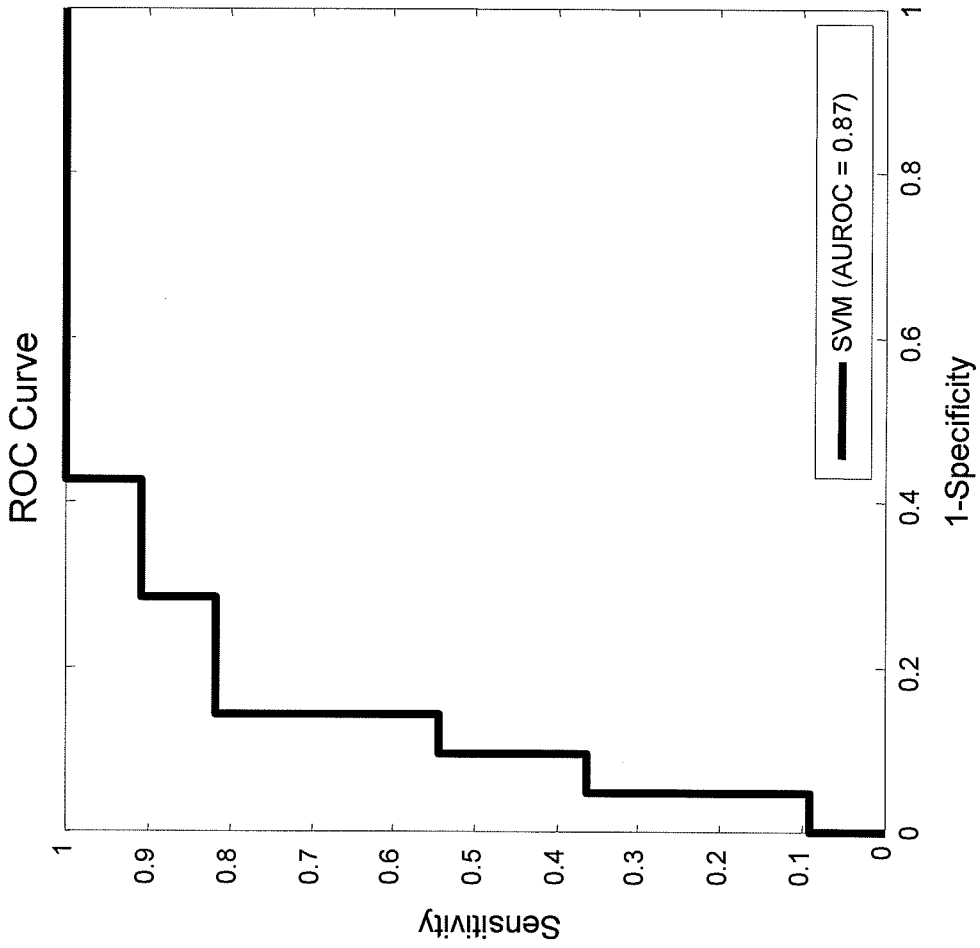

ROC Curve
Sensitivity
1-Specificity
SVM (AUROC = 0.87)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
0.2
0.4
0.6
0.8
1

