(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 January 2004 (15.01.2004)

PCT

(10) International Publication Number
WO 2004/005869 A1

(51) International Patent Classification[7]: G01J 1/58

(21) International Application Number: PCT/US2003/021312

(22) International Filing Date: 8 July 2003 (08.07.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/394,696 9 July 2002 (09.07.2002) US
10/243,535 13 September 2002 (13.09.2002) US

(71) Applicant: **MEDISPECTRA, INC.** [US/US]; 45 Hartwell Avenue, Lexington, MA 02421 (US).

(72) Inventors: **SCHOMACKER, Kevin, T.**; 6 George Street, Maynard, MA 01754 (US). **MEESE, Thomas**; 1195 Beacon Street, Apt.2, Brookline, MA 02446 (US). **OURADNIK, Michael**; 35 Jeffrey Road, Wayland, MA 01778 (US). **FLANAGAN, John**; 735 Plymouth Street, Holbrook, MA 02343 (US). **GAO, Harry**; 19 Garden Road, Stoneham, MA 02180 (US).

(74) Agent: **HAULBROOK, William, R.**; Testa, Hurwitz & Thibeault, LLP, High Street Tower, 125 High Street, Boston, MA 02110 (US).

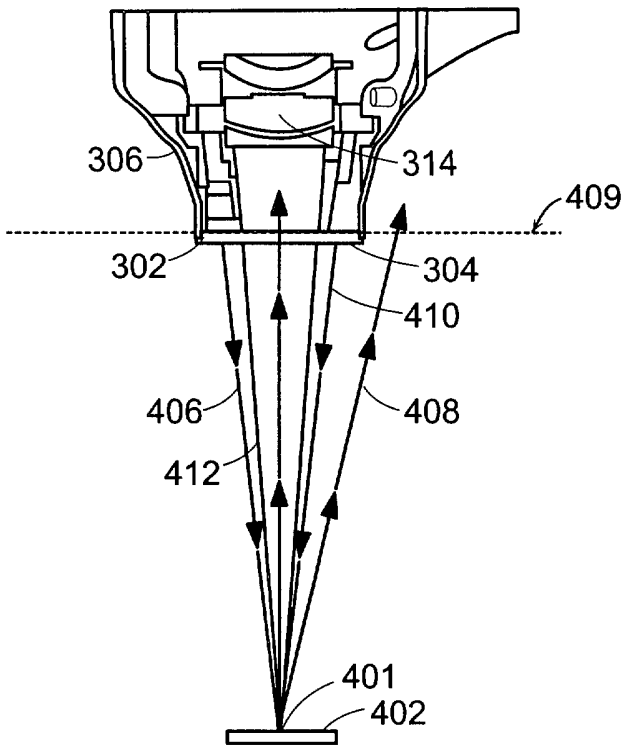
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

[Continued on next page]

(54) Title: METHOD AND APPARATUS FOR IDENTIFYING SPECTRAL ARTIFACTS



(57) Abstract: The invention provides an apparatus and methods for determining whether spectral data obtained from a region of a tissue sample (402) are affected by an artifact. Artifacts include, for example, lighting artifacts such as glare and shadow and obstruction artifacts, such as blood, a speculum, a smoke tube, or other obstruction. Additionally, the invention provides an apparatus and methods for obtaining redundant spectral data of a given region of a sample (402). A redundant set of spectral data is useful where one or more artifacts affect some but not all sets of the spectral data, such that the redundant set of data is unaffected by the artifact and is representative of the tissue. An embodiment of the invention comprises using representative spectral data in diagnosing a condition of a region of tissue (402).

WO 2004/005869 A1

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

METHOD AND APPARATUS FOR IDENTIFYING SPECTRAL ARTIFACTS

Prior Applications

[0001] This application claims the benefit of U.S. Patent Application Serial Number 10/243,535, filed September 13, 2002, and U.S. Provisional Patent Application Serial Number 60/394,696, filed July 9, 2002.

Field of the Invention

[0002] This invention relates generally to spectroscopic methods. More particularly, in certain embodiments, the invention relates to an apparatus and methods for determining whether spectral data obtained from a region of a tissue sample are affected by one or more artifacts, such as glare, shadow, or an obstruction.

Background of the Invention

[0003] Spectral analysis may be used to diagnose disease in tissue. For example, spectral data may be obtained during a diagnostic procedure in which spectral scans are performed on the tissue of a patient. One such diagnostic procedure is an acetowhitening procedure, in which a chemical agent is applied to tissue and the response of the tissue is captured in a spectral scan at some point following the application of the agent. The chemical agent is used, for example, to enhance the detected difference between spectral data obtained from normal tissue and spectral data obtained from abnormal or diseased tissue.

[0004] Spectral measurements of tissue may be non-representative of the actual condition of the tissue when they are affected by one or more artifacts. Artifacts include lighting artifacts such as glare or shadow, and obstructions, such as blood, a speculum, a smoke tube, or other instruments used during the procedure. Artifacts may be located and determined using visual evidence of a region of tissue at the time of the procedure. However, there are currently no other suitable methods of determining whether spectral data obtained from a region of a tissue sample are affected by an artifact. Also, current methods of obtaining spectral data do not allow for the characterization of tissue in the event an artifact adversely affects the data.

Summary of the Invention

[0005] The invention provides an apparatus and methods for obtaining redundant spectral data in order to compensate for artifacts that may be present in optical sample analysis. By illuminating a region of tissue with light incident to the region at more than one angle, it is possible to obtain redundant spectral data for the region. If one set of data for a region is adversely affected by an artifact such as glare, shadow, or an obstruction, then redundant data for

the region, obtained using light incident to the region at a different angle, may be useful. The redundant data may be used to describe the region of tissue, unobscured by the artifact.

[0006] The invention comprises methods for determining if spectral data obtained from a sample region are affected by an artifact, and if so, whether or not redundant data may be used in place of the affected data. Embodiments of the invention comprise the use of metrics to determine whether an artifact is affecting the spectral data from a region of a tissue sample. Methods also comprise determining what kind of artifact is affecting the data from the region. These metrics involve computations using values of the spectral data corresponding to a the region of the tissue sample. Methods of the invention do not rely on any additional visual evidence of the tissue sample, such as human visual inspection, to determine the presence or absence of an artifact. In certain embodiments, the presence of an artifact is desired. However, in preferred embodiments, the presence of an artifact is not desired, for example, because the artifact adversely affects the spectral data.

[0007] If it is determined that an artifact has rendered unusable a given set of data for a region of the sample, then the redundant data corresponding to the region may be considered. Since the redundant data is obtained using light incident to the region at a different angle from that used to obtain the affected data, the artifact may not have affected the redundant data. Multiple sets of redundant data may be used in order to compensate for one or more artifacts. Preferred methods of the invention comprise determining whether redundant data are affected by an artifact or, alternatively, whether redundant data are unaffected by the artifact and representative of the unobscured tissue. If the set of redundant data is representative of an unobscured tissue, such data may be used in place of the affected data in characterizing the region or determining the condition of the region of tissue. As mentioned above, more than one redundant set of data may be obtained. Also, if more than one set of data is determined to be unaffected by an artifact, averages of the unaffected data may be used to characterize the region of tissue.

[0008] Although specific metrics were developed for application to the analysis of *in vivo* cervical tissue subject to artifacts such as glare, shadow, and obstructions, methods for developing analogous metrics are also disclosed as part of the invention. Such methods may be used to create metrics for the analysis of other types of tissue such as *in vivo* or *ex vivo* colorectral, gastroesophageal, urinary bladder, lung, skin tissue, and/or any tissue comprising epithelial cells, for example. These methods may be used to create metrics for tissues that are subject to other states of health and/or other types of artifacts, in addition to those discussed

herein. The invention also comprises methods of determining computational metrics for use in applications employing different types of spectral data than those specifically discussed herein.

[0009] In most embodiments discussed herein, spectral data are obtained as a function of wavelength within a range of between about 360nm and 720nm. However, in some embodiments, the range of wavelengths is from about 190nm to about 1100nm. In the methods discussed herein, where a range of about 360nm to about 720nm is specified, a broader range within about 190nm and about 1100nm is alternately used for some embodiments.

[0010] In one aspect, the invention is directed to a method of determining a condition of a region of a tissue sample using two or more sets of spectral data, each set obtained using light incident to the region at a unique angle. The method comprises the steps of: obtaining a first set of spectral data corresponding to a region of a tissue sample using light incident to the region at a first angle; obtaining a second set of spectral data corresponding to the region using light incident to the region at a second angle; selecting at least one of the two sets that is representative of the region of the tissue sample; and determining a condition of the region of the tissue sample based at least in part on a portion of the representative data.

[0011] Both the first and the second sets of spectral data comprise reflectance spectral data in some embodiments. In other embodiments, at least one of the two sets of spectral data comprises fluorescence spectral data. In some embodiments, the method further comprises obtaining one or more additional sets of spectral data corresponding to a region of interest, each set using light incident to the region at a unique angle.

[0012] The condition to be determined may be a state of health. In one embodiment the state of health comprises at least one of the following conditions: normal squamous tissue, metaplasia, Cervical Intraepithelial Neoplasia Grade I (CIN I), Cervical Intraepithelial Neoplasia Grade II (CIN II), Cervical Intraepithelial Neoplasia Grade III (CIN III), carcinoma in-situ (CIS), and cancer. In some embodiments, the state of health is a combination of two or more of the conditions above, such as Cervical Intraepithelial Neoplasia Grade II or Grade III (CIN II/III).

[0013] In another aspect, the invention is directed to a method of determining whether spectral data obtained from a region of a tissue sample are affected by an artifact. The method comprises the steps of: obtaining a first set of spectral data corresponding to a region of a tissue sample using light incident to the region at a first angle; obtaining a second set of spectral data corresponding to the region using light incident to the region at a second angle; and determining whether the first set of data is affected by an artifact based at least in part on a portion of the data from each of the two sets.

[0014] Both the first and the second sets of spectral data comprise reflectance spectral data in some embodiments. In other embodiments, the method comprises obtaining a third set of spectral data comprising fluorescence spectral data.

[0015] The invention comprises methods of applying various computational metrics in determining whether or not spectral data are affected by an artifact. According to one embodiment, the method of determining whether spectral data are affected by an artifact comprises computing a difference between R_1, a member of the first set of spectral data discussed above, and R_2, a member of the second set of spectral data discussed above, and comparing the difference to a constant, where R_1 and R_2 correspond to at least approximately identical wavelengths. This difference is a percent difference in some preferred embodiments.

[0016] In some preferred embodiments, the method of determining whether spectral data are affected by an artifact comprises computing N differences, $|R_1(X_i)-R_2(X_i)|$, optionally weighting each of the N differences using at least one of $R_1(X_i)$ and $R_2(X_i)$, defining a maximum of a subset of the N optionally-weighted differences, and comparing the maximum to a first constant, where i = 1 to N, N is an integer, X_i is a wavelength between about 360nm and about 720nm, $R_1(X_i)$ is a member of the first set of data corresponding to the wavelength X_i, and $R_2(X_i)$ is a member of the second set of data corresponding to the wavelength X_i.

[0017] The method of determining whether spectral data are affected by an artifact in some embodiments further comprises comparing $R_1(X_1)$ to a second constant, where $R_1(X_1)$ is a member of the first set of data corresponding to a wavelength X_1 between about 409nm and about 429nm.

[0018] The method of determining whether spectral data are affected by an artifact in some embodiments further comprises comparing the quotient $\{(R_1(X_1)/R_2(X_1))/(R_1(X_2)/R_2(X_2))\}$ to a second constant, where X_1 is a wavelength between about 360nm and about 720nm, X_2 is a wavelength between about 360nm and about 720nm, $R_1(X_1)$ is a member of the first set of data corresponding to the wavelength X_1, $R_2(X_1)$ is a member of the second set of data corresponding to the wavelength X_1, $R_1(X_2)$ is a member of the first set of data corresponding to the wavelength X_2, $R_2(X_2)$ is a member of the second set of data corresponding to the wavelength X_2. In one embodiment, X_1 is a wavelength between about 566nm and about 586nm, and X_2 is a wavelength between about 589nm and about 609nm. In one embodiments, the determining step further comprises comparing $R_1(X_3)$ to a third constant, where $R_1(X_3)$ is a member of the first set of data corresponding to a wavelength X_3 between about 689 and about 709nm. In another

embodiment, X_3 is between about 360nm and about 720nm. In yet another embodiment X_3 is between about 409nm and about 429nm.

[0019] The method of determining whether spectral data are affected by an artifact in some embodiments further comprises comparing a value Q to a second constant, where Q is an approximate slope of a plot of $\{R_1(X_i)/R_2(X_i)\}$ with respect to wavelength, over a subset of a wavelength range of about 360nm to about 720nm, X_i is a wavelength between about 360nm and about 720nm, $R_1(X_i)$ is a member of the first set of data corresponding to the wavelength X_i, and $R_2(X_i)$ is a member of the second set of data corresponding to the wavelength X_i.

[0020] In another embodiment, the method of determining whether spectral data are affected by an artifact further comprises comparing $R_1(X_1)$ to a second constant and comparing $R_1(X_1)$ to $R_2(X_1)$, where $R_1(X_1)$ is a member of the first set of data corresponding to a wavelength X_1 between about 360nm and about 720nm, and $R_2(X_1)$ is a member of the second set of data corresponding to the wavelength X_1.

[0021] The method of determining whether spectral data are affected by an artifact in another embodiment further comprises comparing $R_1(X_1)$ to a second constant and comparing $R_1(X_1)$ to $R_2(X_1)$, where $R_1(X_1)$ is a member of the first set of data corresponding to a wavelength X_1 between about 489nm and about 509nm, and $R_2(X_1)$ is a member of the second set of data corresponding to the wavelength X_1.

[0022] According to some embodiments, the method of determining whether spectral data are affected by an artifact comprises comparing $R_1(X_1)$ to a constant, where $R_1(X_1)$ is a member of the first set of data corresponding to a wavelength X_1 between about 409nm and about 429nm. In one embodiment, the determining step further comprises comparing a value Q to a second constant, where the value Q is an approximate slope of a plot of $\{R_1(X_i)/R_2(X_i)\}$ with respect to wavelength, over a subset of a wavelength range of about 576nm to about 599nm, X_i is a wavelength between about 360nm and about 720nm, $R_1(X_i)$ is a member of the first set of data corresponding to the wavelength X_i, and $R_2(X_i)$ is a member of the second set of data corresponding to the wavelength X_i.

[0023] In some embodiments, the method of determining whether spectral data are affected by an artifact comprises comparing the quotient $R_1(X_1)/R_1(X_2)$ to a constant, where $R_1(X_1)$ is a member of the first set of data corresponding to a wavelength X_1 between about 360nm and about 720nm, and $R_1(X_2)$ is a member of the first set of data corresponding to a wavelength X_2 between about 360nm and about 720nm. In one embodiment, X_1 is a wavelength between about 489nm and 509nm and X_2 is a wavelength between about 533nm and about 553nm.

[0024] According to one embodiment, the method of determining whether spectral data are affected by an artifact comprises comparing R_1 to a first constant and comparing R_2 to a second constant, where R_1 is a member of the first set of data corresponding to a wavelength between about 489nm and about 509nm and R_2 is a member of the second set of data corresponding to a wavelength between about 489nm and about 509nm.

[0025] The artifact comprises a lighting artifact in some embodiments. The lighting artifact comprises glare and/or shadow in some embodiments. In other embodiments, the artifact comprises an obstruction. An obstruction comprises blood, mucus, a speculum, and/or a smoke tube in these embodiment. There may be both lighting artifacts and obstruction artifacts in a given embodiment.

[0026] According to one embodiment, the tissue sample comprises cervical tissue. In some embodiments, the tissue sample contains epithelial cells as tissue components. The tissue sample comprises at least one of a group consisting of cervical, colorectal, gastroesophageal, urinary bladder, lung, and skin tissue in some embodiments.

[0027] In another aspect, the invention is directed to a method of determining whether spectral data corresponding to a region of a tissue sample is affected by an artifact using two sets of reflectance spectral data and one set of fluorescence spectral data. The method comprises the steps of: obtaining a first set of reflectance spectral data corresponding to a region of a tissue sample using light incident to the region at a first angle; obtaining a second set of reflectance spectral data corresponding to the region using light incident to the region at a second angle; obtaining a set of fluorescence spectral data corresponding to the region; and determining whether any of the data is affected by an artifact based at least in part on at least one of the following: a subset of the first set of reflectance spectral data, a subset of the second set of reflectance spectral data, and a subset of the set of fluorescence spectral data. In one embodiment, the determining step comprises comparing F to a constant, where F is a member of the set of fluorescence spectral data corresponding to a wavelength between about 469nm and about 489nm.

[0028] In another aspect, the invention is directed to methods of determining a spectral characteristic of an artifact. These include methods of determining computational metrics used to judge whether spectral data obtained from a region are affected by an artifact. A preferred method comprises the steps of: (a) at each of a first plurality of regions of tissue, obtaining a first set of reflectance spectral data known to be affected by a given artifact; (b) at each of a second plurality of regions of tissue, obtaining a second set of reflectance spectral data known

not to be affected by the artifact; and (c) determining a spectral characteristic of the artifact based at least in part on the first and second sets of reflectance spectral data.

[0029] The method of determining a spectral characteristic in some embodiments comprises locating a wavelength at which there is a maximum difference between a mean of one or more members of the first set of reflectance spectral data corresponding to the wavelength and a mean of one or more members of the second set of reflectance spectral data corresponding to the wavelength, relative to a variation measure.

[0030] In some embodiments, the method of determining a spectral characteristic comprises computing N differences, $|\mu_i(A_j(X_i))-\mu_i(B_k(X_i))|$, and defining a maximum of a subset of the N differences, where i = 1 to N, N is an integer, X_i is a wavelength between about 360nm and about 720nm, j = 1 to M1, M1 is an integer, $A_j(X_i)$ represents one of M1 members of the first set of reflectance spectral data corresponding to the wavelength X_i, k = 1 to M2, M2 is an integer, $B_k(X_i)$ represents one of M2 members of the second set of reflectance spectral data corresponding to the wavelength X_i, $\mu_i(A_j(X_i))$ is a mean of the M1 members of the first set of data corresponding to the wavelength X_i, and $\mu_i(B_k(X_i))$ is a mean of the M2 members of the second set of data corresponding to the wavelength X_i.

[0031] According to some embodiments, the method of determining a spectral characteristic comprises computing N quotients, $[|\mu_i(A_j(X_i))-\mu_i(B_k(X_i))| / \{\sigma_i^2(A_j(X_i))+\sigma_i^2(B_k(X_i))\}^{0.5}]$, and defining a maximum of a subset of the N quotients, where i = 1 to N, N is an integer, X_i is a wavelength between about 360nm and about 720nm, j = 1 to M1, M1 is an integer, $A_j(X_i)$ represents one of M1 members of the first set of reflectance spectral data corresponding to the wavelength X_i, k = 1 to M2, M2 is an integer, $B_k(X_i)$ represents one of M2 members of the second set of reflectance spectral data corresponding to the wavelength X_i, $\mu_i(A_j(X_i))$ is a mean of said M1 members of the first set of data corresponding to the wavelength X_i, $\mu_i(B_k(X_i))$ is a mean of the M2 members of the second set of data corresponding to the wavelength X_i, $\sigma_i(A_j(X_i))$ represents a standard deviation of the M1 members of the first set of data corresponding to the wavelength X_i, and $\sigma_i(B_k(X_i))$ represents a standard deviation of the M2 members of the second set of data corresponding to the wavelength X_i.

[0032] The method of determining a spectral characteristic in some embodiments comprises computing N quotients, $[|\mu_i(A_j(X1_i)/A_j(X2_i))-\mu_i(B_k(X1_i)/B_k(X2_i))| / \{\sigma_i(A_j(X1_i)/A_j(X2_i))+ \sigma_i^2(B_k(X1_i)/B_k(X2_i))\}^{0.5}]$, and defining a maximum of a subset of the N quotients, where i = 1 to N, N is an integer, $X1_i$ is a wavelength between about 360nm and about 720nm, $X2_i$ is a wavelength between about 360nm and about 720nm, j = 1 to M1, M1 is an integer, $A_j(X1_i)$

represents one of M1 members of the first set of reflectance spectral data corresponding to the wavelength $X1_i$, $A_j(X2_i)$ represents one of M1 members of the first set of reflectance spectral data corresponding to the wavelength $X2_i$, k = 1 to M2, M2 is an integer, $B_k(X1_i)$ represents one of M2 members of the second set of reflectance spectral data corresponding to the wavelength $X1_i$, $B_k(X2_i)$ represents one of M2 members of the second set of reflectance spectral data corresponding to the wavelength $X2_i$, $\mu_i(A_j(X1_i)/A_j(X2_i))$ is a mean of M1 quotients $A_j(X1_i)/A_j(X2_i)$ for j = 1 to M1, $\mu_i(B_k(X1_i)/B_k(X2_i))$ is a mean of M2 quotients $B_k(X1_i)/B_k(X2_i)$ for k = 1 to M2, $\sigma_i(A_j(X1_i)/A_j(X2_i))$ represents a standard deviation of the M1 quotients $A_j(X1_i)/A_j(X2_i)$, and $\sigma_i(B_k(X1_i)/B_k(X2_i))$ represents a standard deviation of the M2 quotients $B_k(X1_i)/B_k(X2_i)$.

[0033] In another aspect, the invention is directed to a method of determining a characteristic of a region of a tissue sample by obtaining at least two sets of reflectance spectral data, each using light incident to the region at a different angle, and eliminating data that is adversely affected by an artifact. The method comprises the steps of: (a) obtaining a first set of reflectance spectral data corresponding to a region of a tissue sample using light incident to the region at a first angle; (b) obtaining a second set of reflectance spectral data corresponding to the region using light incident to the region at a second angle; (c) determining whether at least one of the first set of reflectance data and the second set of reflectance data is affected by an artifact based at least in part on a subset of the first set of reflectance data and a subset of the second set of reflectance data; (d) rejecting at least one member of at least one of the first set of reflectance data and the second set of reflectance data determined in step (c) to be affected by the artifact; (e) determining a characteristic of the region of the tissue sample based at least in part on at least one member of at least one of the first set of reflectance data and the second set of reflectance data not rejected in step (d).

