US006961599B2

(12) **United States Patent**
Lambert et al.

(10) **Patent No.: US 6,961,599 B2**
(45) **Date of Patent: Nov. 1, 2005**

(54) **IDENTIFYING OR MEASURING SELECTED SUBSTANCES OR TOXINS IN A SUBJECT USING RESONANT RAMAN SIGNALS**

(75) Inventors: **James L. Lambert**, Sunland, CA (US); **Mark S. Borchert**, La Canada, CA (US)

(73) Assignees: **Childrens Hospital Los Angeles**, Los Angeles, CA (US); **California Institute of Technology**, Pasadena, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 205 days.

(21) Appl. No.: **10/396,010**

(22) Filed: **Mar. 25, 2003**

(65) **Prior Publication Data**

US 2004/0127778 A1 Jul. 1, 2004

Related U.S. Application Data

(63) Continuation-in-part of application No. PCT/US02/00649, filed on Jan. 9, 2002, which is a continuation-in-part of application No. 09/756,897, filed on Jan. 9, 2001, now Pat. No. 6,574,501.

(51) **Int. Cl.**^{7} **A61B 5/00**
(52) **U.S. Cl.** **600/318**
(58) **Field of Search** 600/314, 318, 600/319, 321; 382/115, 117

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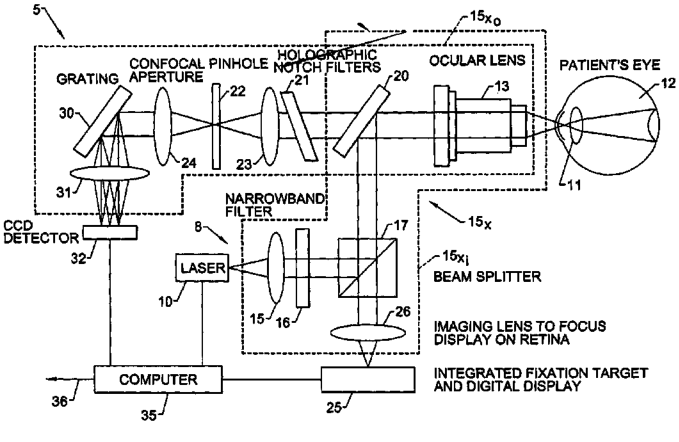
(Continued)

Primary Examiner—Eric F. Winakur
(74) *Attorney, Agent, or Firm*—Myers Bigel Sibley & Sajovec PA

(57) **ABSTRACT**

Methods and systems of the present invention identify the presence of and/or the concentration of a selected analyte in a subject by: (a) illuminating a selected region of the eye of a subject with an optical excitation beam, wherein the excitation beam wavelength is selected to generate a resonant Raman spectrum of the selected analyte with a signal strength that is at least 100 times greater than Raman spectrums generated by non-resonant wavelengths and/or relative to signals of normal constituents present in the selected region of the eye; (b) detecting a resonant Raman spectrum corresponding to the selected illuminated region of the eye; and (c) identifying the presence, absence and/or the concentration of the selected analyte in the subject based on said detecting step. The apparatus may also be configured to be able to obtain biometric data of the eye to identify (confirm the identity of) the subject.

61 Claims, 36 Drawing Sheets


5
15x₀
CONFOCAL PINHOLE
APERTURE
HOLOGRAPHIC NOTCH FILTERS
OCULAR LENS
PATIENT'S EYE
GRATING
30
22
21
20
13
12
24
23
31
11
NARROWBAND FILTER
CCD DETECTOR
8
17
15x
32
LASER
15xᵢ
BEAM SPLITTER
10
15
16
26
IMAGING LENS TO FOCUS DISPLAY ON RETINA
COMPUTER
INTEGRATED FIXATION TARGET AND DIGITAL DISPLAY
36
35
25

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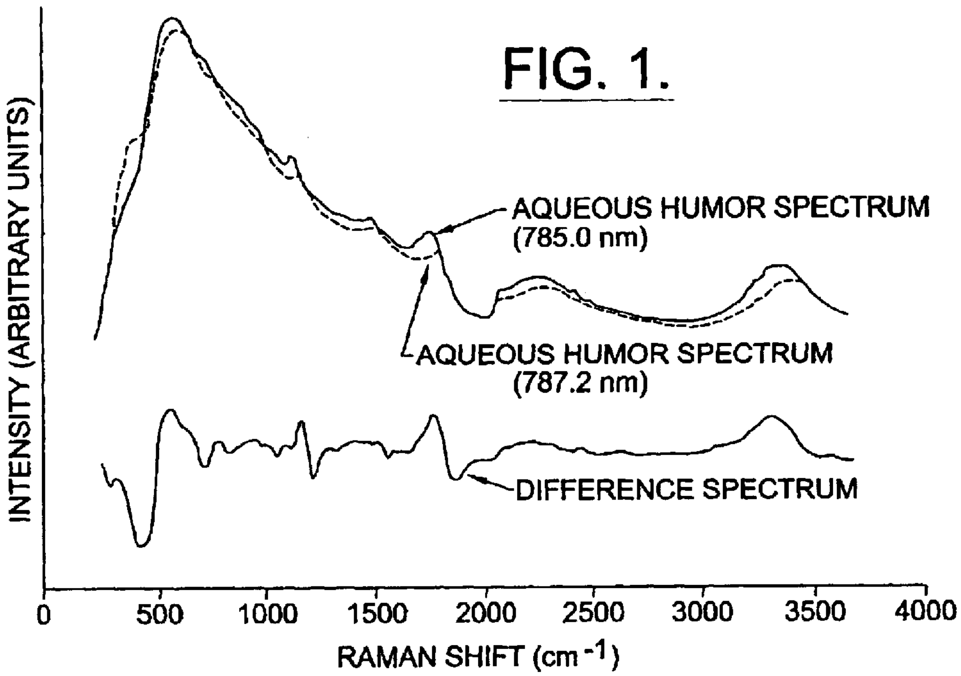
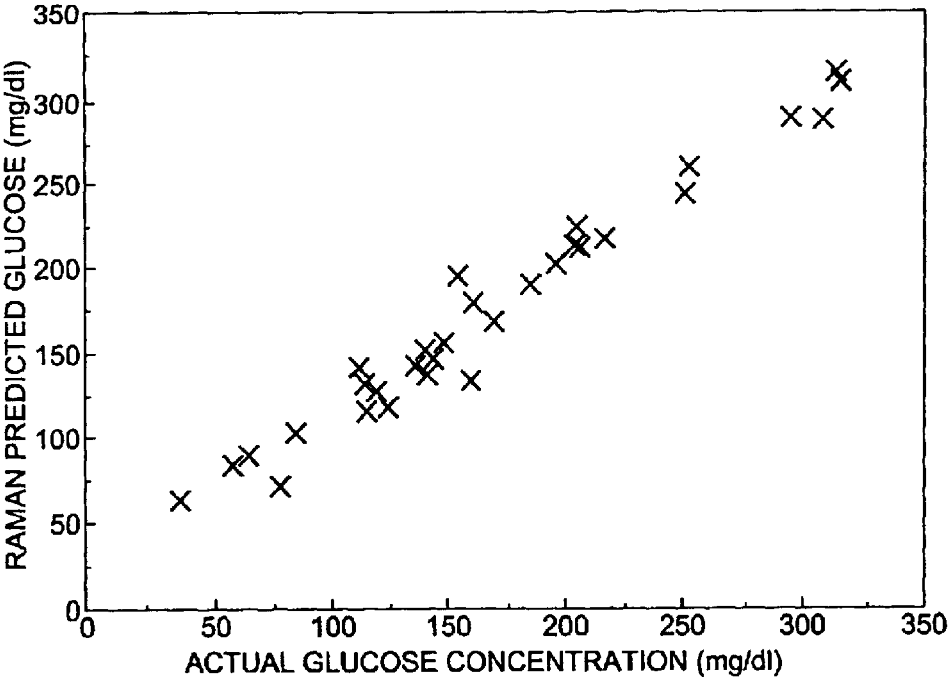


FIG. 1.

INTENSITY (ARBITRARY UNITS)
AQUEOUS HUMOR SPECTRUM (785.0 nm)
AQUEOUS HUMOR SPECTRUM (787.2 nm)
DIFFERENCE SPECTRUM
0
500
1000
1500
2000
2500
3000
3500
4000
RAMAN SHIFT (cm-1)



RAMAN PREDICTED GLUCOSE (mg/dl)
350
300
250
200
150
100
50
0
0
50
100
150
200
250
300
350
ACTUAL GLUCOSE CONCENTRATION (mg/dl)

FIG. 2.

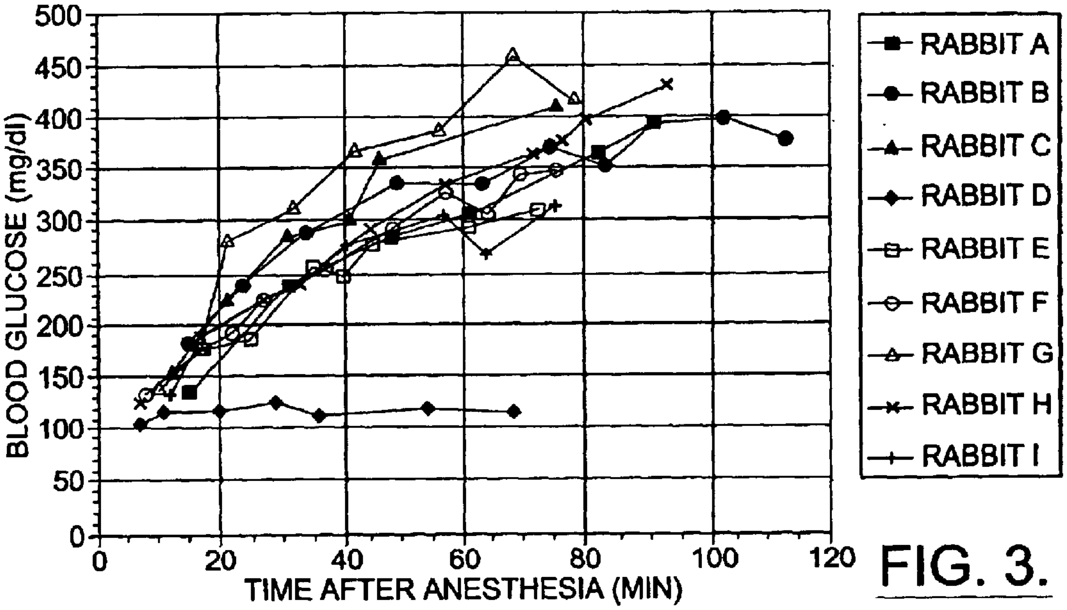

BLOOD GLUCOSE (mg/dl)
500
450
400
350
300
250
200
150
100
50
0
0
20
40
60
80
100
120
TIME AFTER ANESTHESIA (MIN)
RABBIT A
RABBIT B
RABBIT C
RABBIT D
RABBIT E
RABBIT F
RABBIT G
RABBIT H
RABBIT I

FIG. 3.

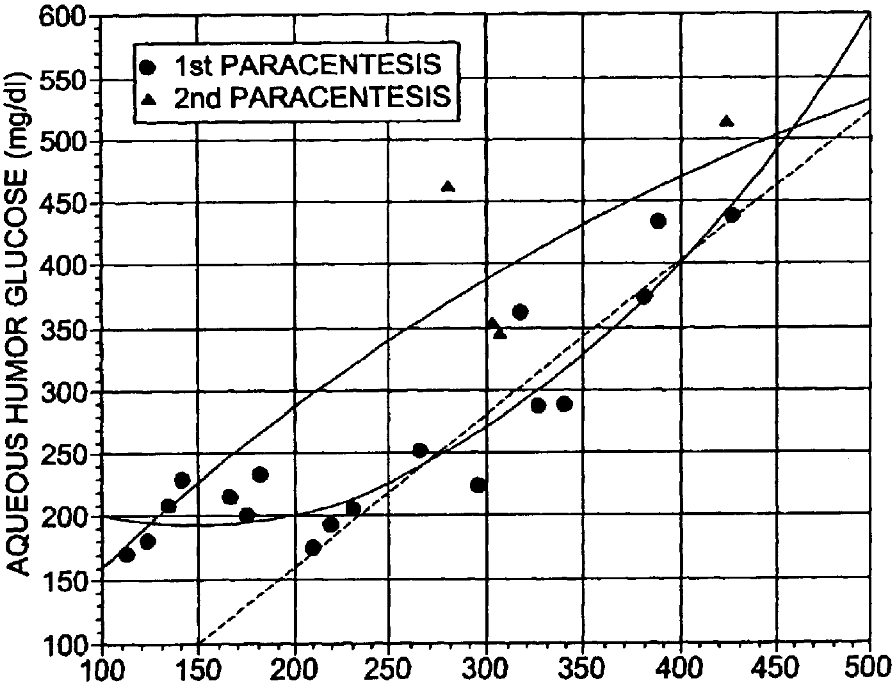

AQUEOUS HUMOR GLUCOSE (mg/dl)
600
550
500
450
400
350
300
250
200
150
100
100
150
200
250
300
350
400
450
500
BLOOD GLUCOSE (mg/dl)
● 1st PARACENTESIS
▲ 2nd PARACENTESIS

FIG. 4.

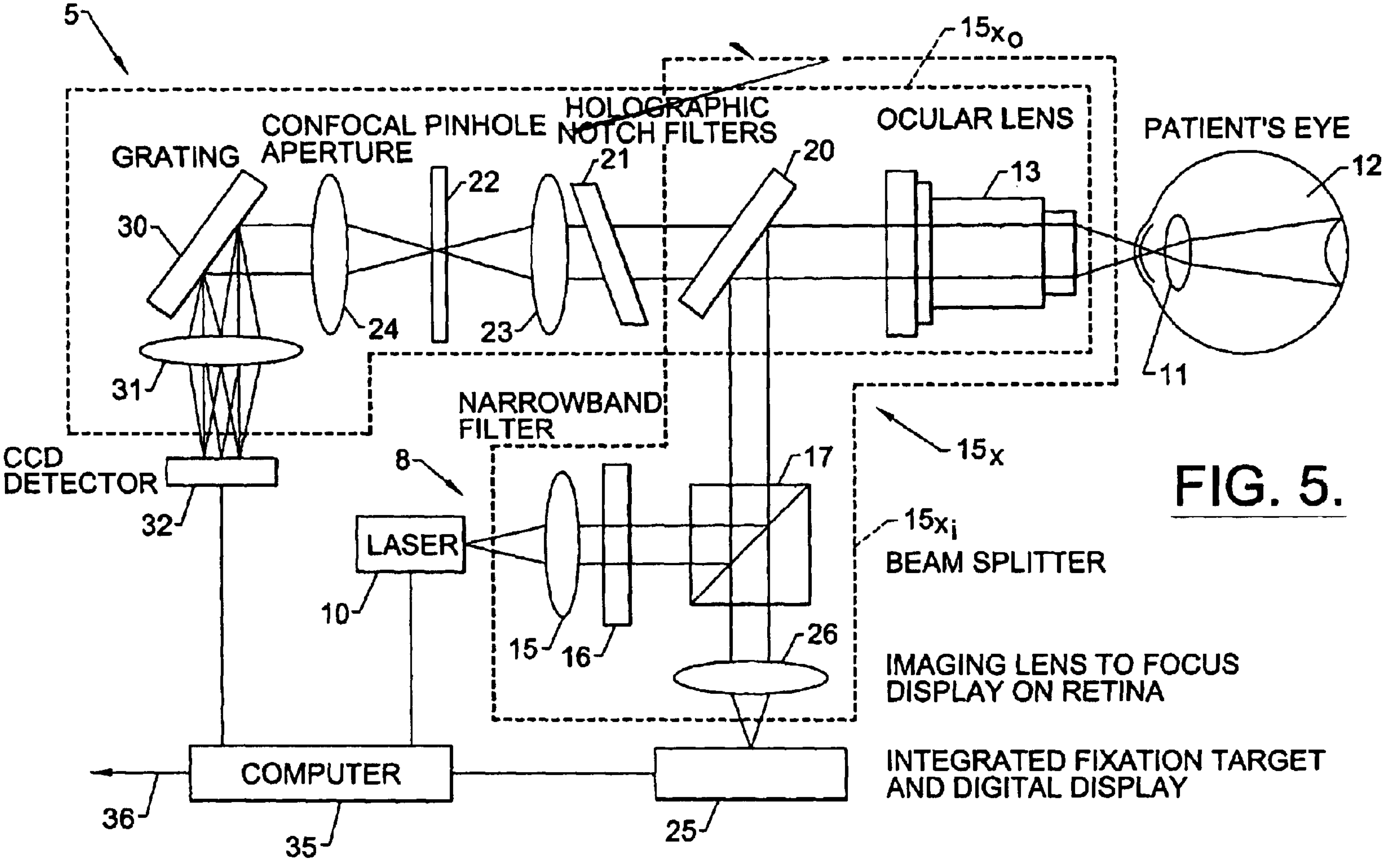

5
$15x_o$
GRATING
CONFOCAL PINHOLE APERTURE
HOLOGRAPHIC NOTCH FILTERS
OCULAR LENS
PATIENT'S EYE
12
13
20
21
22
30
24
23
11
31
NARROWBAND FILTER
CCD DETECTOR
32
8
17
$15x$
LASER
10
$15x_i$
BEAM SPLITTER
15
16
26
IMAGING LENS TO FOCUS DISPLAY ON RETINA
COMPUTER
INTEGRATED FIXATION TARGET AND DIGITAL DISPLAY
36
35
25

FIG. 5.

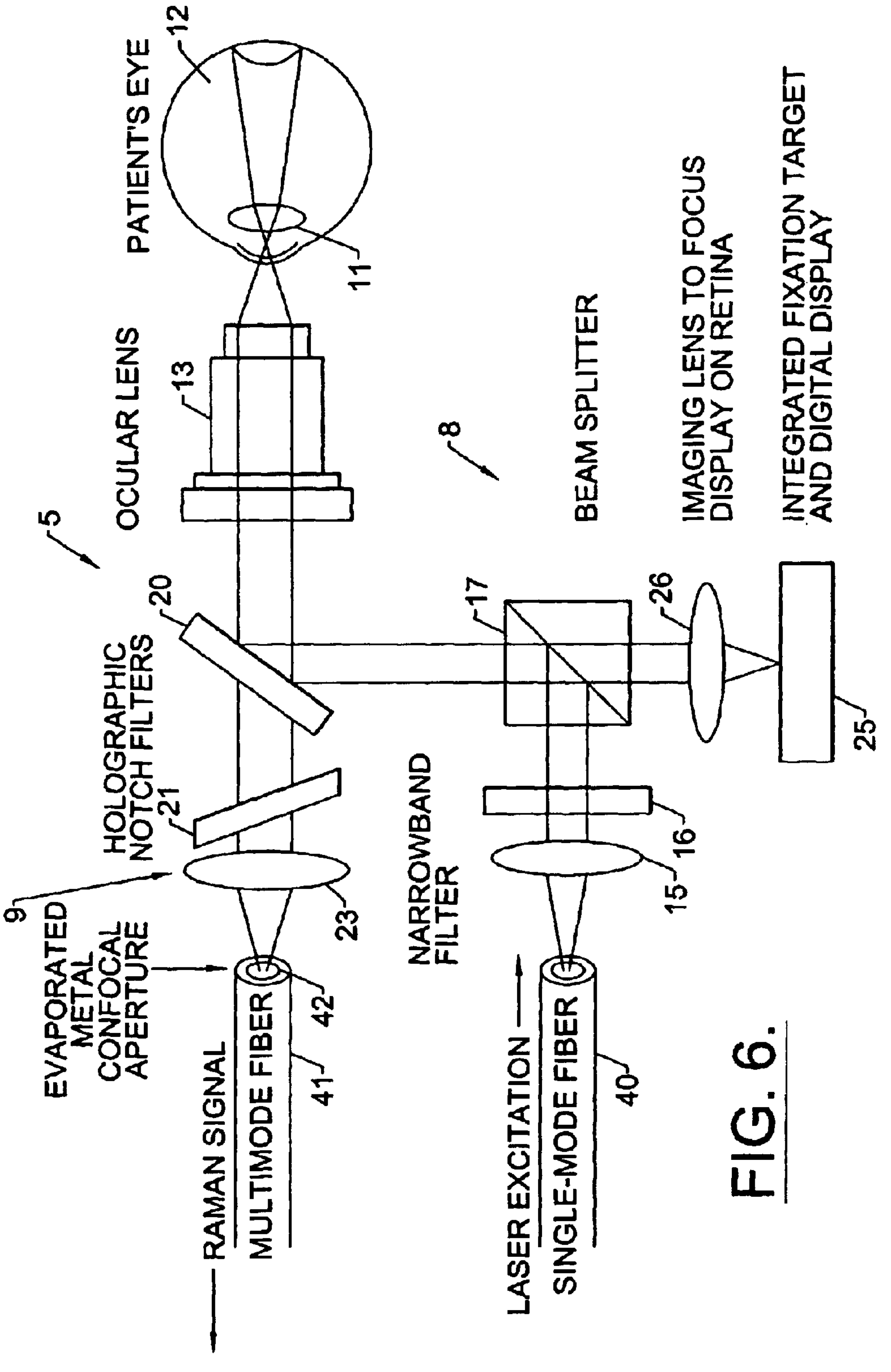

PATIENT'S EYE
12
11
OCULAR LENS
13
5
8
BEAM SPLITTER
IMAGING LENS TO FOCUS DISPLAY ON RETINA
INTEGRATED FIXATION TARGET AND DIGITAL DISPLAY
20
17
26
25
HOLOGRAPHIC NOTCH FILTERS
21
NARROWBAND FILTER
16
15
9
EVAPORATED METAL CONFOCAL APERTURE
23
42
RAMAN SIGNAL
MULTIMODE FIBER
41
LASER EXCITATION
SINGLE-MODE FIBER
40

FIG. 6.

FIG. 7a.