FIG. 9A

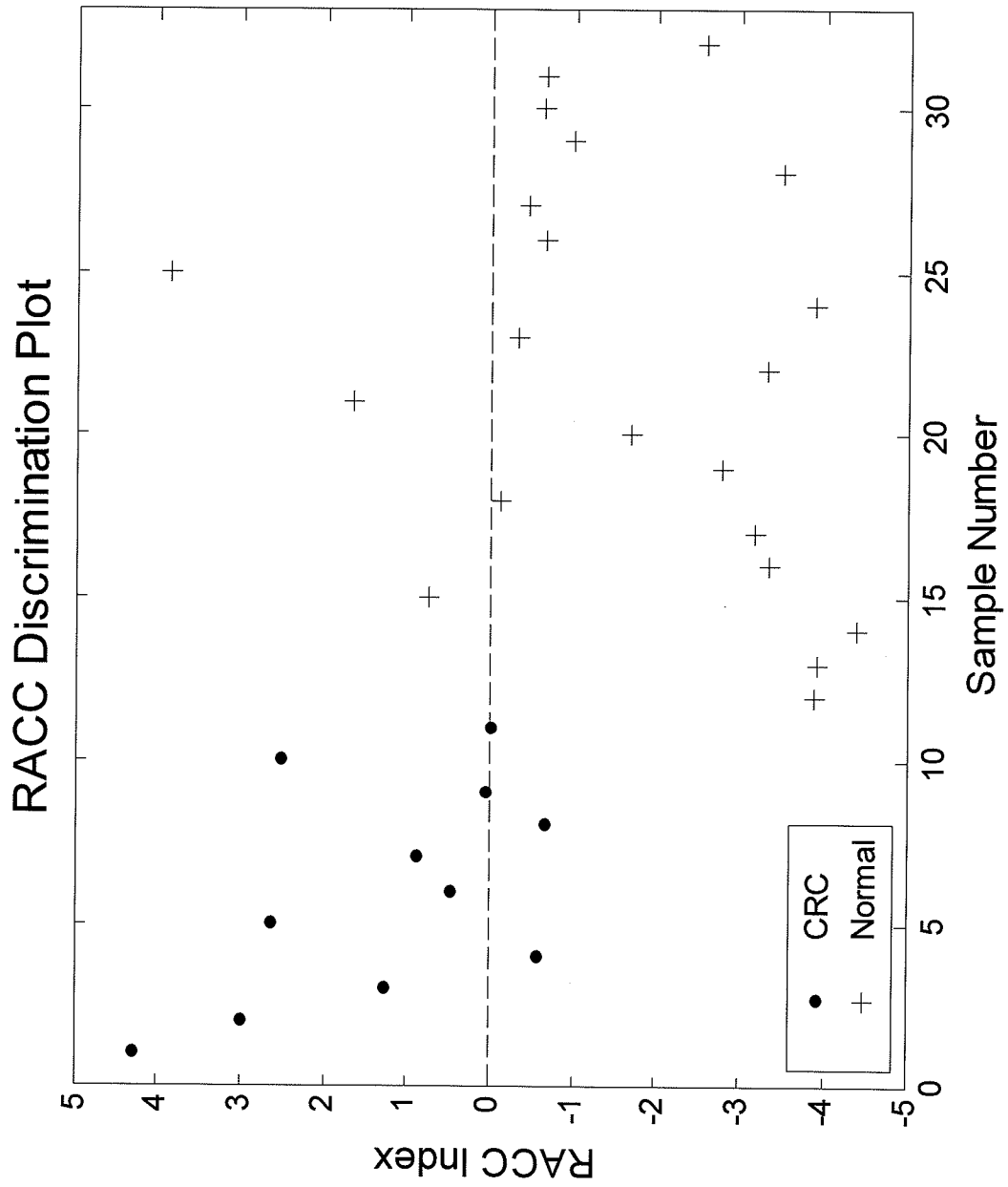

RACC Discrimination Plot
RACC Index
Sample Number
CRC
Normal
5
4
3
2
1
0
-1
-2
-3
-4
-5
0
5
10
15
20
25
30