[0034] In some embodiments, the method further comprises obtaining a set of fluorescence spectral data corresponding to the region, and step (e) comprises determining the condition of the region of the tissue sample based at least in part on at least one member of at least one of the first set and the second set of reflectance data and at least one member of the set of fluorescence spectral data.

[0035] Although certain embodiments of the invention are specifically described with respect to fluorescence spectral data and/or reflectance (backscatter) spectral data, these methods may be adapted for use with other kinds of optical signal data that may be affected by artifacts, including Raman, infrared, video signal data, and combinations thereof.

Brief Description of the Drawings

[0036] The objects and features of the invention can be better understood with reference to the drawings described below, and the claims. The drawings are not necessarily to scale, emphasis instead generally being placed upon illustrating the principles of the invention. In the drawings, like numerals are used to indicate like parts throughout the various views. The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the U.S. Patent and Trademark Office upon request and payment of the necessary fee.

[0037] Figure 1 depicts a spectroscopic system that employs a plurality of spectral types according to an illustrative embodiment of the invention.

[0038] Figure 2 depicts a block diagram of the spectroscopic system of Figure 1 according to an illustrative embodiment of the invention.

[0039] Figure 3A depicts a side view of a probe having top and bottom illumination sources according to an illustrative embodiment of the invention.

[0040] Figure 3B depicts front views of four exemplary arrangements of illumination sources about a probe head according to an illustrative embodiment of the invention.

[0041] Figure 4A depicts exemplary illumination of a region of a tissue sample using light incident to the region at two different angles according to an illustrative embodiment of the invention.

[0042] Figure 4B shows schematic diagrams demonstrating specular and diffuse reflection from a region of a tissue sample according to an illustrative embodiment of the invention.

[0043] Figure 4C depicts illumination of a cervical tissue sample using a probe and a speculum according to an illustrative embodiment of the invention.

[0044] Figure 4D shows a graph depicting exemplary values of reflectance spectral data as a function of wavelength for tissue regions affected by glare, tissue regions affected by shadow, and tissue regions affected by neither glare nor shadow according to an illustrative embodiment of the invention.

[0045] Figure 5A shows a graph depicting mean values and standard deviations of broadband reflectance spectral data using the BB1 channel light source for regions confirmed as being obscured by blood, obscured by mucus, obscured by glare from the BB1 source, obscured by glare from the BB2 source, or unobscured, according to an illustrative embodiment of the invention.

[0046] Figure 5B shows a graph depicting mean values and standard deviations of broadband reflectance spectral data using the BB2 channel light source for regions confirmed as being obscured by blood, obscured by mucus, obscured by glare from the BB1 source, obscured by glare from the BB2 source, or unobscured, according to an illustrative embodiment of the invention.

[0047] Figure 6A shows a graph depicting the weighted difference between the mean reflectance values of glare-obscured regions and unobscured regions of tissue as a function of wavelength, according to an illustrative embodiment of the invention.

[0048] Figure 6B shows a graph depicting the weighted difference between the mean reflectance values of blood-obscured regions and unobscured regions of tissue as a function of wavelength, according to an illustrative embodiment of the invention.

[0049] Figure 6C shows a graph depicting the weighted difference between the mean reflectance values of mucus-obscured regions and unobscured regions of tissue as a function of wavelength, according to an illustrative embodiment of the invention.

[0050] Figure 7A shows a graph depicting a ratio of the weighted differences between the mean reflectance values of glare-obscured regions and unobscured regions of tissue at two wavelengths, according to an illustrative embodiment of the invention.

[0051] Figure 7B shows a graph depicting a ratio of the weighted differences between the mean reflectance values of blood-obscured regions and unobscured regions of tissue at two wavelengths, according to an illustrative embodiment of the invention.

[0052] Figure 7C shows a graph depicting a ratio of the weighted differences between the mean reflectance values of mucus-obscured regions and unobscured regions of tissue at two wavelengths, according to an illustrative embodiment of the invention.

[0053] Figure 8 shows a graph depicting as a function of wavelength mean values and confidence intervals of a ratio of BB1 and BB2 broadband reflectance spectral values for regions confirmed as being either glare-obscured or shadow-obscured tissue, according to an illustrative embodiment of the invention.

[0054] Figure 9A shows a graph depicting BB1 and BB2 broadband reflectance spectral data for a region of tissue where the BB1 data is affected by glare but the BB2 data is not, according to an illustrative embodiment of the invention.

[0055] Figure 9B shows a graph depicting BB1 and BB2 broadband reflectance spectral data for a region of tissue where the BB2 data is affected by shadow but the BB1 data is not, according to an illustrative embodiment of the invention.

[0056] Figure 9C shows a graph depicting BB1 and BB2 broadband reflectance spectral data for a region of tissue that is obscured by blood, according to an illustrative embodiment of the invention.

[0057] Figure 9D shows a graph depicting BB1 and BB2 broadband reflectance spectral data for a region of tissue that is unobscured, according to an illustrative embodiment of the invention.

[0058] Figure 10A shows a graph depicting the reduction in the variability of broadband reflectance measurements of CIN II/III-confirmed tissue produced by filtering, according to an illustrative embodiment of the invention.

[0059] Figure 10B shows a graph depicting the reduction in the variability of broadband reflectance measurements of tissue classified as "no evidence of disease confirmed by pathology" produced by filtering, according to an illustrative embodiment of the invention.

[0060] Figure 10C shows a graph depicting the reduction in the variability of broadband reflectance measurements of tissue classified as "metaplasia by impression" produced by filtering, according to an illustrative embodiment of the invention.

[0061] Figure 10D shows a graph depicting the reduction in the variability of broadband reflectance measurements of tissue classified as "normal by impression" produced by filtering, according to an illustrative embodiment of the invention.

[0062] Figure 11A depicts an exemplary image of cervical tissue divided into regions for which two types of reflectance spectral data and one type of fluorescence spectral data are obtained, according to an illustrative embodiment of the invention.

[0063] Figure 11B is a representation of the regions depicted in Figure 11A and shows the categorization of each region according to an illustrative embodiment of the invention.

[0064] Figure 12A depicts an exemplary image of cervical tissue divided into regions for which two types of reflectance spectral data and one type of fluorescence spectral data are obtained, according to an illustrative embodiment of the invention.

[0065] Figure 12B is a representation of the regions depicted in Figure 11A and shows the categorization of each region according to an illustrative embodiment of the invention.

[0066] Figure 13A depicts an exemplary image of cervical tissue divided into regions for which two types of reflectance spectral data and one type of fluorescence spectral data are obtained, according to an illustrative embodiment of the invention.

[0067] Figure 13B is a representation of the regions depicted in Figure 11A and shows the categorization of each region according to an illustrative embodiment of the invention.

[0068] Figure 14A depicts an exemplary image of cervical tissue divided into regions for which two types of reflectance spectral data and one type of fluorescence spectral data are obtained.

[0069] Figure 14B is a representation of the regions depicted in Figure 11A and shows the categorization of each region according to an illustrative embodiment of the invention.

Description of the Illustrative Embodiment

[0070] In general, the invention relates to an apparatus and methods for determining whether spectral data obtained from a region of a tissue sample are affected by an artifact, such as glare, shadow, or an obstruction. The invention further relates to methods of using the known presence and absence of artifacts in reference sample regions to determine whether artifacts affect spectral data from test sample regions. An embodiment method of the invention comprises obtaining redundant spectral data of a given region of a sample, determining if the redundant spectral data are affected by an artifact, and if not, using the redundant data in place of artifact-affected data to determine a characteristic or condition of the region. The following is a detailed description of preferred embodiments of the invention.

[0071] Preferred methods utilize one or more types of spectral data such as fluorescence spectral data and reflectance spectral data to determine the condition of a tissue sample. Preferred methods of the invention comprise obtaining at least one redundant set of spectral data of a given type for a region of tissue. A redundant set is useful where one or more artifacts affect some but not all sets of the spectral data, such that the redundant set of data is unaffected by the artifact and is representative of the tissue. Preferred methods comprise using this representative data to determine a condition of a region of tissue. Methods of the invention also comprise determining whether an artifact exists by using data from at least two of the sets of spectral data obtained. Multiple redundant sets can be used to analyze multiple artifacts.

[0072] Methods of the invention generally apply to the analysis of a biological sample. For example, preferred methods comprise analyzing a region of cervical tissue during an acetowhitening test conducted on a patient. Other preferred methods comprise analyzing colorectal, gastroesophageal, urinary bladder, lung, skin tissue, and/or any tissue comprising epithelial cells. Some methods involve application of other agents in or on tissue; and some methods involve no application of agents to tissue.

[0073] One embodiment of the invention comprises obtaining spectral data of one or more types, such as reflectance spectral data and fluorescence spectral data, from a region of tissue in

a biological sample. In this embodiment, at least two sets of spectral data are obtained from a given region of tissue, each set being obtained using light incident to the region at a different (average) angle. Then, the method comprises using at least a portion of the data from each of the two sets of data to determine whether either or both of the two sets of data are affected by an artifact. Artifacts include, among others, lighting artifacts, such as glare or shadow, and obstructions, such as blood, a speculum, a smoke tube, or other instruments used during the tissue examination procedure.

[0074] The invention further comprises an apparatus that provides illumination of a given region of a tissue sample using light incident to the region at more than one angle. This allows a user to obtain the multiple sets of spectral data described above. In one embodiment, the apparatus comprises a probe that has multiple illumination sources, variously located about a probe head. In an exemplary application, the angle of the cervix or other tissue being examined may be such that the light from one illumination source of the probe is specularly reflected toward the collection optics, causing glare. The spectral data obtained using this illumination source is therefore corrupted and is likely not representative of the region of tissue being examined. Similarly, if an object such as a speculum or other instrument blocks light from one illumination source, a shadow may be cast on the region of tissue, and the spectral data may therefore be corrupted and non-representative of the region. However, since the apparatus comprises more than one illumination source, spectral data obtained using light from one of the other illumination sources may not be affected by glare or shadow and may be usable in the analysis of the region of the sample.

[0075] Obstructions such as blood or mucus may affect spectral data obtained using an illumination source, and may result in data which is non-representative of the underlying region of tissue. Preferred methods of the invention include determining whether spectral data is affected by such an obstruction using the spectral data itself, without having to rely on other visual evidence of the tissue.

[0076] Additionally, the invention comprises methods of determining a spectral characteristic of an artifact using spectral data of regions of tissue affected by the artifact and spectral data of regions of tissue not affected by the artifact. These methods may be used, for example, to create metrics by which spectral data may be analyzed and determined to be either adversely affected by an artifact or properly representative of the region of tissue.

[0077] Figure 1 depicts an exemplary spectroscopic system 100 employing a plurality of spectral data types according to an illustrative embodiment of the invention. The spectroscopic

system of Figure 1 comprises a console 102 connected to a probe 104 by means of a cable 106. The cable 106 carries electrical and optical signals between the console 102 and the probe 104. The probe 104 accommodates a disposable component 108 which may be used once and discarded. The console 102 and the probe 104 are mechanically connected by an articulating arm 110, which can also support the cable 106. The console 102 contains much of the hardware and the software of the system, and the probe 104 contains the necessary hardware for making suitable spectroscopic observations. The details of the system are further explained in conjunction with Figure 2.

[0078] Figure 2 shows an exemplary operational block diagram 200 of a spectroscopic system of the type depicted in Figure 1. According to an illustrative embodiment, the spectroscopic system of Figure 1 and Figure 2 is substantially the same as single-beam spectrometer devices, but is adapted to include the features of the invention. In other embodiments, the spectroscopic system of Figure 1 and Figure 2 is substantially the same as double-beam spectrometer devices, adapted to include the features of the invention. Still other embodiments use other types of spectroscopic systems. The console 102 comprises a computer 202 which executes software that controls the operation of the spectroscopic system 100. The software comprises one or more modules recorded on machine-readable media such as magnetic disks, magnetic tape, CD-ROM, and semiconductor memory, for example. Preferably, the machine-readable medium is resident within the computer 202. In alternative embodiments, the machine-readable medium can be connected to the computer 202 by a communication link. In alternative embodiments, one can substitute computer instructions in the form of hardwired logic for software, or one can substitute firmware (i.e., computer instructions recorded on devices such as PROMs, EPROMS oe EEPROMs, or the like) for software. The term machine-readable instructions as used herein is intended to encompass software, hardwired logic, firmware and the like.

[0079] The computer 202 in Figure 2 is a general purpose computer. The computer 202 can be an embedded computer, a personal computer such as a laptop or desktop computer, of other type of computer, that is capable of running the software, issuing suitable control commands, and recording information in real time. In one embodiment, the computer 202 has a display 204 for reporting information to an operator of the spectroscopic system 100, a keyboard 206 for enabling the operator to enter information and commands, and a printer 208 for providing a print-out, or permanent record, of measurements made by the spectroscopic system 100 and for printing diagnostic results, for example, for inclusion in the chart of a patient. In an illustrative embodiment of the invention, some commands entered at the keyboard enable a user to select a

particular spectrum for analysis or to reject a spectrum, and to select particular segments of a spectrum for normalization. Other commands enable a user to select the wavelength range for each particular segment and to specify both wavelength contiguous and non-contiguous segments.

[0080] The console 102 in the embodiment shown in Figure 2 also comprises an ultraviolet (UV) source 210 such as a nitrogen laser or a frequency-tripled Nd:YAG laser, one or more white light sources 212 such as one, two, three, four, or more Xenon flash lamps, and control electronics 214 for controlling the light sources both as to intensity and as to the time of onset of operation and the duration of operation. One or more power supplies 216 are included in the console 102 in this embodiment to provide regulated power for the operation of all of the components. In this embodiment, the console 102 of Figure 2 also comprises at least one spectrometer and at least one detector (spectrometer and detector 218) suitable for use with each of the light sources. In some embodiments, a single spectrometer can operate with both the UV light source and the white light source. In some embodiments, the same detector can record UV and white light signals, and in some embodiments different detectors are used for each light source.

[0081] The console 102 in the embodiment shown in Figure 2 also comprises coupling optics 220 to couple the UV illumination from the UV light source 210 to one or more optical fibers in the cable 106 for transmission to the probe 104, and coupling optics 222 for coupling the white light illumination from the white light sources 212 to one or more optical fibers in the cable 106 for transmission to the probe 104. The spectral response of a specimen to UV illumination from the UV light source 210 observed by the probe 104 is carried by one or more optical fibers in the cable 106 for transmission to the spectrometer and detector 218 in the console 102. The spectral response of a specimen to the white light illumination from the white light source 212 observed by the probe 104 is carried by one or more optical fibers in the cable 106 for transmission to the spectrometer and detector 218 in the console 102. In the embodiment shown in Figure 2, the console 102 comprises a footswitch 224 to enable an operator of the spectroscopic system 100 to signal when it is appropriate to commence a spectral observation by stepping on the switch. In this manner, the operator has his or her hands free to perform other tasks, for example, aligning the probe 104.

[0082] The console 102 of Figure 2 comprises a calibration port 226 for calibrating the optical components of the spectrometer system. In an embodiment, an operator places the probe 104 in registry with the calibration port 226 and issues a command that starts the calibration operation.

In one embodiment of the calibration operation, a calibrated light source provides a calibration signal in the form of illumination of known intensity over a range of wavelengths, and/or at a number of discrete wavelengths. The probe 104 detects the calibration signal. The probe 104 transmits the detected signal through the optical fiber in the cable 106 to the spectrometer and detector 218. A test spectral result is obtained. A calibration of the spectral system is computed as the ratio of the amplitude of the known illumination at a particular wavelength divided by the test spectral result at the same wavelength. In an embodiment, calibration is performed before use of the probe 104 on a patient. Here, calibration would account for patient-to-patient variation.

[0083] In an embodiment, the probe 104 comprises probe optics 230 for illuminating a specimen to be analyzed with UV light from the UV source 210 and for collecting the fluorescent and reflectance (backscatter) illumination from the specimen that is being analyzed. The probe 104 in the embodiment shown in Figures 1 and 2 comprises a scanner assembly 232 that provides illumination from the UV source 210, for example, in a raster pattern over a target area of the specimen of cervical tissue to be analyzed. The probe 104 comprises a video camera 234 for observing and recording visual images of the specimen under analysis. The probe 104 comprises a targeting souce 236, which can be used to determine where on the surface of the specimen to be analyzed the probe 104 is pointing. The probe 104 also comprises white light optics 238 to deliver white light from white light sources 212 for recording the reflectance data and to assist the operator in visualizing the specimen to be analyzed. In an embodiment, once the operator aligns the spectroscopic system and depresses the footswitch 224, the computer 202 controls the actions of the light sources 210, 212, the coupling optics 220, 222, the transmission of light signals and electrical signals through the cable 106, the operation of the probe optics 230 and the scanner assembly 232, the retrieval of observed spectra, the coupling of the observed spectra into the spectrometer and detector 218 via the cable 106, the operation of the spectrometer and detector 218, and the subsequent signal processing and analysis of the recorded spectra.

[0084] Figure 3A depicts a side view of a probe 104 having top and bottom illumination sources 302, 304 according to an illustrative embodiment of the invention. In this embodiment, the illumination sources 302, 304 are situated at an upper and a lower location about the perimeter of a probe head 306 such that at any region on the surface of a tissue sample 308 there is illuminating light incident to the region at each of two different angles. Here, the illuminating light is depicted by the upper and lower intersecting cones 310, 312. The probe head 306

contains probe optics 230 for illuminating regions of tissue and for collecting illumination reflected or otherwise emitted from regions of tissue. In this embodiment, probe optics for collecting illumination 314 are located between the top and bottom illumination sources 302, 304. In some embodiments, other arrangements of illuminating and collecting probe optics 230 which allow the illumination of a given region of tissue with light incident to the region at more than one angle are used. One such arrangement includes collecting optics 314 positioned around illuminating optics.

[0085] In one embodiment, the top and bottom illumination sources 302, 304 are alternately turned on and off in order to sequentially illuminate the tissue at equal and opposite angles relative to the collection axis. For example, the top illumination source 302 is turned on while the bottom illumination source 304 is turned off, such that spectral measurements may be obtained for light reflected from a region of the tissue sample 308 illuminated with light incident to the region at a first angle. This angle is relative to the surface of the tissue sample at a point on the region, for example. Then, the top illumination source 302 is turned off while the bottom illumination source 304 is turned on, such that spectral measurements may be obtained using light incident to the region at a second angle. If data obtained using one of the illumination sources is adversely affected by an artifact, such as glare or shadow, then data obtained using another illumination source, with light incident to the region at a different angle, may be unaffected by the artifact and may still be useful.

[0086] In some embodiments, the spectral measurements include reflectance data obtained over a range of wavelengths. In some embodiments, the spectral measurements include fluorescence data obtained over a range of wavelengths.

[0087] Embodiment methods include different illumination alternation schemes. For example, the top and the bottom illumination sources 302, 304 may be alternately cycled on and off more than once while obtaining data for a given region. Also, the illumination sources may overlap, such that more than one illumination source is on at one time for at least part of the illumination collection procedure. Other illumination alternation schemes are possible, depending at least in part on the arrangement of illumination sources in relation to the probe head 306.

[0088] After data are obtained from one region of the tissue using light incident to the region at more than one angle, data may likewise be obtained from another region of the tissue. An embodiment method comprises illuminating a target area of the tissue sample region-by-region using a scanner assembly 232. An embodiment comprises alternately illuminating a first region using light incident to the region at more than one angle as described above, then adjusting the

probe optics 230 to repeat the illumination sequence at a different region within the target area of the tissue sample. In an embodiment, the process is repeated until a desired subset of the entire target area has been scanned. In one embodiment, five hundred regions are scanned within a target area having a diameter of about 25-mm. In an embodiment, the scan of the aforementioned five hundred regions takes about 12 seconds. In other embodiments, the number of regions scanned, the size of the target area, and/or the duration of the scan vary from the above.

[0089] Figure 3B depicts front views of four exemplary arrangements 320, 322, 324, 326 of illumination sources about a probe head 306 according to an illustrative embodiment of the invention. The drawings are not to scale; they simply serve to illustrate exemplary relative arrangements of illumination sources about the perimeter of a probe head 306. Other embodiment arrangements include collecting optics 314 positioned around the perimeter of the probe head 306. The first arrangement 320 of Figure 3B has one top illumination source 328 and one bottom illumination source 330, which are alternately cycled on and off as described above. The illumination sources are arranged about the collecting optics 331, which are located in the center of the probe head 306. Light from an illumination source is reflected from the tissue and captured by the collecting optics 331.

[0090] The second arrangement 322 of Figure 3B is similar to the first arrangement 320, except that there are two illumination sources 332, 334 in the top half of the probe head 306 and two illumination sources 336, 338 in the bottom half of the probe head 306. In one embodiment, the two lights above the midline 340 are turned on and the two lights below the midline 340 are turned off while obtaining a first set of spectral data; then the lights above the midline 340 are turned off and the lights below the midline 340 are turned on while obtaining a second set of spectral data. An alternate embodiment comprises turning only one of the four illumination sources on at a time to obtain four sets of spectral data for a given region. Another embodiment comprises turning the illumination sources on and off in various other patterns. Some embodiments comprise using noncircular or otherwise differently shaped illumination sources, and/or using a different number of illumination sources. Some embodiments comprise using arrangements where the collecting optics are positioned about the illuminating optics. Some embodiments comprise arrangements where the collecting optics are otherwise positioned with respect to the illuminating optics.

[0091] The third arrangement 324 of Figure 3B includes each illumination source 342, 344 positioned on either side of the probe head 306. An embodiment comprises alternating these lights in a manner analogous to those described for the first arrangement 320.

[0092] The fourth arrangement 326 of Figure 3B is similar to the second arrangement 322, except that the lights 348, 350 on the right side of the probe head 306 are turned off and on together, alternately with lights 352, 354 on the left side of the probe head 306. Thus, two sets of spectral data may be obtained for a given region, one set using lights on the right of the midline 346, and the other set using lights on the left of the midline 346.