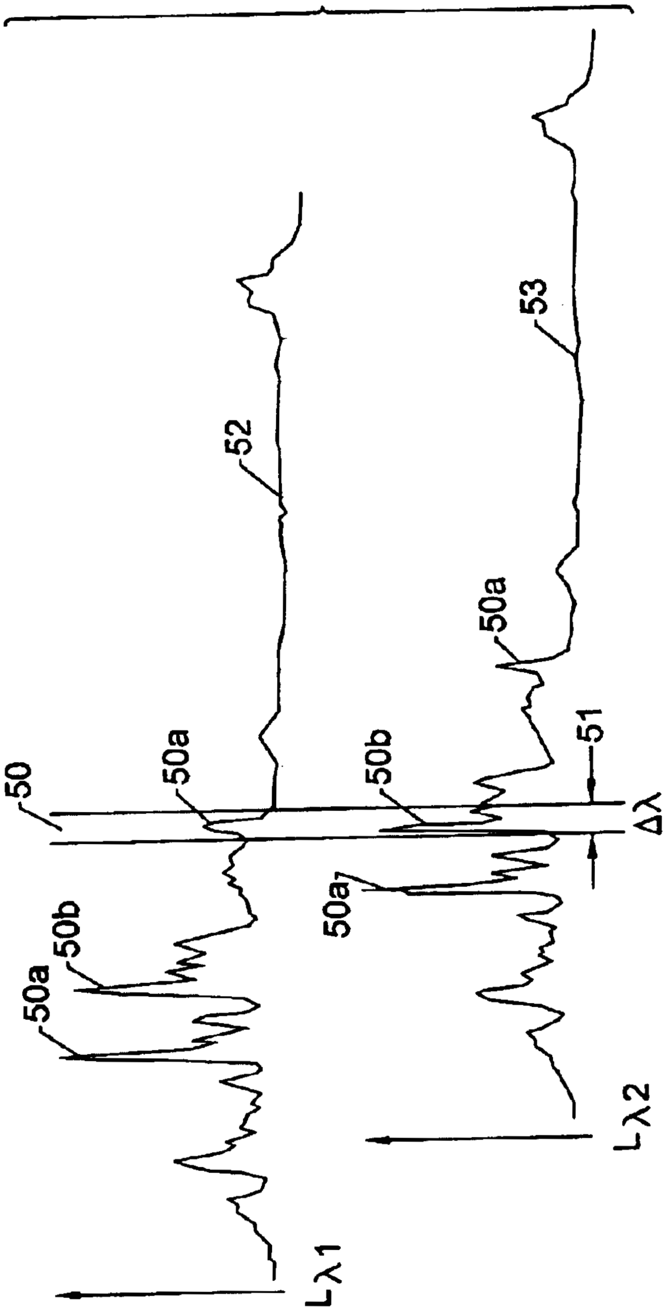
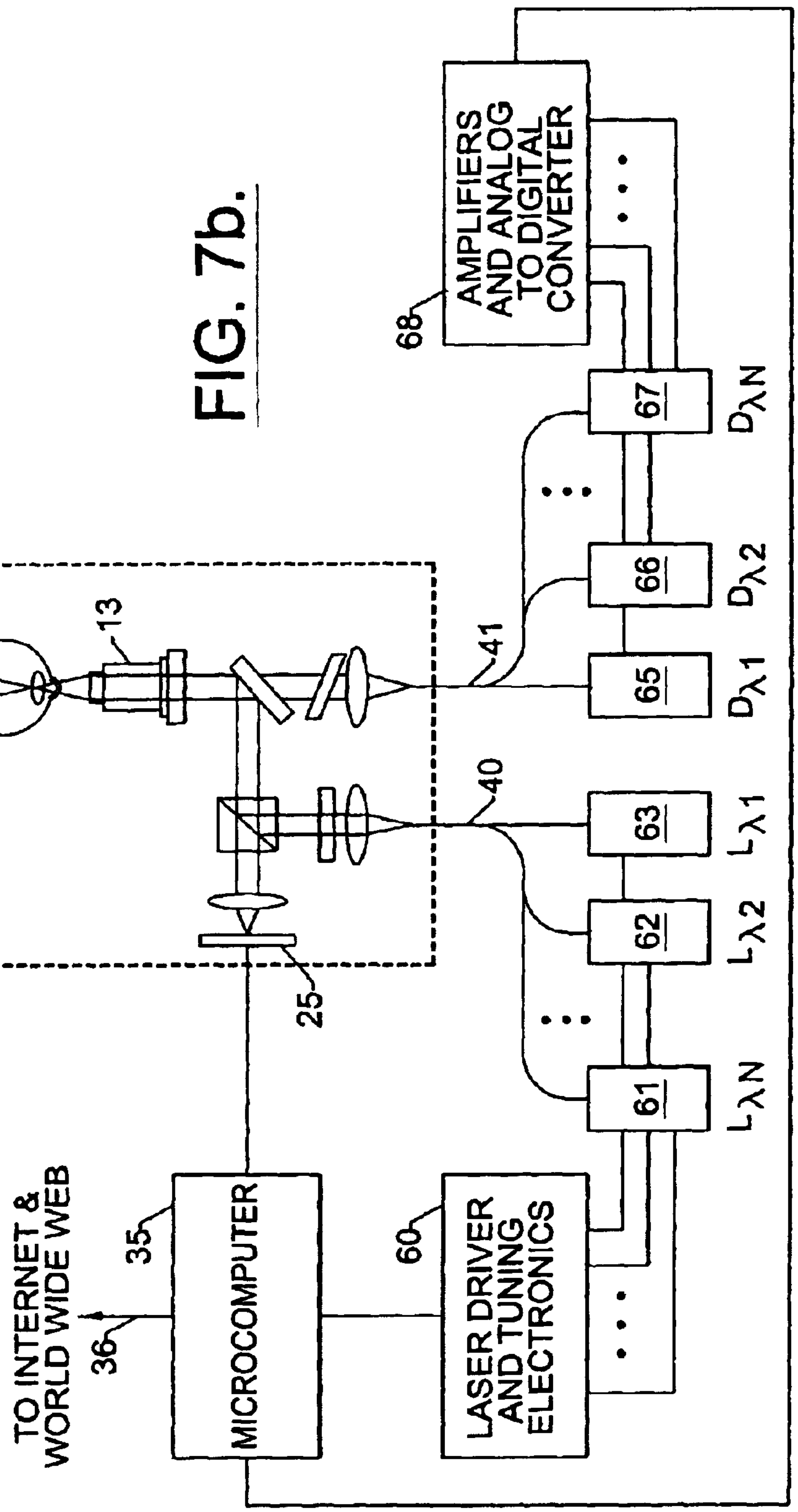


FIG. 7b.


FROM FIG. 6
12
13
25
40
41
63
62
61
65
66
67
$L_{\lambda 1}$
$L_{\lambda 2}$
$L_{\lambda N}$
$D_{\lambda 1}$
$D_{\lambda 2}$
$D_{\lambda N}$
68
AMPLIFIERS AND ANALOG TO DIGITAL CONVERTER
60
LASER DRIVER AND TUNING ELECTRONICS
35
MICROCOMPUTER
36
TO INTERNET & WORLD WIDE WEB

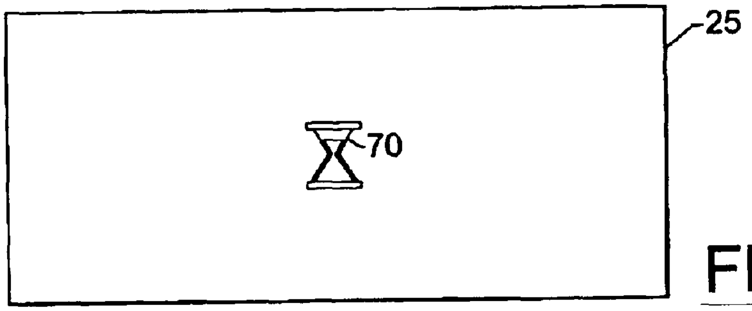


FIG. 8.

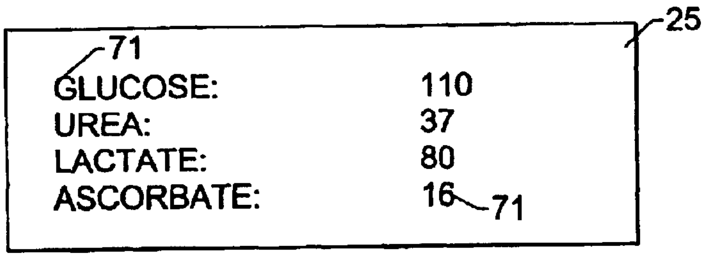

25
71
GLUCOSE: 110
UREA: 37
LACTATE: 80
ASCORBATE: 16
71

FIG. 9.

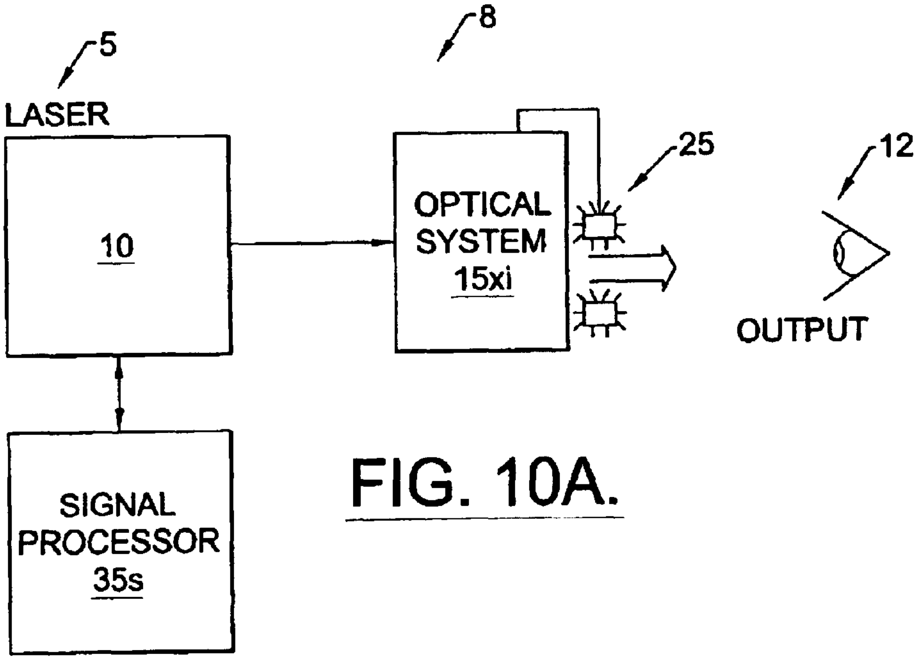

5
LASER
10
8
OPTICAL SYSTEM 15xi
25
12
OUTPUT
SIGNAL PROCESSOR 35s

FIG. 10A.

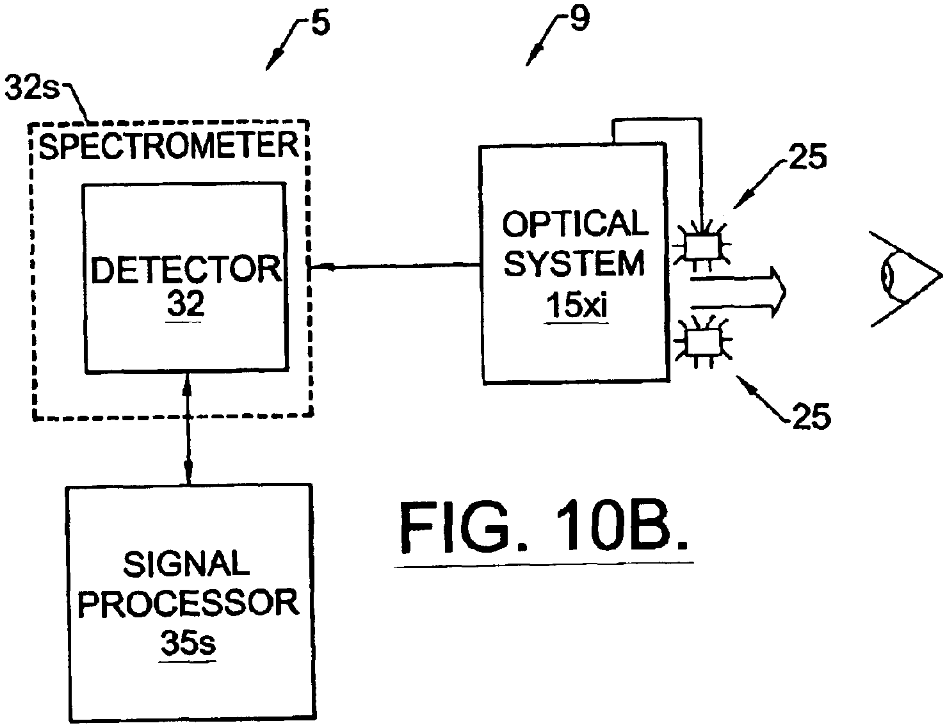

5
9
32s
SPECTROMETER
DETECTOR
32
OPTICAL
SYSTEM
15xi
25
25
SIGNAL
PROCESSOR
35s

FIG. 10B.

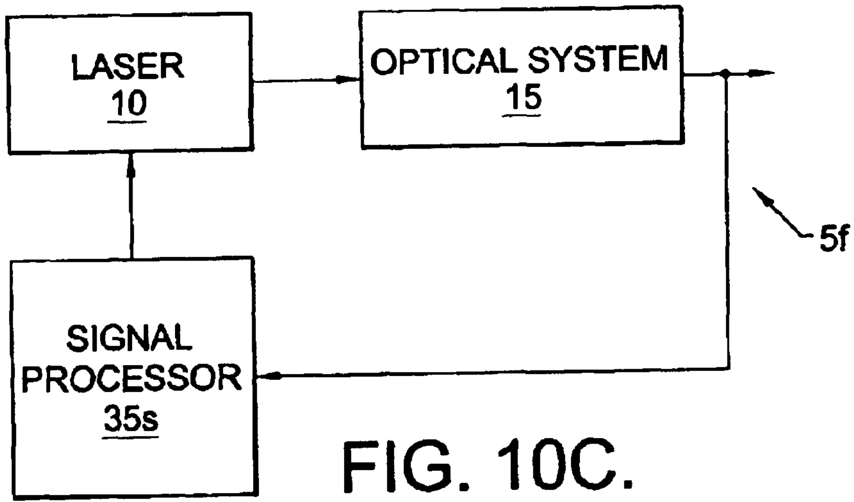

LASER
10
OPTICAL SYSTEM
15
5f
SIGNAL
PROCESSOR
35s

FIG. 10C.

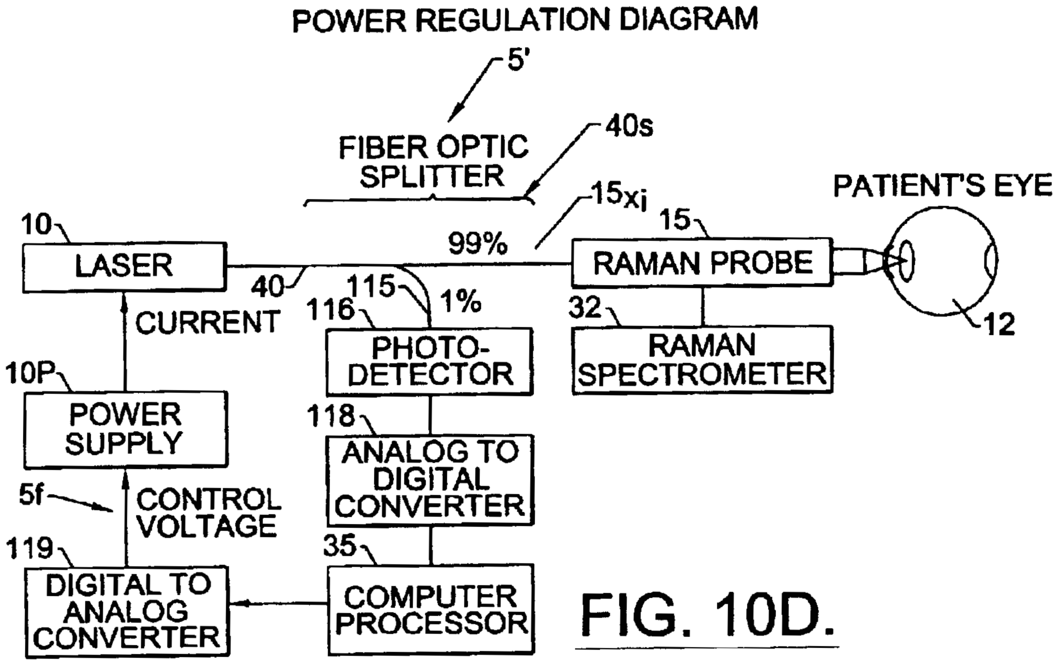

POWER REGULATION DIAGRAM
5'
FIBER OPTIC SPLITTER
40s
15xi
PATIENT'S EYE
15
10
LASER
99%
RAMAN PROBE
40
115
1%
116
CURRENT
PHOTO-DETECTOR
32
RAMAN SPECTROMETER
12
10P
POWER SUPPLY
118
ANALOG TO DIGITAL CONVERTER
5f
CONTROL VOLTAGE
119
35
DIGITAL TO ANALOG CONVERTER
COMPUTER PROCESSOR

FIG. 10D.

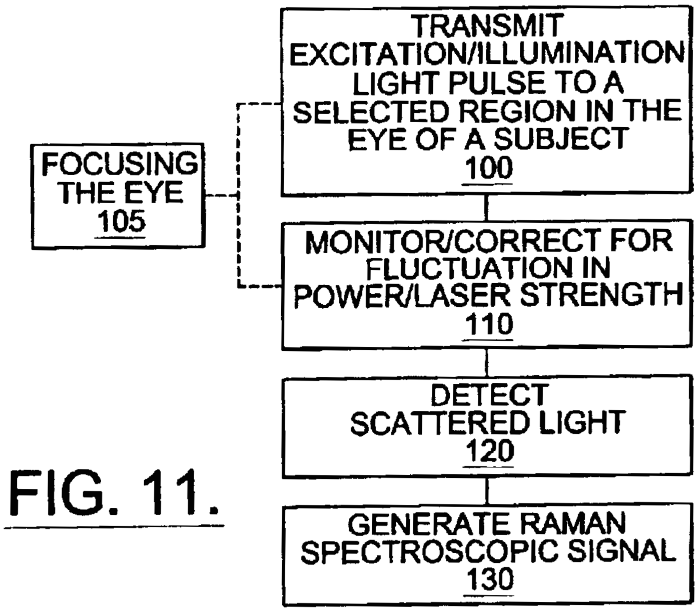

TRANSMIT EXCITATION/ILLUMINATION LIGHT PULSE TO A SELECTED REGION IN THE EYE OF A SUBJECT
100
FOCUSING THE EYE
105
MONITOR/CORRECT FOR FLUCTUATION IN POWER/LASER STRENGTH
110
DETECT SCATTERED LIGHT
120
GENERATE RAMAN SPECTROSCOPIC SIGNAL
130

FIG. 11.

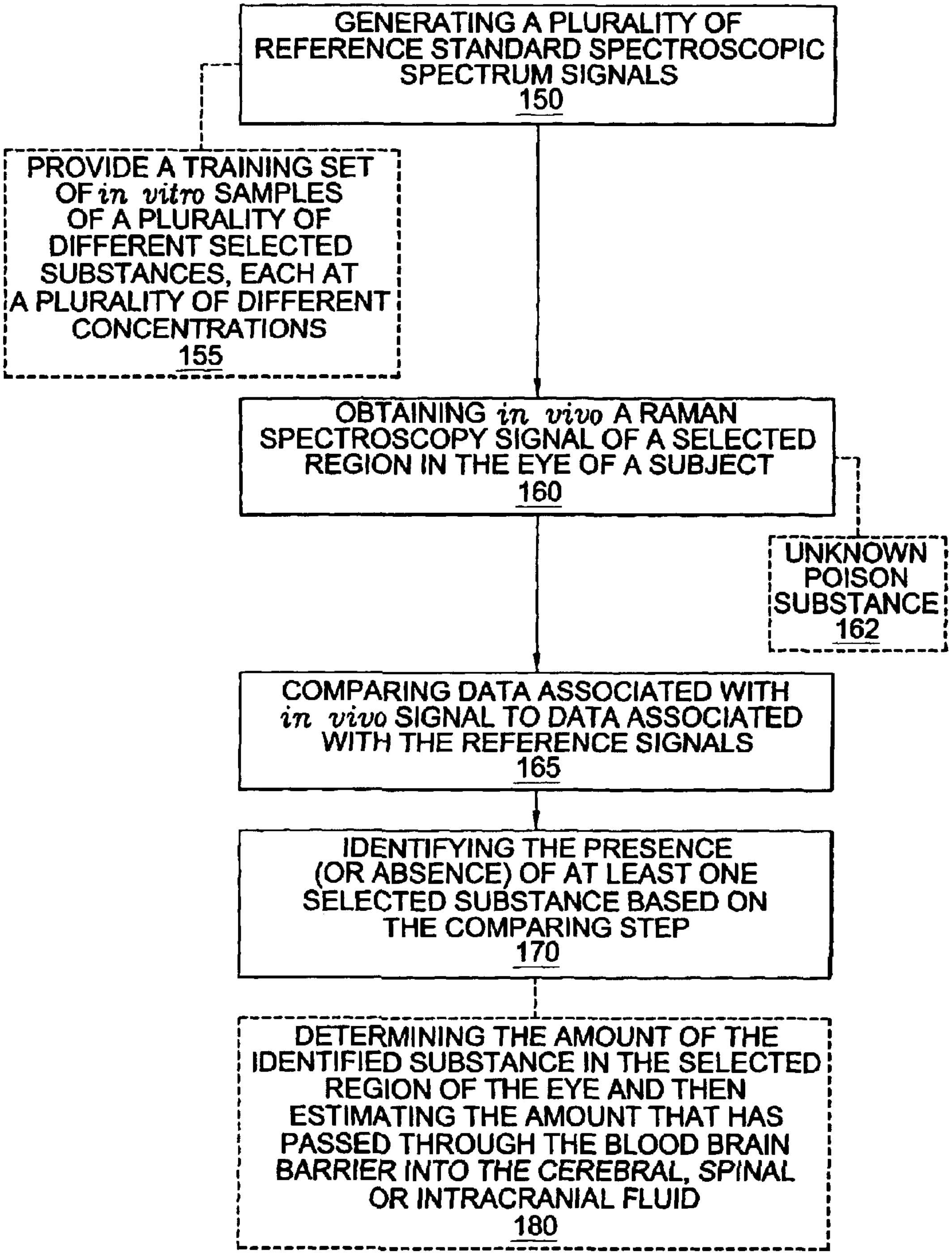

GENERATING A PLURALITY OF REFERENCE STANDARD SPECTROSCOPIC SPECTRUM SIGNALS
150
PROVIDE A TRAINING SET OF *in vitro* SAMPLES OF A PLURALITY OF DIFFERENT SELECTED SUBSTANCES, EACH AT A PLURALITY OF DIFFERENT CONCENTRATIONS
155
OBTAINING *in vivo* A RAMAN SPECTROSCOPY SIGNAL OF A SELECTED REGION IN THE EYE OF A SUBJECT
160
UNKNOWN POISON SUBSTANCE
162
COMPARING DATA ASSOCIATED WITH *in vivo* SIGNAL TO DATA ASSOCIATED WITH THE REFERENCE SIGNALS
165
IDENTIFYING THE PRESENCE (OR ABSENCE) OF AT LEAST ONE SELECTED SUBSTANCE BASED ON THE COMPARING STEP
170
DETERMINING THE AMOUNT OF THE IDENTIFIED SUBSTANCE IN THE SELECTED REGION OF THE EYE AND THEN ESTIMATING THE AMOUNT THAT HAS PASSED THROUGH THE BLOOD BRAIN BARRIER INTO THE CEREBRAL, SPINAL OR INTRACRANIAL FLUID
180

FIG. 12.

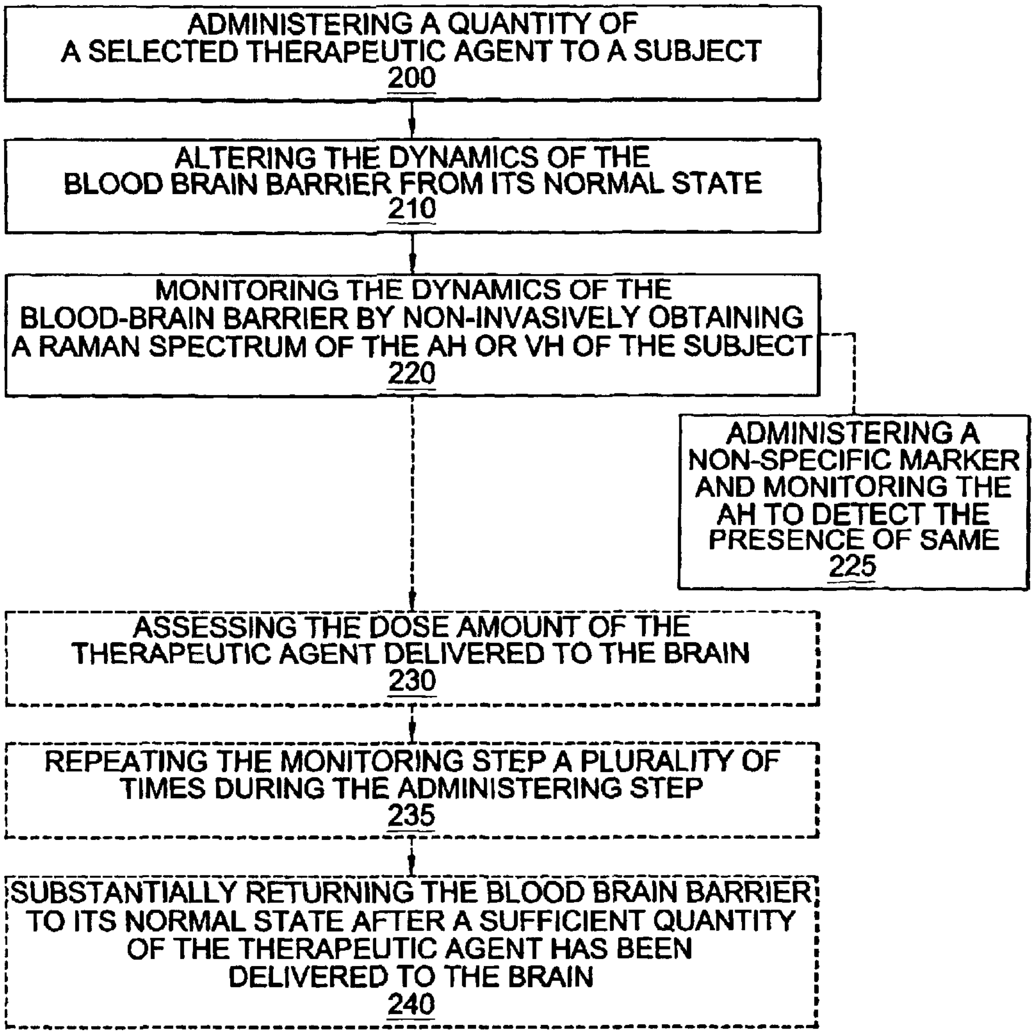

ADMINISTERING A QUANTITY OF A SELECTED THERAPEUTIC AGENT TO A SUBJECT
200
ALTERING THE DYNAMICS OF THE BLOOD BRAIN BARRIER FROM ITS NORMAL STATE
210
MONITORING THE DYNAMICS OF THE BLOOD-BRAIN BARRIER BY NON-INVASIVELY OBTAINING A RAMAN SPECTRUM OF THE AH OR VH OF THE SUBJECT
220
ADMINISTERING A NON-SPECIFIC MARKER AND MONITORING THE AH TO DETECT THE PRESENCE OF SAME
225
ASSESSING THE DOSE AMOUNT OF THE THERAPEUTIC AGENT DELIVERED TO THE BRAIN
230
REPEATING THE MONITORING STEP A PLURALITY OF TIMES DURING THE ADMINISTERING STEP
235
SUBSTANTIALLY RETURNING THE BLOOD BRAIN BARRIER TO ITS NORMAL STATE AFTER A SUFFICIENT QUANTITY OF THE THERAPEUTIC AGENT HAS BEEN DELIVERED TO THE BRAIN
240

FIG. 13.