FIG. 9B

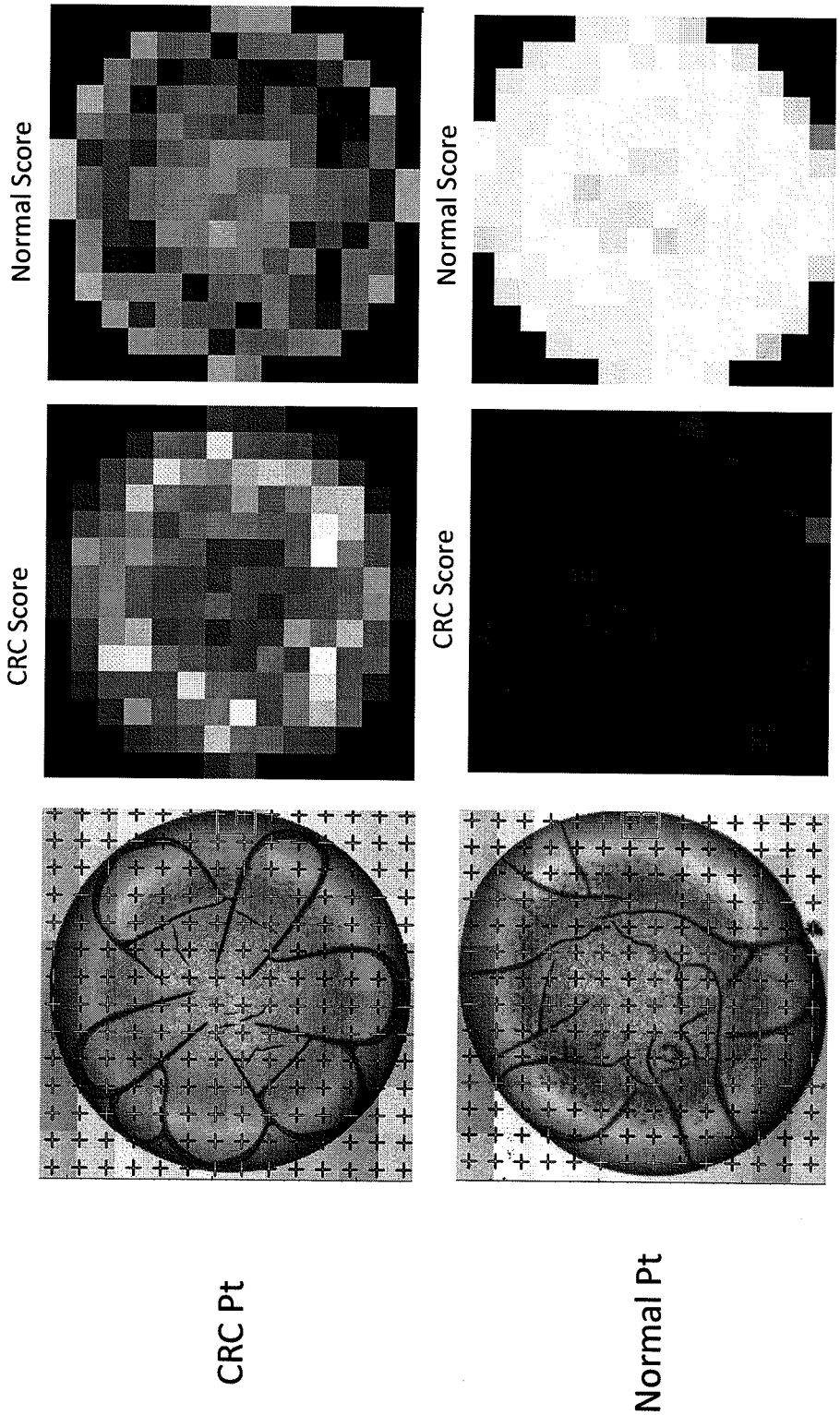

Normal Score
Normal Score
CRC Score
CRC Score
CRC Pt
Normal Pt

FIG. 10

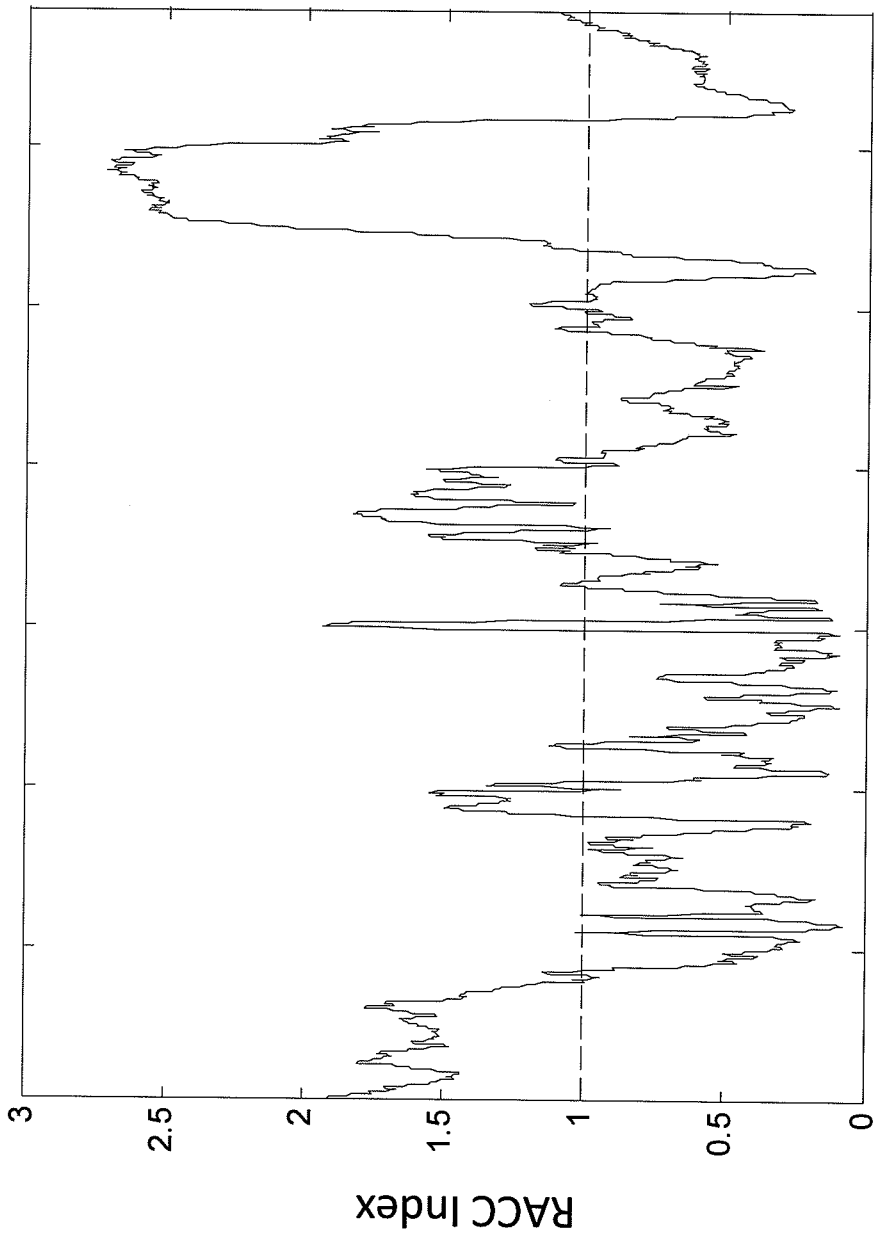

RACC Index
0
0.5
1
1.5
2
2.5
3
600
800
1000
1200
1400
1600
Wavenumber (cm-1)