[0093] Figure 4A depicts exemplary illumination of a region 401 of a tissue sample 402 using light incident to the region 401 at two different angles 406, 408 according to an illustrative embodiment of the invention. Figure 4A demonstrates that source light position may affect whether or not data is affected by glare. Note the probe head 306 of Figure 4A has been rotated from its position depicted in Figure 3A for illustrative purposes. In an embodiment, the top illumination source 302 and bottom illumination source 304 are turned on sequentially and illuminate the surface of a tissue sample 402 at equal and opposite angles relative to the collection axis 409. Arrows represent the light 406 emitted from the top illumination source 302, and the light 408 specularly reflected from the surface of the region 401 of the tissue sample 402. In preferred embodiments, it is desired to collect diffusely reflected light, as opposed to specularly reflected light 408 (glare). Since the specularly reflected light 408 from the top illumination source 302 does not enter the collecting optics 314 in the example illustrated in Figure 4A, a set of data obtained using the top illumination source 302 would not be affected by glare.

[0094] However, in the example illustrated in Figure 4A, the emitted light 408 from the bottom illumination source 304 reaches the surface of the region 401 of the tissue 402 and is specularly reflected into the collecting optics 314, shown by the arrow 412. Data obtained using the bottom illumination source 304 in the example pictured in Figure 4A would be affected by glare. This data may not be useful, for example, in determining a characteristic or a condition of the region 401 of the tissue 402. In this example, it would be advantageous to instead use the set of data obtained using the top illumination source 302 since it is not affected by glare.

[0095] Figure 4B shows schematic diagrams demonstrating specular and diffuse reflection from a region 416 of a tissue sample 424 according to an illustrative embodiment of the invention. Figure 4B demonstrates that position of the collection optics may affect whether or not data is affected by glare. The first diagram 420 demonstrates the specular reflection of light

incident 430 to the surface 428 of a region 416 of tissue 424 with collection optics centered to provide an acceptance cone 433 as shown. This is analogous to the reflection of light from the top illumination source 302 illustrated in Figure 4A. There is an interface 428 between the tissue 424 and the surrounding air 426. Light 430 with illumination intensity $I_o(\lambda)$ strikes the air-tissue interface 428 at the region 416. Light 432 with a fraction of the initial illumination intensity, $\alpha I_o(\lambda)$, is specularly reflected from the surface 428, where α is a real number between 0 and 1. The acceptance cone 433 is the space through which light is diffusely reflected from the tissue 424 into the collecting optics 314, in this embodiment. In other embodiments, light may also be emitted or otherwise transmitted from the surface of the tissue. In the embodiment illustrated in Figure 4B, it is the diffusely reflected light that is of interest, since spectral data obtained from diffusely reflected light can be used to determine the condition of the region of the sample. Since there is no specular reflection within the acceptance cone 433, only diffusely reflected light is collected, and the collected signal corresponds to $I_t(\lambda)$, where $I_t(\lambda)$ is the intensity of light diffusely reflected from the region 416 on the surface 428 of the tissue 424.

[0096] The second diagram 422 of Figure 4B demonstrates the specular reflection of light incident to the surface 428 of a region 416 of tissue 424 with collection optics off-center, providing an acceptance cone 438 as shown. In the second diagram 422 of Figure 4B, light 434 with illumination intensity $I_o(\lambda)$ strikes the surface 428 of the tissue 424. Light 436 with a fraction of the initial illumination intensity, $\alpha I_o(\lambda)$, is specularly reflected from the surface 428, where α is a real number between 0 and 1. Unlike in the first diagram 420 of Figure 4B, there is specular reflection within the acceptance cone 438 in the second diagram 422, and so both diffusely reflected light and specularly reflected light reach the collecting optics 314. Thus, in the example illustrated in the second diagram 422, the collected signal corresponds to an intensity represented by the sum $I_t(\lambda) + \alpha I_o(\lambda)$. It may be difficult or impossible to separate the two components of the measured intensity, thus, the data may not be helpful in determining the condition of the region 416 of the tissue sample 424, due to the glare effect.

[0097] Figure 4C is a diagram 450 depicting illumination of a region 460 of a cervical tissue sample 462 using a probe 104 and a vaginal speculum 454 according to an illustrative embodiment of the invention. In a preferred embodiment, the probe 104 operates without physically contacting the tissue being analyzed. In one embodiment, a disposable sheath is used to cover the probe head 306, for example, in case of incidental contact of the probe head 306 with the patient's body. In an embodiment, the sheath is disposed of after a single use on a patient. In an embodiment, the disposable sheath has a unique identifier, such as a two-

dimensional bar code. The apparatus and methods described herein are not limited to use in the analysis of vaginal tissue. Other tissue types may be analyzed using these methods, including colorectal, gastroesophageal, urinary bladder, lung, skin tissue, and/or any tissue comprising epithelial cells, for example.

[0098] The diagram 450 of Figure 4C demonstrates that a misalignment of the probe 104 may create conditions where either or both of the top and bottom speculum blades 456, 458 block part or all of the illumination path from either or both of the intersecting upper and lower cones of illuminating light 310,312, thereby affecting the spectral data obtained for the region 460 of the tissue sample 462. The speculum blades, or other obstructions such as a smoke tube or other implements used during examination, may physically obstruct the region 460 being analyzed, or may partially obstruct the light illuminating the region 460 causing a shadow. In either case, the spectral data obtained may be adversely affected and rendered unusable for characterizing the region of the tissue sample. Obtaining multiple sets of spectral data using illumination from sources at various positions and angles improves the chances of obtaining at least one set of spectral data that is not affected by glare, shadow, and/or obstructions.

[0099] Figure 4D shows a graph 470 depicting exemplary values of reflectance spectral data 472 as a function of wavelength 474 for tissue regions affected by glare 476, tissue regions affected by shadow 478, and tissue regions affected by neither glare nor shadow 480 according to an illustrative embodiment of the invention. The reflectance spectral data 472 represent the fraction of incident light that is reflected from the sample. The intensity of the incident light may be determined using a NIST-traceable diffuse reflectance target, such as a 10% diffuse reflectance target. The graph 470 shows that the reflectance values of a region of tissue affected by glare 476 are higher at all measured wavelengths than the reflectance of a region of tissue not affected by glare 480. The graph 470 also shows that the reflectance values of a region of tissue with illumination partially blocked by a speculum blade such that the region is in shadow, are lower at all measured wavelengths than the reflectance of a region of tissue not affected by shadow 480. The shapes of all three curves 476, 478, 480 are different. In this example, the data affected by glare or shadow may not be usable to determine a condition or characteristic of the region of the sample, if the data are not representative of the region of the tissue sample. There is no simple way to accurately separate out the effect of the glare or shadow in order to determine the diffuse reflection at the region of the tissue, in this case. Hence, glare and shadow may adversely affect spectral data obtained for a region of a tissue sample.

[0100] An illustrative embodiment of the invention comprises obtaining one fluorescence spectrum and two broadband reflectance spectra at each of a plurality of scan locations of the sample tissue. Here, a spectrum refers to a collection of spectral data over a range of wavelengths. In one embodiment method, spectral data are collected over a range of wavelengths between 360 and 720 nm in 1 nm increments. In other embodiments, the range of wavelengths is between about 190nm and 1100nm. Here, the two reflectance spectra are referred to as the BB1 (broadband one) and BB2 (broadband two) spectra. BB1 and BB2 differ in the way that the tissue is illuminated at the time the spectral data are obtained as described below. In one embodiment, the probe head 306 has 4 illumination sources located circumferentially about the collection optics. Two sources are above and two are below the horizontal plane, as illustrated in the second arrangement 322 of Figure 3B. The two upper sources are used to obtain BB1 spectra and the two lower sources are used to obtain BB2 spectra. Since the upper and lower sources illuminate a region of the tissue sample using light incident to the region at different angles, an artifact – for example, or shadow – may affect one of the two reflectance spectra obtained for the region, while the other reflectance spectrum is unaffected. For example, during acquisition of spectral data, the BB1 spectrum may be unaffected by an artifact even if the BB2 spectrum is adversely affected by the artifact. In such a case, BB1 spectral data may be used to characterize the condition of the region of tissue even though the BB2 data is not representative of the region. In other embodiments, the BB1 and BB2 spectra comprise one or more other types of spectral data, such as absorbance spectra, adsorption spectra, transmission spectra, fluorescence spectra, and/or other types of optical and atomic emission spectra. The skilled artisan is aware of other ways in which BB1 and BB2 can be made to differ with respect to the ways in which tissue can be illuminated or otherwise contacted with electromagnetic radiation.

[0101] Figure 5A shows a graph depicting mean values and standard deviations of broadband reflectance spectral data using the BB1 channel light source for regions confirmed as being obscured by blood, obscured by mucus, obscured by glare from the BB1 source, obscured by glare from the BB2 source, or unobscured, according to an illustrative embodiment of the invention. Various sample test points corresponding to regions of tissue from patient scans were visually identified as having blood, mucus, or glare present. A sample point was identified as having blood present if it was completely covered by blood and if there was no glare. A sample point was identified as having mucus present if it was completely covered by mucus and if there was no glare. A sample point was identified as having glare based on visual evidence of glare

and large reflectance values in at least one of the two sets of reflectance spectral data (the BB1 spectrum or the BB2 spectrum). Figure 5A shows the range of BB1 reflectance values 502 for a given category of the sample test points which lie within one standard deviation of the mean for the category, plotted as a function of wavelength 504. Figure 5A shows ranges of BB1 reflectance values 502 for each of the following categories of sample test points: those identified as having blood present 506, those identified as having mucus present 508, those identified as having glare from the BB1 illumination source 510, those identified as having glare from the BB2 illumination source 512, and those identified as unobstructed tissue 514.

[0102] Similarly, Figure 5B shows a graph depicting mean values and standard deviations of broadband reflectance spectral data using the BB2 channel light source for regions confirmed as being obscured by blood 524, obscured by mucus 526, obscured by glare from the BB1 source 528, obscured by glare from the BB2 source 530, or unobscured 532, according to an illustrative embodiment of the invention. Figure 5B shows the range of BB2 reflectance values 520 for a given category of the sample test points which lie within one standard deviation of the mean for the category, plotted as a function of wavelength 504. Figure 5B shows ranges of BB2 reflectance values 520 for each of the following categories of sample test points: those identified as having blood present 524, those identified as having mucus present 526, those identified as having glare from the BB1 illumination source 528, those identified as having glare from the BB2 illumination source 530, and those identified as unobstructed tissue 532.

[0103] Figures 5A and 5B show that a region with glare from one illumination source does not necessarily have high reflectance values corresponding to data obtained using the other illumination source. For example, in Figure 5A, the range of BB1 reflectance values 502 of points with visual evidence of glare from the BB2 source 512 is similar to the range of BB1 reflectance values 502 of unobstructed tissue 514. Similarly, in Figure 5B, the range of BB2 reflectance values 520 of points demonstrating glare from the BB1 source 528 is similar to the range of BB2 reflectance values 520 of unobstructed tissue 532. Therefore, one of the two sets of reflectance spectral data may be useful in characterizing the tissue even if the other of the two sets is corrupted by an artifact, such as glare.

[0104] In one embodiment, it is desired to determine spectral characteristics caused by various artifacts so that data corresponding to a region affected by a given artifact may be identified. It is further desired to determine a spectral characteristic of an artifact based on the spectral data itself, without having to rely on other visual evidence of a given artifact. In order to determine these spectral characteristics, an embodiment of the invention comprises using spectral data

known to be affected by a given artifact based on visual evidence, as well as spectral data known not to be affected by an artifact. Techniques that may be used to identify spectral characteristics and/or to develop classification rules determining whether given data are affected by an artifact include, for example, discriminant analysis (linear, nonlinear, multivariate), neural networks, principal component analysis, and decision tree analysis. One embodiment comprises determining a particular wavelength that gives the greatest difference between the artifact-affected spectral data (the outlier) and spectral data from corresponding nearby tissue that is known to be unaffected by the artifact (the tissue). Alternatively, the embodiment comprises determining a wavelength that gives the largest difference between the outlier and the tissue, as weighted by a measure of variability of the data. In one embodiment, this method locates where the difference between the mean reflectance for the outlier and the tissue is at a maximum relative to the difference between the standard deviations for the outlier data and the tissue data. In one embodiment, the method determines a maximum value of D as a function of wavelength, where D is the difference given in Equation 1 below:

$$D(\lambda) = \frac{\left|\mu(BB(\lambda))_{Outlier} - \mu(BB(\lambda))_{Tissue}\right|}{\sqrt{\sigma^2(BB(\lambda))_{Outlier} + \sigma^2(BB(\lambda))_{Tissue}}}, \qquad (1)$$

where $\mu(BB(\lambda))_{Outlier}$ is the mean of a set of reflectance spectral data at wavelength λ known to be affected by a given artifact, $\mu(BB(\lambda))_{Tissue}$ is the mean of a set of reflectance spectral data at wavelength λ that is known not to be affected by the artifact, $\sigma(BB(\lambda))_{Outlier}$ is the standard deviation of the set of reflectance spectral data at wavelength λ known to be affected by the given artifact, and $\sigma(BB(\lambda))_{Tissue}$ is the standard deviation of the set of reflectance spectral data at wavelength λ known not to be affected by the given artifact.

[0105] Figure 6A shows a graph depicting the weighted difference 602 between the mean reflectance values of glare-obscured regions and unobscured regions of tissue as a function of wavelength 604, according to an illustrative embodiment of the invention. The weighted difference is as given in Equation 1. For the data sets used in Figure 6A, the wavelength providing the maximum value 606 of D in Equation (1) is about 420 nm. Thus, exemplary spectral characteristics identifiable with this set of glare-obscured "outlier" data include the reflectance spectral data at around 420nm, and any deviation of this data from reflectance spectral "tissue" data for unobscured regions of correspondingly similar tissue at around 420nm. This embodiment uses reflectance spectral data. Other embodiments may use other types of spectral data, including fluorescence data.

[0106] Figure 6B shows a graph depicting the weighted difference 602 between the mean reflectance values of blood-obscured regions and unobscured regions of tissue as a function of wavelength 604, according to an illustrative embodiment of the invention. The weighted difference is as given in Equation 1. For the data sets used in Figure 6B, the wavelength providing the maximum value 608 of D in Equation (1) is about 585 nm. **[0107]** Thus, exemplary spectral characteristics identifiable with this set of blood-obscured "outlier" data include the reflectance spectral data at about 585nm, and any deviation of this data from reflectance spectral "tissue" data for unobscured regions of correspondingly similar tissue at about 585nm. This embodiment uses reflectance spectral data. Other embodiments may use other types of spectral data, including fluorescence spectral data.

[0108] Figure 6C shows a graph depicting the weighted difference 602 between the mean reflectance values of mucus-obscured regions and unobscured regions of tissue as a function of wavelength 604, according to an illustrative embodiment of the invention. The weighted difference is as given in Equation 1. For the data sets used in Figure 6C, the wavelength providing the maximum value 610 of D in Equation (1) is about 577nm. Thus, exemplary spectral characteristics identifiable with this set of mucus-obscured "outlier" data include the reflectance spectral data at about 577nm, and any deviation of this data from reflectance spectral "tissue" data for unobscured regions of correspondingly similar tissue at about 577nm. This embodiment uses reflectance spectral data. Other embodiments may use other types of spectral data, including fluorescence spectral data.

[0109] In some embodiments, it may be desired to find a pair of wavelengths that would provide an acceptable spectral characteristic of an artifact "outlier." One illustrative embodiment comprises determining two wavelengths where the ratio of spectral data at the two wavelengths is most different for the artifact-affected spectral data (the "outlier") and spectral data from corresponding nearby tissue that is known to be unaffected by the artifact (the "tissue"). Alternatively, the method comprises determining two wavelengths where the ratio of spectral data at the two wavelengths weighted by a measure of variability is most different for the outlier data and the tissue data. In one embodiment, the method comprises determining a maximum value of D as a function of wavelength, where D is the difference given in Equation 2 below:

$$D = \frac{\left|\mu\left(BB(\lambda)/BB(\lambda')\right)_{Outlier} - \mu\left(BB(\lambda)/BB(\lambda')\right)_{Tissue}\right|}{\sqrt{\sigma^2\left(BB(\lambda)/BB(\lambda')\right)_{Outlier} + \sigma^2\left(BB(\lambda)/BB(\lambda')\right)_{Tissue}}}, \qquad (2)$$

where $\mu(BB(\lambda)/BB(\lambda'))_{Outlier}$ is the mean of the ratios of reflectance at wavelength λ and reflectance at wavelength λ' for a set of reflectance spectral data known to be affected by a given artifact, $\mu(BB(\lambda)/BB(\lambda'))_{Tissue}$ is the mean of the ratios of reflectance at wavelength λ and reflectance at wavelength λ' for a set of reflectance spectral data that is known not to be affected by the given artifact, $\sigma(BB(\lambda)/BB(\lambda'))_{Outlier}$ is the standard deviation of the ratios of reflectance at wavelength λ and reflectance at wavelength λ' for a set of reflectance spectral data known to be affected by the given artifact, and $\sigma(BB(\lambda)/BB(\lambda'))_{Tissue}$ is the standard deviation of the ratios of reflectance at wavelength λ and reflectance at wavelength λ' for a set of reflectance spectral data known not to be affected by the given artifact.

[0110] Figure 7A shows a graph depicting a ratio of the weighted differences 702 between the mean reflectance values of glare-obscured regions and unobscured regions of tissue at two wavelengths, a numerator wavelength 704 and a denominator wavelength 706, according to an illustrative embodiment of the invention. The weighted difference 702 is as given in Equation 2. For the data sets used in Figure 7A, the two wavelengths providing the maximum value of D in Equation (2) are about 401 nm (numerator) and about 404 nm (denominator). Thus, exemplary spectral characteristics identifiable with this set of glare-obscured "outlier" data include the ratio of reflectance spectral data at about 401nm and the reflectance spectral data at about 404nm, as well as any deviation of this ratio from those of corresponding regions of similar but unobscured tissue. This embodiment uses reflectance spectral data. Other embodiments may use other types of spectral data, including fluorescence data.

[0111] Figure 7B shows a graph depicting a ratio of the weighted differences 702 between the mean reflectance values of blood-obscured regions and unobscured regions of tissue at two wavelengths, a numerator wavelength 704 and a denominator wavelength 706, according to an illustrative embodiment of the invention. The weighted difference is as given in Equation 2. For the data sets used in Figure 7B, the two wavelengths providing the maximum value of D in Equation (2) are about 595 nm (numerator) and about 718 nm (denominator). Thus, an exemplary spectral characteristic identifiable with this set of blood-obscured "outlier" data includes the ratio of the reflectance spectral data at about 595nm and the reflectance spectral data about 718nm. This embodiment uses reflectance spectral data. Other embodiments may use other types of spectral data, including fluorescence data.

[0112] Figure 7C shows a graph depicting a ratio of the weighted differences702 between the mean reflectance values of mucus-obscured regions and unobscured regions of tissue at two wavelengths, a numerator wavelength 704 and a denominator wavelength 706, according to an

illustrative embodiment of the invention. The weighted difference is as given in Equation 2. For the data sets used in Figure 7B, the two wavelengths providing the maximum value of D in Equation (2) are about 545 nm (numerator) and about 533 nm (denominator). Thus, an exemplary spectral characteristic identifiable with this set of mucus-obscured "outlier" data includes the ratio of the reflectance spectral data at about 545nm and the reflectance spectral data at about 533nm. This embodiment uses reflectance spectral data. Other embodiments may use other types of spectral data, including fluorescence data.

[0113] Another type of lighting artifact which may obscure spectral data is shadow, which may be caused, for example, by an obstruction blocking part of the light from an illumination source on the optical probe 104 of the embodiment apparatus. It may be important to differentiate between glare and shadow, so that spectral data representing unobstructed tissue can be properly identified. In an embodiment, broadband reflectance is expressed as the intensity of light diffusely reflected from a region of the tissue, I_t, over the intensity of incident light, I_o, at the region. When glare is measured in addition to light diffusely reflected from the tissue, a percentage of the original intensity of incident light is included in the tissue reflectance measurement, so that the "reflectance" reading of a region of a sample experiencing glare, $R_g(\lambda)$, may be expressed as in Equation 3:

$$R_g(\lambda) = (I_t(\lambda) + \alpha I_o(\lambda))/I_o(\lambda) \quad , \qquad (3)$$

where α is a real number between 0.0 and 1.0; $I_t(\lambda)$ is the intensity of light diffusely reflected from the region of tissue at wavelength λ, and $I_o(\lambda)$ is the intensity of light incident on the region of the sample at wavelength λ. The intensity of the specularly-reflected light is $\alpha I_o(\lambda)$. When the region of the sample is shadowed, only a portion of the incident intensity reaches the region. Thus, the "reflectance" reading of a region of a sample experiencing shadow, $R_s(\lambda)$, may be expressed as in Equation 4:

$$R_s(\lambda) = \beta I_t(\lambda)/I_o(\lambda). \qquad (4)$$

where β is a real number between 0.0 and 1.0; $I_t(\lambda)$ is the intensity of light at wavelength λ diffusely reflected from the region of tissue with an incident light intensity of $I_o(\lambda)$, and $I_o(\lambda)$ is the intensity of light at wavelength λ that would be incident on the region of the sample if unshadowed.

[0114] In one embodiment of the invention, the method comprises determining if only one set of a pair of sets of spectral data is affected by a lighting artifact, such as glare or shadow, each

set having been obtained using light incident on the sample at a unique angle. If it is determined that only one set of a pair of sets of spectral data is affected by the artifact, then the other set of spectral data may be used in the determination of a characteristic of the region of the sample, for example. In one embodiment, it is determined that there is evidence of a lighting artifact in the spectral data. Such evidence may be a large difference between the reflectance measurements of the two sets of spectral data. If such evidence exists, then one of the reflectance measurements will either be R_g or R_s, as given by Equation 3 and Equation 4. In cases where members of only one set are affected by a lighting artifact, the remaining set of reflectance measurements may be expressed as R, the intensity of light diffusely reflected from the region of the tissue, I_t, divided by the intensity of light incident on the region of the tissue, I_o. In an embodiment method, the larger of the two reflectance measurements corresponding to a given wavelength is divided by the smaller. In cases where only one of the sets is affected by a lighting artifact, the resulting quotient will be either R_g/R, which is equal to $1+\alpha I_o(\lambda)/I_t(\lambda)$, or R/R_s, which is equal to the constant, $1/\beta$. If glare is present, the value of the quotient will depend on wavelength and the plot of the quotient as a function of wavelength should look like an inverted unobstructed tissue broadband signal because of the $\alpha I_o(\lambda)/I_t(\lambda)$ term. If shadow is present, the plot of the quotient should be constant across the spectrum.