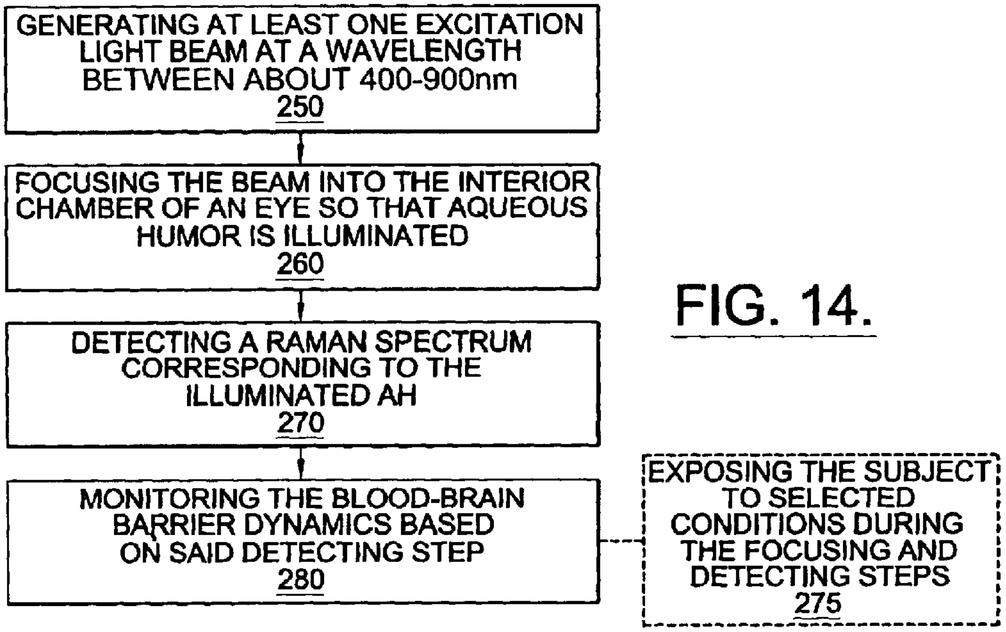

GENERATING AT LEAST ONE EXCITATION LIGHT BEAM AT A WAVELENGTH BETWEEN ABOUT 400-900nm
250
FOCUSING THE BEAM INTO THE INTERIOR CHAMBER OF AN EYE SO THAT AQUEOUS HUMOR IS ILLUMINATED
260
DETECTING A RAMAN SPECTRUM CORRESPONDING TO THE ILLUMINATED AH
270
MONITORING THE BLOOD-BRAIN BARRIER DYNAMICS BASED ON SAID DETECTING STEP
280
EXPOSING THE SUBJECT TO SELECTED CONDITIONS DURING THE FOCUSING AND DETECTING STEPS
275

FIG. 14.

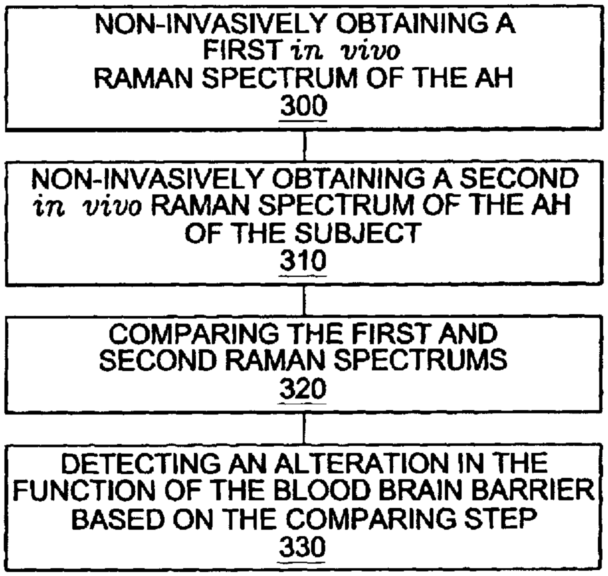

NON-INVASIVELY OBTAINING A FIRST *in vivo* RAMAN SPECTRUM OF THE AH
300
NON-INVASIVELY OBTAINING A SECOND *in vivo* RAMAN SPECTRUM OF THE AH OF THE SUBJECT
310
COMPARING THE FIRST AND SECOND RAMAN SPECTRUMS
320
DETECTING AN ALTERATION IN THE FUNCTION OF THE BLOOD BRAIN BARRIER BASED ON THE COMPARING STEP
330

FIG. 15.

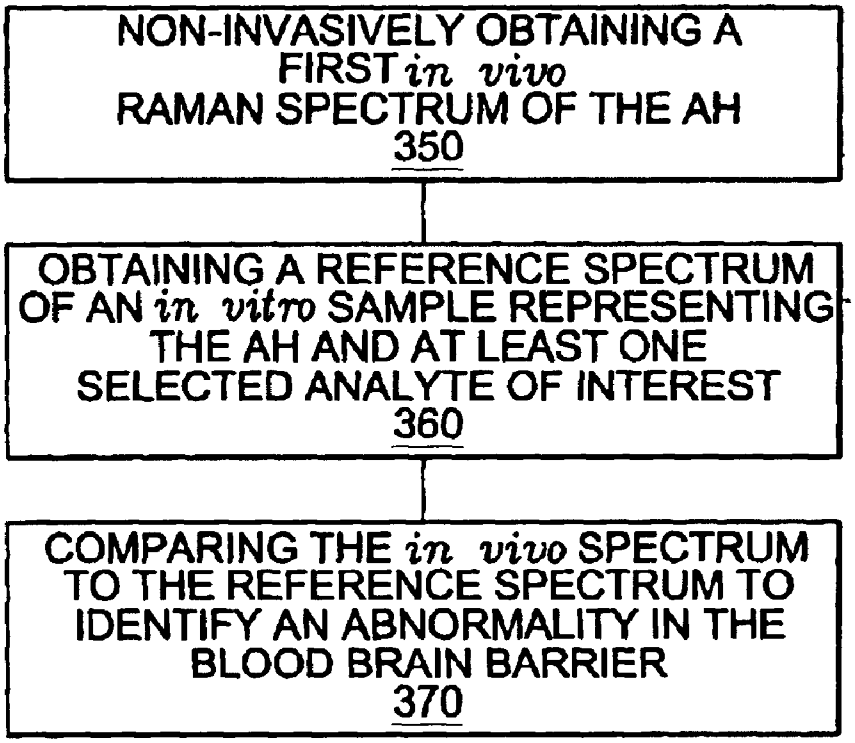

NON-INVASIVELY OBTAINING A FIRST *in vivo* RAMAN SPECTRUM OF THE AH
350
OBTAINING A REFERENCE SPECTRUM OF AN *in vitro* SAMPLE REPRESENTING THE AH AND AT LEAST ONE SELECTED ANALYTE OF INTEREST
360
COMPARING THE *in vivo* SPECTRUM TO THE REFERENCE SPECTRUM TO IDENTIFY AN ABNORMALITY IN THE BLOOD BRAIN BARRIER
370

FIG. 16.

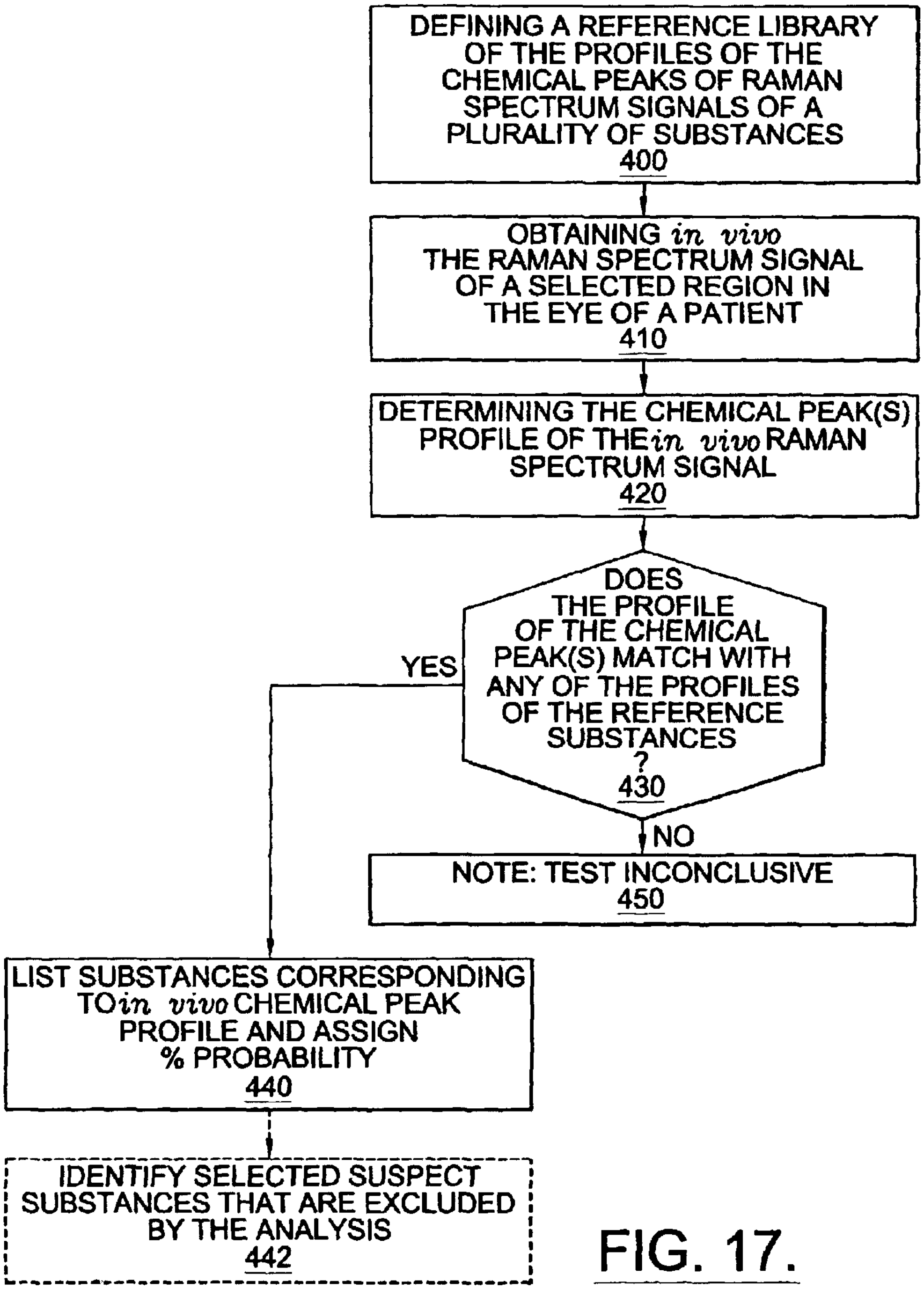

DEFINING A REFERENCE LIBRARY OF THE PROFILES OF THE CHEMICAL PEAKS OF RAMAN SPECTRUM SIGNALS OF A PLURALITY OF SUBSTANCES
400
OBTAINING *in vivo* THE RAMAN SPECTRUM SIGNAL OF A SELECTED REGION IN THE EYE OF A PATIENT
410
DETERMINING THE CHEMICAL PEAK(S) PROFILE OF THE *in vivo* RAMAN SPECTRUM SIGNAL
420
DOES THE PROFILE OF THE CHEMICAL PEAK(S) MATCH WITH ANY OF THE PROFILES OF THE REFERENCE SUBSTANCES ?
430
YES
NO
NOTE: TEST INCONCLUSIVE
450
LIST SUBSTANCES CORRESPONDING TO *in vivo* CHEMICAL PEAK PROFILE AND ASSIGN % PROBABILITY
440
IDENTIFY SELECTED SUSPECT SUBSTANCES THAT ARE EXCLUDED BY THE ANALYSIS
442

FIG. 17.

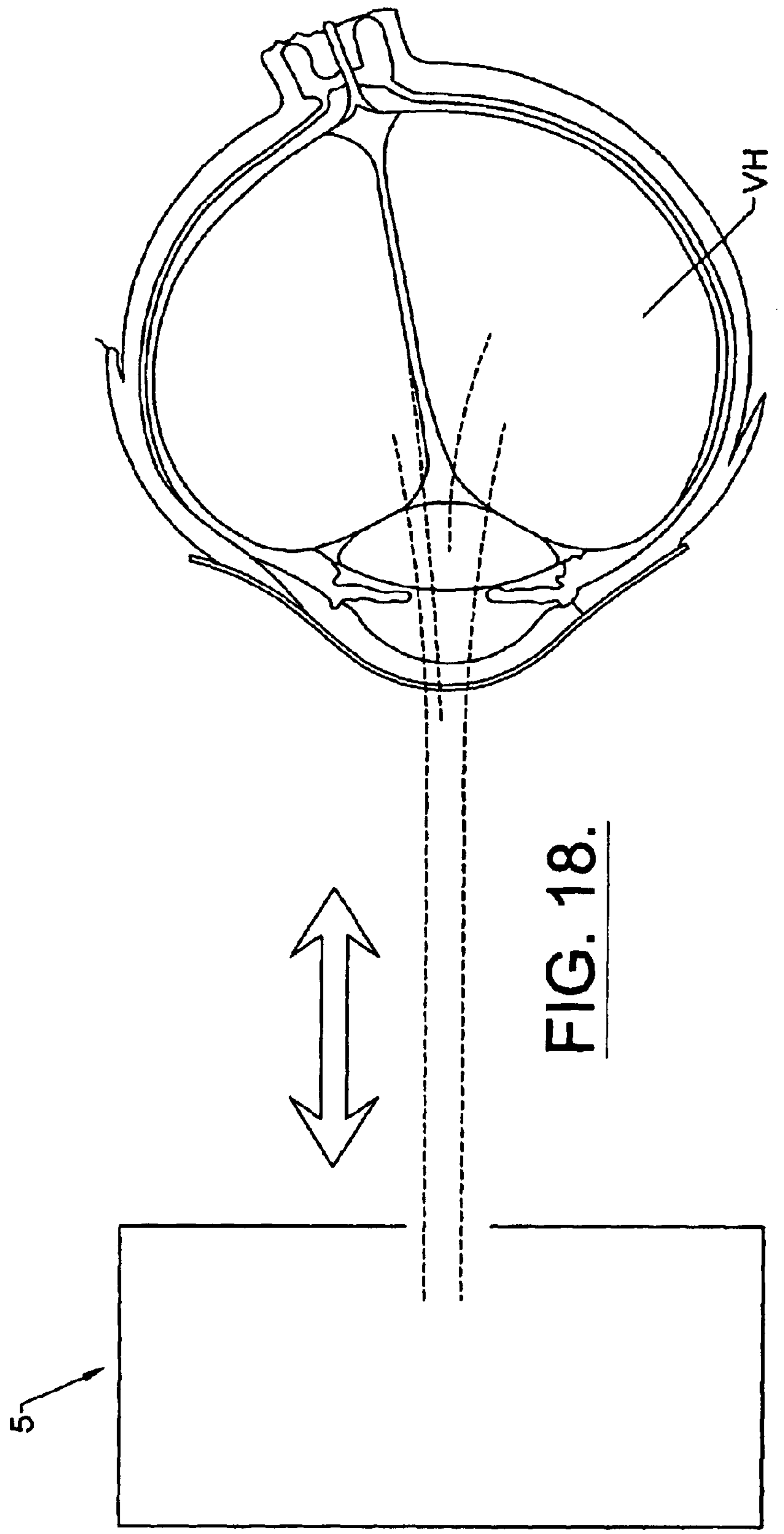


FIG. 18.

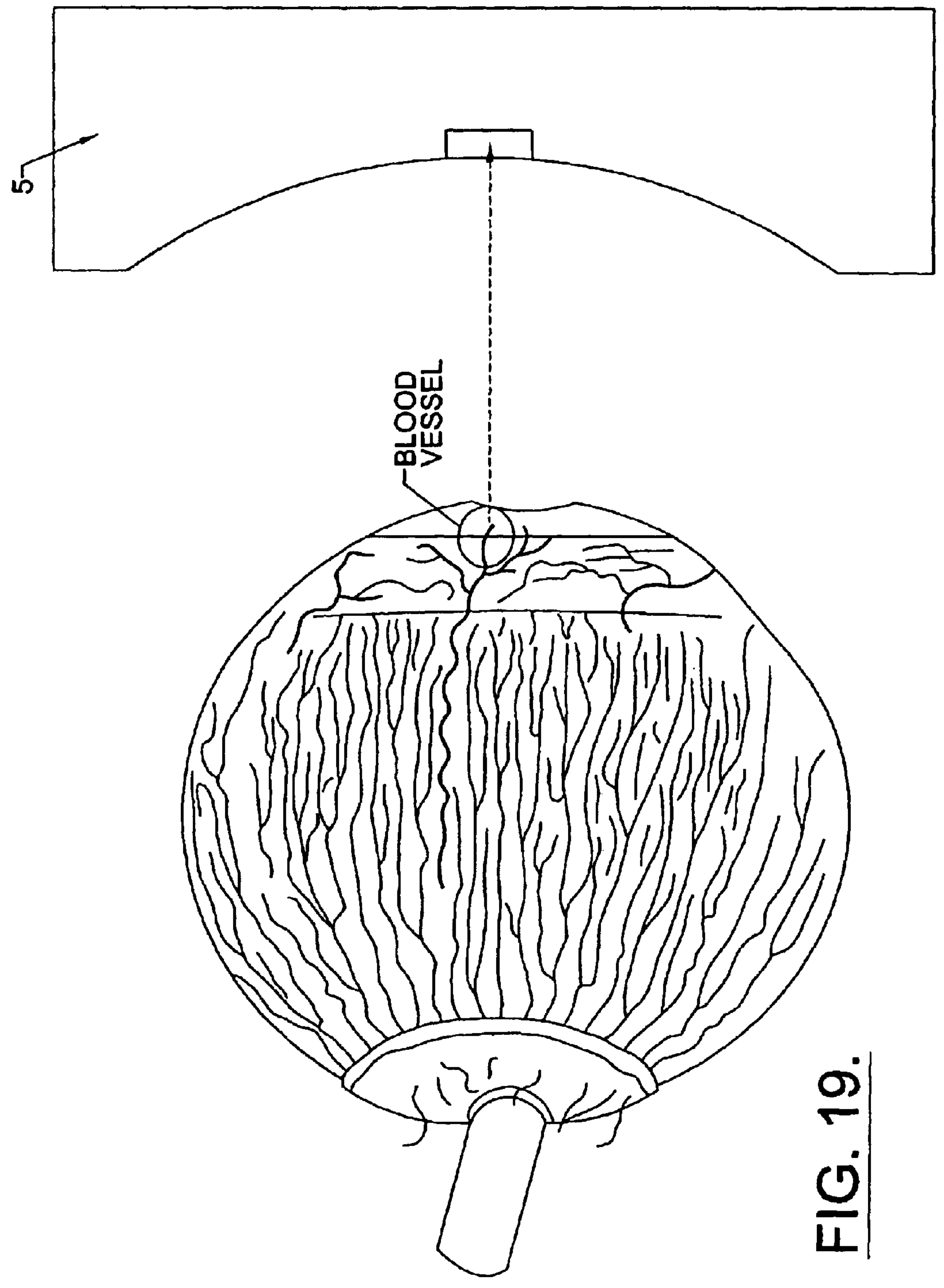
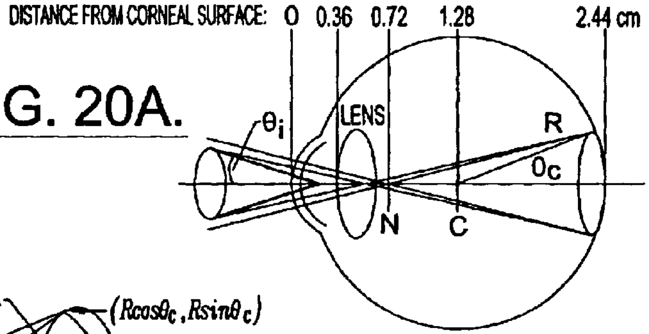

5
BLOOD VESSEL

FIG. 19.

Optical power density calculations are performed using a canonical model of the adult eye (20A). a ray-tracing technique is used to calculate the area of retina exposed, A, by treating the incident light as if it emanated from node point N. Equation 1 is an expression of A as a function of θ_c and the radius of the globe R (20B). Equation 2 is derived inOorder to express θ_c in terms of the angle of incidence θ_i. Equation 2 must be solved numerically for θ_c. Substituting θ_c into Equation 1 is used to determine A. Power density is easily calculated for a given input power once A is determined. Solutions for both the exposed retinal area and the power density (assuming 30 mW input) are shown as a function of the angle of incidence (20C).

FIG. 20A.


DISTANCE FROM CORNEAL SURFACE: 0 0.36 0.72 1.28 2.44 cm
θi
LENS
R
θc
N
C

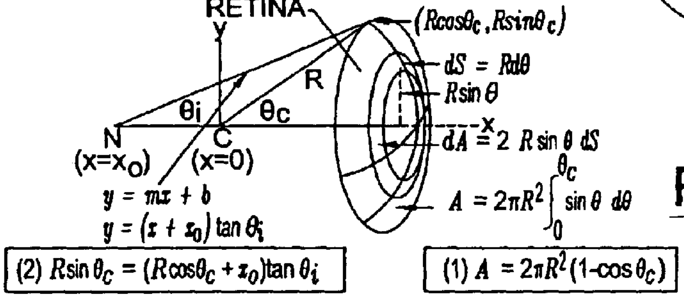

RETINA
Y
(Rcosθc, Rsinθc)
dS = Rdθ
Rsin θ
R
θi
θc
N
C
X
(x=x0)
(x=0)
dA = 2 R sin θ dS
y = mx + b
y = (x + x0) tan θi
A = 2πR² ∫0^θc sin θ dθ
(2) R sin θc = (R cos θc + x0) tan θi
(1) A = 2πR²(1-cos θc)

FIG. 20B.