FIG. 11B

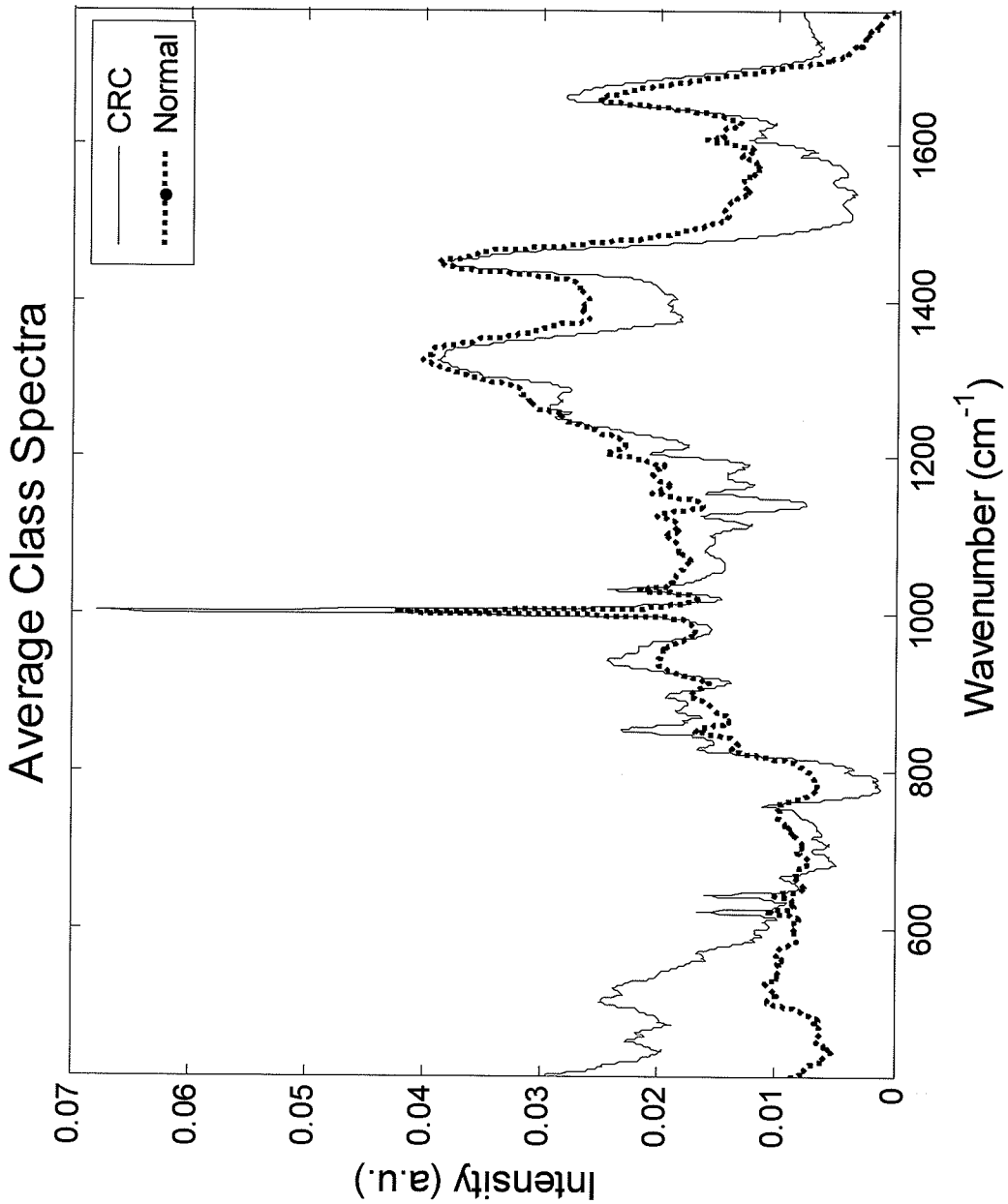
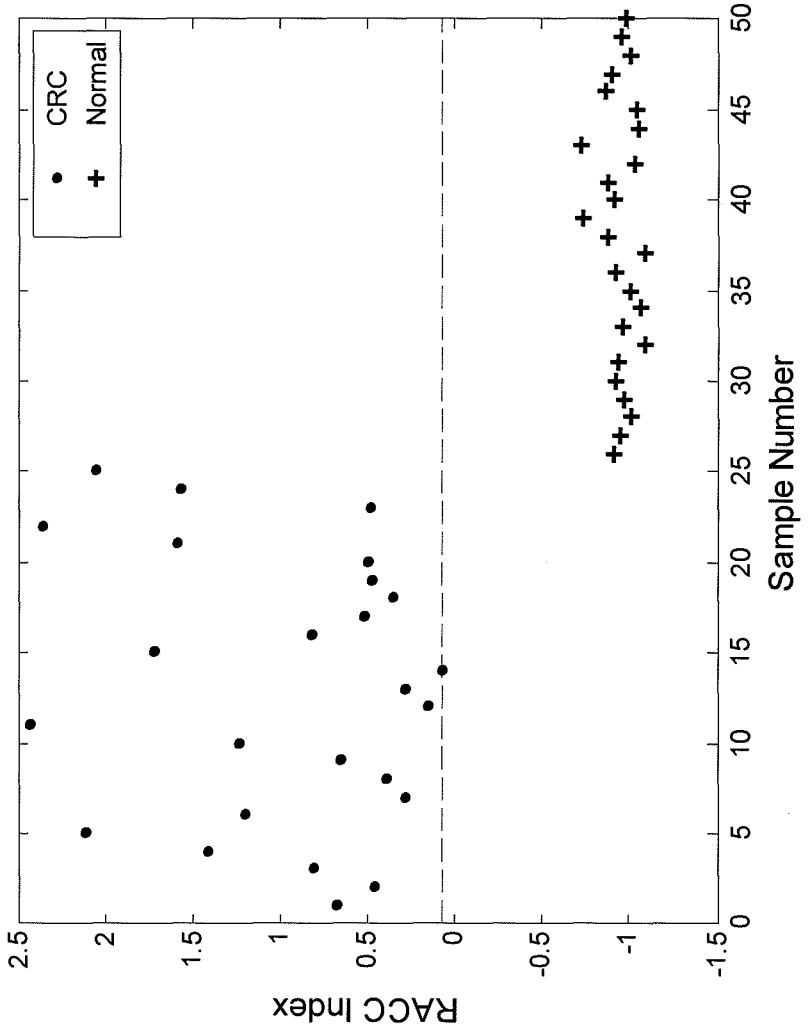

Average Class Spectra
CRC
Normal
Intensity (a.u.)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
600
800
1000
1200
1400
1600
Wavenumber (cm-1)

FIG. 11A


CRC
Normal
RACC Index
Sample Number
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
0
5
10
15
20
25
30
35
40
45
50