[0115] Figure 8 shows a graph depicting as a function of wavelength 804 mean values and confidence intervals of a ratio 802 of BB1 and BB2 broadband reflectance spectral values (larger value divided by smaller value) for regions confirmed as being either glare-obscured or shadow-obscured tissue, according to an illustrative embodiment of the invention. The shadow points 806 yield a nearly constant value, while the glare points 808 vary over the range of wavelength 804 in a manner that resembles the inverse of unobstructed tissue reflectance. Thus, Figure 8 illustrates an embodiment in which it is determined whether only one set of a pair of sets of spectral data is affected by either glare or shadow, such that the other set is unaffected by glare or shadow and may be used to determine a characteristic of the tissue, for example. In an embodiment, the method comprises differentiating between glare and shadow by observing the steep slope of glare-affected reflectance spectral measurements between about 577nm and 599nm, for example, compared to the nearly flat slope of shadow-affected reflectance spectral measurements at those wavelengths, as seen in Figure 8.

[0116] In one embodiment, the method comprises developing spectral artifact classification rules (metrics) using spectral data, including one or more sets of fluorescence and broadband reflectance data obtained using light at one or more angles. In one embodiment, one set of

fluorescence data and two sets of reflectance data are used for a given region of a tissue sample, where each of the two sets of reflectance data are obtained using light incident on the region at a different angle. These metrics determine what data is representative of a given region of tissue. By varying the metrics, desired levels of sensitivity and selectivity of a resulting tissue characterization using tissue-representative data may be achieved.

[0117] The following metrics for an exemplary preferred embodiment were determined using the embodiments discussed above, as well as other techniques. These metrics were developed using one set of fluorescence data and two sets of reflectance data, BB1 and BB2, for samples of cervical tissue. Other embodiments use other combinations of spectral data sets. Each of the two sets of reflectance data used in the following metrics were obtained using light incident to a region of a sample at different angles. An embodiment of the invention uses any or all of the metrics listed below to determine if any set of data should be eliminated from use in determining a characteristic of a region of tissue, due to the presence of a spectral artifact. In an embodiment of the invention, wavelengths within a range of the wavelengths shown below are used. In one embodiment, this range about the wavelengths is about ±10 nm. In an embodiment of the invention, only certain parts of the metrics shown below are used. In one embodiment, only a portion of a given set of spectral data are eliminated, not the entire set. In one embodiment, BB1 and BB2 reflectance data are obtained, but fluorescence data is not. Here, "eliminate data" means to eliminate data from consideration in an analysis, for example, an analysis to determine a condition of a region. It is possible to change sensitivity and selectivity of a tissue diagnostic algorithm by varying the metrics below, for instance by changing one or more of the threshold constants. Such variations are within an embodiment of this invention. The metrics for the exemplary preferred embodiment are as follows:

Glare Metric #1: Eliminate BB1 data IF:

I. BB1(419) > 0.25 **OR** BB1(499)/BB1(543)<1.05;

OR II. Max{|ΔBB|/avgBB}(370-710) > 0.25 **AND** BB1(419) > 0.18;

OR III. Max{|ΔBB|/avgBB}(370-710) > 0.4 **AND** {BB1(576)/BB2(576)}/{BB1(599)/BB2(599)}>1.1 **AND** BB2(699) > 0.3.

Glare Metric #2: Eliminate BB2 data IF:

I. BB2(419) > 0.25 **OR** BB2(499)/BB2(543)<1.05;

OR II. Max{|ΔBB|/avgBB}(370-710) > 0.25 **AND** BB2(419) > 0.18;

OR III. Max{|ΔBB|/avgBB}(370-710) > 0.4 **AND** {BB2(576)/BB1(576)}/{BB2(599)/BB1(599)}>1.1 **AND** BB1(699) > 0.3.

Shadow Metric #1: Eliminate BB1 data IF:

I. BB2(499)>BB1(499) **AND** Max{|ΔBB|/avgBB}(370-710) > 0.25 **AND** BB1(499) < 0.05;

OR II. Max{|ΔBB|/avgBB}(370-710) > 0.5 **AND** {BB2(576)/BB1(576)}/{BB2(599)/BB1(599)}<1.1 **AND** BB2>BB1 **AND** BB2(419) < 0.2.

Shadow Metric #2: Eliminate BB2 data IF:

I. BB1(499)>BB2(499) **AND** Max{|ΔBB|/avgBB}(370-710) > 0.25 **AND** BB2(499) < 0.05;

OR II. Max{|ΔBB|/avgBB}(370-710) > 0.5 **AND** {BB1(576)/BB2(576)}/{BB1(599)/BB2(599)}<1.1 **AND** BB1>BB2 **AND** BB1(419) < 0.2.

Low Signal: Eliminate BB1, BB2, and Fl data IF:

I. Fl(479) < 3.5 counts/μJ;

OR II. BB1(499) < 0.035 & BB2(499) < 0.035.

Mucus Metric: Eliminate BB1, BB2, and Fl data IF:

I. Max{|ΔBB|/avgBB}(370-710) < 0.25 **AND** avgBB(577) > 0.11;

AND II. BB1(406) / BB1(541) < 1.0 **AND** BB2(406) / BB2(535) < 1.0;

AND III. BB1(544) / BB1(532) > 0.95 **AND** BB2(544) / BB2(532) > 0.95.

where BB1(X) is the BB1 reflectance spectrum measurement at wavelength X, BB2(X) is the BB2 reflectance spectrum measurement at wavelength X, Max{|ΔBB|/avgBB}(370-710) indicates the maximum of {the absolute value of the difference between the BB1 and BB2 reflectance spectrum measurements divided by the average of the BB1 and BB2 measurements at a given wavelength} over the range of about 370 to 710nm, and Fl(X) is the fluorescence spectrum measurement at wavelength X. The following are notes regarding the Metrics listed above and apply to a preferred embodiment, subject to the variations described above:

Glare Metric #1 and Glare Metric #2:

Level I: Broadband measurements are generally greater than about 0.25 at about 419nm only when there is glare in the channel (i.e. BB1 or BB2). The lack of a downward slope between about 499 and about 543 nm is also a strong indication that the broadband measurements are affected by glare.

Level II: Large percentage differences in the broadband measurements combined with higher than average reflectance at about 419 nm also indicates the presence of glare.

Level III: A maximum broadband percent difference that is larger than about 0.4 indicates that there is a lighting artifact present. The presence of a slope when the broadband measurements at about 576 and about 599 nm are divided and an off-channel broadband greater than about 0.3 at about 699 nm reveals that the lighting artifact is due to glare instead of shadow.

If a point is identified as glare in one channel, then subsequently identified as glare in both channels, both broadband measurements should be eliminated.

Shadow Metric #1 and Shadow Metric #2:

Level I: Broadband measurements that are shadowed generally will have a large percent difference between BB1 and BB2 and a low reflectance at about 499 nm.

Level II: A maximum broadband percent difference that is larger than about 0.5 indicates that there is a lighting artifact present. Lacking a large slope when the broadband measurements at about 576 and about 599 nm are divided and an off-channel broadband less than about 0.2 at about 419 nm reveals that the point is shadow instead of glare.

Cases where both BB and Fl measurements should be eliminated:

Low Signal:

Broadband measurements lower than about 0.035 at about 449 nm or fluorescence measurements lower than about 3.5 at about 479 nm indicate that the measurements are not coming from tissue, but rather from blood, the Os, smoke tube, speculum, or another obstruction. Sites with significant shadowing in both broadband channels are also identified with this metric. Because of the uncertainty of the tissue being measured, the reflectance and fluorescence data from that point are assumed invalid, regardless of whether it was identified by fluorescence or the broadband channels.

Mucus Metric:

Level I: Mucus broadband measurements generally will have a lower percent difference than glare, and a greater average reflectance than (unobstructed) tissue.

Level II: The shape of the mucus broadband curves can help to identify a point as mucus. A smaller change in the broadband measurements between about 406 nm and about 541 nm indicates a smaller hemoglobin β-band and therefore a greater chance of the observed point being mucus.

Level III: A smaller change from about 532 nm to about 544 nm is also an indication of a smaller hemoglobin β-band, and helps to differentiate mucus from glare.

Points that are obstructed by mucus will give inaccurate readings for fluorescence and both broadband reflectance channels.

[0118] The metrics used in this embodiment include a low signal metric, which detects spectral data affected by obstruction artifacts such as blood, a speculum, a smoke tube, or other obstruction. These were combined into one low signal metric in this embodiment, since regions affected by these artifacts exhibit similar characteristics, such as low fluorescence and low broadband reflectance measurements.

[0119] Figure 9A shows a graph depicting broadband reflectance 902 as a function of wavelength 904 for the BB1 channel 906 and the BB2 channel 908 measurements for a region of tissue where the BB1 data is affected by glare but the BB2 data is not, according to an illustrative embodiment of the invention. The glare leads to a higher value of reflectance 902 than that of surrounding unaffected tissue. By applying the metrics listed above according to an embodiment of this invention, it is determined that the BB1 set of spectral data is affected by glare and is thus not suitably representative of this region of the tissue sample. Applying the method of this embodiment also determines that the BB2 set of spectral data is representative of this region of the sample (unaffected by an artifact), since it is not eliminated. One embodiment comprises using this representative data to determine a condition of this region of the sample, for example, the state of health.

[0120] Figure 9B shows a graph depicting broadband reflectance 902 as a function of wavelength 904 for the BB1 channel 910 and the BB2 channel 912 broadband reflectance spectral data for a region of tissue where the BB2 data is affected by shadow but the BB1 data is not, according to an illustrative embodiment of the invention. The shadow leads to a lower value of reflectance 902 than that of surrounding unaffected tissue. By applying the metrics listed above in this embodiment, it is determined that the BB2 set of spectral data is affected by shadow and is therefore not suitably representative of this region of the tissue sample. However, applying this method also leads to the determination that the BB1 set of spectral data is representative of this region of the sample, since the BB1 set of data is not eliminated. One embodiment comprises using this representative data to determine a condition of this region of the sample, for example, the state of health.

[0121] Figure 9C shows a graph depicting broadband reflectance 902 as a function of wavelength 904 for the BB1 channel 914 and the BB2 channel 916 measurements for a region of tissue that is obscured by blood, according to an illustrative embodiment of the invention. By applying the metrics listed above, it is determined that blood is present, and that both the BB1

and the BB2 sets of spectral data are considered unrepresentative of this region of the tissue sample.

[0122] Figure 9D shows a graph depicting broadband reflectance 902 as a function of wavelength 904 for the BB1 channel 918 and the BB2 channel 920 measurements for a region of tissue that is unobscured, according to an illustrative embodiment of the invention. Applying this method determines that neither set of spectral data is affected by an artifact, and, therefore, either is representative of the tissue sample. One embodiment comprises using an average value 922 of the BB1 and BB2 measurements at each wavelength to represent the region of the tissue sample in determining a condition of this region, for example, the state of health of the region.

[0123] Application of the metrics listed above was performed using various tissue types to verify the sensitivity and specificity of the metrics. While it is undesirable in preferred embodiments to eliminate good spectral data of normal tissue, it is worse in preferred embodiments to eliminate good spectral data of diseased tissue, particularly if it is desired to use the data in the classification of the state of health of a region of tissue. The following tissue types were used in the verification: tt-132 (metaplasia by impression), tt-155 (normal by impression), tt-115 (mucus), tt-117 (blood), and NEDpath (no evidence of disease confirmed by pathology), and cin23all (CIN II/CIN III diseased tissue). Table 1 shows the number of points (regions) corresponding to each of these tissue types, the determinations from the metrics listed above for these points, and the number of points where one set of broadband reflectance spectral data were eliminated, where both sets of broadband reflectance spectral data were eliminated, and where both reflectance and fluorescence spectral data were eliminated.

Table 1: Verification of Metrics

Tissue Type	cin23all	nedpath	tt-115	tt-117	tt-132	tt-155
Total pts.	230	460	584	26	3909	1356
Low Signal	2	4	10	16	2	1
Mucus	0	3 (2)	85	0	5	0
Glare in BB1	5	21	19	0	115	11
Glare in BB2	4	22	9	2	113	20
Glare in both	6	5	42	1	45	3
Shadow in BB1	17	10	7	2	165	46
Shadow in BB2	6	24 (1)	44	0	291	30
One BB Removed	32 (13.9%)	77 (16.7%)	79 (13.5%)	4 (15.4%)	685 (17.5%)	107 (1.3%)
Both BB Removed	8 (3.5%)	12 (2.6%)	137 (23.5%)	17 (65.4%)	52 (1.3%)	3 (0.2%)
Fl and BB Removed	2 (0.9%)	7 (1.5%)	95 (16.27%)	16 (61.5%)	7 (0.18%)	1 (0.07%)

[0124] For the regions (points) corresponding to CIN II/ CIN III diseased tissue, no broadband reflectance measurements were unnecessarily eliminated from the set. The points identified as being low signal were all located on the Os. All points that were identified by the metric as glare or shadow were verified as being correct.

[0125] For the nedpath points (no evidence of disease), only two tissue points were unnecessarily eliminated after being misidentified as mucus. A point that was actually dark red tissue with glare was incorrectly identified as shadow in BB2. The points that were identified as glare were verified as being correct.

[0126] Out of the 584 mucus points, 85 were correctly identified as mucus. The points that were identified as glare or shadow (and mucus) were verified as being correct.

[0127] Out of the 26 blood points, 16 were identified as being low signal. The glare points and shadow points were accurate.

[0128] Out of the 3909 points in the metaplasia by impression group, there were no valid tissue points lost. The data set was improved by eliminating over 700 readings of points affected by either glare or shadow.

[0129] Out of the 1358 normal by impression points, no measurements were unnecessarily removed from the set.

[0130] Figure 10A shows a graph depicting the reduction in the variability of broadband reflectance measurements 1002 of CIN II/III-confirmed tissue produced by filtering (eliminating non-representative spectral data) using the metrics described above, according to an illustrative embodiment of the invention. The graph depicts mean values and standard deviations of broadband reflectance spectral data before and after filtering.

[0131] Figure 10B shows a graph depicting the reduction in the variability of broadband reflectance measurements 1002 of tissue classified as "no evidence of disease confirmed by pathology" produced by filtering using the metrics described above, according to an illustrative embodiment of the invention. The graph depicts mean values and standard deviations of broadband reflectance spectral data before and after filtering.

[0132] Figure 10C shows a graph depicting the reduction in the variability of broadband reflectance measurements 1002 of tissue classified as "metaplasia by impression" produced by filtering using the metrics described above, according to an illustrative embodiment of the invention. The graph depicts mean values and standard deviations of broadband reflectance spectral data before and after filtering.

[0133] Figure 10D shows a graph depicting the reduction in the variability of broadband reflectance measurements 1002 of tissue classified as "normal by impression" produced by filtering using the metrics described above, according to an illustrative embodiment of the invention. The graph depicts mean values and standard deviations of broadband reflectance spectral data before and after filtering.

[0134] Figure 11A depicts an exemplary image of cervical tissue 1102 divided into regions for which two types of reflectance spectral data and one type of fluorescence spectral data are obtained, according to an illustrative embodiment of the invention. Figure 11B is a representation of the regions depicted in Figure 11A and shows the categorization of each region according to an illustrative embodiment of the invention. The black-highlighted sections 1104 of the image 1102 in Figure 11A correspond to points (regions) that had both reflectance measurements eliminated by application of the embodiment method. Many of the lower points 1106, as seen in both Figures 11A and 11B, are in shadow because the speculum obstructs the view of one of the channels. Glare is correctly identified prominently at the upper one o'clock position 1108. Since there are blood points on the shadowed section, some are labeled blood (low signal) and others are treated as shadow. The mucus metric identifies several points 1110 around the Os that have a bubbly, mucus-like liquid.

[0135] Figure 12A depicts an exemplary image of cervical tissue 1202 divided into regions for which two types of reflectance spectral data and one type of fluorescence spectral data are obtained, according to an illustrative embodiment of the invention. Figure 12B is a representation of the regions depicted in Figure 11A and shows the categorization of each region according to an illustrative embodiment of the invention. Figures 12A and 12B show an example of a cervix that has a large portion of the lower half 1204 affected by shadow. However, only one of the sets of reflectance spectral data (BB2) is affected by the shadow artifact. The BB1 reflectance spectral data is not affected by shadow. Applying the metrics above, the BB1 data are used to describe these regions, while the BB2 data are eliminated from consideration. The accuracy of the reflectance measurements should be improved significantly for this patient using the metrics of the embodiment discussed above, since the more accurate broadband measurements will be used instead of simply averaging the two broadband measurements, which would skew the measurements due to a lighting artifact.

[0136] Figure 13A depicts an exemplary image of cervical tissue 1302 divided into regions for which two types of reflectance spectral data and one type of fluorescence spectral data are obtained, according to an illustrative embodiment of the invention. Figure 13B is a representation of the regions depicted in Figure 13A and shows the categorization of each region according to an illustrative embodiment of the invention. Figures 13A and 13B show an image with a portion 1304 that is shadowed and off of the cervix. Due to an obstruction from the smoke tube in the upper part of the image, there are many low signals. Even though much of the cervix is shadowed in BB1 1306, there are still some usable BB2 and fluorescence readings.

[0137] Figure 14A depicts an exemplary image of cervical tissue 1402 divided into regions for which two types of reflectance spectral data and one type of fluorescence spectral data are obtained, according to an illustrative embodiment of the invention. Figure 14B is a representation of the regions depicted in Figure 14A and shows the categorization of each region according to an illustrative embodiment of the invention. The image in Figure 14A shows a large mucus obstruction 1404. The metrics of the embodiment do a good job of identifying mucus that is thick and light colored. The metrics correctly mark the dark red tissue as tissue instead of blood or shadow. The top edge of the cervix is identified as shadow, and glare points are correctly flagged.

Equivalents

[0138] While the invention has been particularly shown and described with reference to specific preferred embodiments, it should be understood by those skilled in the art that various changes in form and detail may be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

What is claimed is:

1. A method of determining a condition of a region of a tissue sample, said method comprising the steps of:

(a) obtaining a first set of spectral data corresponding to a region of a tissue sample using light incident to said region at a first angle;

(b) obtaining a second set of spectral data corresponding to said region using light incident to said region at a second angle;

(c) selecting at least one of said first set of spectral data and said second set of spectral data that is representative of said region of said tissue sample; and

(d) determining a condition of said region of said tissue sample based at least in part on a subset of said at least one set of spectral data selected in step (c).

2. The method of claim 1, wherein said first set of spectral data comprises reflectance spectral data and said second set of spectral data comprises reflectance spectral data.

3. The method of claim 1, wherein said at least one of said first set of spectral data and said second set of spectral data comprises fluorescence spectral data.

4. The method of claim 1, further comprising obtaining a third set of spectral data corresponding to said region using light incident to said region at a third angle.

5. The method of claim 1, further comprising obtaining each of a plurality of sets of spectral data in addition to said first set and said second set using light incident to said region at a unique angle.

6. The method of claim 1, wherein said condition is a state of health.

7. The method of claim 6, wherein said state of health comprises at least one of the conditions of normal squamous tissue, metaplasia, CIN I, CIN II, CIN III, CIS, and cancer.

8. A method of determining whether spectral data obtained from a region of a tissue sample are affected by an artifact, said method comprising the steps of:

obtaining a first set of spectral data corresponding to a region of a tissue sample using light incident to said region at a first angle;

obtaining a second set of spectral data corresponding to said region using light incident to said region at a second angle; and

determining whether said first set of data is affected by an artifact based at least in part on a subset of said first set of data and a subset of said second set of data.

9. The method of claim 8, wherein said first set of spectral data comprises reflectance spectral data and said second set of spectral data comprises reflectance spectral data.

10. The method of claim 8, further comprising obtaining a third set of spectral data, where said third set of spectral data comprises fluorescence spectral data.

11. The method of claim 8, wherein said determining step comprises computing a difference between R_1, a member of said first set of spectral data, and R_2, a member of said second set of spectral data, and comparing said difference to a constant, where R_1 and R_2 correspond to at least approximately identical wavelengths.

12. The method of claim 11, wherein said difference is a percent difference.

13. The method of claim 8, wherein said determining step comprises computing N differences, $|R_1(X_i)-R_2(X_i)|$, optionally weighting each of said differences using at least one of $R_1(X_i)$ and $R_2(X_i)$, defining a maximum of a subset of said N optionally-weighted differences, and comparing said maximum to a first constant, where i = 1 to N, N is an integer, X_i is a wavelength between about 360nm and about 720nm, $R_1(X_i)$ is a member of said first set of data corresponding to said wavelength X_i, and $R_2(X_i)$ is a member of said second set of data corresponding to said wavelength X_i.

14. The method of claim 13, wherein said determining step further comprises comparing $R_1(X_1)$ to a second constant, where $R_1(X_1)$ is a member of said first set of data corresponding to a wavelength X_1 between about 409nm and about 429nm.

15. The method of claim 8, wherein said determining step comprises comparing $R_1(X_1)$ to a constant, where $R_1(X_1)$ is a member of said first set of data corresponding to a wavelength X_1 between about 409nm and about 429nm.

16. The method of claim 8, wherein said determining step comprises comparing the quotient $R_1(X_1)/R_1(X_2)$ to a constant, where $R_1(X_1)$ is a member of said first set of data corresponding to a wavelength X_1 between about 360nm and about 720nm, and $R_1(X_2)$ is a member of said first set of data corresponding to a wavelength X_2 between about 360nm and about 720nm.

17. The method of claim 16 wherein X_1 is a wavelength between about 489nm and 509nm and X_2 is a wavelength between about 533nm and about 553nm.