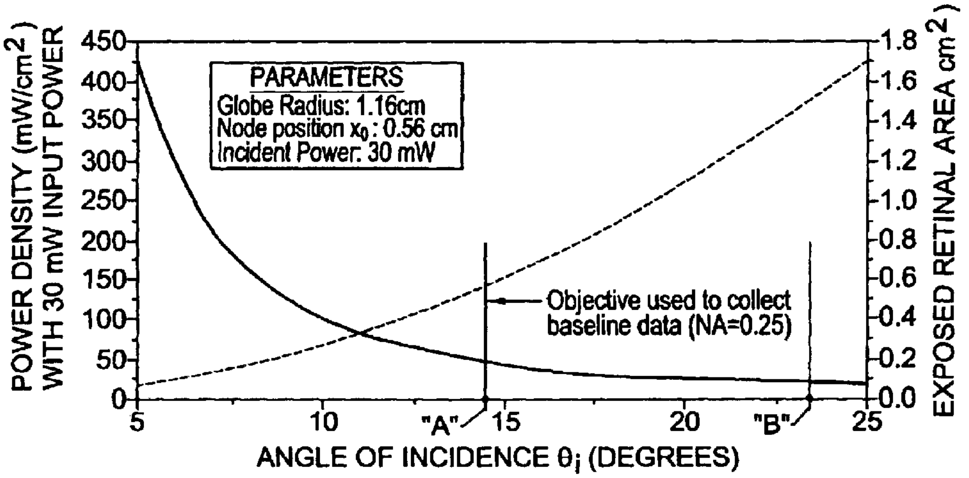
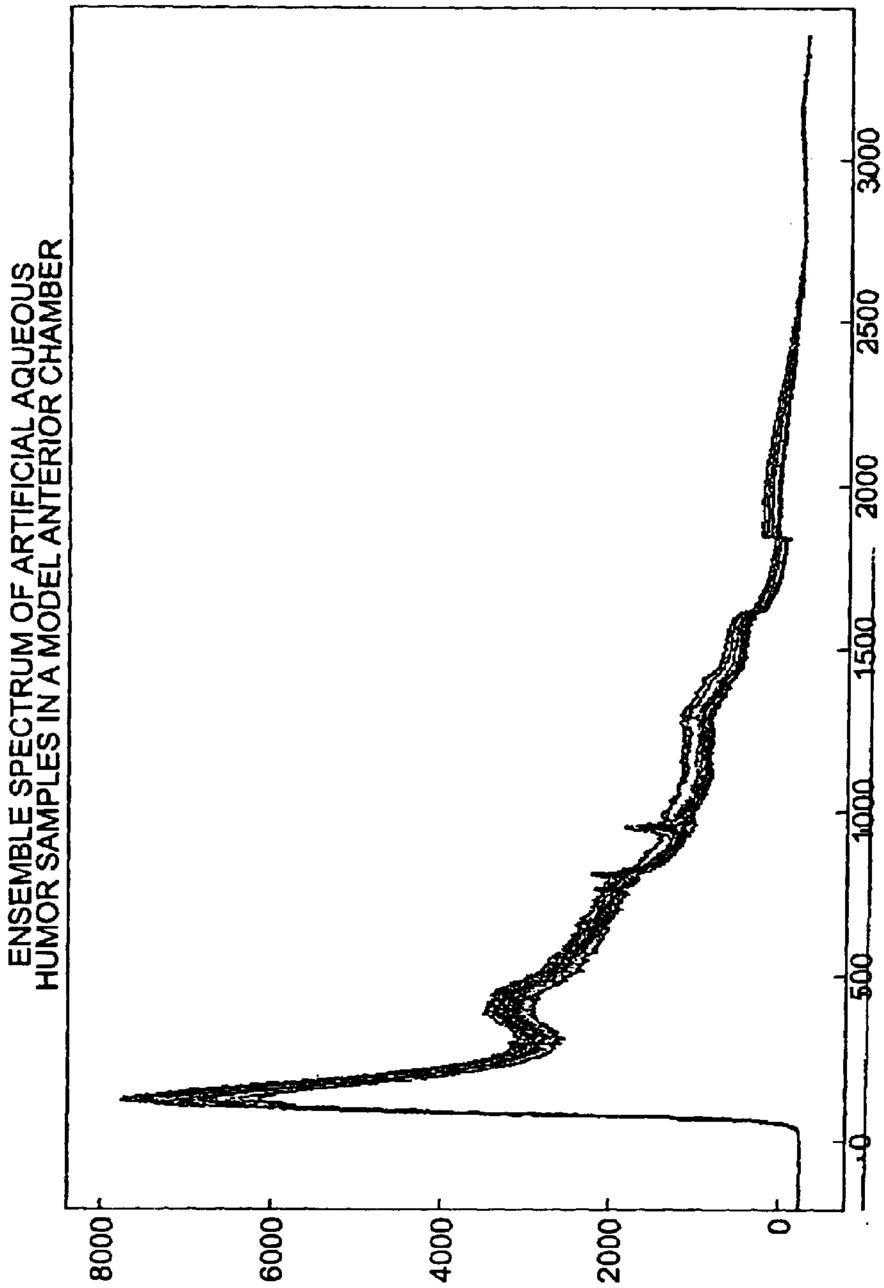


FIG. 20C.



ENSEMBLE SPECTRUM OF ARTIFICIAL AQUEOUS HUMOR SAMPLES IN A MODEL ANTERIOR CHAMBER

FIG. 21A.

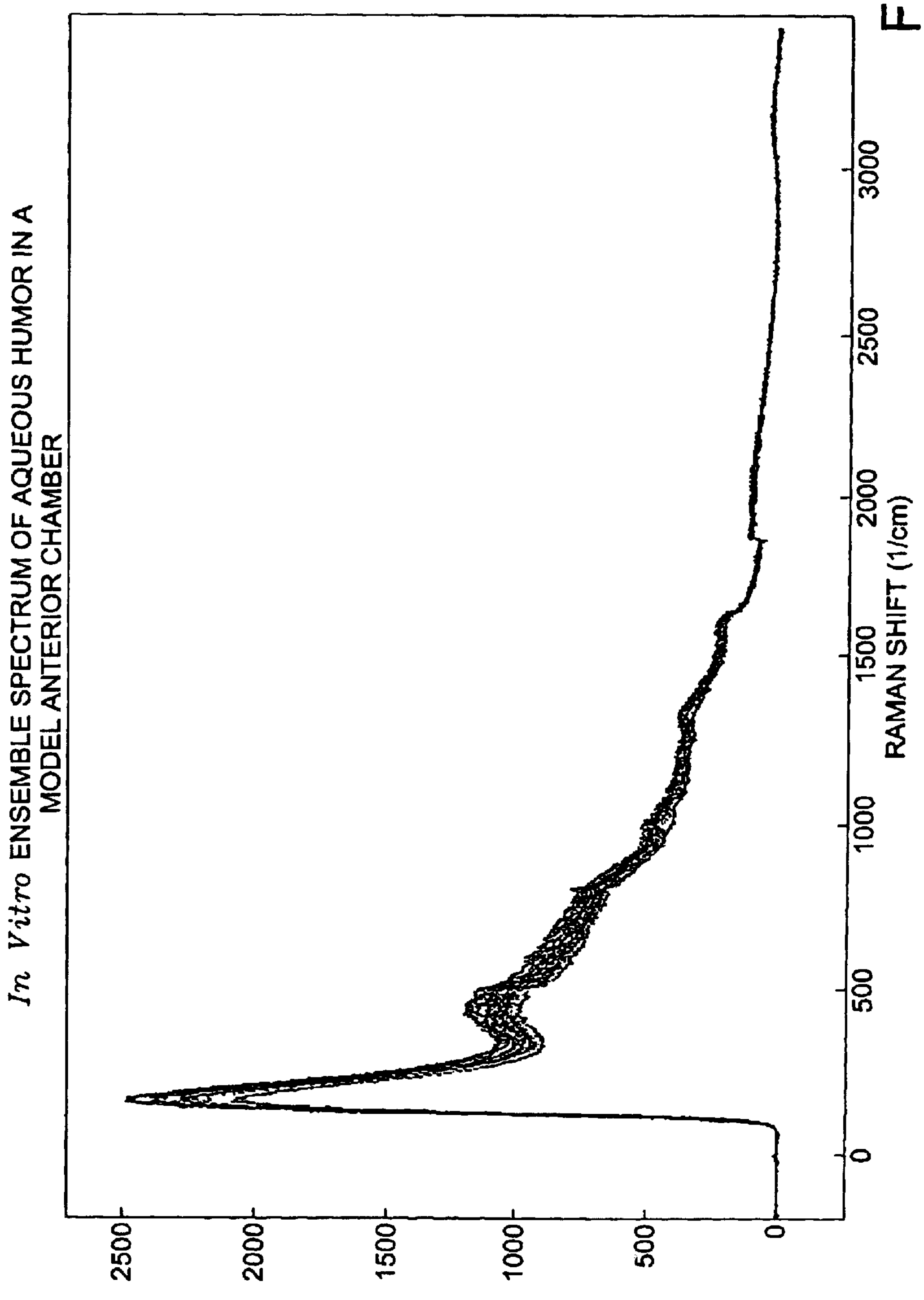

In Vitro ENSEMBLE SPECTRUM OF AQUEOUS HUMOR IN A MODEL ANTERIOR CHAMBER
2500
2000
1500
1000
500
0
0
500
1000
1500
2000
2500
3000
RAMAN SHIFT (1/cm)

FIG. 21B.

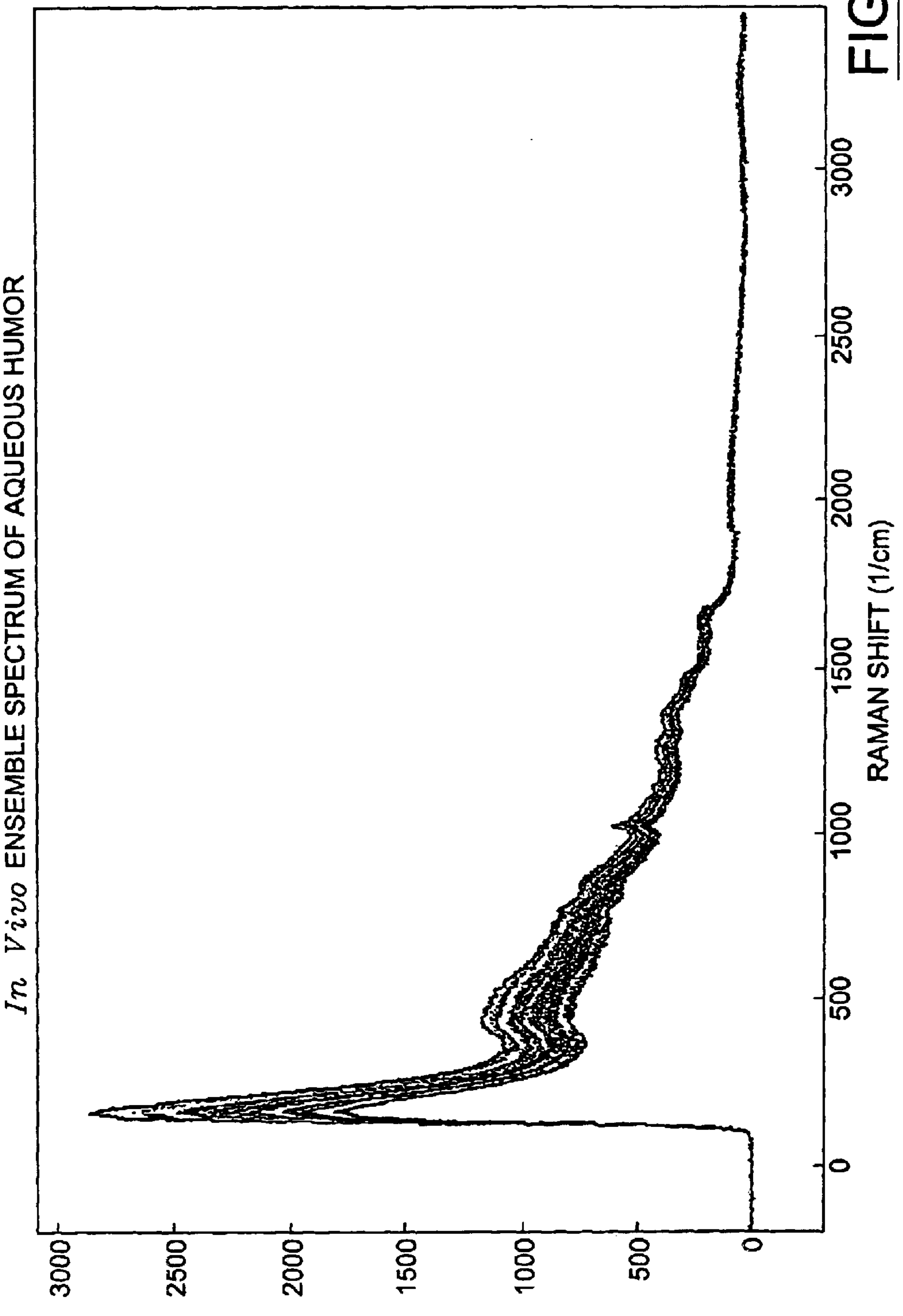

In Vivo ENSEMBLE SPECTRUM OF AQUEOUS HUMOR
3000
2500
2000
1500
1000
500
0
0
500
1000
1500
2000
2500
3000
RAMAN SHIFT (1/cm)

FIG. 21C.

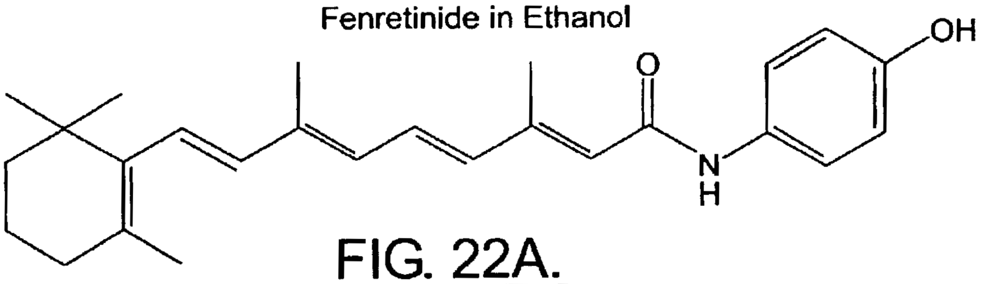

Fenretinide in Ethanol
O
N
H
OH

FIG. 22A.

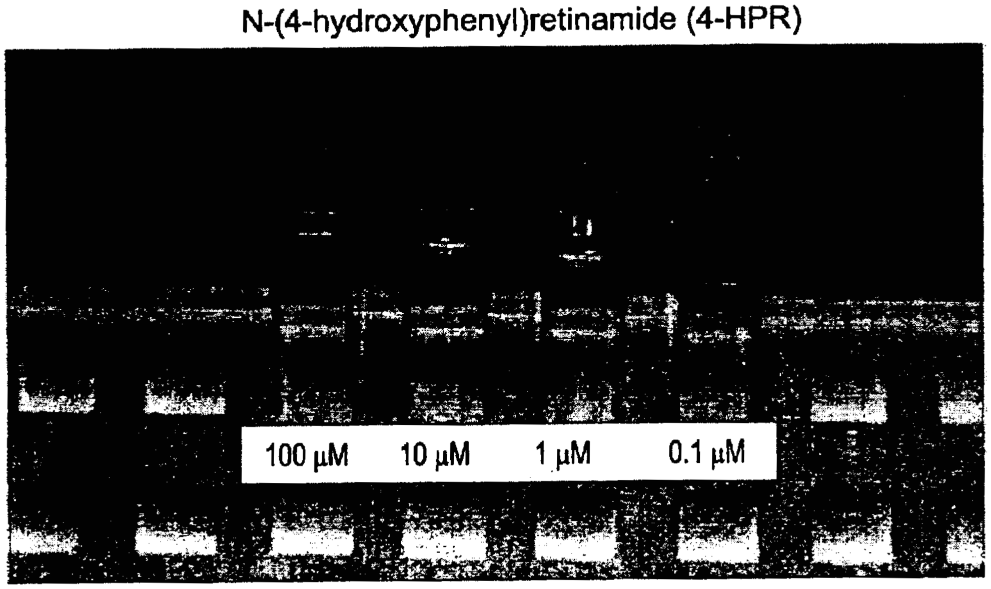

N-(4-hydroxyphenyl)retinamide (4-HPR)
100 µM
10 µM
1 µM
0.1 µM

FIG. 22B.

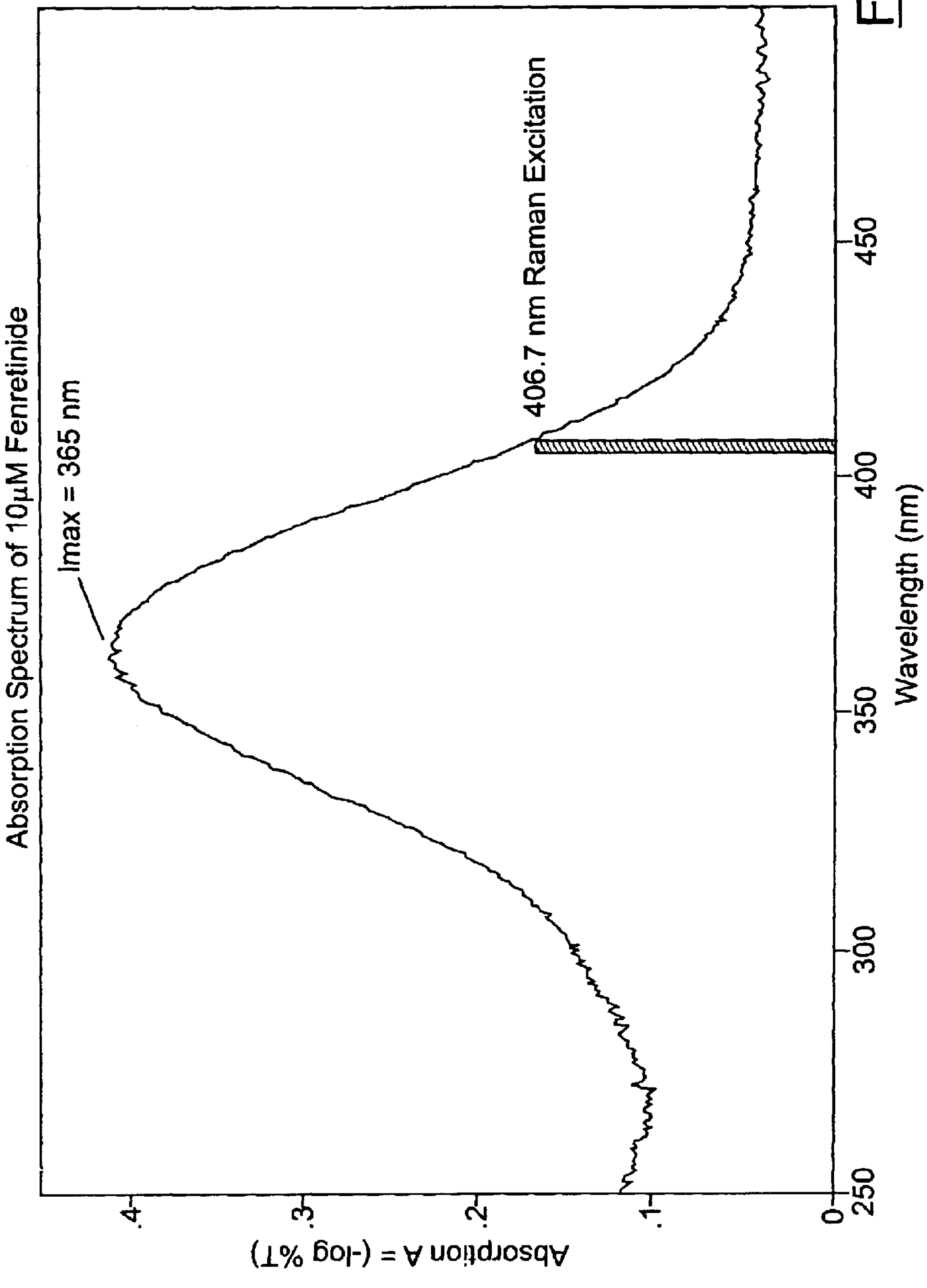

Absorption Spectrum of 10μM Fenretinide
lmax = 365 nm
406.7 nm Raman Excitation
Absorption A = (-log %T)
0
.1
.2
.3
.4
250
300
350
400
450
Wavelength (nm)

FIG. 23.

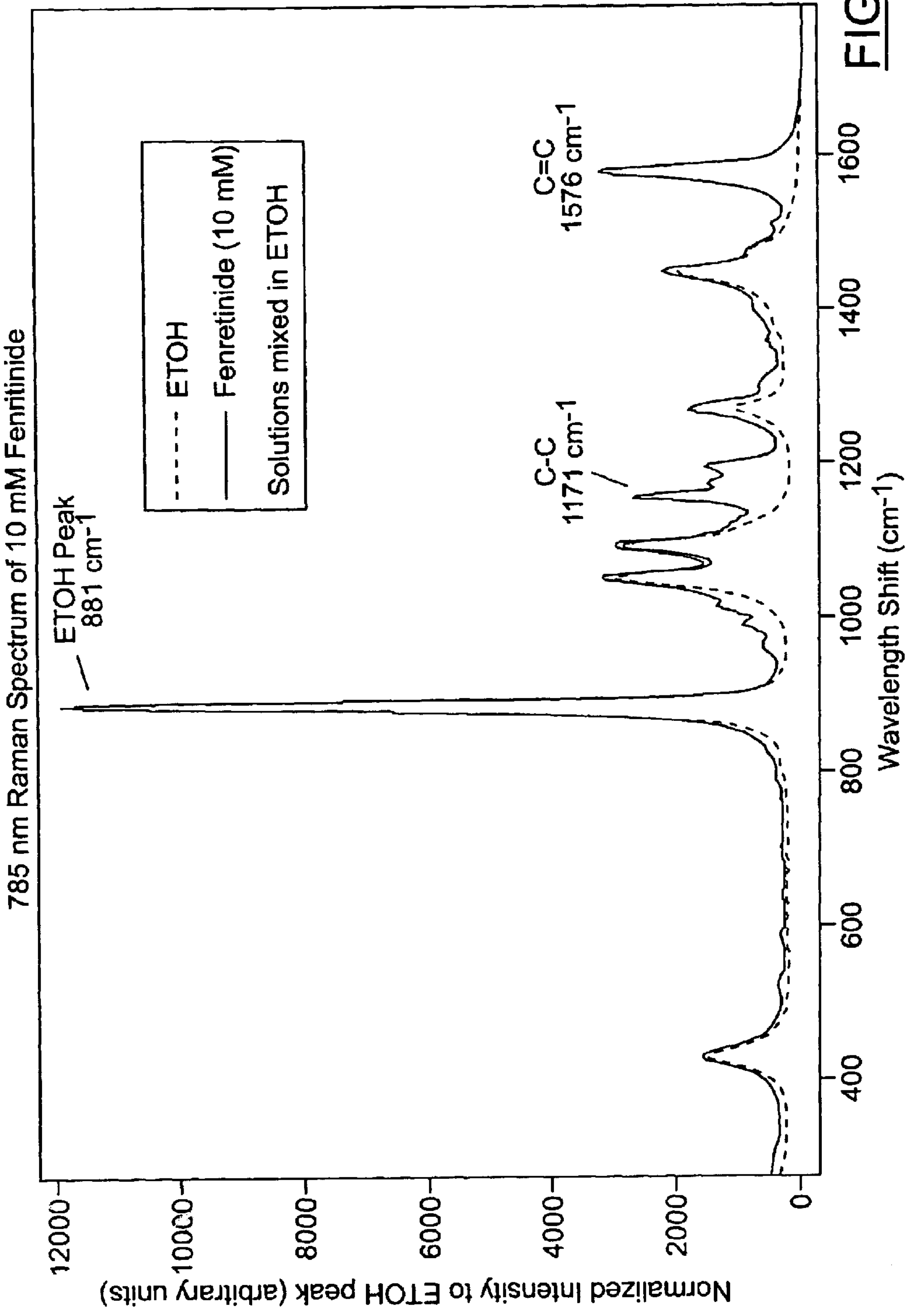

785 nm Raman Spectrum of 10 mM Fenritinide
ETOH Peak
881 cm-1
C-C
1171 cm-1
C=C
1576 cm-1
- - - - ETOH
——— Fenretinide (10 mM)
Solutions mixed in ETOH
Normalized Intensity to ETOH peak (arbitrary units)
12000
10000
8000
6000
4000
2000
0
400
600
800
1000
1200
1400
1600
Wavelength Shift (cm-1)

FIG. 24.