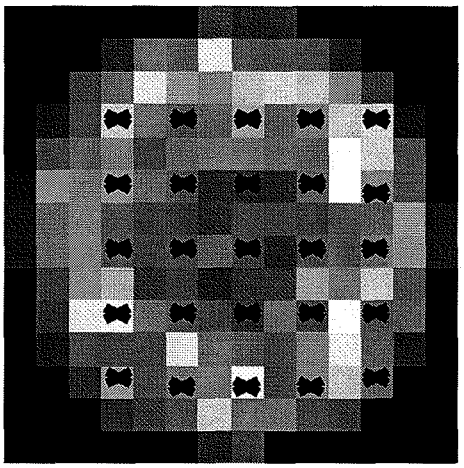
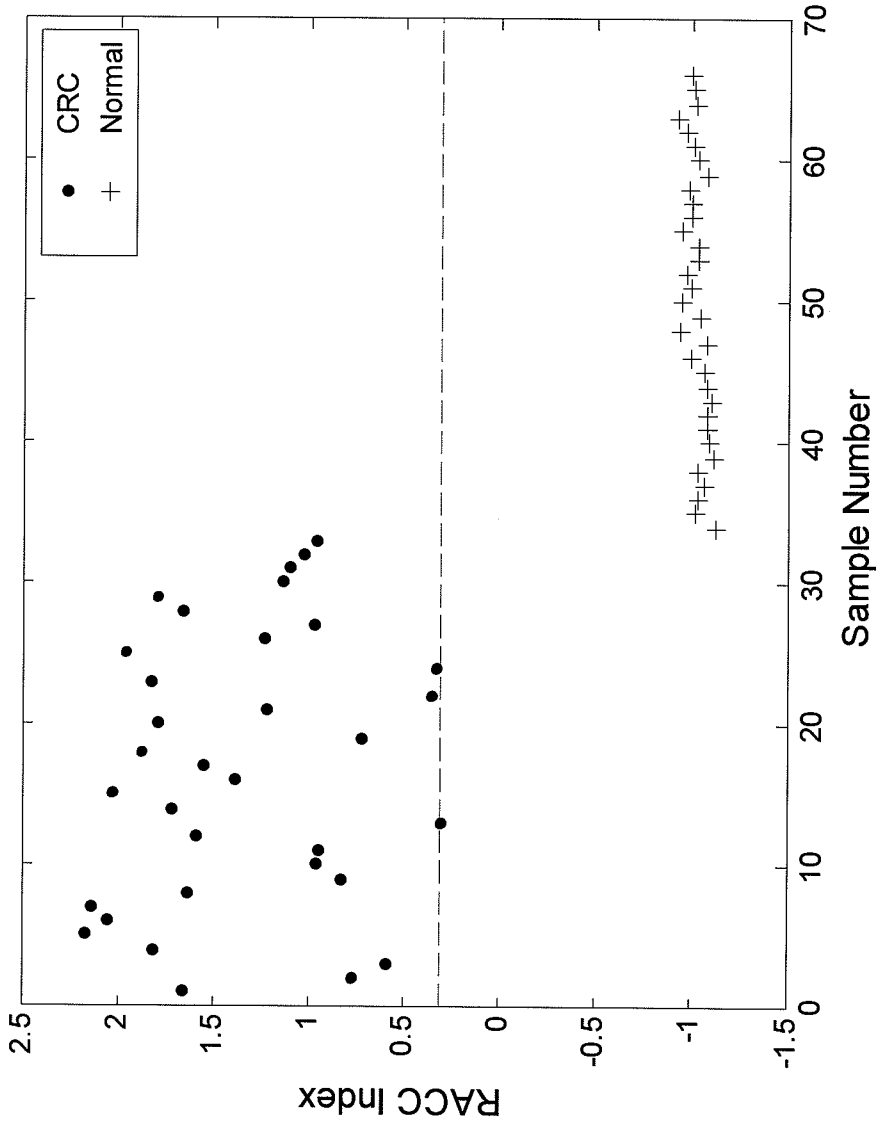


FIG. 12A


CRC
Normal
RACC Index
Sample Number
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
0
10
20
30
40
50
60
70

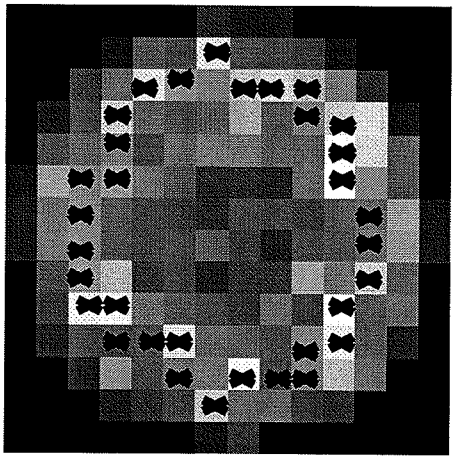
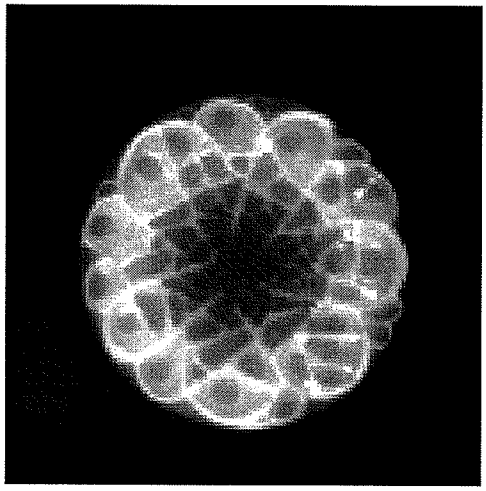


FIG. 12B

	Class	Mean	Std Dev	Difference Between Means	% Difference Between Means	Difference Between Std Dev	% Difference Between Std Dev
Grid Pattern	CRC	0.9863	0.7286	1.9399	22%	0.634	-21%
	Normal	-0.9536	0.0946				
Ring Pattern	CRC	1.3393	0.5522	2.3664		0.4995	
	Normal	-1.0271	0.0527				