18. The method of claim 13, wherein said determining step further comprises comparing the quotient $\{(R_1(X_1)/R_2(X_1))/(R_1(X_2)/R_2(X_2))\}$ to a second constant, where X_1 is a wavelength between about 360nm and about 720nm, X_2 is a wavelength between about 360nm and about 720nm, $R_1(X_1)$ is a member of said first set of data corresponding to said wavelength X_1, $R_2(X_1)$ is a member of said second set of data corresponding to said wavelength X_1, $R_1(X_2)$ is a member of said first set of data corresponding to said wavelength X_2, $R_2(X_2)$ is a member of said second set of data corresponding to said wavelength X_2.

19. The method of claim 18 wherein X_1 is a wavelength between about 566nm and about 586nm, and X_2 is a wavelength between about 589nm and about 609nm.

20. The method of claim 19, wherein said determining step further comprises comparing $R_1(X_3)$ to a third constant, where $R_1(X_3)$ is a member of said first set of data corresponding to a wavelength X_3 between about 689 and about 709nm.

21. The method of claim 13, wherein said determining step further comprises comparing a value Q to a second constant, where Q is an approximate slope of a plot of $\{R_1(X_i)/R_2(X_i)\}$ with respect to wavelength, over a subset of a wavelength range of about 360nm to about 720nm.

22. The method of claim 15, wherein said determining step further comprises comparing a value Q to a second constant, where said value Q is an approximate slope of a plot of $\{R_1(X_i)/R_2(X_i)\}$ with respect to wavelength, over a subset of a wavelength range of about 576nm to about 599nm.

23. The method of claim 13, wherein said determining step further comprises comparing $R_1(X_1)$ to a second constant and comparing $R_1(X_1)$ to $R_2(X_1)$, where $R_1(X_1)$ is a member of said first set of data corresponding to a wavelength X_1 between about 360nm and about 720nm, and $R_2(X_1)$ is a member of said second set of data corresponding to said wavelength X_1.

24. The method of claim 13, wherein said determining step further comprises comparing $R_1(X_1)$ to a second constant and comparing $R_1(X_1)$ to $R_2(X_1)$, where $R_1(X_1)$ is a member of said first set of data corresponding to a wavelength X_1 between about 489nm and about 509nm, and $R_2(X_1)$ is a member of said second set of data corresponding to said wavelength X_1.

25. The method of claim 18, wherein said determining step further comprises comparing $R_1(X_3)$ to a third constant, where $R_1(X_3)$ is a member of said first set of data corresponding to a wavelength X_3 between about 360nm and about 720nm.

26. The method of claim 18, wherein said determining step further comprises comparing $R_1(X_3)$ to a third constant, where $R_1(X_3)$ is a member of said first set of data corresponding to a wavelength X_3 between about 409nm and about 429nm.

27. The method of claim 8, wherein said determining step comprises comparing R_1 to a first constant and comparing R_2 to a second constant, where R_1 is a member of said first set of data corresponding to a wavelength between about 489nm and about 509nm and R_2 is a member of said second set of data corresponding to a wavelength between about 489nm and about 509nm.

28. The method of claim 8, wherein said artifact comprises a lighting artifact.

29. The method of claim 28, wherein said lighting artifact comprises glare.

30. The method of claim 28, wherein said lighting artifact comprises shadow.

31. The method of claim 8, wherein said artifact comprises an obstruction.

32. The method of claim 31, wherein said obstruction comprises blood.

33. The method of claim 31, wherein said obstruction comprises a portion of at least one of a group consisting of a speculum and a smoke tube.

34. The method of claim 31, wherein said obstruction comprises mucus.

35. The method of claim 8, wherein said tissue sample comprises cervical tissue.

36. The method of claim 8, wherein said tissue sample comprises epithelial cells.

37. The method of claim 8, wherein said tissue sample comprises at least one of a group consisting of colorectal, gastroesophageal, urinary bladder, lung, and skin tissue.

38. A method of determining whether spectral data corresponding to a region of a tissue sample is affected by an artifact, said method comprising the steps of:

obtaining a first set of reflectance spectral data corresponding to a region of a tissue sample using light incident to said region at a first angle;

obtaining a second set of reflectance spectral data corresponding to said region using light incident to said region at a second angle;

obtaining a set of fluorescence spectral data corresponding to said region; and

determining whether any of said first set of reflectance spectral data, said second set of reflectance spectral data and said set of fluorescence spectral data are affected by an artifact based at least in part on at least one of the following: a subset of said first set of reflectance spectral data, a subset of said second set of reflectance spectral data, and a subset of said set of fluorescence spectral data.

39. The method of claim 38, wherein said determining step comprises comparing F to a constant, where F is a member of said set of fluorescence spectral data corresponding to a wavelength between about 469nm and about 489nm.

40. A method of determining a spectral characteristic of an artifact, said method comprising the steps of:

(a) at each of a first plurality of regions of tissue, obtaining a first set of reflectance spectral data affected by a known artifact;

(b) at each of a second plurality of regions of tissue, obtaining a second set of reflectance spectral data not affected by said known artifact; and

(c) determining a spectral characteristic of said known artifact based at least in part on said first set of spectral data and said second set of spectral data.

41. The method of claim 40, wherein said determining step comprises locating a wavelength at which there is a maximum difference between a mean of one or more members of said first set

corresponding to said wavelength and a mean of one or more members of said second set corresponding to said wavelength, relative to a variation measure.

42. The method of claim 40, wherein said determining step comprises computing N differences, $|\mu_i(A_j(X_i))-\mu_i(B_k(X_i))|$, and defining a maximum of a subset of said N differences, where i = 1 to N, N is an integer, X_i is a wavelength between about 360nm and about 720nm, j = 1 to M1, M1 is an integer, $A_j(X_i)$ represents one of M1 members of said first set of reflectance spectral data corresponding to said wavelength X_i, k = 1 to M2, M2 is an integer, $B_k(X_i)$ represents one of M2 members of said second set of reflectance spectral data corresponding to said wavelength X_i, $\mu_i(A_j(X_i))$ is a mean of said M1 members of said first set of data corresponding to said wavelength X_i, and $\mu_i(B_k(X_i))$ is a mean of said M2 members of said second set of data corresponding to said wavelength X_i.

43. The method of claim 40, wherein said determining step comprises computing N quotients, $[|\mu_i(A_j(X_i))-\mu_i(B_k(X_i))| / \{\sigma_i^2(A_j(X_i))+\sigma_i^2(B_k(X_i))\}^{0.5}]$, and defining a maximum of a subset of said N quotients, where i = 1 to N, N is an integer, X_i is a wavelength between about 360nm and about 720nm, j = 1 to M1, M1 is an integer, $A_j(X_i)$ represents one of M1 members of said first set of reflectance spectral data corresponding to said wavelength X_i, k = 1 to M2, M2 is an integer, $B_k(X_i)$ represents one of M2 members of said second set of reflectance spectral data corresponding to said wavelength X_i, $\mu_i(A_j(X_i))$ is a mean of said M1 members of said first set of data corresponding to said wavelength X_i, $\mu_i(B_k(X_i))$ is a mean of said M2 members of said second set of data corresponding to said wavelength X_i, $\sigma_i(A_j(X_i))$ represents a standard deviation of said M1 members of said first set of data corresponding to said wavelength X_i, and $\sigma_i(B_k(X_i))$ represents a standard deviation of said M2 members of said second set of data corresponding to said wavelength X_i.

44. The method of claim 40, wherein said determining step comprises computing N quotients, $[|\mu_i(A_j(X1_i)/A_j(X2_i))-\mu_i(B_k(X1_i)/B_k(X2_i))| / \{\sigma_i(A_j(X1_i)/A_j(X2_i))+ \sigma_i^2(B_k(X1_i)/B_k(X2_i))\}^{0.5}]$, and defining a maximum of a subset of said N quotients, where i = 1 to N, N is an integer, $X1_i$ is a wavelength between about 360nm and about 720nm, $X2_i$ is a wavelength between about 360nm and about 720nm, j = 1 to M1, M1 is an integer, $A_j(X1_i)$ represents one of M1 members of said first set of reflectance spectral data corresponding to said wavelength $X1_i$, $A_j(X2_i)$ represents one of M1 members of said first set of reflectance spectral data corresponding to said wavelength $X2_i$, k = 1 to M2, M2 is an integer, $B_k(X1_i)$ represents one of M2 members of said second set of reflectance spectral data corresponding to said wavelength $X1_i$, $B_k(X2_i)$ represents one of M2 members of said second set of reflectance spectral data corresponding to said wavelength $X2_i$, $\mu_i(A_j(X1_i)/A_j(X2_i))$ is a mean of M1 quotients

$A_j(X1_i)/A_j(X2_i)$ for j = 1 to M1, $\mu_i(B_k(X1_i)/B_k(X2_i))$ is a mean of M2 quotients $B_k(X1_i)/B_k(X2_i)$ for k = 1 to M2, $\sigma_i(A_j(X1_i)/A_j(X2_i))$ represents a standard deviation of said M1 quotients $A_j(X1_i)/A_j(X2_i)$, and $\sigma_i(B_k(X1_i)/B_k(X2_i))$ represents a standard deviation of said M2 quotients $B_k(X1_i)/B_k(X2_i)$.

45. A method of determining a characteristic of a region of a tissue sample, said method comprising the steps of:

(a) obtaining a first set of reflectance spectral data corresponding to a region of a tissue sample using light incident to said region at a first angle;

(b) obtaining a second set of reflectance spectral data corresponding to said region using light incident to said region at a second angle;

(c) determining whether at least one of said first set of reflectance data and said second set of reflectance data is affected by an artifact based at least in part on a subset of said first set of reflectance data and a subset of said second set of reflectance data;

(d) rejecting at least one member of at least one of said first set of reflectance data and said second set of reflectance data determined in step (c) to be affected by said artifact; and

(e) determining a characteristic of said region of said tissue sample based at least in part on at least one member of at least one of said first set of reflectance data and said second set of reflectance data not rejected in step (d).

46. The method of claim 45, further comprising obtaining a set of fluorescence spectral data corresponding to said region, and wherein step (e) comprises determining said condition of said region of said tissue sample based at least in part on

at least one member of at least one of said first set of reflectance data and said second set of reflectance data

and at least one member of said set of fluorescence spectral data.

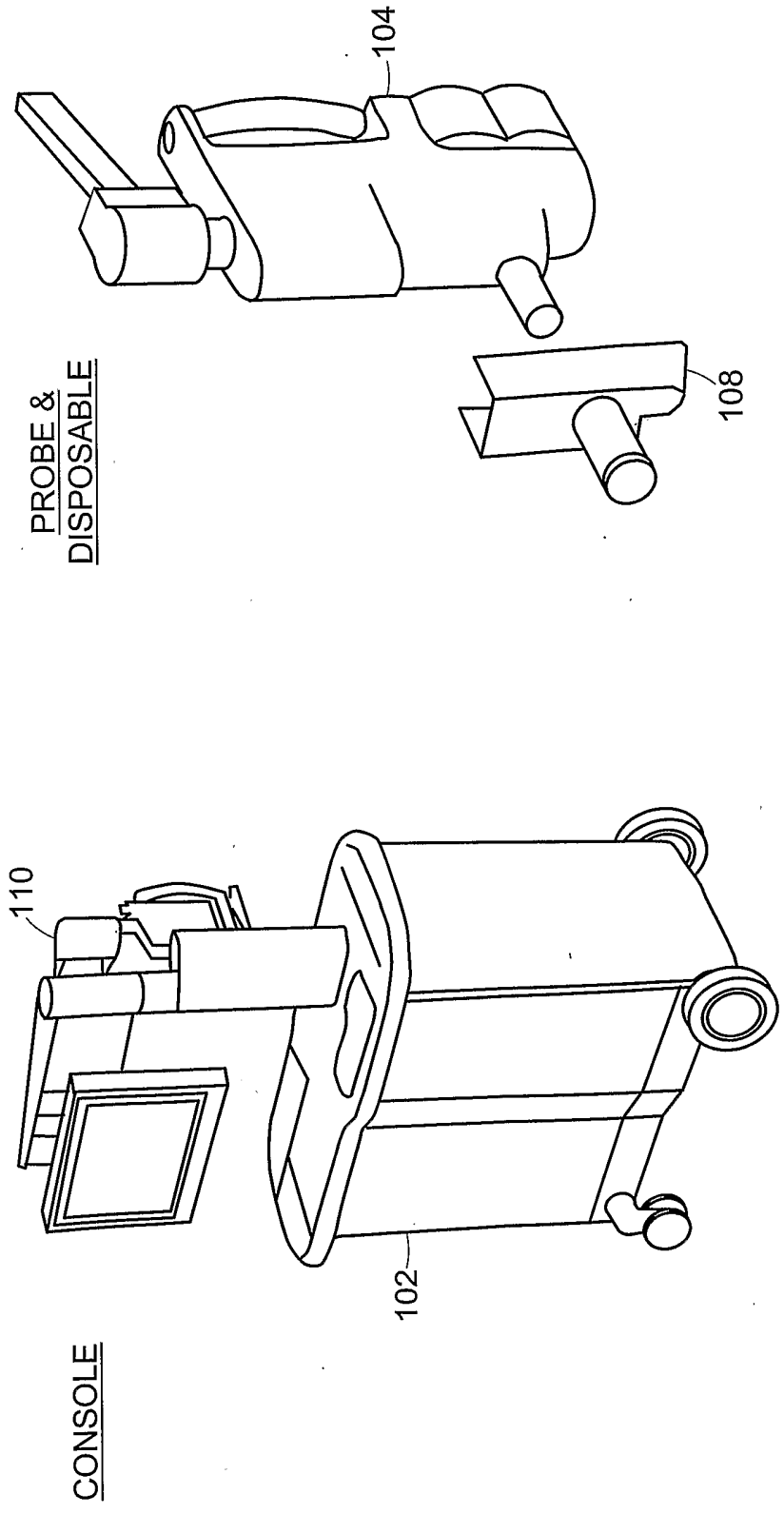


FIG. 1

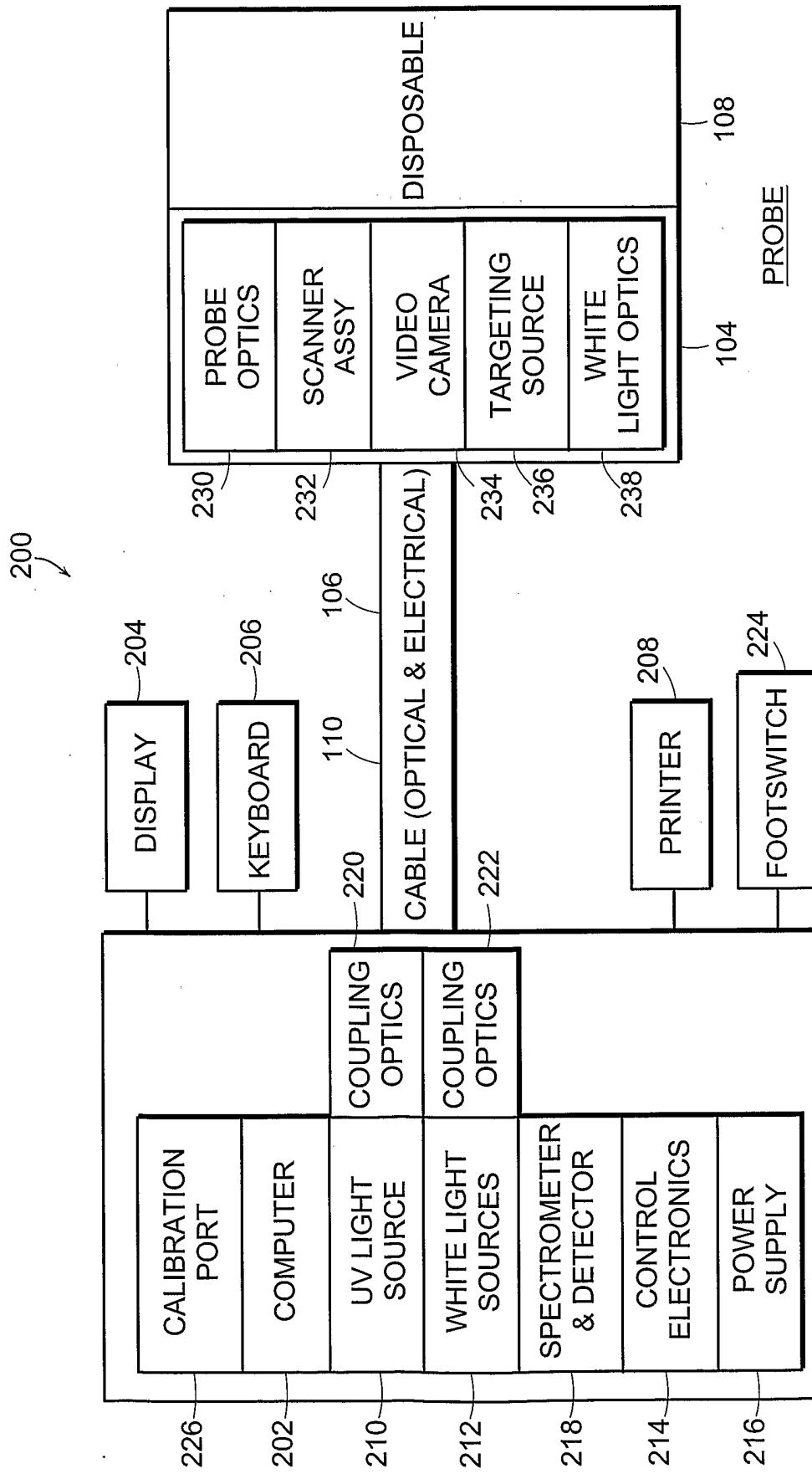
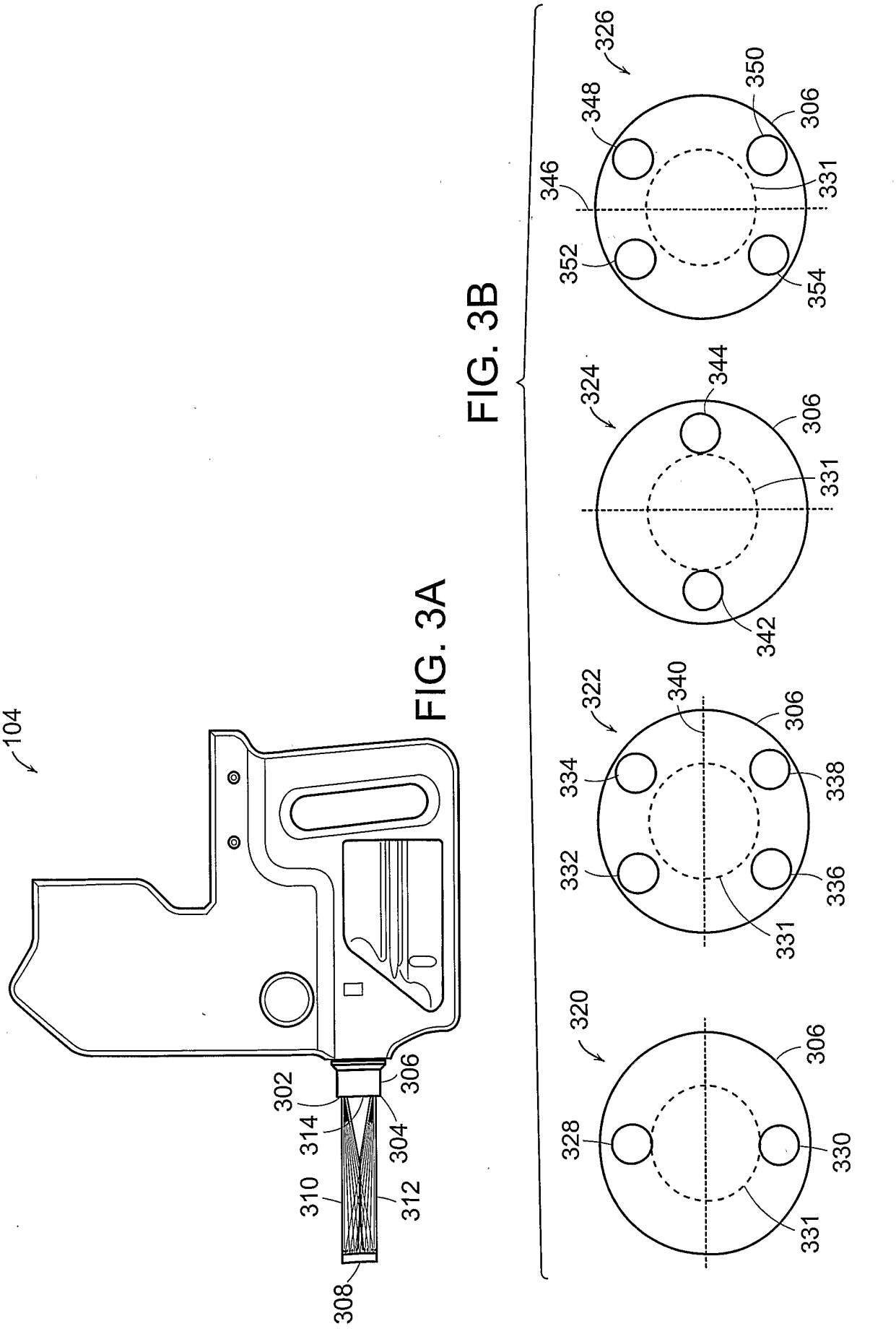

200
DISPOSABLE
108
PROBE OPTICS
SCANNER ASSY
VIDEO CAMERA
TARGETING SOURCE
WHITE LIGHT OPTICS
PROBE
104
230
232
234
236
238
106
CABLE (OPTICAL & ELECTRICAL)
110
220
222
204
DISPLAY
206
KEYBOARD
208
PRINTER
224
FOOTSWITCH
COUPLING OPTICS
COUPLING OPTICS
CALIBRATION PORT
COMPUTER
UV LIGHT SOURCE
WHITE LIGHT SOURCES
SPECTROMETER & DETECTOR
CONTROL ELECTRONICS
POWER SUPPLY
CONSOLE
102
226
202
210
212
218
214
216

FIG. 2


104
302
306
314
304
310
312
308
FIG. 3A
FIG. 3B
320
328
330
306
331
322
334
332
340
338
336
324
342
344
326
346
348
352
350
354