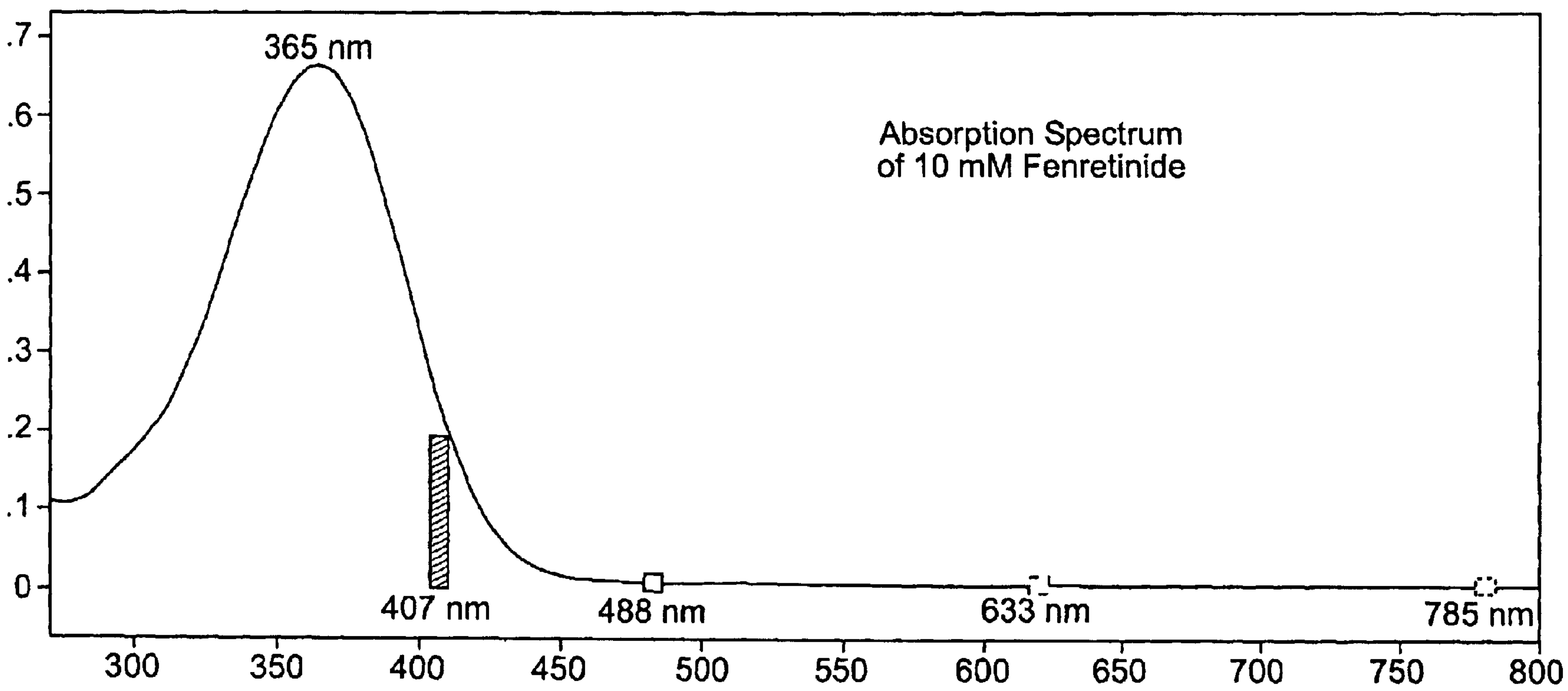

.7
.6
.5
.4
.3
.2
.1
0
365 nm
Absorption Spectrum
of 10 mM Fenretinide
407 nm
488 nm
633 nm
785 nm
300
350
400
450
500
550
600
650
700
750
800

FIG. 25A.

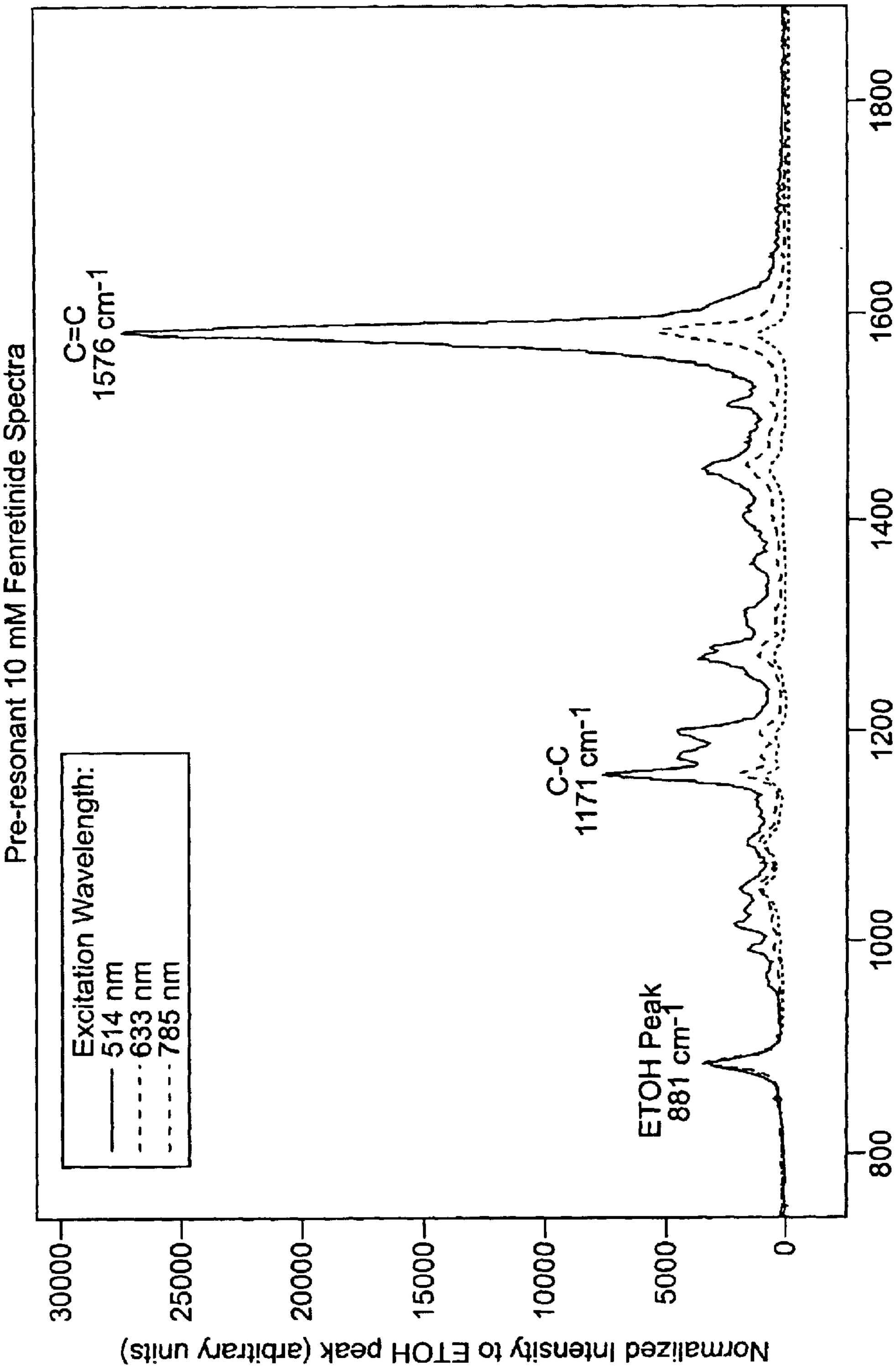

Pre-resonant 10 mM Fenretinide Spectra
Excitation Wavelength:
514 nm
633 nm
785 nm
ETOH Peak
881 cm-1
C-C
1171 cm-1
C=C
1576 cm-1
Normalized Intensity to ETOH peak (arbitrary units)
30000
25000
20000
15000
10000
5000
0
800
1000
1200
1400
1600
1800
Wavelength Shift (cm-1)

FIG. 25B.

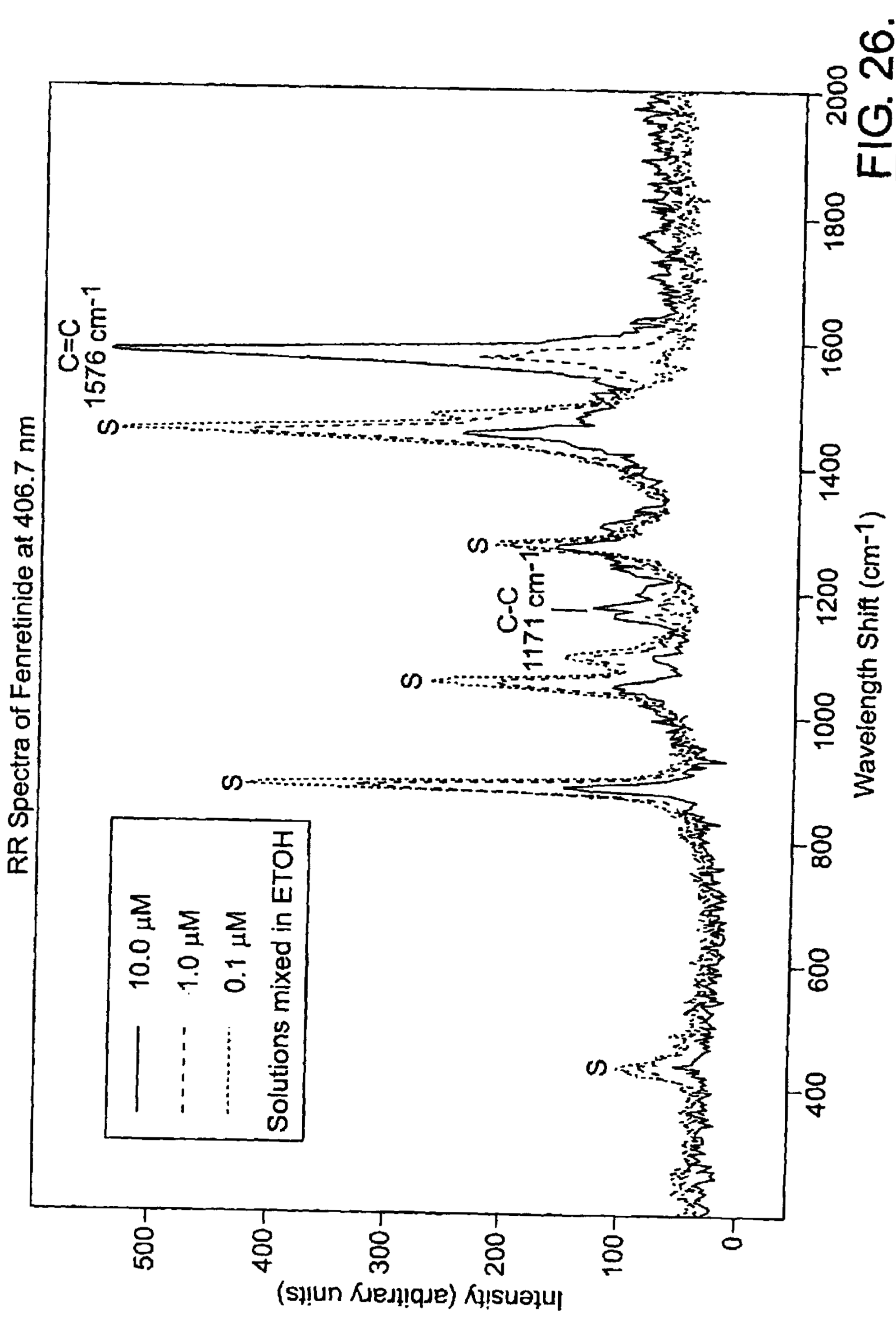
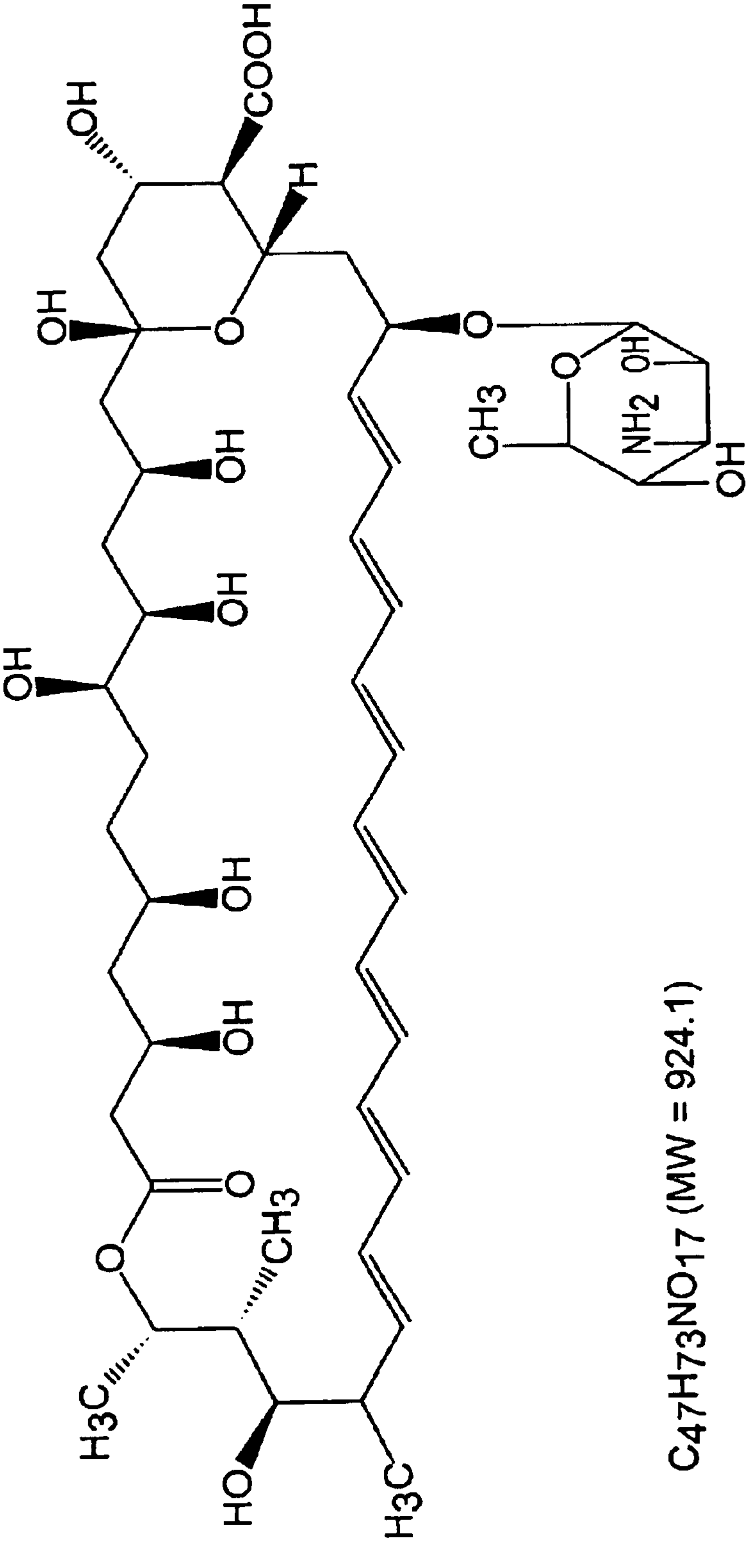

RR Spectra of Fenretinide at 406.7 nm
10.0 μM
1.0 μM
0.1 μM
Solutions mixed in ETOH
S
S
S
S
S
C-C
1171 cm-1
C=C
1576 cm-1
Intensity (arbitrary units)
0
100
200
300
400
500
400
600
800
1000
1200
1400
1600
1800
2000
Wavelength Shift (cm-1)

FIG. 26.


OH
COOH
H
OH
O
O
CH3
O
OH
NH2
OH
OH
OH
OH
OH
OH
O
CH3
O
H3C
HO
H3C

$C_{47}H_{73}NO_{17}$ (MW = 924.1)

FIG. 27.

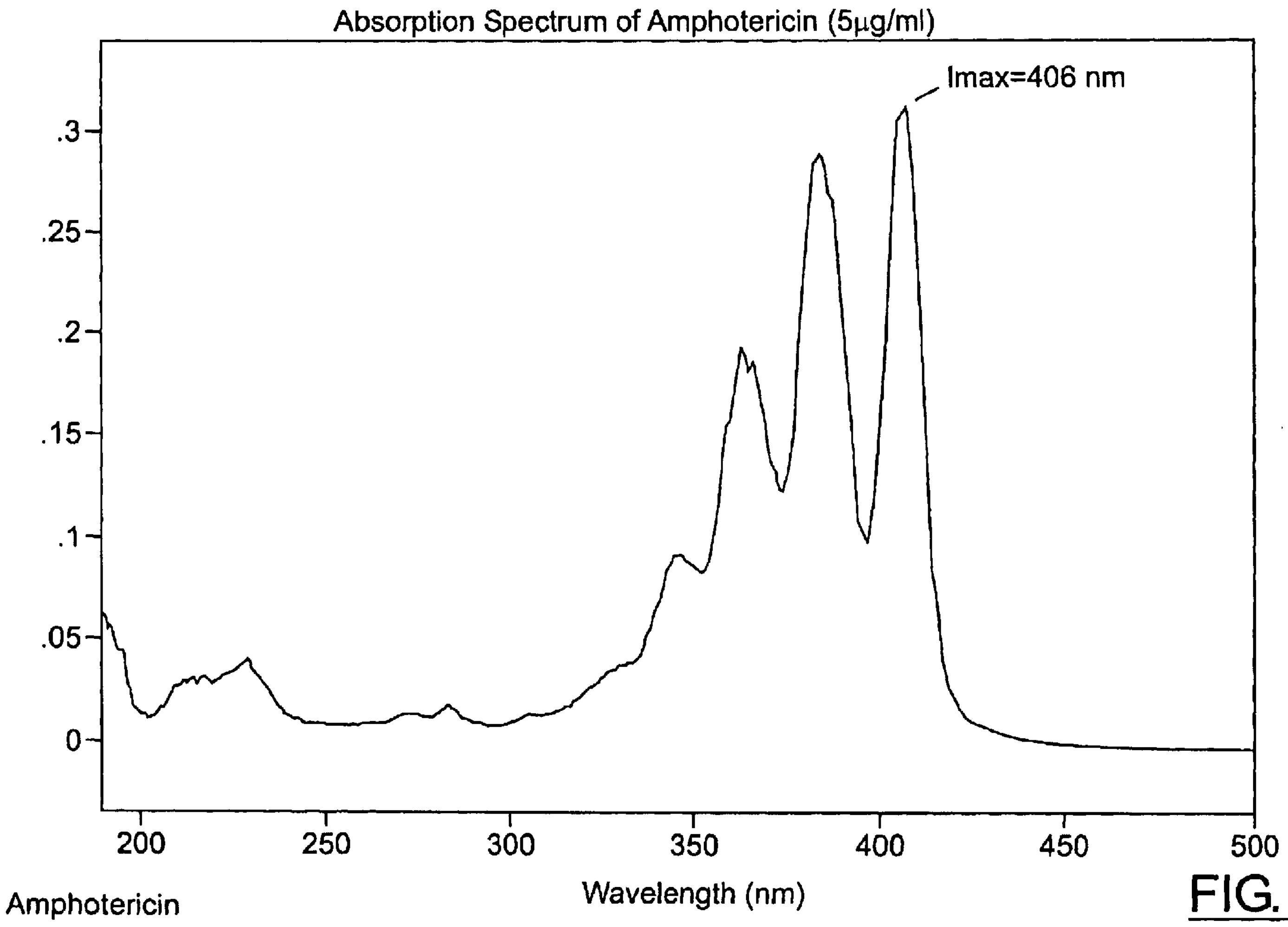

Absorption Spectrum of Amphotericin (5μg/ml)
Imax=406 nm
.3
.25
.2
.15
.1
.05
0
200
250
300
350
400
450
500
Wavelength (nm)
Amphotericin

FIG. 28.

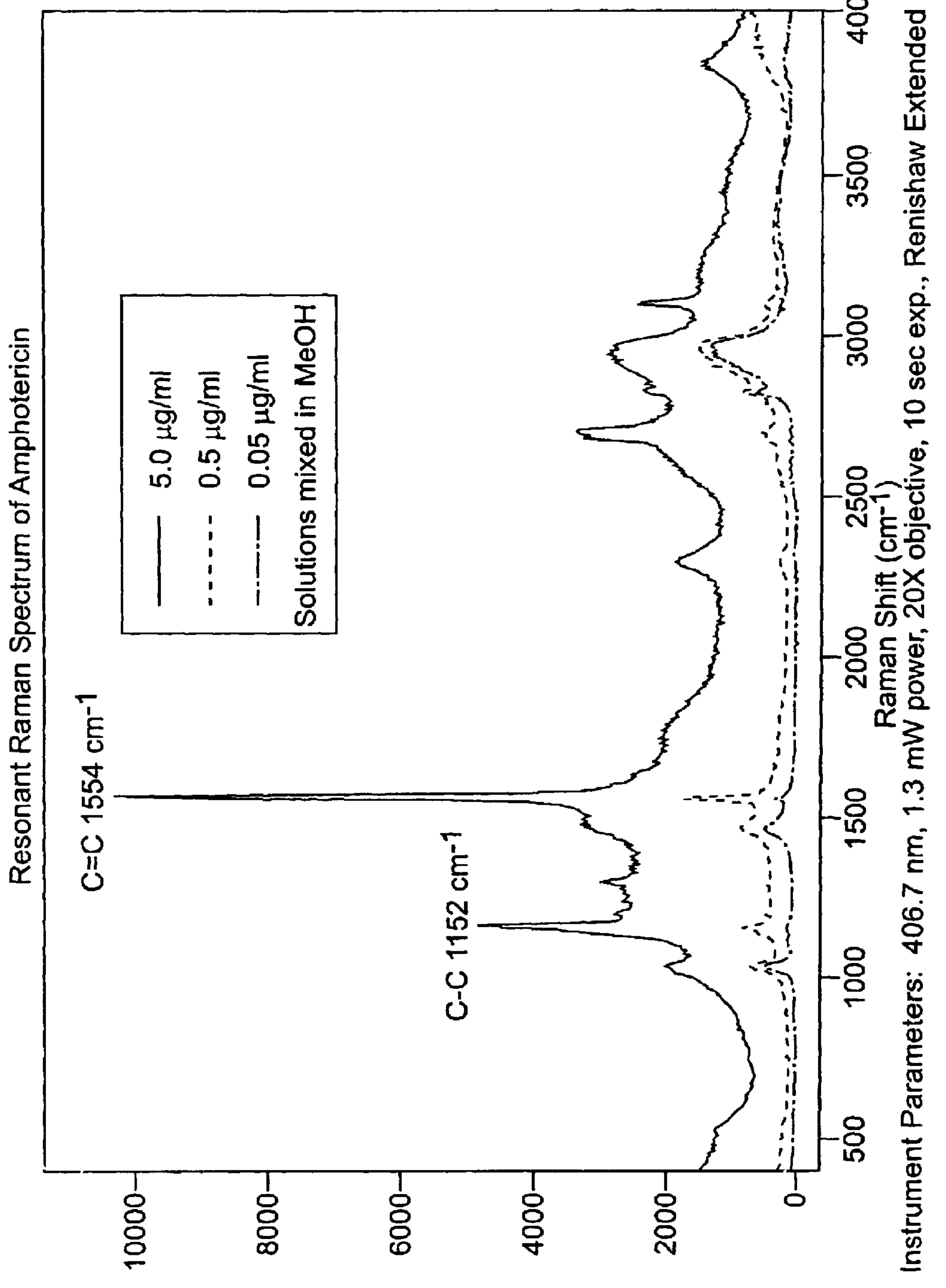

Resonant Raman Spectrum of Amphotericin
C=C 1554 cm-1
C-C 1152 cm-1
5.0 µg/ml
0.5 µg/ml
0.05 µg/ml
Solutions mixed in MeOH
10000
8000
6000
4000
2000
0
500
1000
1500
2000
2500
3000
3500
4000
Raman Shift (cm-1)
Instrument Parameters: 406.7 nm, 1.3 mW power, 20X objective, 10 sec exp., Renishaw Extended Scan

FIG. 29.