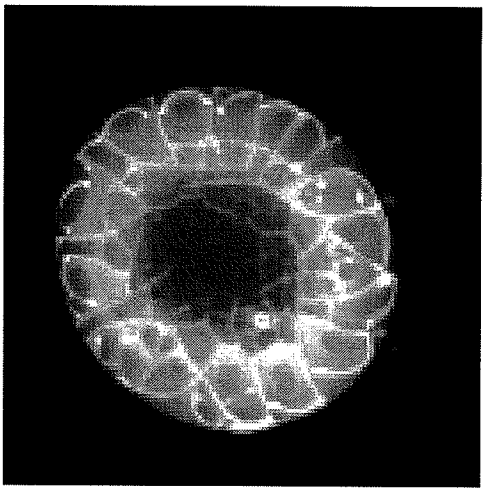
FIG. 12C

FIG. 13A



CRC Pt

FIG. 13B



Normal Pt

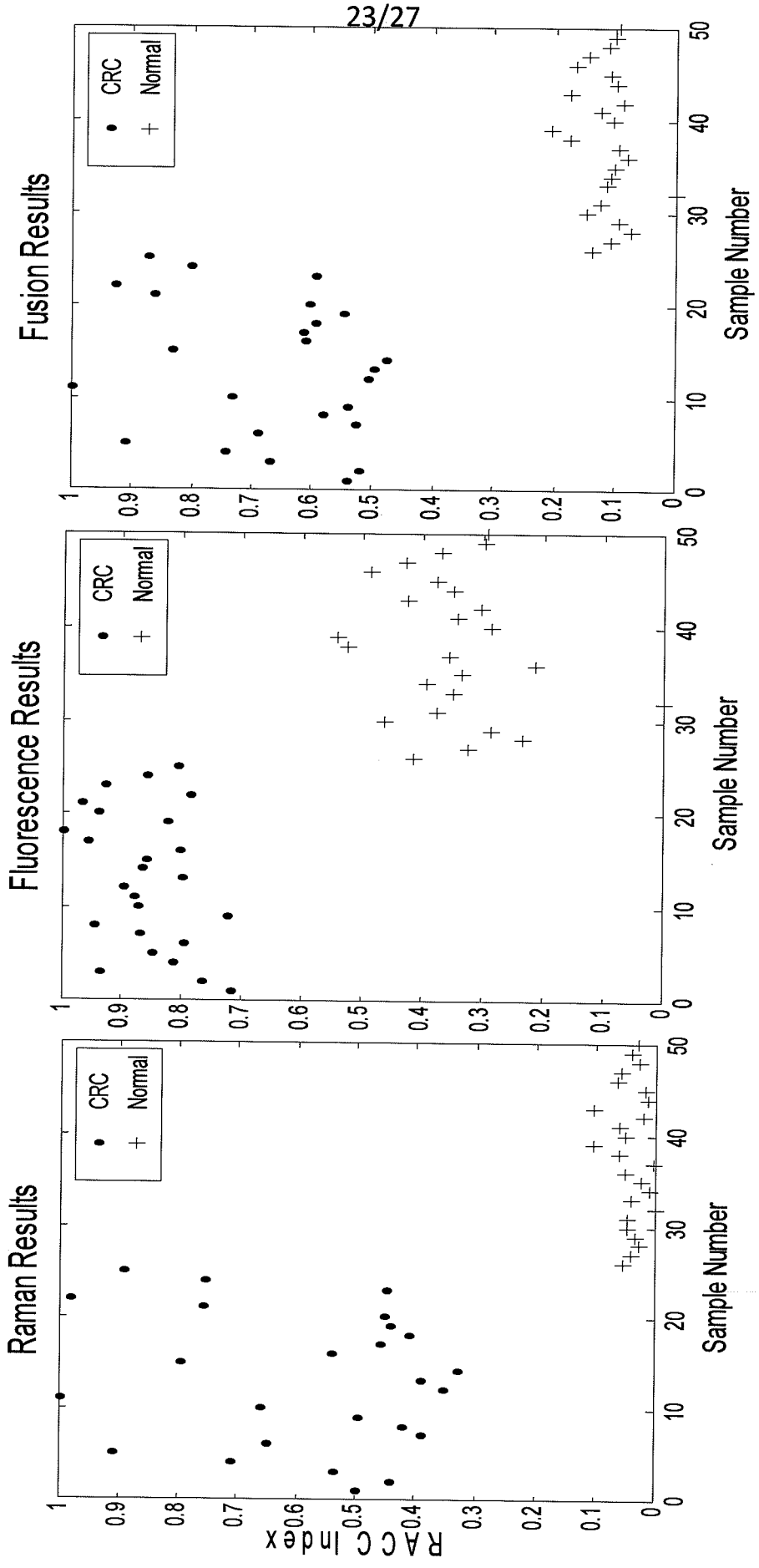

Raman Results
Fluorescence Results
Fusion Results
CRC
Normal
RACC Index
Sample Number

FIG. 14

Spectral Feature (cm^{-1})	Assignment	α-Helix Conformation	Random Coil Conformation
1660	Amide I	Decreases	Increases
1230-1300	Amide III	Increase at 1263	No change at 1263
941	C-C Stretch of Polypeptide Backbone	Increase at 938	No change at 938
857/827	Tyrosine Fermi Resonance Doublet	Decrease ratio (1.1)	Increase ratio (1.2)