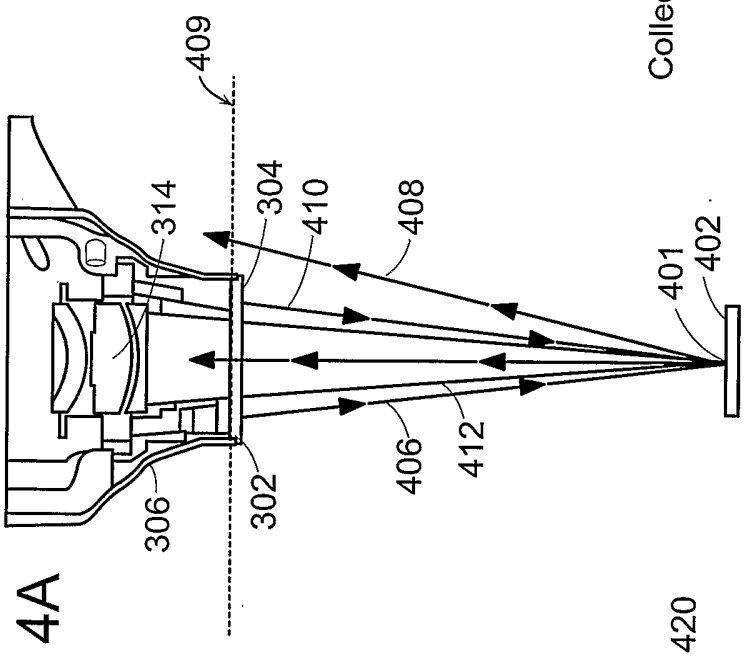


FIG. 4A

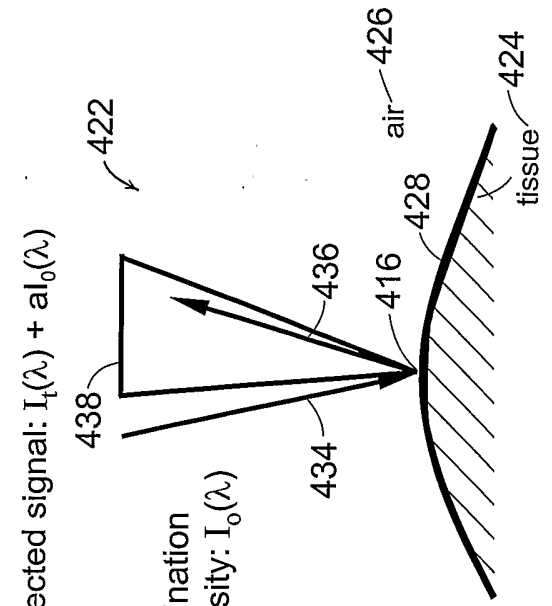


FIG. 4B-2

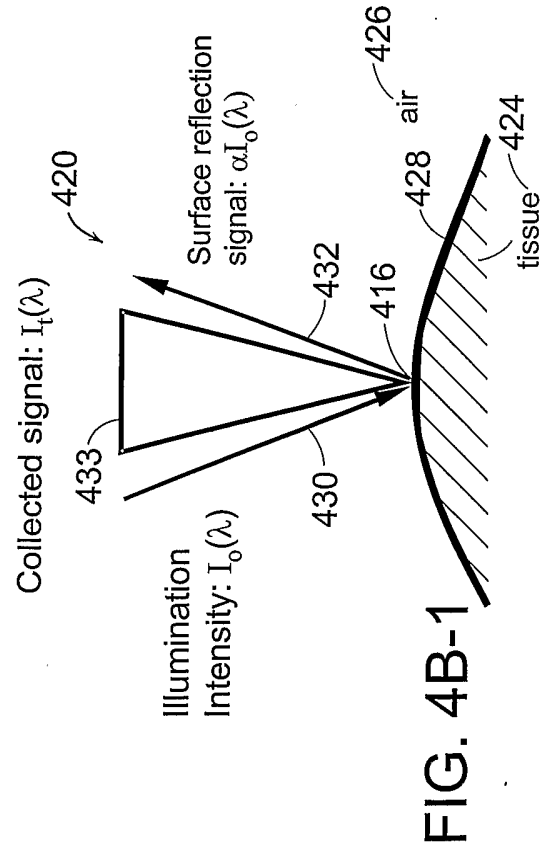


FIG. 4B-1

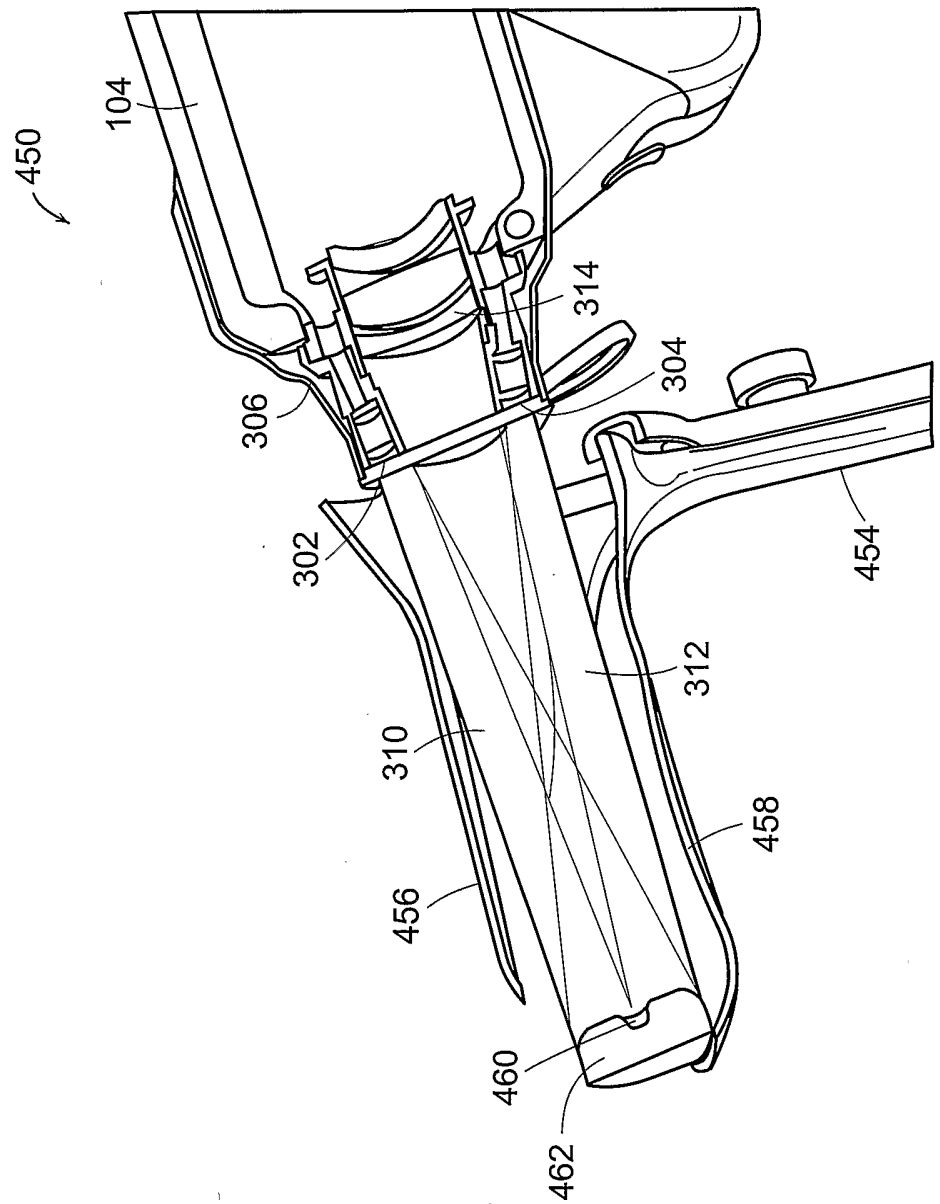


FIG. 4C

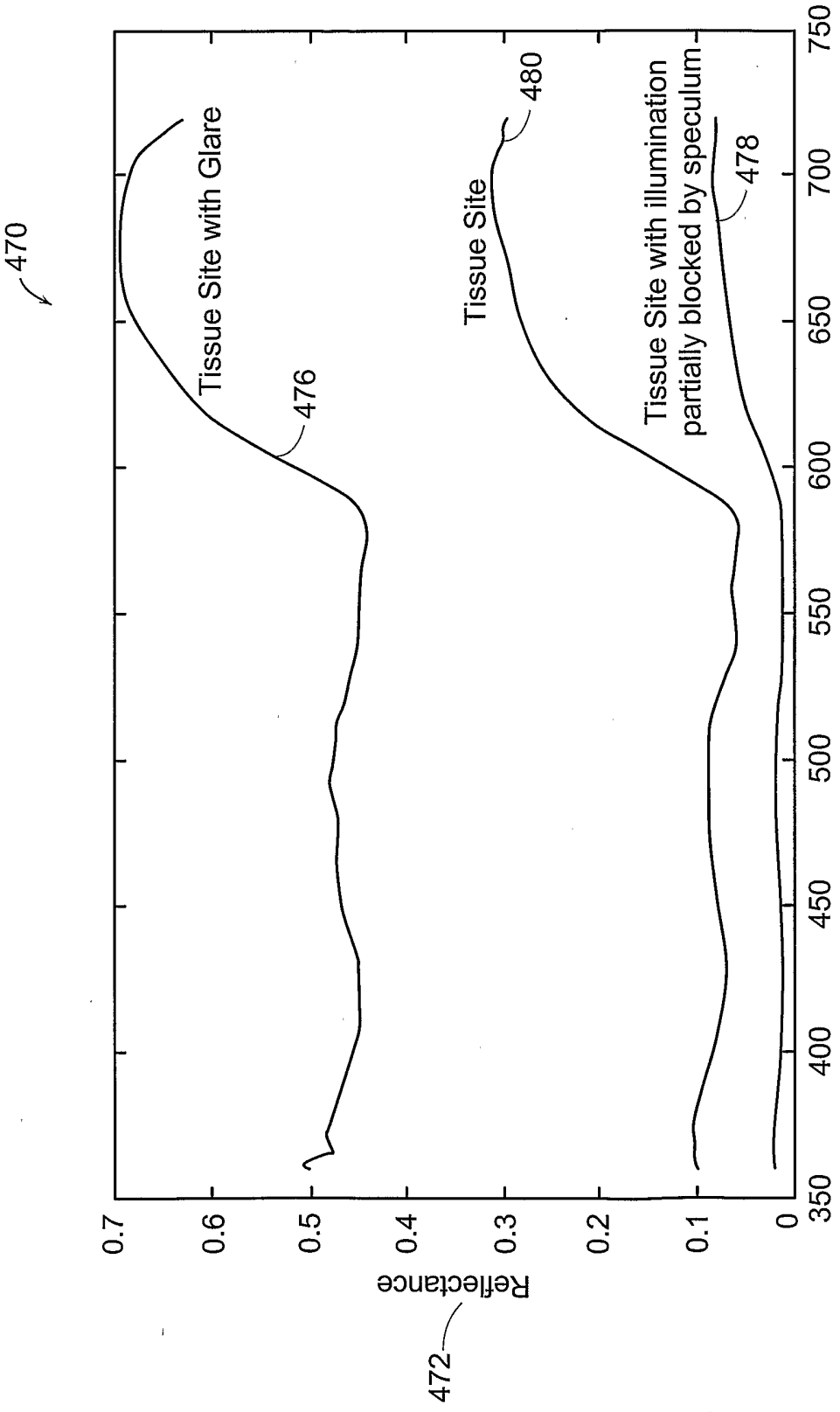

470
Tissue Site with Glare
476
Tissue Site
480
Tissue Site with illumination
partially blocked by speculum
478
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Reflectance
472
350
400
450
500
550
600
650
700
750
Wavelength (nm)
474

FIG. 4D

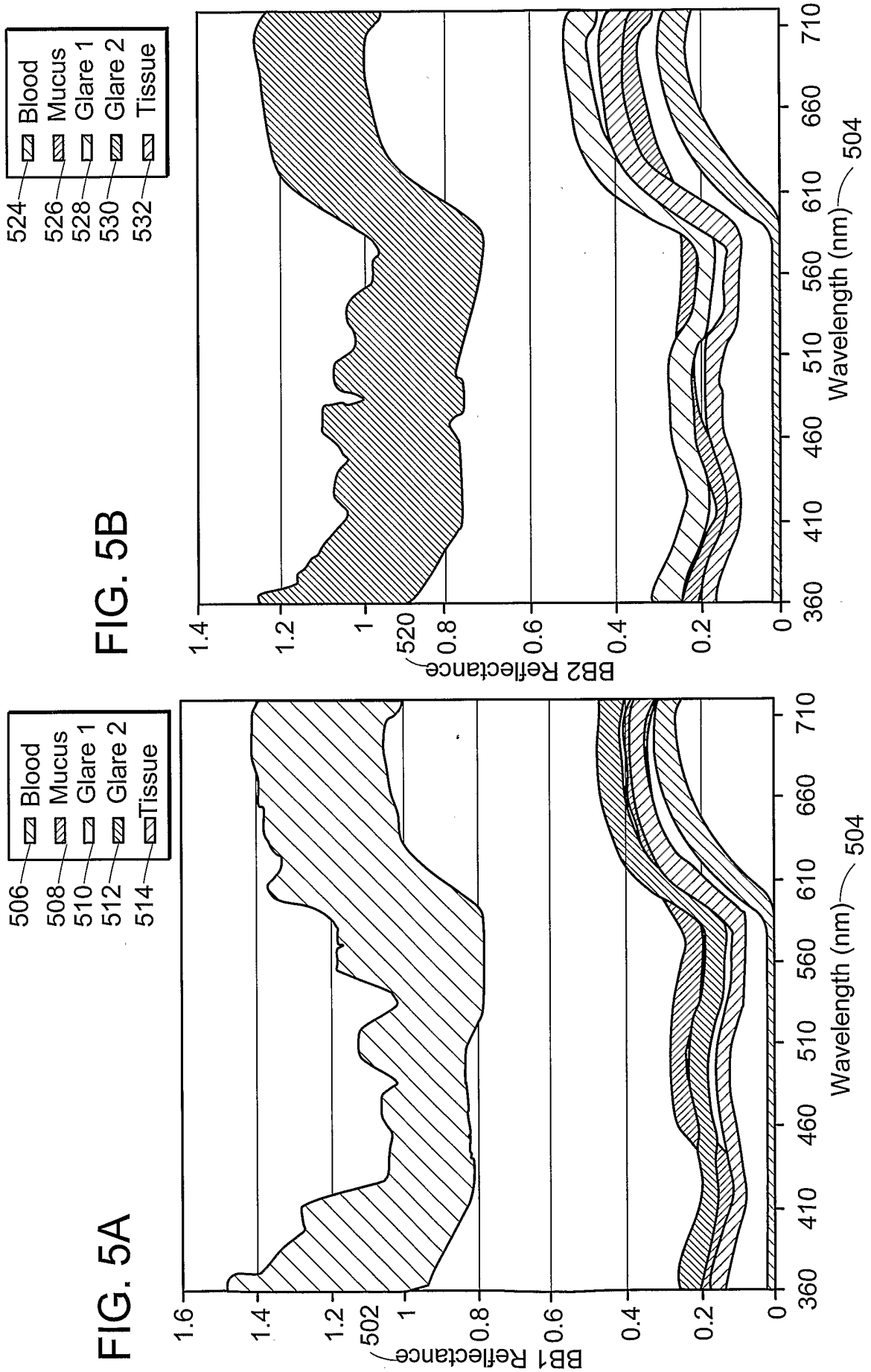

506 Blood
508 Mucus
510 Glare 1
512 Glare 2
514 Tissue
BB1 Reflectance
502
Wavelength (nm)
504
524 Blood
526 Mucus
528 Glare 1
530 Glare 2
532 Tissue
BB2 Reflectance
520
Wavelength (nm)
504