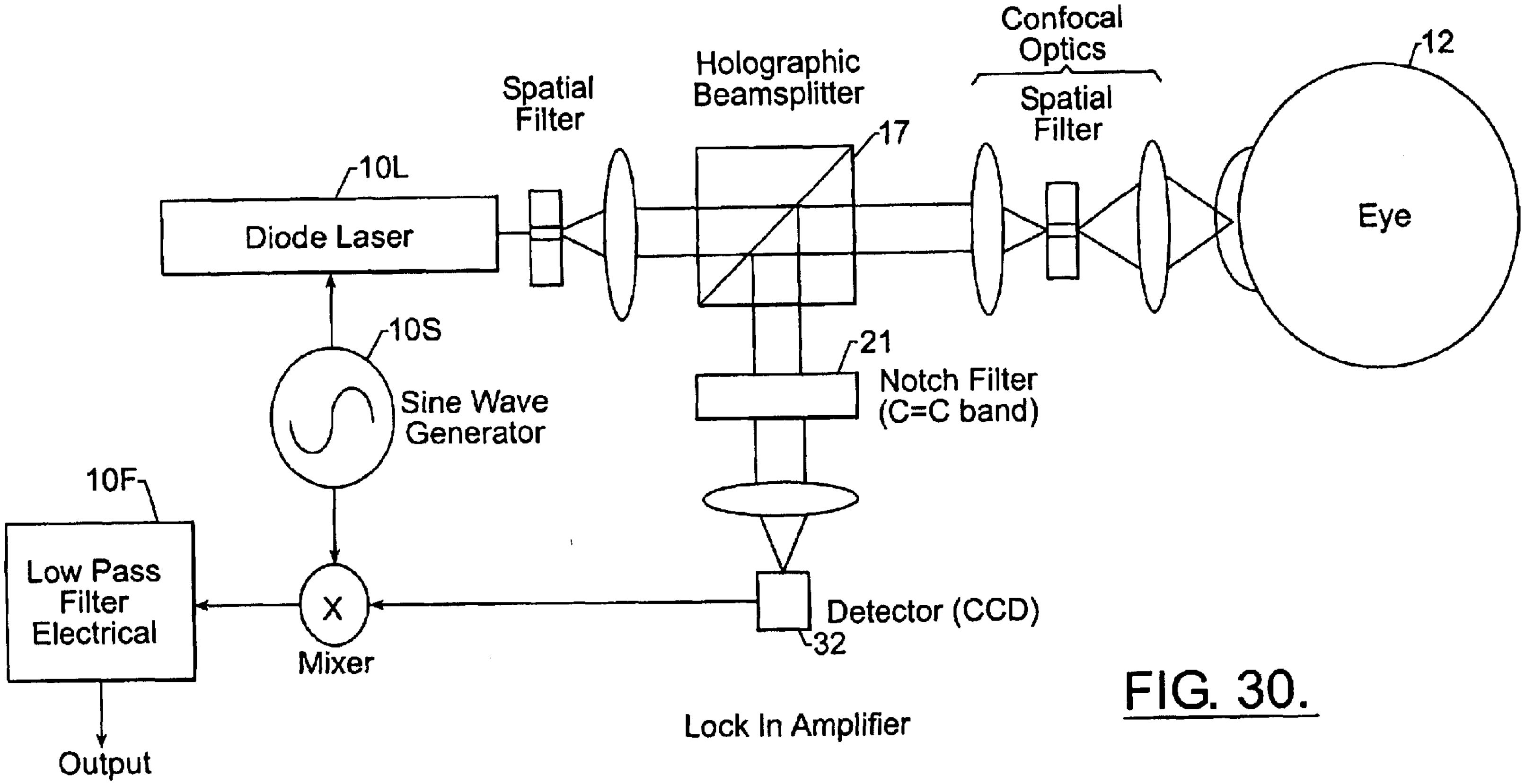

Confocal
Optics
Spatial
Filter
12
Eye
Holographic
Beamsplitter
17
Spatial
Filter
10L
Diode Laser
10S
Sine Wave
Generator
21
Notch Filter
(C=C band)
10F
Low Pass
Filter
Electrical
X
Mixer
Detector (CCD)
32
Output
Lock In Amplifier

FIG. 30.

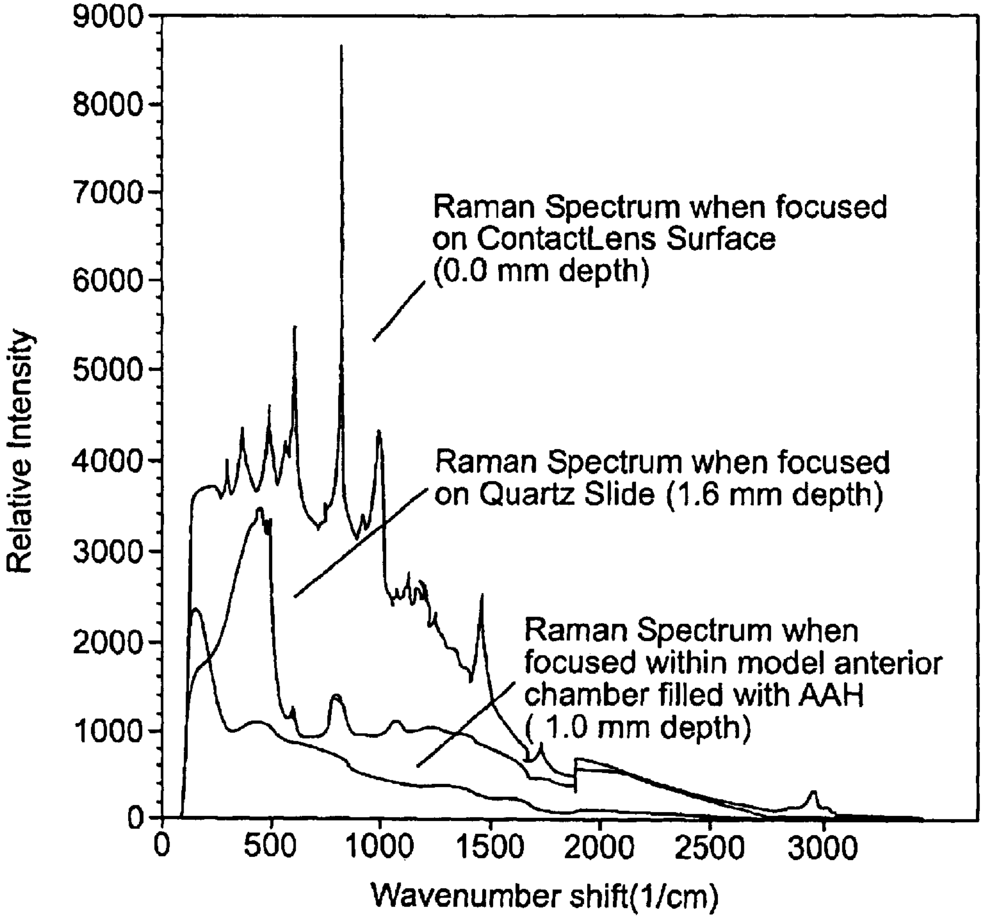


FIG. 31A.

Raman spectra of artificial aqueous humor (AAH) and components of a physical model of the anterior chamber collected using confocal optics.

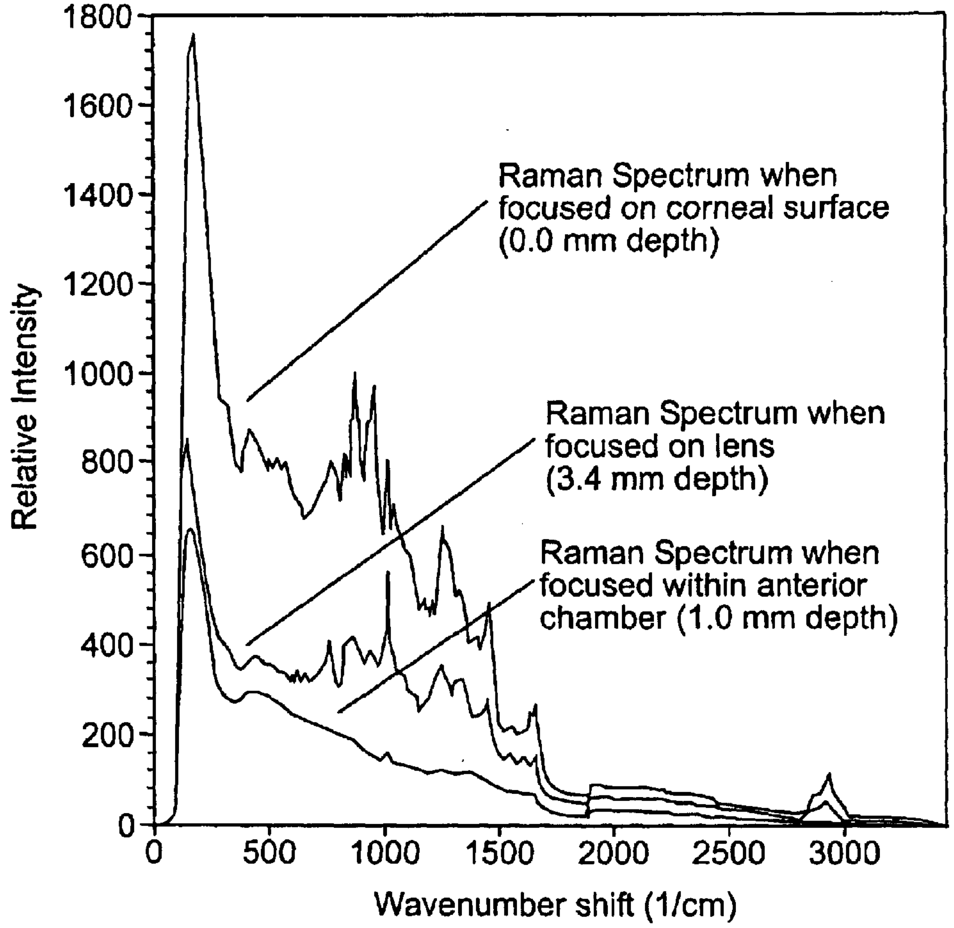
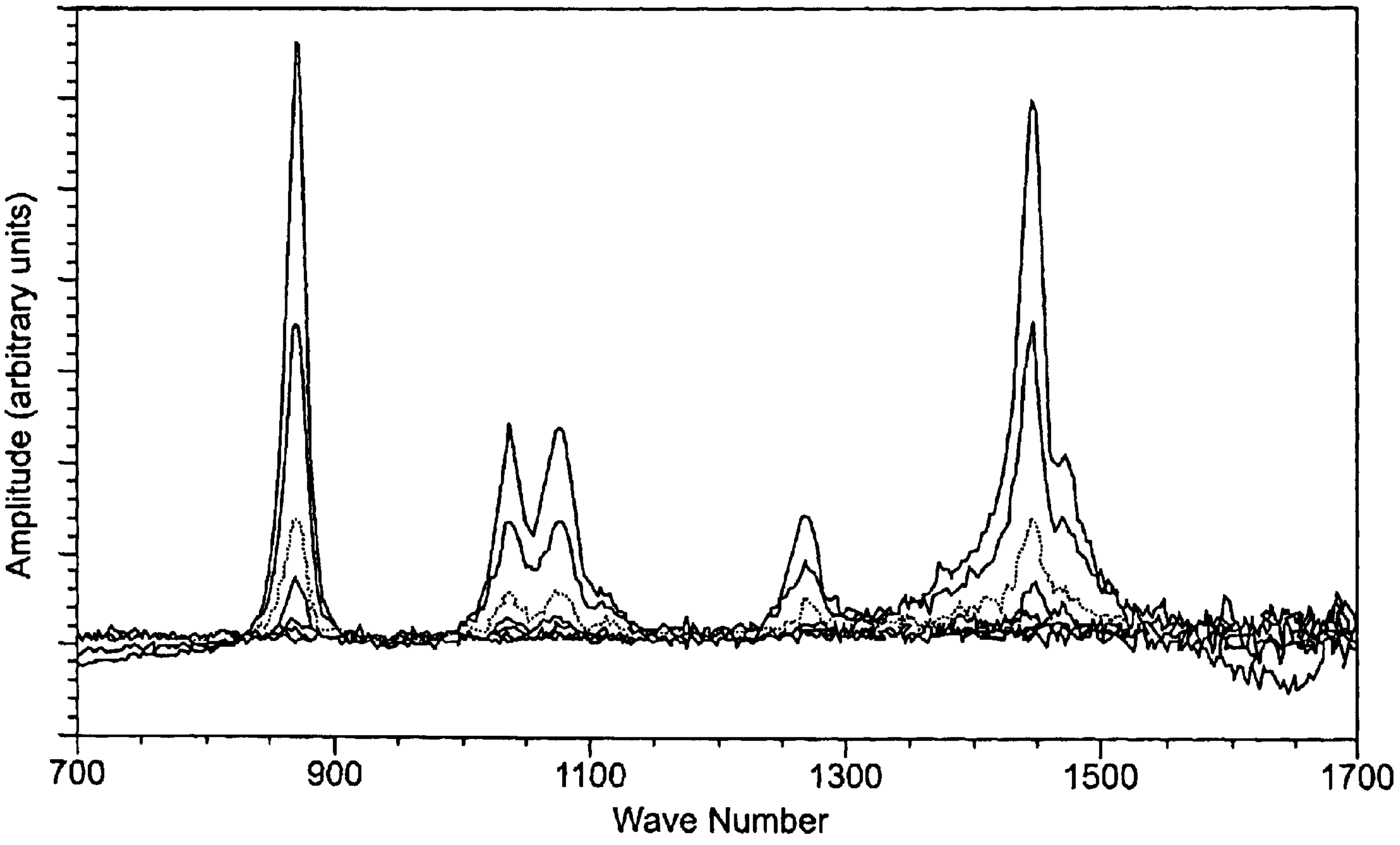


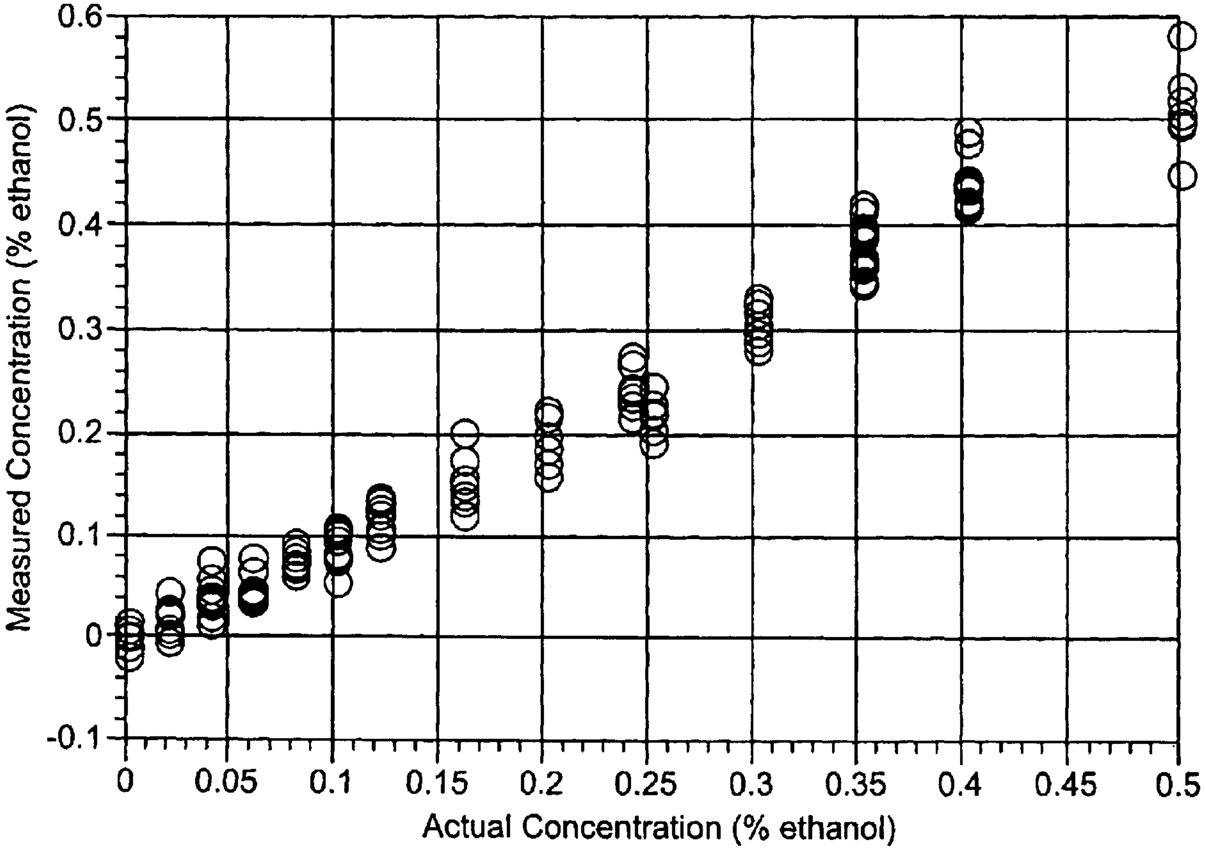
FIG. 31B.

In vivo Raman spectra of structures of the anterior chamber of a rabbit. Raman spectra of AH can clearly be distinguised from those of nearby cornea and lens.



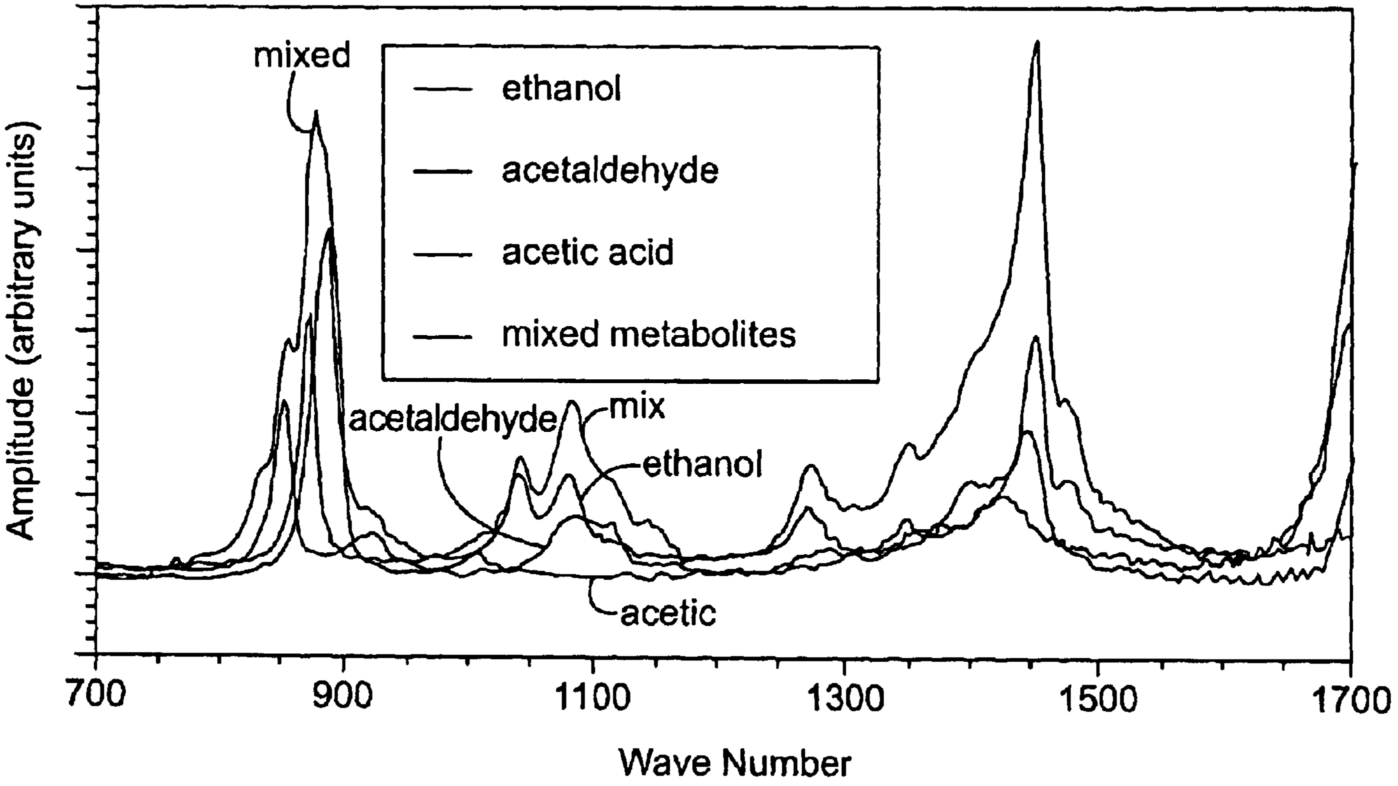
Normalized spectra for various levels of ethanol in artificial aqueous humor.

FIG. 32.



PLS-measured ethanol concentration plotted with respect to actual ethanol concentration in aqueous solution.

FIG. 33.



The Raman spectra of 0.333M aqueous solutions of ethanol, acetaldehyde, and acetic acid each have similar Raman cross-sections. The spectrum of a mixture of the three metabolites is a sum of the individual spectra, demonstrating adherence to the Beer-Lambert law.

FIG. 34.

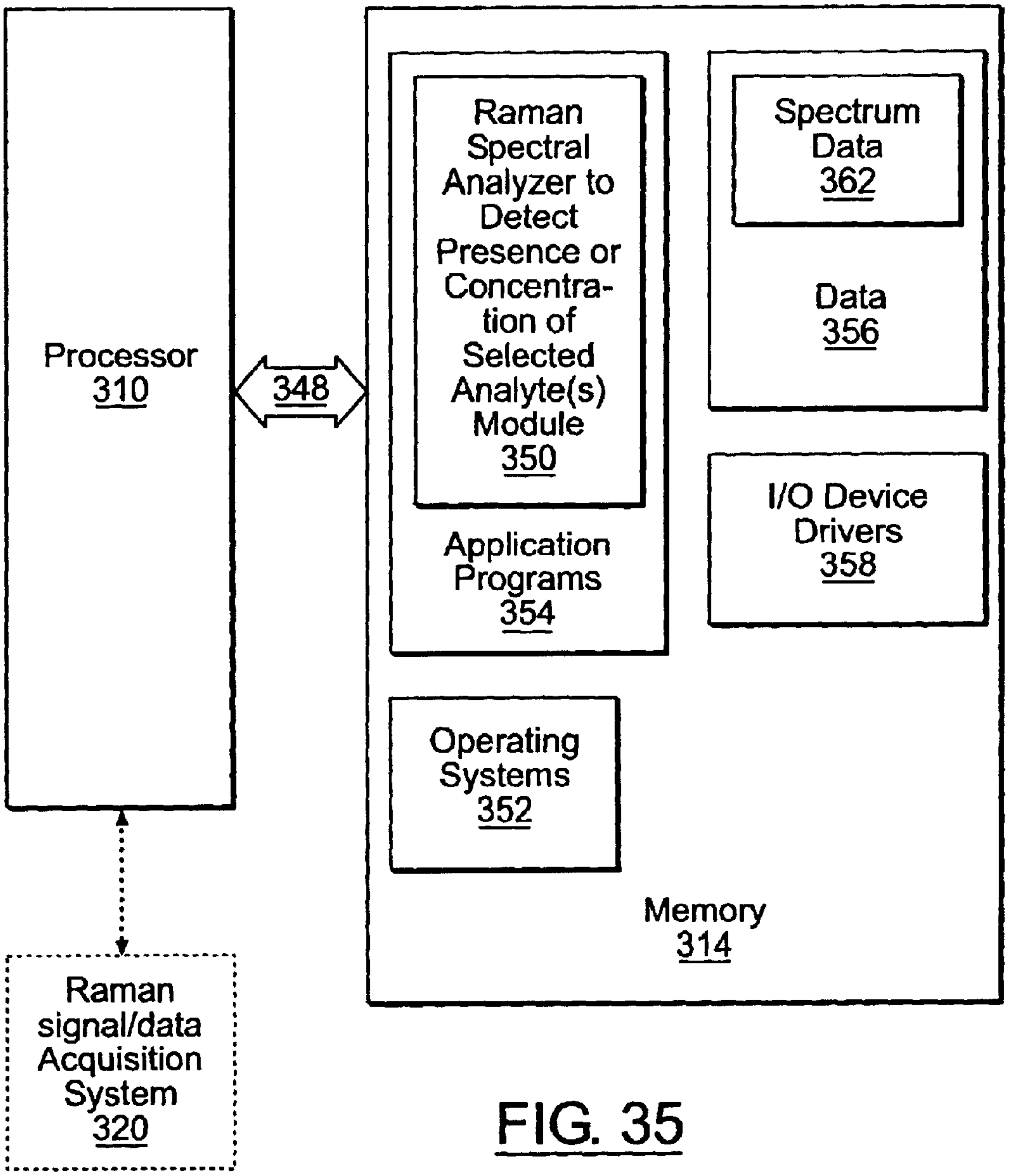

Processor
310
348
Raman Spectral Analyzer to Detect Presence or Concentration of Selected Analyte(s) Module
350
Application Programs
354
Spectrum Data
362
Data
356
I/O Device Drivers
358
Operating Systems
352
Memory
314
Raman signal/data Acquisition System
320

FIG. 35

IDENTIFYING OR MEASURING SELECTED SUBSTANCES OR TOXINS IN A SUBJECT USING RESONANT RAMAN SIGNALS

RELATED APPLICATIONS

This application is a continuation-in-part of International Patent Application Serial No. PCT/US/02/00649, filed Jan. 9, 2002, which is a continuation-in-part of U.S. application Ser. No. 09/756,897, filed Jan. 9, 2001 now U.S. Pat. No. 6,574,501, the disclosures of which are hereby incorporated by reference as if restated in their entireties herein.