FIG. 16

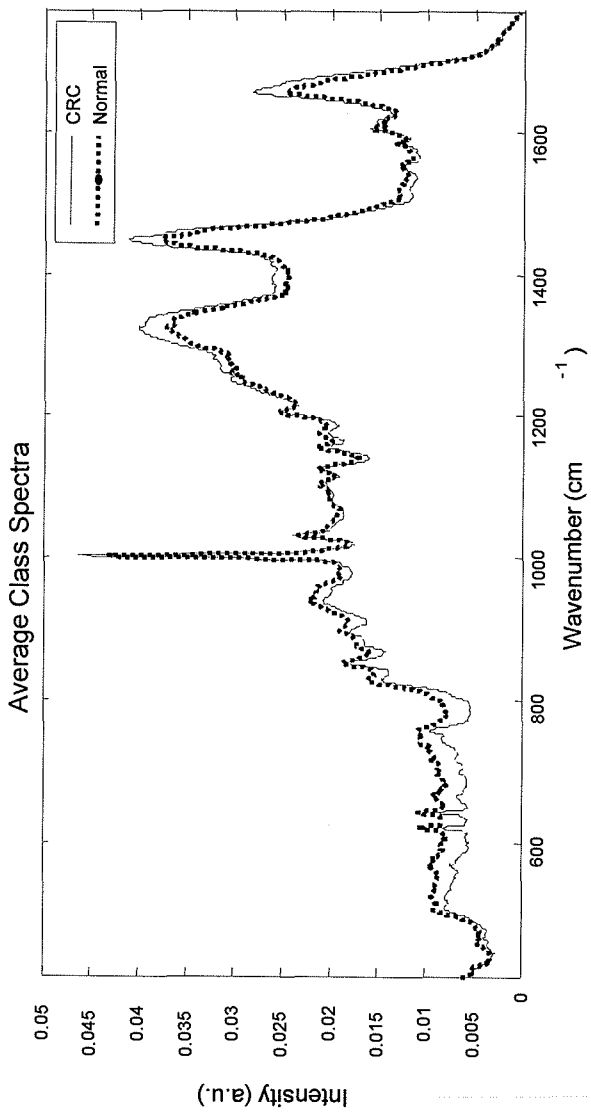

Average Class Spectra
CRC
Normal
Intensity (a.u.)
0.05
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
600
800
1000
1200
1400
1600
Wavenumber (cm -1)

FIG. 15

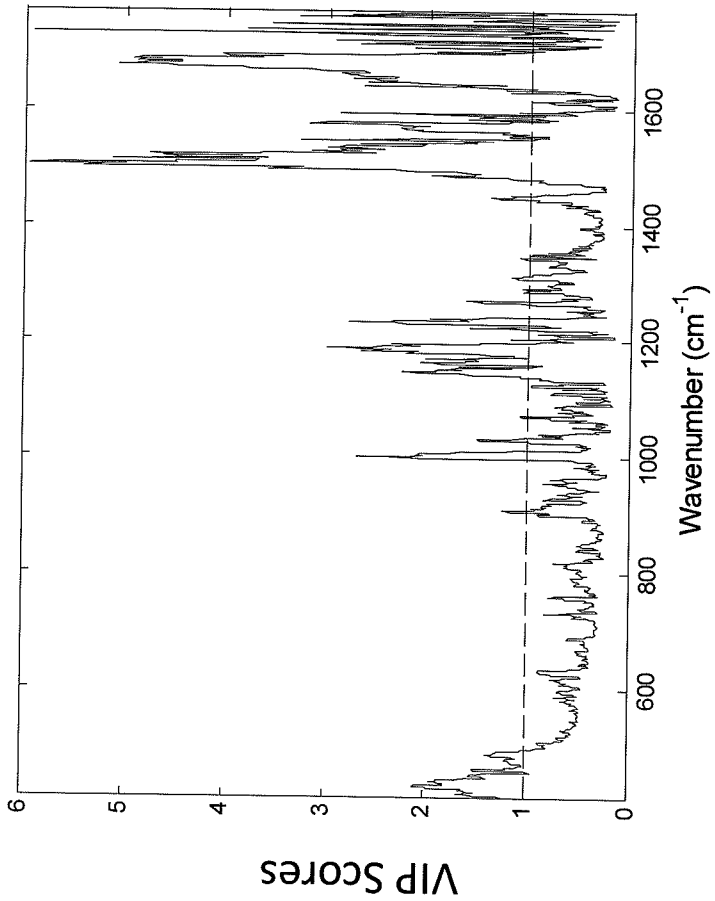

VIP Scores
0
1
2
3
4
5
6
600
800
1000
1200
1400
1600
Wavenumber (cm-1)