FIG. 5A

FIG. 5B

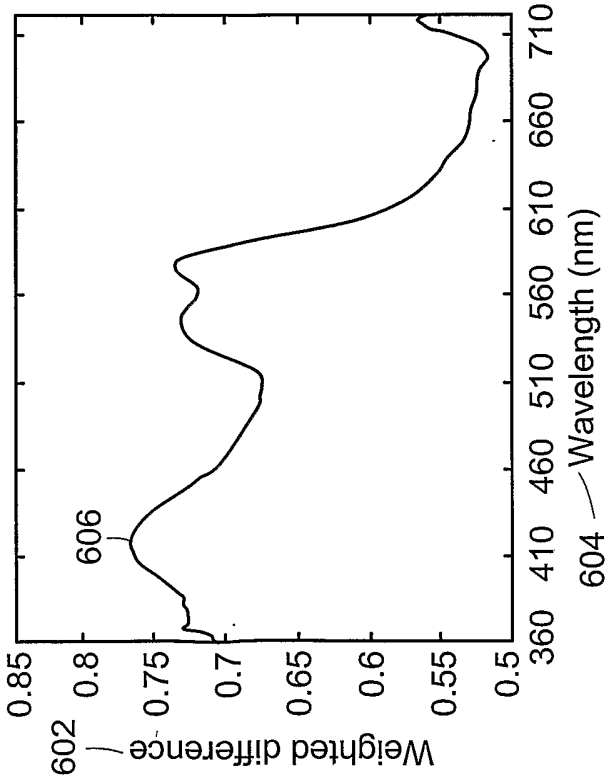


FIG. 6A

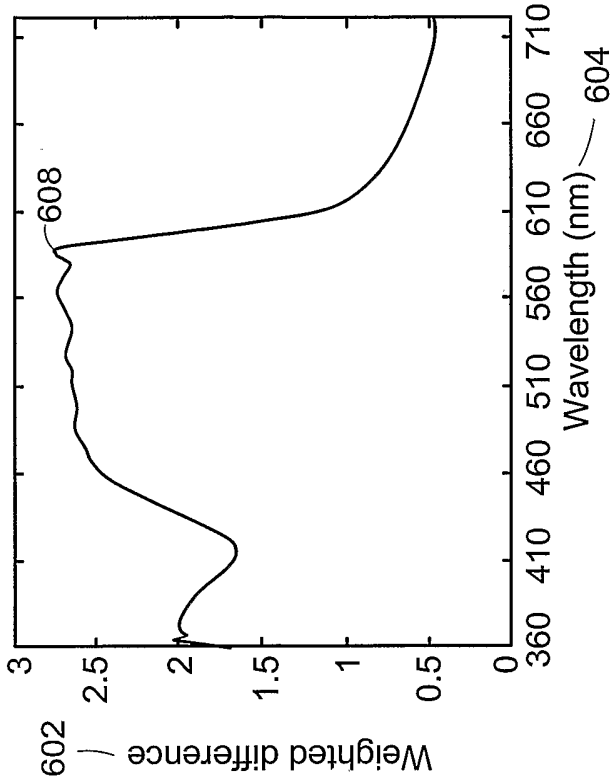


FIG. 6B

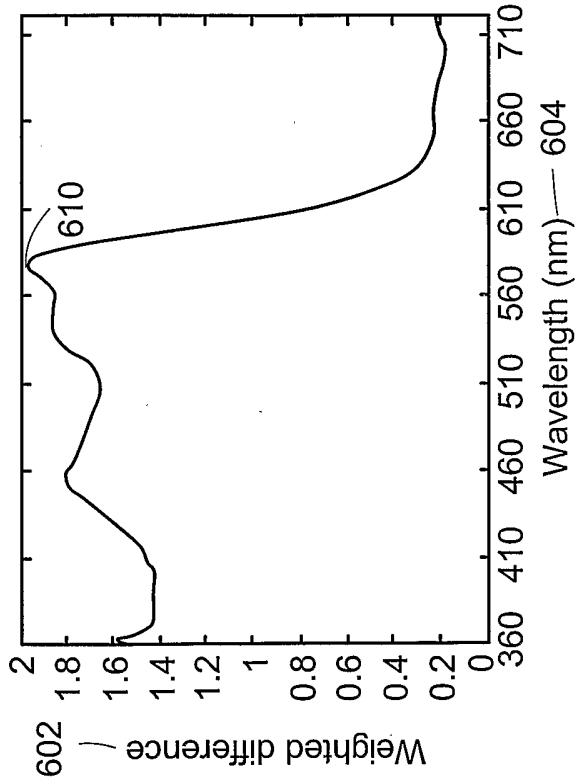


FIG. 6C

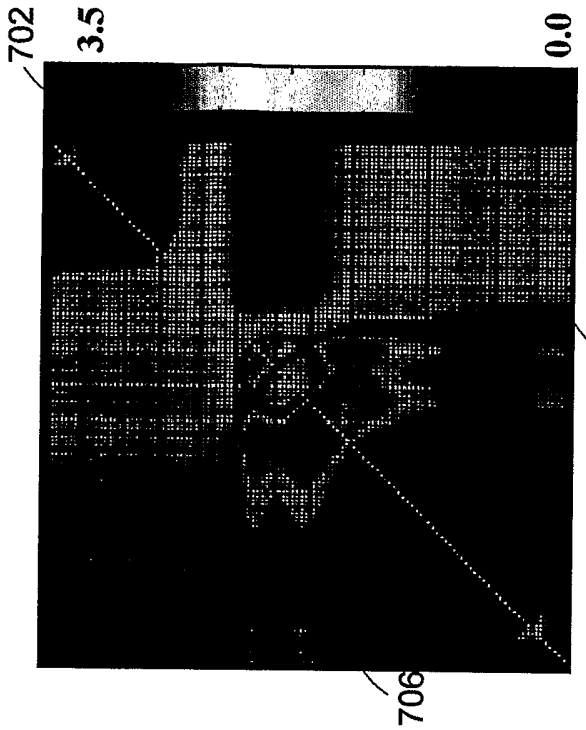


FIG. 7B

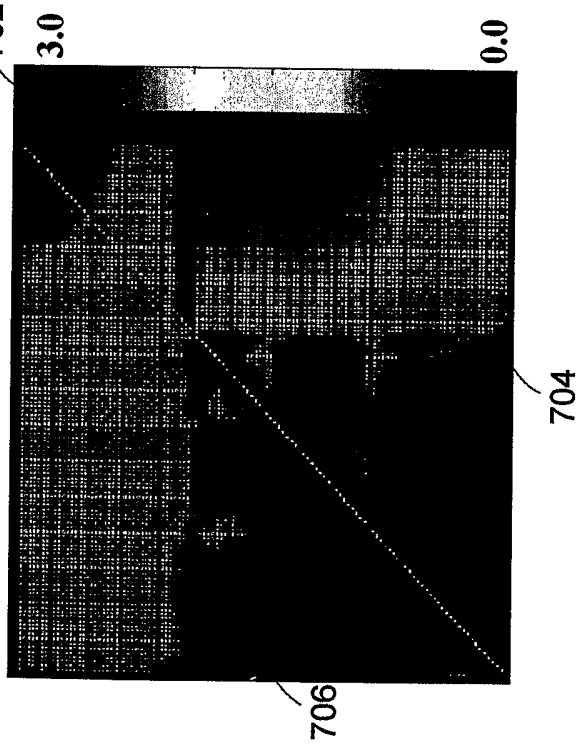


FIG. 7C

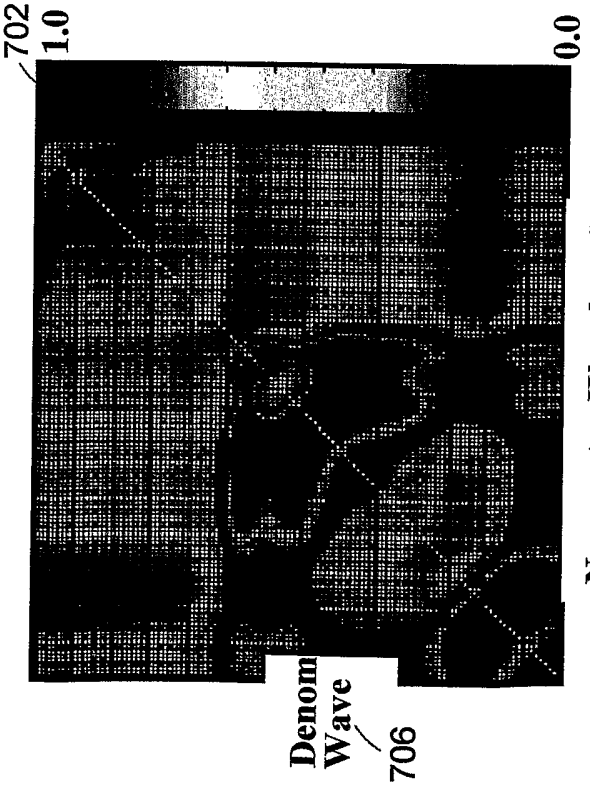


FIG. 7A

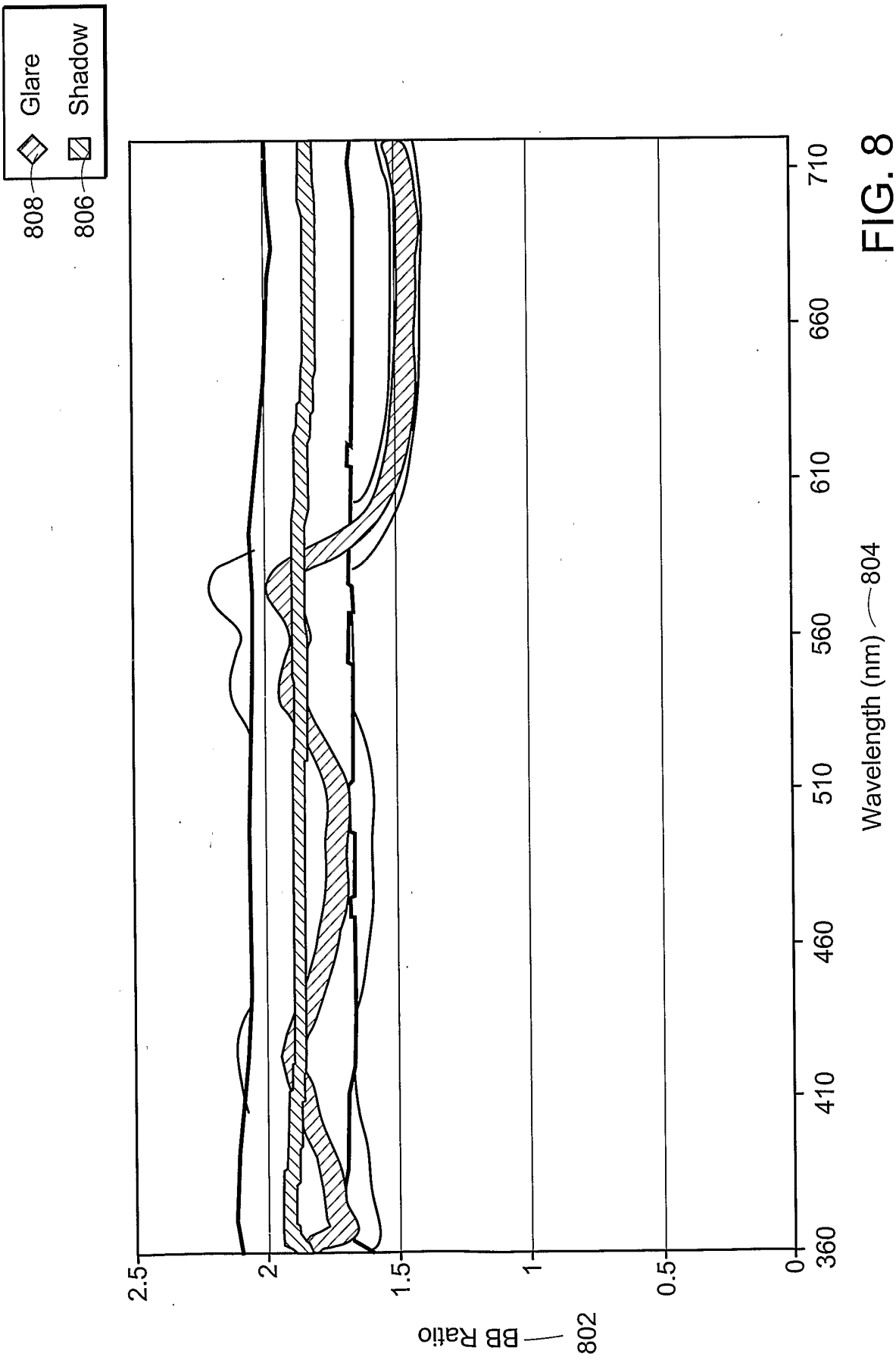

Glare
Shadow
808
806
2.5
2
1.5
1
0.5
0
BB Ratio
802
360
410
460
510
560
610
660
710
Wavelength (nm)
804

FIG. 8

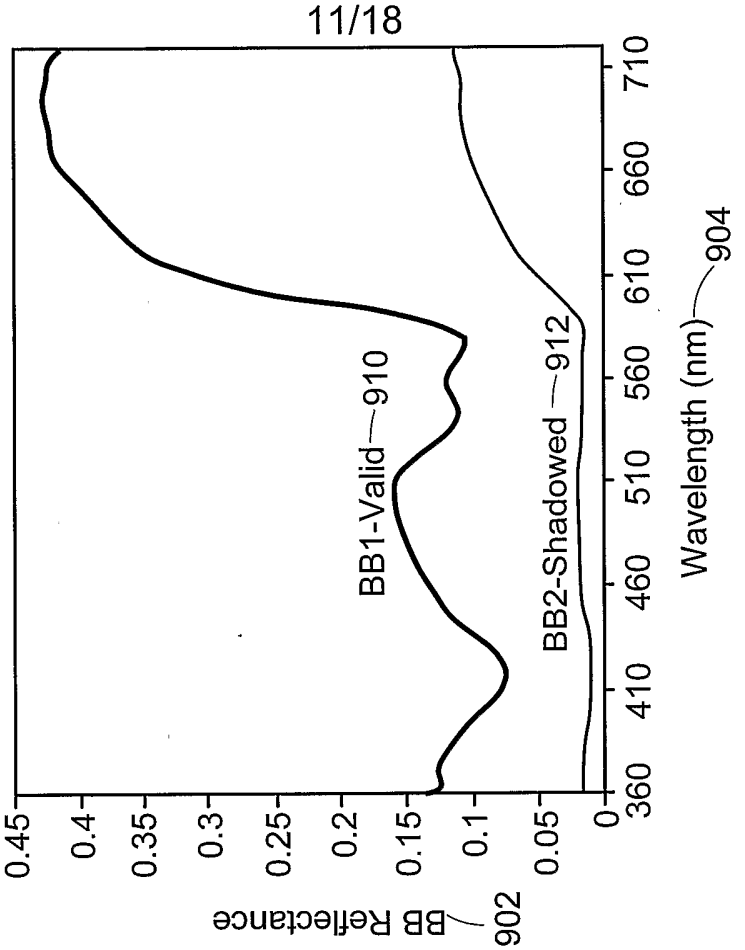

BB Reflectance
902
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
360
410
460
510
560
610
660
710
Wavelength (nm)
904
BB1-Valid
910
BB2-Shadowed
912

FIG. 9B

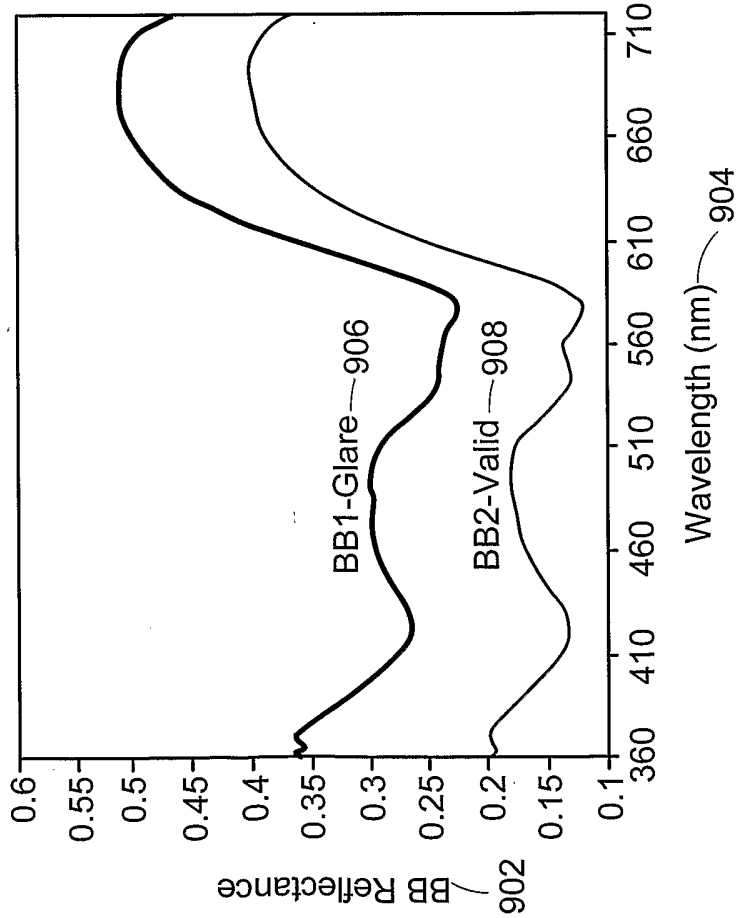

BB Reflectance
902
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
360
410
460
510
560
610
660
710
Wavelength (nm)
904
BB1-Glare
906
BB2-Valid
908

FIG. 9A

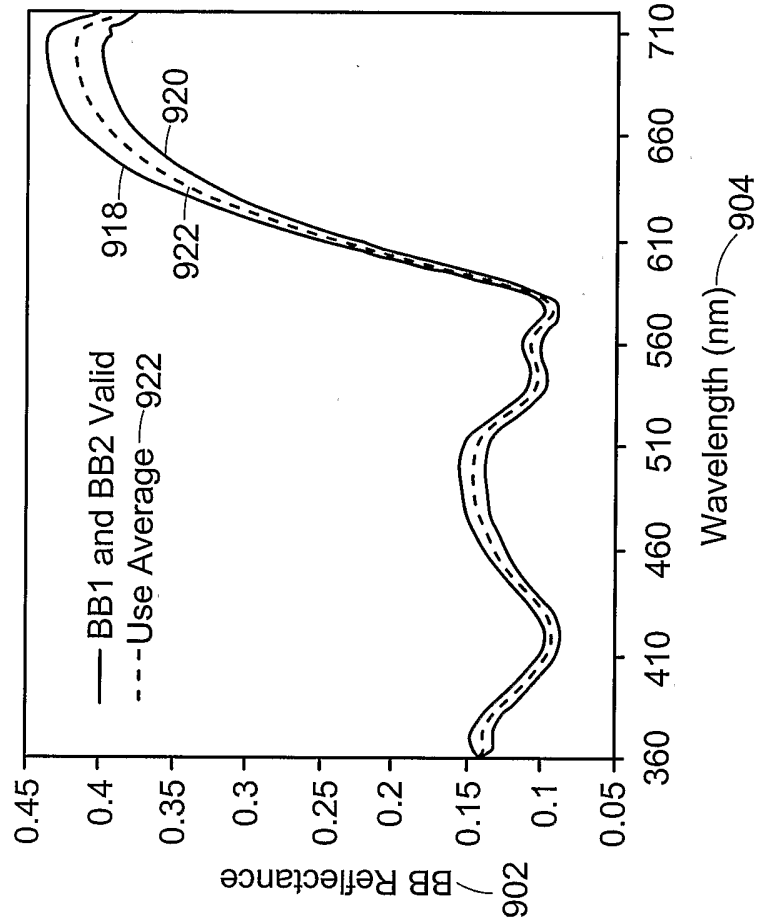

BB1 and BB2 Valid
Use Average
922
918
920
922
BB Reflectance
902
Wavelength (nm)
904
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
360
410
460
510
560
610
660
710

FIG. 9D

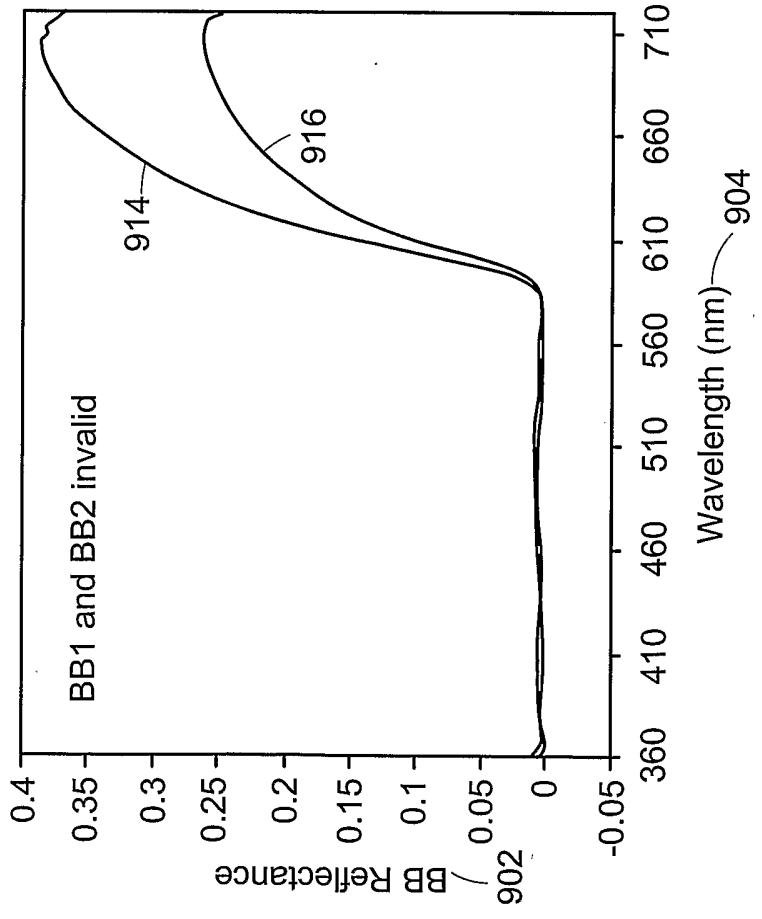

BB1 and BB2 invalid
914
916
BB Reflectance
902
Wavelength (nm)
904
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
360
410
460
510
560
610
660
710