The invention described herein was made in the performance of work under a NASA contract, and is subject to the provisions of Public Law 96-517 (35 USC § 202) in which the Contractor has elected to retain title. The United States Government has certain rights to this invention.

FIELD OF THE INVENTION

The present invention concerns methods and apparatus for measuring or identifying the presence of selected substances in the body of a subject via non-invasive spectrographic analysis of certain regions of the eye, such as the aqueous humor in the anterior chamber of the eye.

BACKGROUND OF THE INVENTION

Non-invasive measurement of physiological and foreign substances, including blood glucose, by optical spectroscopy techniques has remained an elusive target for at least two decades. Blood, tissue, and most excreted fluids contain numerous substances which confound many spectral signatures. On the other hand, the aqueous humor (AH), which fills the anterior chamber of the eye (between the lens and cornea), contains relatively few molecules capable of interfering with the spectroscopic detection of glucose. These are primarily lactate, ascorbate, and urea. This fact, along with its optically accessible location behind the cornea, makes the AH an attractive choice as a site on which to attempt non-invasive analysis of many substances present in a biological subject, including glucose.

Pohjola (*Acta Ophthalmologica Suppl.* 88, 1–80 (1996)) showed that the ratio of aqueous glucose to plasma glucose in normal euglycemic individuals is related to age and ranges from 0.6 to 0.9. He further showed in seven humans with steady-state hyperglycemia that similar ratios applied. There is little, if any, data regarding the equilibration time of aqueous humor glucose with changes in plasma glucose in humans. Some recent research suggests that the glucose content of the AH compared with that in the capillary blood in man is about 0.75 regardless of the glycemic state of the person. See e.g., Schrader et al., *The glucose content of the aqueous humour compared with capillary blood in man*, Invest. Ophthalmol. Vis. Sci. (Suppl.) 44:404 (2000).

Numerous investigators over the years have suggested that the ratio of aqueous glucose to plasma glucose in the normoglycemic rabbit ranges from 0.42 to 1.01 (S. Pohjola, supra; D. Reddy and V. Kinsey, *Arch. Ophthalmol.* 63, 715–720 (1960); M. Reim et al., *Ophthalmologica* 154, 39–50 (1967); W. March et al., *Diabetes Care* 5, 259 (1982)). It is uncertain whether this variability is normal or could be attributed to differences in glucose measurement techniques, collection techniques, sample storage, and anesthesia. It is believed that the relationship of aqueous glucose to rising, or falling, plasma glucose has not been previously studied in rabbits.

Coté has reviewed the relative strengths and weaknesses of optical glucose sensing techniques (*J. Clin. Engineering* 22, 253 (1997)). Raman spectroscopy is potentially attractive because it can distinguish glucose in water solutions containing various levels of other optically active metabolites (S. Wang et al., *Applied Optics* 32, 925 (1993)). Raman spectroscopy measures the shift in the wavelength of incident light as it is scattered by molecules. Any given molecule typically causes a characteristic shift in the spectrum of scattered light, which is dependent upon its intermolecular and intramolecular bonds. This is in contradistinction to fluorescence, which is caused by changes in electron energy states, and does not shift relative to the wavelength of incident light.

Wicksted et al, (*Appl. Sectroscop.* 49, 987 (1995)) suggest that the Raman signature for glucose can be identified in aqueous humor samples, and Goetz et al. (*IEEE Trans. Biomed. Eng.* 42, 728 (1995)) have demonstrated that higher than physiologic levels of glucose can be measured with Raman spectroscopy in water solutions. J. Lambert et al. (*LEOS Newsletter* 12, 19–22 (1998)) suggest that measurement of glucose at physiologic levels is possible in water solutions containing other analytes normally found in the aqueous humor. In certain situations, when solutions containing fluorescent substances are studied, however, the fluorescence signal may overwhelm the relatively weak Raman-shifted signal. This is a potential problem if Raman spectroscopy is applied to certain regions in the eye, such as the conjunctiva or vitreous or aqueous humor (and/or depending upon what the Raman signal is attempting to identify or measurer), which can contain proteins that fluoresce.

U.S. Pat. No. 5,243,983 to Tarr et al. proposes a non-invasive blood glucose measurement system using stimulated Raman spectroscopy. Stimulated Raman spectroscopy can require the use of both a pump and a probe laser beam. In operation, the probe laser beam is used to measure the stimulated Raman light at a single wavelength after transmission across the anterior chamber of the eye. Commercially, this may be undesirable, since an optical component contacting the eye is used to direct the beam across the anterior chamber. In addition, use of a single wavelength may limit the ability to measure glucose at physiologic levels within tissue containing many other Raman scattering chemicals.

Others have also proposed various glucose measurement devices. For example, U.S. Pat. No. 5,433,197 to Stark suggests a non-invasive glucose measurement apparatus that employs broadband, infrared light stimulation. In addition, U.S. Pat. No. 5,553,617 to Barkenhagen proposes a non-invasive method for measuring body chemistry from the eye of a subject by measuring a spectral response such as a Raman scattering response. While the latter reference alleges that it may be used for medical applications (such as the determination of sugar in diabetics), specific details on how this might be accurately carried out are not provided. Another example is found in U.S. Pat. No. 5,710,30 to Essenpreis, which proposes a method for measuring the concentration of glucose in a biological sample such as the eye (see FIG. 4 therein) with interferometric measurement procedures. Still another example is proposed in U.S. Pat. No. 5,666,956 to Buchert et al., wherein it is proposed that an instrument for the non-invasive measurement of a body analyte can be based on naturally emitted infrared radiation.

In spite of the foregoing efforts, a commercially viable, non-invasive monitor which can successfully employ a non-invasive optical analysis of certain regions of the eye, including the aqueous humor of the eye, has not yet been developed. Difficulties in developing such a device include:

(a) determining reliable correlations of the typical millimolar quantities of selected substances or chemicals; (b) obtaining accurate measurements of selected substances; and (c) inhibiting damaging effects to the eye which may be caused by excessive exposure to light in an instrument that is used to generate the analysis signal spectrum in the AH. Accordingly, there is a continued need for improved systems, methods, and devices for the non-invasive in vivo analysis of foreign and natural physiologic substances in a biological subject via analysis of certain regions of the eye.

SUMMARY OF THE INVENTION

The present invention provides methods and systems for monitoring or evaluating the blood aqueous barrier and, thus, the blood brain barrier dynamics of the subject. The present invention also provides methods and systems which can detect the presence or absence of one or more selected substances or analytes of interest in the body by optically analyzing certain regions of the eye, including at least one of the AH, the vitreous humor ("VH"), and one or more blood vessels in the conjunctiva. In certain embodiments, the analysis can provide information regarding the presence of and/or quantify a detected substance in the body such as in the cerebral spinal or intracranial fluid of the subject (indirectly, through a correlation with the presence or quantification of the substance in the blood vessel in the conjunctiva, or the AH or the vitreous humor).

Embodiments of the invention can employ Raman spectroscopy to non-invasively obtain, in vivo, at least one signature spectroscopic signal to identify and/or measure the level or concentration of a substance or substances of interest in the subject (either in the blood and/or brain) based on the signal.

In certain embodiments, the present invention can be used to monitor or evaluate the blood brain barrier dynamics, which may be intentionally altered (such as through the administration of chemicals or exposure to certain environmental conditions such as increased pressure) during such evaluation. By intentionally breaking down the blood brain barrier, medicines which are normally inhibited from crossing the barrier may be allowed to more readily cross and enter into the brain. To monitor such a change in the operation or dynamics of the blood brain barrier, nonspecific markers can be introduced or injected into the subject. The non-specific marker is selected based on its molecular size and/or its normal reluctance to cross the blood brain barrier. The blood brain barrier can then be intentionally altered (broken down or opened) so that the non-specific marker is able to cross therethrough. An optical reading of a selected region of the eye can be taken, and the present invention can assess whether the marker is present (either at all or in an increased amount over a pre-alteration state) in the blood aqueous barrier. Further, in some embodiments, the concentration of the marker in the eye can be determined (such as in the AH or vitreous humor) in the blood aqueous compartment. If the marker is identified as being present, this indicates that the blood brain barrier has been altered. Once the blood brain barrier is altered, a desired treatment regimen can be administered to the subject (such as drug used for chemotherapy) to treat tumors or other conditions in the eyes or brain. In some embodiments, as an alternative to the use of non-specific markers, the present invention can monitor the presence or concentration of the treatment drug itself in the blood aqueous compartment in the eye. Examples of suitable markers include large molecule natural and synthetic substances that do not normally cross the blood brain barrier, including, but not limited to, antibiotics including antifungal agents and/or erythromycin, and conjugated substances such as conjugated billirubin.

Typically, the treatment drug is configured such that it is inhibited from crossing the blood brain barrier. Thus, in certain applications, in order to deliver a sufficient quantity of the drug to the brain, the amount of drug, which is systemically delivered, can be undesirably toxic to the patient away from the targeted treatment region in the brain. The present invention can now assess or assure either that the barrier is sufficiently altered to allow the drug to pass more efficiently therethrough and/or quantify or assess that a sufficient amount of the treatment drug is getting into the brain so that the systemic amount can be more closely regulated and reduced. After the desired treatment (or dose) is indicated as delivered to the brain, the blood brain barrier can be restored such that it is substantially in its pre-altered state. The return to the pre-altered state can be confirmed by taking another optical reading to confirm that the marker is in a reduced concentration in the blood aqueous compartment in the eye.

By identifying and/or quantifying the amount of the non-specific marker present in the aqueous humor (and, thus in the blood aqueous barrier), an estimate or determination of the concentration in the brain can be established. Typically, the concentration in the blood aqueous humor can be presumed to be similar to that in the blood brain compartment. Further, the two concentrations can be correlated so that a quantitative value of the amount in the cerebral spinal or intracranial fluid can be determined based on that found in the blood aqueous compartment so that a titrated dose of the treatment drug in the brain may be able to be determined. The correlation relationship or ratio may vary within certain population segments. In certain embodiments, the correlation relationship can be determined based on data collected across a representative population (by age, size, weight, gender, race, disease or physiological impairment or abnormality, or national origin). Thus, the amount of the selected treatment drug actually getting into the brain can be correlated to the systemic dose delivered to the patient so that the dose or level in the spinal fluid is sufficiently high for treatment of the tumor or other condition but the systemic dose is sized to provide reduced toxicity to the subject (by avoiding administering unnecessary quantities to the subject).

In certain embodiments, the present invention can provide methods, similar to that discussed for intentionally altering the dynamics of the blood brain barrier, which use a non-specific marker with a new drug to evaluate the impact that a new drug or therapy regimen has on the blood brain barrier for safety or other considerations.

In other embodiments, the optical detection of an analyte, agent, or drug itself, in certain regions of the eye, without the use of a marker, may be sufficient to indicate the drug's impact on the blood brain barrier.

In other embodiments, the environmental conditions surrounding the patient or subject, can be altered and the dynamics of the blood brain barrier monitored. For example, subjects which are exposed to different elevations, gravity conditions, or to increased intracranial pressure, may exhibit different or altered blood brain barrier characteristics, either transiently, or more chronically, than persons not so exposed. These subjects may include astronauts, pilots, divers, trauma victims, and the like. Evaluating the blood brain barrier dynamics can identify whether larger molecules or pathogens are able to cross the blood brain barrier, which may, under normal circumstances, be inhibited or prevented from entering the brain. In certain embodiments, the present invention can be used to assess which constituents in the blood cross into the intracranial fluid via the blood brain barrier.

As generally described above, in one embodiment, a patient can undergo a treatment regimen to deliberately or intentionally alter the blood brain barrier dynamics so that an identified treatment agent(s) is allowed to cross the barrier. For example, an osmotic agent such as a drug (for example, MANNITOL) can be delivered to a subject being treated for cancer to force the blood brain barrier to open (preferably for a limited-time treatment window) to successfully allow a selected chemotherapeutic agent (such as a cytotoxic agent) to be able to more readily cross the blood brain barrier into the brain. Non-invasive monitoring of the tumor dose according to the present invention, can allow monitoring of the barrier dynamics and may, in some embodiments, be able to assess when an adequate, but not excessive, tumor dose is delivered to the brain. The blood brain barrier can be reestablished after the appropriate tumor dosing is delivered. This monitoring of the blood brain barrier dynamics during a treatment regimen may now inhibit or reduce systemic damage in the subject associated with the cytotoxic treatment.

In other embodiments, the present invention provides systems and methods for detecting the presence of a predetermined substance or identifying the presence of an unknown substance in the body of a subject. The substances which can be measured or identified are numerous and can be (a) natural physiologic analytes or chemicals, such as glucose, amino acids, peptides, antibodies, blood (typically using light outside the red spectrum), and/or (b) foreign substances such as medicaments, drugs, or poisons (whether legal or illegal, and whether prescription or over the counter). For example, the present invention can be used to assess the presence of targeted illegal substances, such as alcohol or illegal narcotics such as cocaine, pcp, marijuana, or to identify what toxin or poison a subject has injested out of a number of household or environmental toxins and/or poisons such as herbicides, pesticides, household cleaning products, petroleum products or other common house hold chemicals including benzene, ethylene glycol. The methods and systems of the instant invention may even be used to identify the presence of poisonous plants, insect toxins, and reptile or snake venom. The present invention may be configured to identify whether an unknown substance in a subject is one or more of toxins/agents associated with the most prevalent poison-related emergency room visits. For example, ethylene glycol, methanol, and acetaminophen.

In certain embodiments, the present invention can be used to quantify the amount of a substance in the subject. Typically this embodiment may be particularly suitable for those substances ingested or existing in relatively large quantities or those present in sufficient quantity in the selected region of the eye so as to be detectable in vivo, or so that the substance or analyte is present in physiological levels (in the blood or blood aqueous compartment) of above about 0.001% or above about 0.01–10 μmolars. In certain embodiments, the system and methods can assess low levels of analytes, such as between nano-micromolar levels, depending on the Raman active characteristics of the analyte of interest. For example, for certain substances having molecules that generate resonant Raman spectra and, the signal strength can be greatly enhanced for certain excitation wavelengths that, in turn, may reduce the amount of the analyte that is detectable over non-resonant Raman spectra at that wavelength.

In addition, in certain embodiments, the devices and methods of the present invention may be used to detect increased or decreased levels of physiologic analytes such as caused by system impairments or reactions associated with dehydration, allergic reactions, or physiologic analytes associated with bacterial infections such as spinal meningitis, or to identify whether proteins or antibodies are present in elevated levels to identify a systemic response or a localized infection or disease in the eye or an immune system response, in the subject. Certain particular embodiments of the invention may also be used to treat the eye.

In certain embodiments, the systems and methods of the present invention may be able to detect or identify toxins released or emitted from foods contaminated with food poisoning bacteria such as *E coli*, salmonella (either in vivo or in vitro). Further, in some embodiments, the methods and systems of the present invent may be used to identify the presence of mad cow disease by analyzing certain regions of the eye (either in vitro or in vivo) such as by obtaining a Raman spectrum of a desired region of the eye analyzing the spectrum to detect the presence of small peptides or other markers associated with the disease. Other diseases may be able to be identified in vivo by the presence of a systemic reaction (such as an increased constituent level of a natural physiologic substance) in the subject. It is anticipated that such a method may be potentially used to assess whether the subject has contracted Lyme disease associated with deer tick bites or Rocky Mountain spotted fever.

In some embodiments, the invention can identify the presence of one or a plurality of household or environmental poisons in the subject in a relatively fast "triage" assessment to allow clinicians to determine the appropriate treatment in a timely manner. This can be particularly important for pediatric applications where the substance ingested may be difficult to ascertain for young children, and a relatively quick identification of a particular toxin or toxins ingested may allow more reliable or faster treatment decisions to be established.

One embodiment of the invention is directed to an in vivo method for monitoring the blood brain barrier dynamics of a subject, comprising the step of monitoring the dynamics of the blood brain barrier by non-invasively obtaining the Raman spectrum of a selected region in the eye of the subject. The method may also include the step administering a non-specific marker to the subject selected for its normal reluctance to cross the blood brain barrier under the normal condition. The monitoring step can comprise detecting the presence of the non-specific marker in the selected region of the eye of the subject.

In certain embodiments, the method can include the steps of: altering the dynamics of the blood brain barrier of the subject from a normal condition; and administering a quantity of a selected therapeutic agent to a subject for treatment of condition in the brain or neurological system after the altering step. It can also include the step of substantially returning the blood brain barrier to its normal state after a sufficient quantity of the therapeutic agent has been delivered to the brain. Similarly, the monitoring step can be performed before the therapeutic drug is administered to the subject and subsequently to confirm that the blood brain barrier is substantially returned to its normal condition. The method may also include the step of assessing the dose amount of the therapeutic agent delivered to the brain.

Certain embodiments of the present invention are directed to an in vivo a non-invasive method for determining the level of an analyte of interest in a biological subject. Raman spectroscopy can be used to obtain the signature of the substance in the eye (such as in the AH, VH, or blood vessel in the conjunctiva) and, in some embodiments, to measure the concentration of a natural physiologic or foreign substance, such as glucose and/or proteins, or drugs, alcohol, environmental or household toxins, in the subject. The method can include the steps of: (a) generating an excitation laser beam (e.g., at a wavelength of from about 400 to 900 nanometers); (b) focusing the excitation laser beam into the eye of the subject so that a selected region of the eye is illuminated; (c) detecting (preferably confocally detecting) a Raman spectrum from the illuminated region of the eye; (d) comparing the Raman spectrum from the detecting step to predetermined spectrums corresponding to different analytes of different concentrations; and (e) identifying the presence of an analyte of interest based on the detecting and comparing steps.

In some embodiments, an additional step (f) can be performed to determine the blood or brain level of an analyte of interest for the subject from the Raman spectrum. The blood or brain level may be indirectly computated based on the concentration or amount of the analyte in the blood aqueous compartment (or can be directly measured in the blood itself for the conjunctiva vessel measurement). For the indirect measurement, that value can be correlated (or adjusted/corrected) to provide an assessment of the amount of the substance in the cerebral spinal fluid or blood. The correlation may be such that the amount of the substance directly measured in the AH is substantially similar to that in the cerebral spinal fluid. Alternatively, data correlating the relationship can be established and an empirical or statistical model established.

Although not required, in some embodiments, the detecting step can be followed by the step of subtracting a confounding fluorescence spectrum from the Raman spectrum to produce a difference spectrum; and determining the blood level of the analyte of interest for the subject from that difference spectrum, preferably using linear or nonlinear multivariate analysis such as partial least squares or artificial neural network algorithms. This technique may be particularly suitable where fluorescence is problematic for optical measurements taken directly of the blood level (i.e., by focusing at the blood vessels in the conjunctiva or at the vitreous humor).

In certain embodiments, a low energy excitation wave can be used to generate the Raman signal spectrum. "Low energy", as used herein, means power which is on the order of about 10–400 mJ or less, and typically between about 70–330 mJ. The energy exposure will depend on the power and pulse length of the excitation pulse. Longer wavelength pulses (i.e., above 700) may be used, typically with energy levels closer to the higher end of the scale, while lower wavelengths (400–700) may be used with lower energy exposure levels. In one embodiment, a wavelength of about 633 nm can be used for a pulse of about 5–10 seconds corresponding to about a 2–5 mW power exposure level (and between a 10–20 or 25–50 mJ energy exposure to the patient's eye (or eyes)) for each measurement or monitoring signal obtained. In other embodiments, an optical excitation pulse may have a 785 nm wavelength, a pulse length of about 20 ms–5 s and a power rating of about 14–16 mW. In one embodiment, a 5 sec, 16 mW pulse can be used to obtain the in vivo reading of a cancer agent in the selected region of the eye (typically the AH). In yet other embodiments, for detecting and/or quantifying the presence of substances comprising particular molecules, an excitation wavelength can be selected that substantially corresponds to the absorption spectrum wavelength (for predetermined molecules that generate resonant Raman) with a reduced power requirement of between about 1–5 mW and an exposure time of about 10 seconds or less.

In some embodiments, the excitation beam can be transmitted such that it presents a reduced energy/density exposure rating to the tissue of the eye by shaping the beam to increase the cone angle or span of the excitation beam as it enters the eye to expose more of the area of the retina and reduce the energy/area rating of the excitation pulse to provide improved margins of safety (placing the energy/area rating sufficiently below the threshold of damage). In other embodiments, the transmission path numerical aperture is substantially matched to the return path numerical aperture (of the spectrometer).

A second aspect of the present invention is an apparatus for the non-invasive in vivo determination of the blood level of an analyte of interest in a subject. The apparatus includes a laser source for generating an excitation laser beam (e.g., at a wavelength of from about 400 to 900 nanometers) and an optical system (e.g., a confocal optical system) operatively associated with the laser for focusing the excitation laser beam into a selected region of the eye, including one or more blood vessels in the conjunctiva of the eye, the vitreous humor, or the anterior chamber of an eye (or eyes) of the subject so that the aqueous humor in the desired region of the eye is sufficiently illuminated. The apparatus also includes a detector operatively associated with the optical system and configured to detect a Raman spectrum from the selected illuminated region of the eye and a processor with computer program code for identifying the presence of one or more selected substances or analytes of interest. The computer code may also include code for determining the in vivo level of the analyte of interest in the selected region of the eye and to establish an estimate or measure of the analyte in the blood or cerebral spinal fluid to be established based on a correlation thereto for the subject from the Raman spectrum.

Focusing the optical analysis on the blood vessels in the conjunctiva can allow for a direct measurement of the substance in the blood, while the measurements taken from other portions of the eye can be correlated to provide an estimate or quantification of the substance in the blood and/or in the cerebral spinal fluid (i.e., indirect measurements). The correlation's can be established based on empirical models or actual measurements taken in vitro or in vivo on a representative animal or human population as is well known to those of skill in the art.

In certain embodiments, the apparatus can be configured as a low energy unit to inhibit the exposure of the tissue during the operation of the apparatus. The excitation wavelength at the low power may be less than 700 nm, such as about 406 nm or 633 nm.