FIG. 17

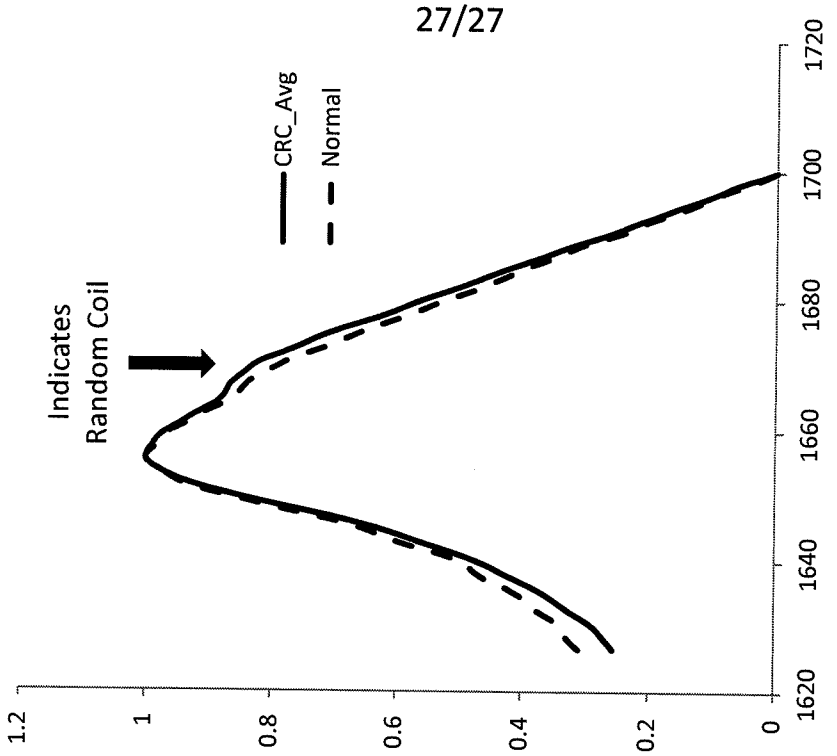


FIG. 18B

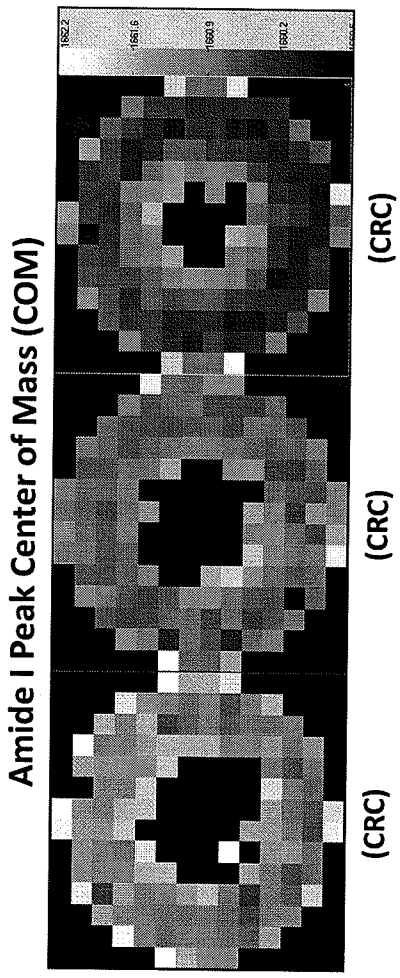


FIG. 18A

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/068671

A. CLASSIFICATION OF SUBJECT MATTER

G01N 33/574(2006.01)i, G01N 33/48(2006.01)i, G01N 33/53(2006.01)i

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)
G01N 33/574; G01J 1/32; G01J 3/44; A61B 6/00; A61B 1/267; G01N 21/65; G01N 33/48; G01N 33/53

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & keywords: illumination, interacted photons, Raman data, disease state

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 7990533 B2 (MAIER et al.) 2 August 2011 See abstract, claims 1, 9, 10, 15 and columns 2-4, 6-10, 12.	1-54
A	WO 2011-088580 A1 (BRITISH COLUMBIA CANCER AGENCY BRANCH) 28 July 2011 See abstract, claims 37, 38 and paragraph [0010].	1-54
A	US 2008-0117416 A1 (HUNTER et al.) 22 May 2008 See abstract and claims 1, 7, 17.	1-54
A	US 2012-0200850 A1 (STEWART et al.) 9 August 2012 See abstract and claim 1.	1-54
A	US 7060955 B1 (WANG) 13 June 2006 See abstract and claims 1, 12.	1-54

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

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"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
05 February 2014 (05.02.2014)	**05 February 2014 (05.02.2014)**

Name and mailing address of the ISA/KR
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 302-701, Republic of Korea
Facsimile No. +82-42-472-7140

Authorized officer
KIM, Seung Beom
Telephone No. +82-42-481-3371

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2013/068671

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 7990533 B2	02/08/2011	US 2009-0002702 A1	01/01/2009
		US 2010-0280762 A1	04/11/2010
		US 7755757 B2	13/07/2010
		WO 2009-035942 A1	19/03/2009
WO 2011-088580 A1	28/07/2011	CA 2786740 A1	28/07/2011
		CN 102740762 A	17/10/2012
US 2008-0117416 A1	22/05/2008	EP 2076172 A2	08/07/2009
		EP 2076172 A4	21/03/2012
		WO 2008-052221 A2	02/05/2008
		WO 2008-052221 A3	28/08/2008
		WO 2008-052221 A9	26/06/2008
US 2012-0200850 A1	09/08/2012	None	
US 7060955 B1	13/06/2006	CN 101072993 A	14/11/2007
		JP 2008-529000 A	31/07/2008
		US 2007-0007444 A1	11/01/2007
		US 7045757 B1	16/05/2006
		US 7268330 B2	11/09/2007

专利名称(译)	用于基于血清的癌症检测的系统和方法		
公开(公告)号	EP2917734A4	公开(公告)日	2016-09-07
申请号	EP2013853852	申请日	2013-11-06
[标]申请(专利权)人(译)	TREADO PATRICK STEWART绍纳语 KIRSCHNER HEATHER RYAN的Priore WILSON ALAN		
申请(专利权)人(译)	CHEMIMAGE CORPORATION TREADO，PATRICK STEWART，绍纳语 KIRSCHNER，希瑟 的Priore，RYAN WILSON，ALAN		
当前申请(专利权)人(译)	CHEMIMAGE CORPORATION TREADO PATRICK STEWART绍纳语 KIRSCHNER HEATHER RYAN的Priore WILSON ALAN		
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摘要(译)

一种用于分析生物样品，诸如干燥的人血清的系统和方法，以确定疾病状态例如结肠直肠癌（CRC）。使用干燥的样品可以保持用于增强局部浓度和/或样品成分的分割电位。该方法可包括照射生物样品中的至少一个位置，以产生多个相互作用的光子，收集相互作用的光子，并生成至少一个拉曼数据集代表的生物样品。一种系统可以包括照明源照亮的生物样品中的至少一个位置，并产生至少一个所述多个相互作用的光子，至少一个反射镜，用于引导相互作用光子检测器。该检测器可以被配置为产生至少一个拉曼数据

集代表的生物样品。该系统和方法可以利用用于多点分析FAST装置或可经配置以分析使用线扫描结构的样品。