FIG. 9C

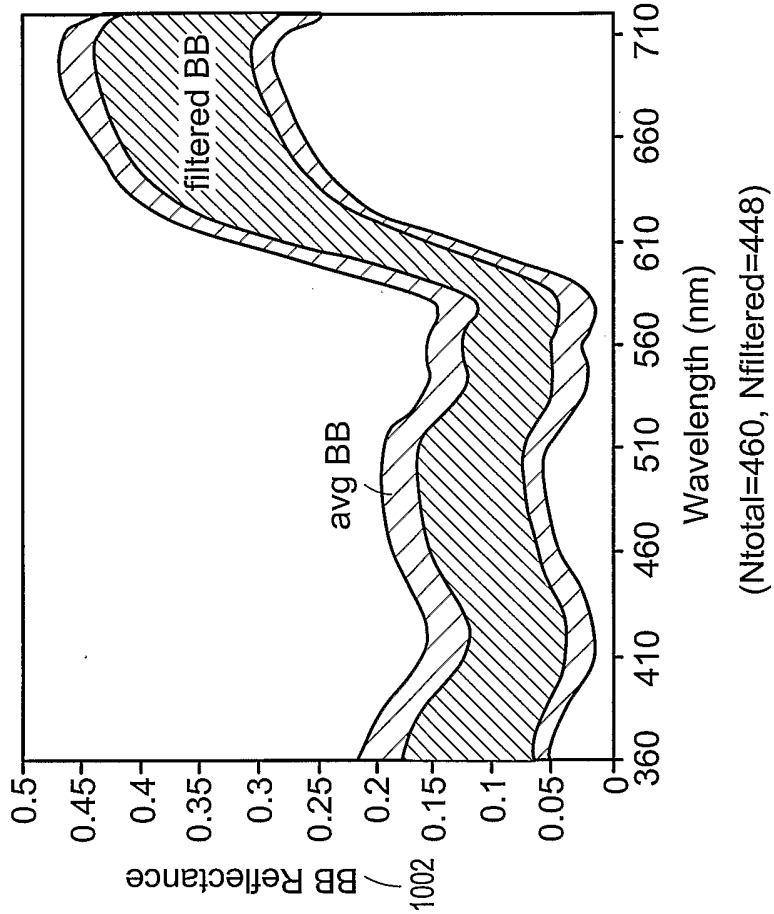


FIG. 10B

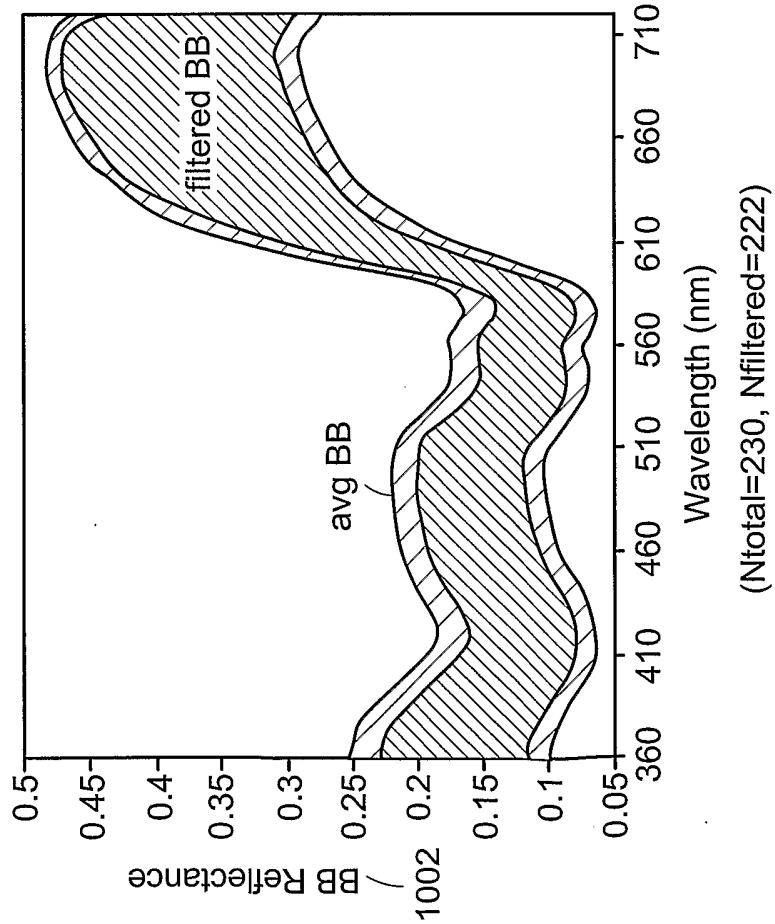


FIG. 10A

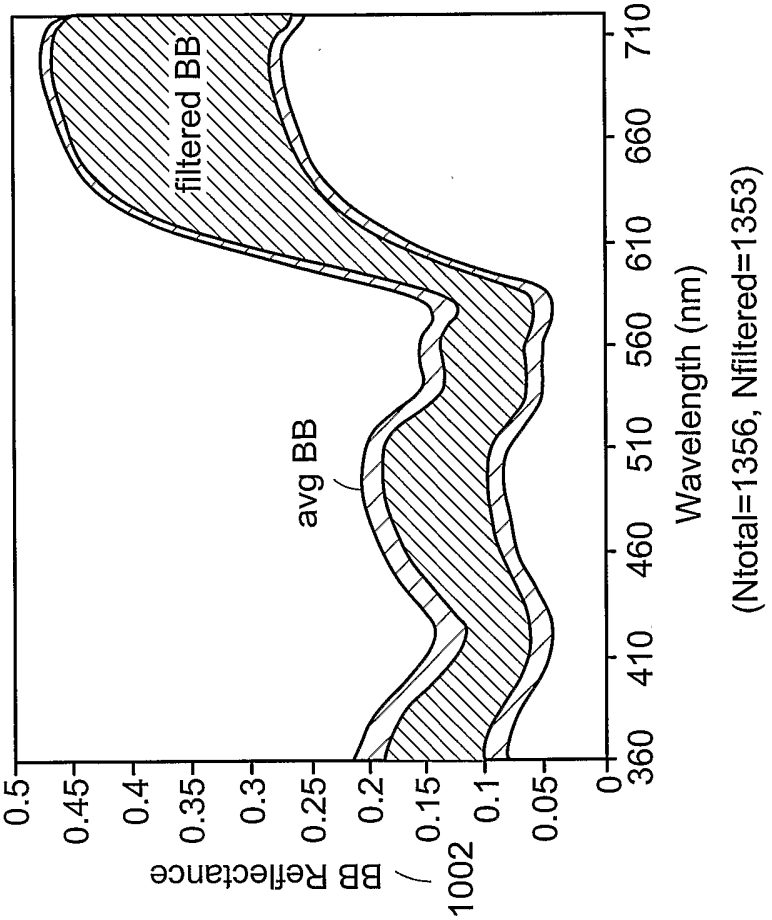


FIG. 10D

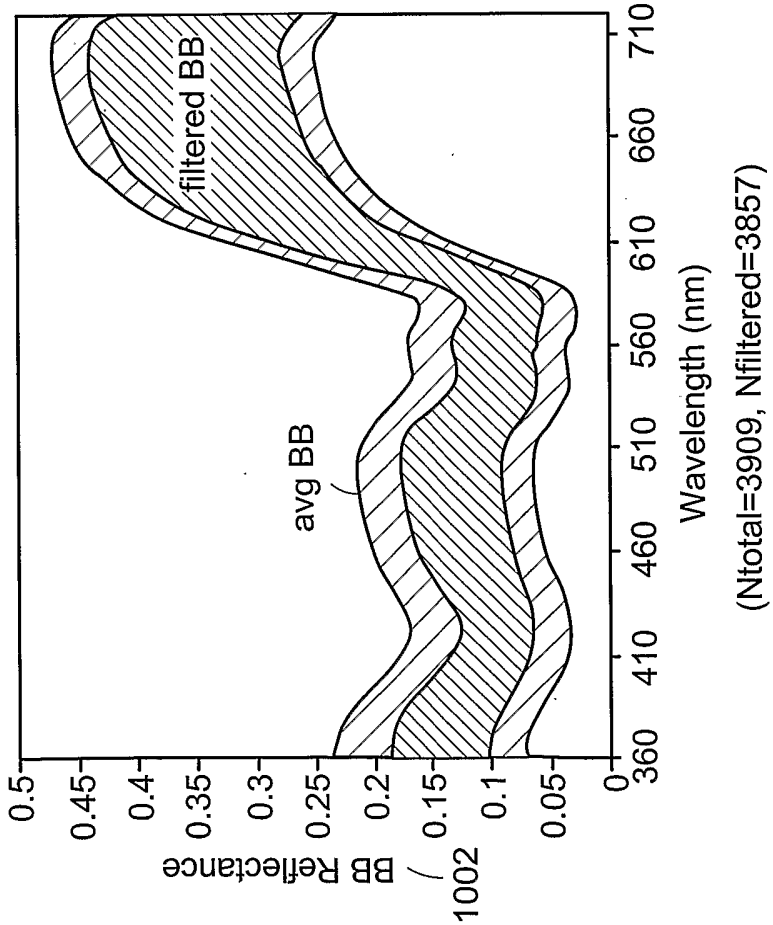


FIG. 10C

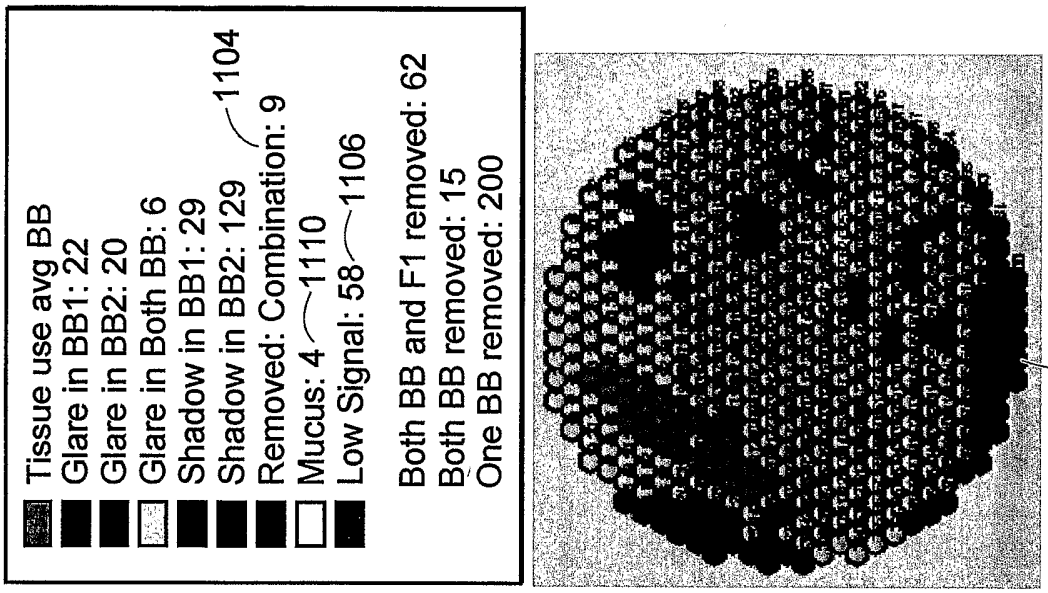


FIG. 11B

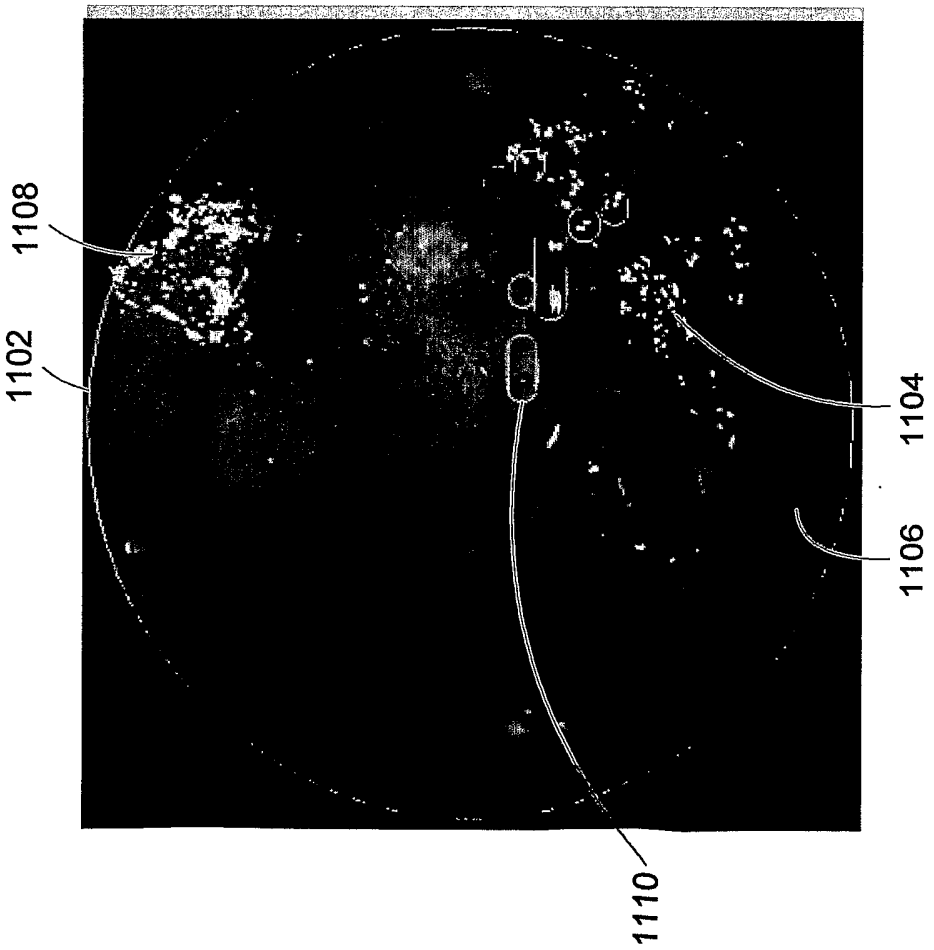
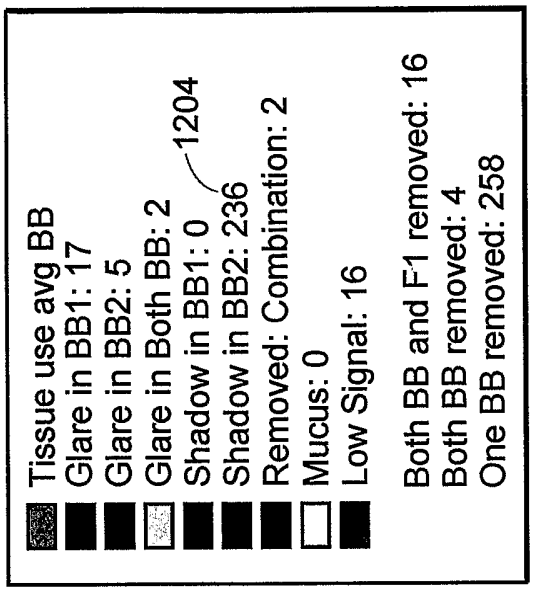


FIG. 11A



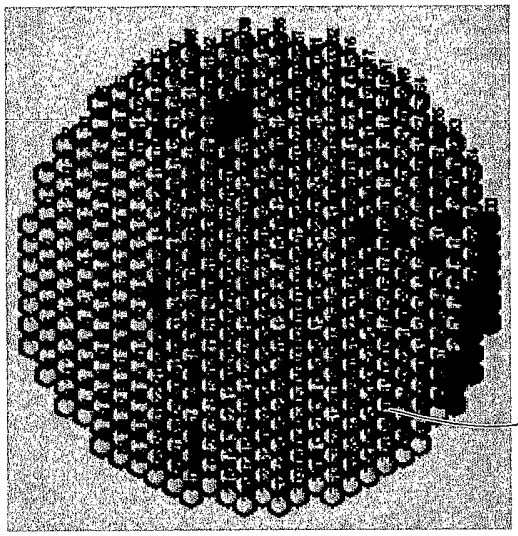


FIG. 12B

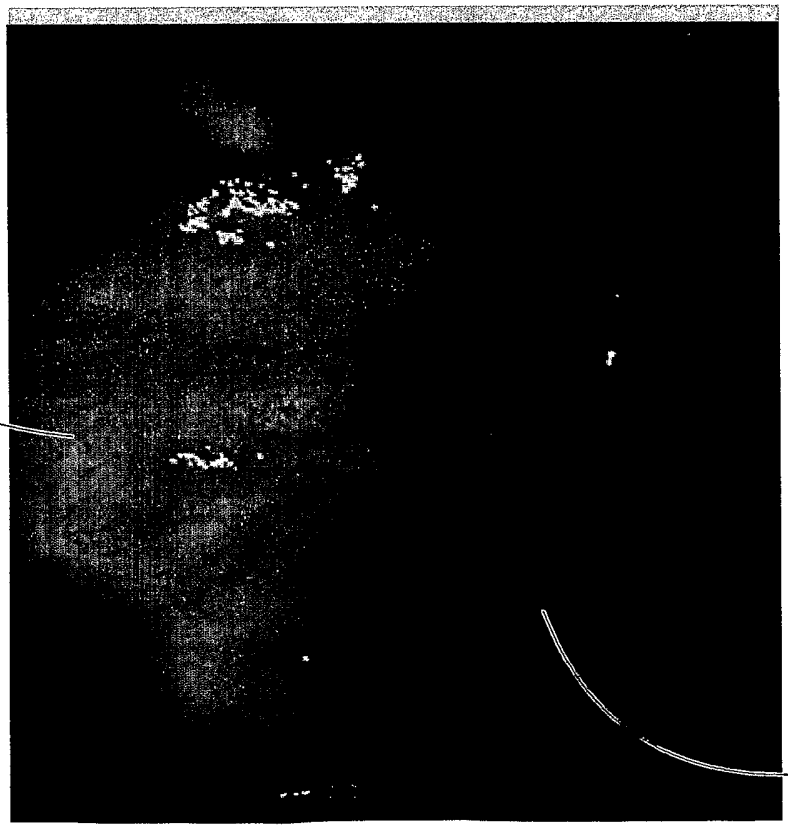


FIG. 12A

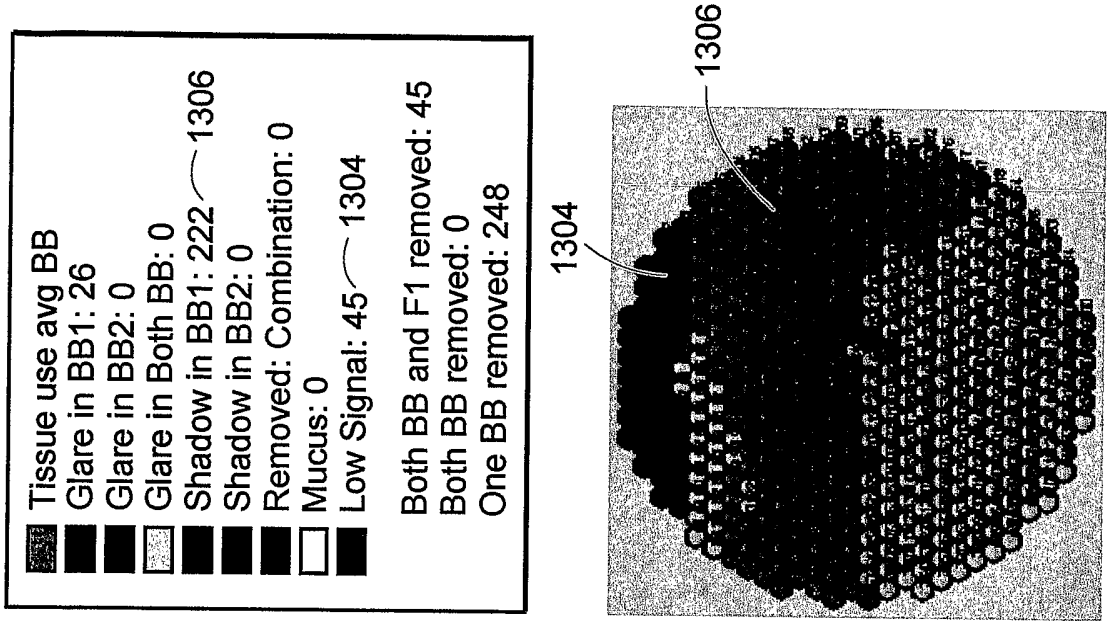


FIG. 13B

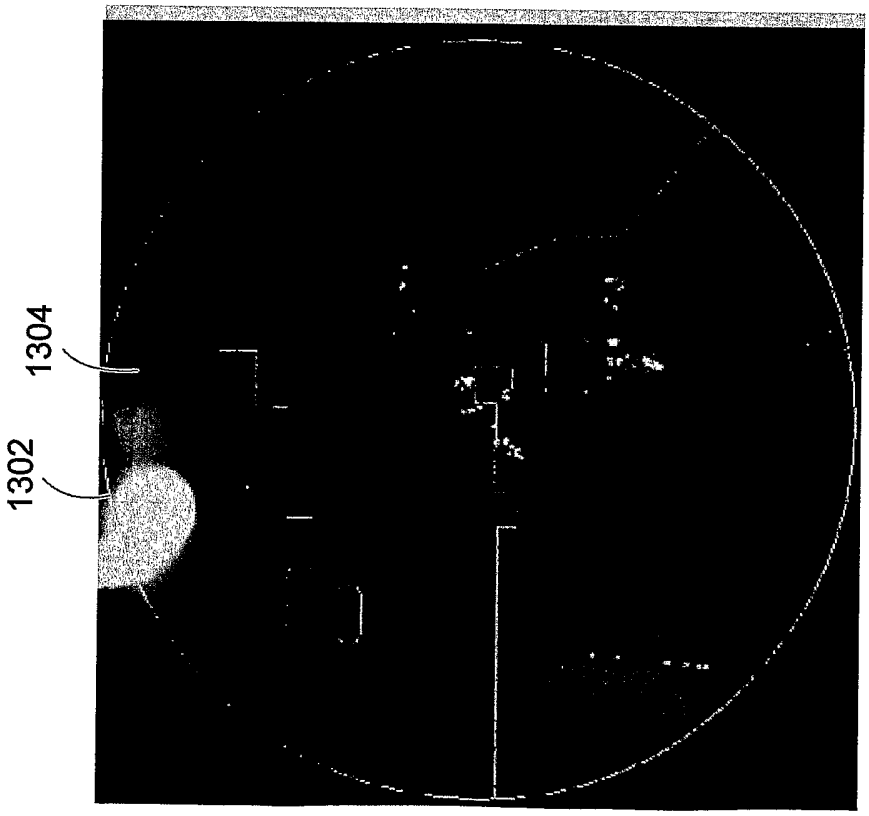
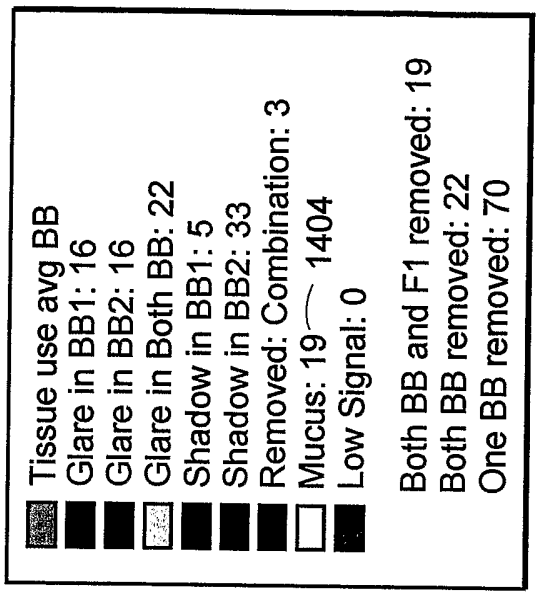


FIG. 13A



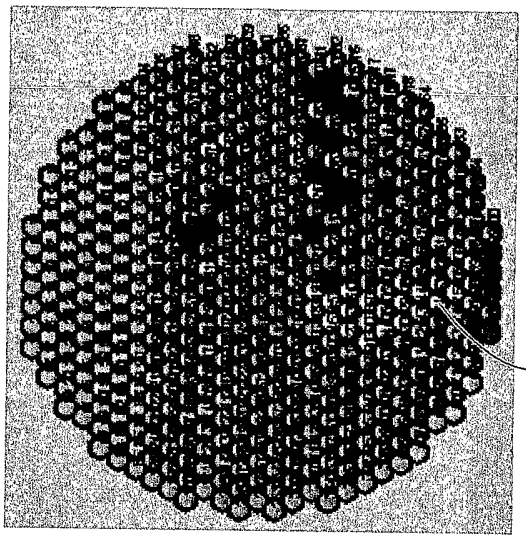


FIG. 14B

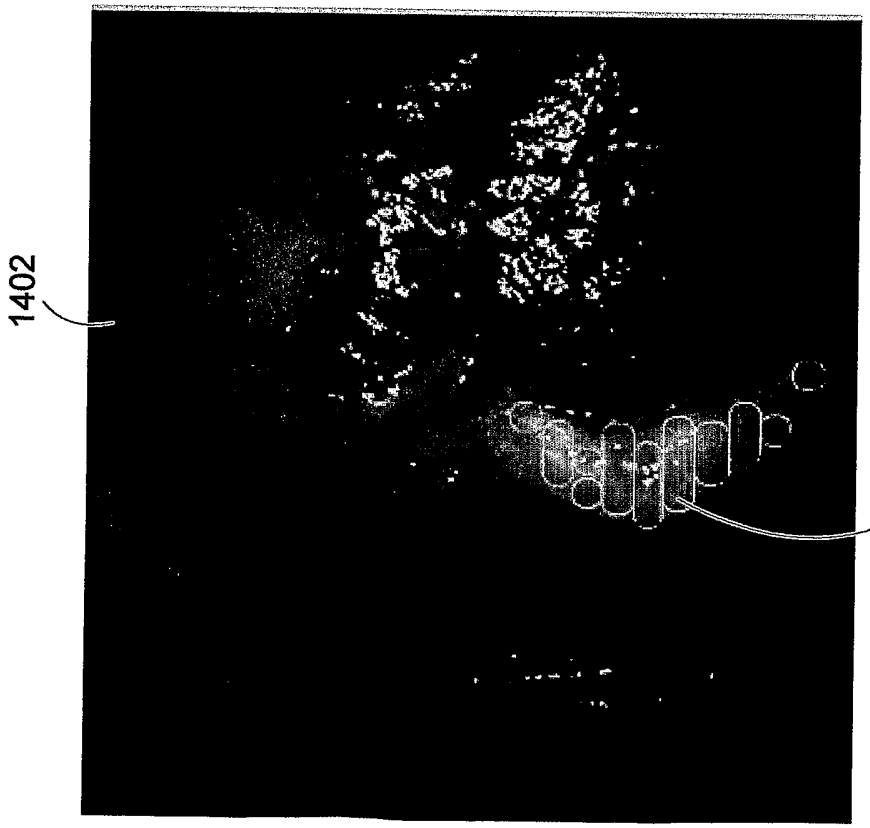


FIG. 14A

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/21312

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : G01J 1/58
US CL : 250/458.1

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)
U.S. : 250/458.1, 461.2, 341.1, 341.2, 341.7, 341.8; 356/317

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,735,276 A (Lemelson) 07 April 1998 (07.04.1998), Figure 4, column 6, lines 21-30,	1-7
---	column 7, lines 34-41, claims 1-83.	---
A		8-46
A	US 6,124,597 A (Shehada et al.) 26 September 2000 (26.09.2000).	1-46
A, P	US 6,574,502 B2 (Hayashi) 03 June 2003 (03.06.2003).	1-46

☐ 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 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
11 September 2003 (11.09.2003)	05 NOV 2003
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703)305-3230	Authorized officer David Porta Telephone No. 703-308-0956

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

PCT/US03/21312

Continuation of B. FIELDS SEARCHED Item 3:
East 1.04
Search Terms: Spectr$ and fluoresc$ and reflect$