Numerous additional features may be incorporated into the apparatus. The device may include a visual display screen for presenting visual indicia to the user, which can be individually adjusted and focused to the particular visual acuity of the subject (similar to vision screening focusing procedures). The apparatus may include a visual display screen for visually displaying the results of the test to the subject (such as through the same aperture or adjacent active matrix screen) as which the test is conducted. It may include a visual fixation target or device, also visible through the test aperture, which controls movement of the eye and simultaneously insures that focusing of the laser beam is properly directed into the anterior chamber of the eye. The processor may contain empirical models of actual testing experience to either determine the blood level or concentration of the analyte of interest or to identify the presence of selected substances. The apparatus may employ a laser of fixed wavelength, a tunable laser (which can sample a plurality of Raman scattered light (at different wavelengths) concurrently), a plurality of fixed wavelength lasers, or other light source means some of which can include means for sliding the Raman spectrum passed a plurality of different wavelength detectors to obviate the need for a full grating based Raman spectrometer (by taking a plurality of samples). The apparatus may include a wireless or remote communication line operably associated with the processor for transmitting the blood level of the analyte of interest to a remote location (such as for emergency home calls to an ER room).

Other devices may be particularly configured to detect the presence of a selected substance and thus, configured to detect the ensemble profile of the spectrum or a peak or peaks of interest. A compact, inexpensive device can include a fixed wavelength diode laser excitation source (configured to operate so as to generate a resonant Raman spectrum for a molecule of interest), and a filter, a lens, and detector.

Another aspect of the present invention is an in vivo method for administering drug or chemical therapy to a subject (such as for treatment of a cancerous tumor in the brain). The method includes the steps of: (a) administering a dose of a selected therapeutic agent to a subject; (b) altering the dynamics of the blood brain barrier from its normal state; (c) monitoring the dynamics of the blood brain barrier by non-invasively obtaining the Raman spectrum of a selected region in the eye (such as the vitreous or aqueous humor) and determining the quantity of the agent therein. The method may also include one or more of (d) estimating the dose of the therapeutic agent delivered to the brain (indirectly, based on the amount detected in the selected region of the eye) (e) repeating said monitoring step a plurality of times during the administering step; and (f) returning the blood brain barrier to its normal state after a sufficient quantity of agent has been delivered to the brain.

In one embodiment, the altering step can be carried out by introducing a chemical to the subject to temporarily open the blood brain barrier to allow larger molecules to pass therethrough. Further, the method can include the step of administering a non-specific marker which is reluctant to or does not normally pass through the blood brain barrier (i.e., is typically inhibited from passing therethrough). The optical analysis can monitor any increase (or the presence) of the non-specific marker in the selected region of the eye to confirm that the blood brain barrier dynamics has been altered.

In another embodiment, the altering step may be carried out by increasing the intracranial pressure of the subject.

Another aspect of the present invention is a method of non-invasively monitoring the blood brain barrier. The method comprises the steps of: (a) generating an excitation beam at a wavelength of from 400 to 900 nanometers; (b) focusing the excitation beam of said generating step into the anterior chamber of an eye of the subject so that aqueous humor in the anterior chamber is illuminated; (c) detecting a Raman spectrum corresponding to the illuminated aqueous humor; and (d) monitoring the AH to predict the behavior of the blood brain barrier dynamics during exposure to selected conditions based on the detecting step (based on the AH Raman spectrum analysis' indication of the presence or concentration of selected substances therein). It is anticipated that the correlation between the blood-aqueous and blood brain barrier is such that the presence and/or concentration in one can be extrapolated to that in the other.

Other embodiments focus the excitation beam such that it has an increased or wider cone angle to spread the light across more area of the retina. Still other embodiments are configured to focus to one or more blood vessels on the conjunctiva or to focus deeper to the vitreous humor.

In certain embodiments, the monitoring step can be used to assess whether the dynamics thereof alter sufficiently to allow selected analytes, which would normally be inhibited from traveling through the blood brain barrier, to pass into the intracranial spinal fluid through the blood brain barrier. In other embodiments, the monitoring step can be carried out when a person is under or exposed to extreme conditions such as when diving, flying, or mountain climbing, or when suffering from a traumatic head or brain injury, or high stress, and the like.

The method can also include the steps of comparing the Raman spectrum from the detecting step to reference spectrums corresponding to at least one selected analyte of interest; and identifying the presence of the least one analyte of interest in the subject based on the detecting and comparing steps in the selected region of the eye. The method may also be able to estimate the dose or affirm the presence of the analyte in the subject's cerebral spinal fluid.

Other embodiments are directed to non-invasive methods for identifying the presence of and/or the concentration of a selected analyte in a subject. The methods include: illuminating a selected region of the eye with an optical excitation beam, wherein the excitation beam wavelength is selected to generate a resonant Raman spectrum of the selected analyte with a signal strength that is at least 100 times greater than Raman spectrums generated by non-resonant wavelengths and/or relative to signals of normal constituents present in the selected region of the eye; detecting a resonant Raman spectrum corresponding to the selected illuminated region of the eye; and identifying the presence and/or the concentration of the selected analyte in the subject based on the detecting step.

Still other embodiments are directed to operations (methods or apparatus) that can determine the presence of and/or the concentration of a selected analyte in a subject and identify the subject. The operations can include: (a) illuminating a selected region of the eye of a subject with an optical excitation beam; (b) detecting a response spectrum corresponding to the selected illuminated region of the eye; (c) determining the presence, absence and/or the concentration of the selected analyte in the subject based on the detecting step; and (d) obtaining a biometric reading of the iris or retinal structure to identify the subject based on the biometric reading.

In certain embodiments, the obtaining step can be carried out by obtaining an image of the iris and comparing the image to predetermined iris characteristics of that subject. The detected spectrum can be, but is not required to be, a Raman spectrum.

Each of the embodiments of the invention may include computer program products (or computer code that implements certain operations) and/or computational and look-up tables associated therewith to identify the presence of the selected substance or substances of interest (and/or calculate the amount or concentration thereof) and to operate or control (regulate) the power of the excitation pulse emitted from the laser, and the illumination and detection of the scattered light.

For example, in certain embodiments, the present invention can include a computer program product for determining the identity of an unknown substance in a subject. The product can comprise computer-readable program code comprising: (a) computer readable program code for defining at least one signature reference spectrum for at least one selected substance of interest; (b) computer readable program code for analyzing an in vivo obtained Raman spectrum of the aqueous humor of the subject; and (c) computer readable program code for based evaluating whether the in vivo Raman spectrum corresponds to at least one of the at lest one signature reference spectrums by comparing selected characteristics between the reference spectrum and the in vivo spectrum.

In various embodiments, the computer readable program code for defining the different reference spectrums can be for a particular one or a plurality of different selected substances. Examples of the selected substance(s) include, but are not limited to: drugs or analytes of interest including, but not limited to, alcohol, a substance banned for athletes in competition, a plurality of illegal narcotic substances, a plurality of household products or common poisons for humans or animals which are potentially poisonous to a subject when ingested, a chemotherapy agent, an antifungal agent, antibiotics, naturally occurring in vivo analytes, and the like. A master look-up reference data base providing Raman spectrum data for one or a large quantity of different poisons or substances can be generated and stored at a central database or at local or regional offices, clinics or the like. The computer program can include means for remotely accessing the data such as via the use of an intranet or Internet.

Other embodiments are directed to a non-invasive method for identifying the presence of and/or the quantity of a selected analyte in a subject. The methods include: (a) providing an excitation beam at a wavelength that is selected to generate a resonant Raman spectrum of the selected analyte with a signal strength that is at least 100 times greater than Raman spectrums generated by excitation wavelengths that are not resonant or strongly pre-resonant; (b) focusing the excitation beam of said generating step into a selected region in the eye of the subject so that the selected region is illuminated; (c) detecting a Raman spectrum corresponding to the illuminated selected region; and (d) identifying the presence of and/or the quantity of the selected analyte in the subject based on said detecting and comparing steps.

The wavelength can be between about 400 nm–900 nm. The selected analyte can be a chemotherapeutic agent, an antifungal agent, or other analyte of interest. The method may also include monitoring at least one peak of interest in the detected Raman spectrum associated with a carbon-carbon bond and/or a double carbon bond associated with the chemical structure of the selected analyte.

In particular embodiments, the selected analyte comprises fenretinide or its substantial chemical equivalent and the excitation wavelength may be between about 400–514 nm. In other embodiments, the selected analyte comprises amphotecin B or its substantial chemical equivalent and the excitation wavelength may also between about 400–514 nm.

Other analytes may be resonant (or strongly pre or post-resonant) at other excitation wavelength ranges.

Still other embodiments are directed to methods of monitoring ethanol and/or its constituents in a subject using Raman spectroscopy. The methods include: (a) obtaining a plurality of reference Raman spectrums of varying concentrations of ethanol and/or its metabolites; (b) transmitting an excitation beam at a wavelength of from 400 to 900 nanometers to an eye of a subject; (c) obtaining a Raman spectrum of ethanol and/or its metabolites in the eye of the subject; (d) comparing the Raman spectrum from said obtaining step to at least one of the reference spectrums; and (e) determining the presence of and/or the concentration of the ethanol and/or its metabolites in the subject based on said obtaining and comparing steps.

The transmitting step may be carried out by confocally delivering the excitation beam to the aqueous humor of the eye of the subject.

The present invention will now be described further and includes other features and analytes that can be included in the methods and apparatus described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a graph which illustrates the broad spectrum of water in the aqueous humor, which, in some situations, can obscure the underlying peaks of interest in the Raman spectrum. The raw spectrum of rabbit aqueous humor is shown taken at 2 slightly different wavelengths (top). The difference spectrum (bottom) achieved by subtracting 1 raw spectrum from the other reveals a resultant bipolar Raman signature. Linear and/or nonlinear multivariate analysis can then be applied.

FIG. **2** is a graph plotting Raman predicted glucose as a function of actual glucose concentration. The glucose concentration in aqueous humor from 16 rabbits was estimated with Raman spectroscopy and compared to the actual glucose concentration measured with a commercial glucometer. The graph shows the Raman predicted glucose concentration after subtracting fluorescence and applying a linear partial least square algorithm followed by nonlinear back propagation with an artificial neural network. This resulted in a high degree of correlation (r^2=0.98) of predicted with actual glucose concentration. Applying the partial least squares algorithm alone resulted in lesser correlation (r^2=0.90).

FIG. **3** is a graph plotting blood glucose as a function of time. Blood glucose steadily rises at variable rates in rabbits after administration of xylazine anesthesia. One animal (rabbit D, filled diamonds) had little change in blood glucose for unknown reasons.

FIG. **4** is a graph plotting aqueous humor glucose as a function of blood glucose. Best-fit second-order polynomial curves demonstrate the relationship between aqueous humor glucose and plasma glucose while plasma glucose is rising in 9 animals. Aqueous glucose measurements from the first paracentesis of an eye correlate well with simultaneous plasma glucose (dark curve). When plasma glucose exceeds 200 mg/dL the relationship is nearly linear (dashed line). Aqueous humor glucose exceeds plasma glucose when plasma glucose is less than 200 mg/dL. The relationship of aqueous humor glucose with plasma glucose is different when the aqueous humor sample is obtained as a second paracentesis (light curve) suggesting that the initial paracentesis disrupts normal glucose homeostasis.

FIG. **5** schematically illustrates a first embodiment of an apparatus of the invention.

FIG. **6** schematically illustrates a second embodiment of an apparatus of the invention.

FIG. **7***a* schematically illustrates the method of sliding Raman spectra features through a particular detector window by changing excitation frequency.

FIG. **7***b* schematically illustrates an apparatus of the invention that utilizes the method illustrated in FIG. **7***a*.

FIG. **8** illustrates a visual fixation display that can be utilized in an apparatus of the invention.

FIG. **9** illustrates a visible readout display that can be utilized in an apparatus of the invention.

FIG. **10***a* is a schematic illustration of one embodiment of a light generation or light pulse system according to the present invention.

FIG. **10***b* is a schematic illustration of one embodiment of a detection system according to the present invention.

FIG. **10***c* is a schematic of one embodiment of a system with a closed loop feedback control system according to the present invention.

FIG. **10***d* is a schematic of an alternate closed loop feedback system according to embodiments of the present invention.

FIG. **11** is a block diagram of a method for determining the presence or the blood level concentration of a selected analyte according to certain embodiments of the present invention.

FIG. **12** is a block diagram of a method for identifying the presence of at least one selected substance in a selected region of the eye of a subject to evaluate the quantity of same in the cerebral spinal or intracranial fluid (it being assumed that the mechanics of the blood aqueous barrier can be representative to the blood brain barrier, the same having passed through the blood brain barrier) according to certain embodiments of the present invention.

FIG. **13** is a block diagram of a method for monitoring or treating a patient by administering a therapeutic agent during a chemotherapy session patient according to embodiments of the present invention.

FIG. **14** is a block diagram of a method of monitoring the blood brain barrier dynamics of a subject during exposure to selected conditions according to embodiments of the present invention.

FIG. **15** is a block diagram of a method for detecting an alteration of the function of the blood brain barrier according to certain embodiments of the present invention.

FIG. **16** is a block diagram of a method for identifying an abnormality in the blood brain barrier of a subject according to embodiments of the present invention.

FIG. **17** is a flow chart of a method according to one embodiment of the present invention.

FIG. **18** is a schematic illustration of an apparatus configured to illuminate and obtain the Raman spectrum from the vitreous humor of the eye according to embodiments of the invention.

FIG. **19** is a schematic illustration of an apparatus configured to illuminate and obtain the Raman spectrum of one or more blood vessels in the conjunctiva of the eye according to embodiments of the invention.

FIGS. **20**A–**20**C illustrate optical power density calculations performed using a canonical model of the adult eye of a human. FIG. **20**A is a model of the human eye. FIG. **20**B is a schematic of a portion of the eye illustrating the angles an equations associated with power density. FIG. **20**C is a graph of the power for exposed retinal area and the power density (assuming a 30 mW input) are shown as a function of the angle of incidence.

FIGS. **21**A–**21**C are graphs of the Raman spectrum of artificial and actual aqueous humor samples. FIG. **21**A corresponds to an ensemble spectrum of artificial aqueous humor samples in a model (artificial or test) anterior chamber. FIG. **21**B corresponds to an in vitro ensemble spectrum of actual aqueous humor in a model anterior chamber. FIG. **21**C corresponds to in vivo ensemble spectrum of aqueous humor.

FIG. **22**A is a drawing of the chemical structure of a chemotherapeutic agent known as fenretinide. FIG. **22**B illustrates the agent in ethanol in different concentrations (shown in decreasing concentration from left to right) of 100 μM (micro-molars), 10 μM, 1 μM, and 0.1 μM.

FIG. **23** is a graph of the absorption spectrum (absorption versus wavelength) of a solution of 10 μM of fenretinide illustrating the peak at 365 nm.

FIG. **24** is graph of the Raman spectrum of intensity verses wavelength shift of a non-resonant Raman spectroscopy (using an excitation wavelength of 785 nm) of fenretinide (dissolved in ethanol). There are two distinct lines, one for ethanol and one for fenretinide that shows key peaks of fenretinide as distinct from those of ethanol.

FIG. **25**A is a graph of the absorption spectrum (absorption versus wavelength) of a solution of 10 mM of fenretinide. FIG. **25**A is inset within FIG. **25**B.

FIG. **25**B is a graph of pre-resonant spectra (excitation wavelengths above the resonant wavelength) of a 10 mM fenretinide corresponding to the substance with the absorption spectrum shown in FIG. **25**A.

FIG. **26** is a graph of intensity versus wavelength shift of the resonant Raman (RR) spectra of fenretinide at three different concentrations (mixed in ETOH), 10.0 μM, 1.0 μM, and 0.1 μM. The peaks from the solvent (S), ethanol are diminished at the higher fenretinide concentrations due to the absorption of excitation light by the fenretinide itself.

FIG. **27** is a drawing of the chemical conjugated structure of amphotericin, an exemplary antifungal agent.

FIG. **28** is a graph of the absorption spectrum of amphotericin B.

FIG. **29** is a graph of the RR spectrum of (generated at an excitation wavelength of 406.7 nm) of amphotericin at three different concentrations (mixed in MeOH), 5.0 μg/ml, 0.5 μg/ml, and 0.05 μg/ml.

FIG. **30** is a schematic of a compact portable detector suitable for detecting the presence of a particular substance according to embodiments of the present invention.

FIGS. **31**A and **31**B are graphs of intensity (arbitrary units) versus wave number of Raman spectrums. FIG. **31**A is a graph of Raman spectra of an artificial aqueous humor and components of a physical model of the anterior chamber collected using confocal optics. FIG. **31**B is a graph of Raman spectra of structures of the anterior chamber of a rabbit. The Raman spectra of AH can be clearly distinguished from those of nearby cornea and lens.

FIG. **32** is a graph of amplitude (arbitrary units) versus wave number of normalized Raman spectra of various levels of ethanol in artificial aqueous humor according to embodiments of the present invention.

FIG. **33** is a graph of PLS-measured ethanol concentration versus actual ethanol concentration in aqueous solution according to embodiments of the present invention.

FIG. **34** is a graph of the Raman spectra for 0.333M aqueous solutions of ethanol, acetaldehyde, acetic acid, and mixed metabolites according to embodiments of the present invention.

FIG. **35** is a block diagram of a computer program module and/or a data processing system according to embodiments of the present invention.

DESCRIPTION OF EMBODIMENTS OF THE INVENTION

The present invention will now be described more fully hereinafter with reference to the accompanying figures, in which embodiments of the invention are shown. This invention may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein. Like numbers refer to like elements throughout. In the figures, layers, components, or features may be exaggerated for clarity and broken lines indicate optional features or operations unless described otherwise.

The term "glucose" as used herein preferably refers to D-glucose. The term "subject" as used herein refers to both human subjects and animal subjects having circulatory systems including domestic large and small animals such as dogs, cats, rabbits, horses, cows, pigs, and the like. Animal subjects may be used in the present invention for veterinary or pharmaceutical drug study purposes. The present invention may be particularly suitable for human subjects. The optical analysis may be performed on selected regions of the eye, including the blood vessels in the conjunctiva (i.e., one or more of the conjunctiva blood vessels located on the surface of the eye), the vitreous humor (the "VH") or the aqueous humor (the "AH").

In certain embodiments, the present invention is concerned with the determination of (a) the blood levels or (b) the brain fluid level, meaning the intracranial or cerebral spinal fluid levels, of selected substances or analytes of interest such as may be predicted by the presence and/or concentration of the substance or analyte in the selected region of the eye of a subject (typically, the AH or the conjuctiva vessel(s)).

In certain embodiments, the present invention recognizes that the mechanics of the blood aqueous barrier and the blood brain barrier are substantially the same such that, if the substance of analyte passes through the blood aqueous barrier, it can be presumed to also pass through the blood brain barrier. The correlation of the amount that passes through the blood brain barrier may be substantially equivalent to that detected in the eye or may be at a lesser or greater concentration. However, the blood concentration of other Raman-active molecules, analytes, or substances which do not typically pass through the blood brain barrier may also be determined by these techniques. Similarly, the amount or presence of the selected substance or molecule, etc., in the eye itself can be determined by these techniques (such as to treat localized in the eye or non-systemic diseases such as cancers of the eye).

As used herein, the term "analyte" is used interchangeably with "substance" to identify a selected target chemical, molecule or molecules, either in its blood-absorbed or partially absorbed, uptaken, or constituent form, or in an ex vivo formulation, where applicable. As such, the substance or analyte can include a chemical compound or composition, whether synthetic or natural, as well as a constituent thereof or molecule of interest. In some embodiments, the analyte or substance can be one that is emitted by the body (sometimes in an elevated amount) in response to a disease or physiologic reaction to a product or toxin and may also be a byproduct of the body's reaction to a substance. As such, as noted in the summary of the invention above, the substances which can be measured or identified according to the present invention are numerous and can include (a) natural physiologic substances, analytes or chemicals, such as glucose, amino acids, peptides, blood, or other components of or in blood, or in the eye and/or (b) foreign or synthetic or artificial substances such as medicaments, drugs, or poisons (whether legal or illegal, and whether prescription or over the counter) which may be present in the subject. For example, the present invention can be used to: (a) identify or assess the presence (or absence) of and/or the blood level or concentration of or (b) the movement through or alteration of the blood-brain barrier of a selected or desired substance. The substances include lactate, urea, benzene, ascorbate alcohol, ethanol, methanol, ethylene glycol, steroids, nicotine, or illegal narcotics such as cocaine, other opiate-based drugs, or prevalent or pervasive designer drugs, or metabolites, or household, organic, or environmental toxins and/or poisons such as herbicides, pesticides, household cleaning products, petroleum products, therapeutic agents such as chemotherapeutic agents, antifungal or other antibiotic agents or other chemicals.

In certain embodiments, the present invention may be particularly suitable for identifying the presence or amount of substances, which present in the selected region of the eye in sufficient quantities to allow Raman based detection. For example, in certain embodiments, the present invention may be able to detect a substance that is present in an amount of at least about 0.01–10 μmolars. In other embodiments, the present invention can detect a substance that is present in at least about 1–100 or 10–100 μmolars. The lower quantities may be more reliably detectable if the substance is a Raman active molecule with enhanced signal strength obtained by using an excitation wavelength that approaches the substance or molecule of interests absorption spectrum or RR excitation wavelength. In some embodiments, the targeted analyte may be present in the selected or targeted region of the eye in an amount of at least about 1 milli-molar or in a physiological concentration level of about at least 0.001%. The present invention may also be able to identify the presence of, or a physiologic reaction to, substances associated with poisonous plants, insect or arachnid poison, and reptile or snake venom, in the subject.

In addition, in certain embodiments, the devices and methods of the present invention may be used (to analyze either in vitro or in vivo) to detect increased or decreased levels of physiologic analytes in the blood or to detect certain disease processes of known conditions and/or to treat conditions of the eye. For example, by identifying increased or decreased levels of certain substances in the body or the presence in the subject of certain peptides or toxins associated with a particular condition, such as those caused by system impairments or reactions associated with dehydration, allergic reactions, toxins from bacterial infections such as spinal meningitis, or elevated or decreased levels of antibodies to identify an immune system response, or even hormones evoked in response to a pregnancy.

In certain embodiments, the methods and devices of the present invention can be configured to (a) identify the presence of and/or (b) quantitatively estimate or determine the blood and/or cerebral spinal or intracranial concentration level of one or more selected substances in the subject. The quantitative assessment can include determining how much of the selected substance is crossing the blood brain barrier (based on a correlation to the amount present in the selected region of the eye, typically the AH). In other embodiments, the methods and devices of the invention can be used to dynamically monitor changes in the operation of the blood brain barrier (which may be altered intentionally for some embodiments of the invention).

In any event, generally stated, in operation, a laser excitation light signal is focused to and transmitted into a selected region in the eye. Typically, this will be the AH, but, in some embodiments, can also include either the blood vessel(s) in the conjunctiva or the vitreous humor. Illumination of the blood vessel(s) in the conjunctiva of the eye will not require as great a penetration depth as either the AH or the vitreous humor, while the vitreous humor will require the excitation light pulse to have an increased penetration depth over that of the AH and the conjunctiva to reach the vitreous humor in the eye.

In any event, after the selected region of the eye is illuminated, a corresponding Raman spectroscopic signal is obtained. Each selected substance can generate a "signature" spectrographic signal with different signature peaks at different frequencies. Higher concentrations will yield stronger signals. The subject's spectroscopic signal can then be digitally compared to a plurality of reference signals stored in computer memory. The reference signals correspond to one or more selected substances which can be predetermined from an in vitro analysis of the substance as it exists in a corresponding sample. For example, for the AH measurement, a representative eye AH can be a representative target phantom such as in artificial AH's, or test vials with known concentrations of the substance in a known solvent, such as a water based or saline solution.

In certain embodiments, the actual signal of the subject can then be compared to the stored "signature" profiles or signals of Raman data corresponding to predetermined substances or analytes to identify the presence of one or a plurality of the selected substances and/or to estimate or quantitatively determine the concentration of that substance(s) in the subject. Thus, the present invention is able to assess, non-invasively, at physiologic levels (typically in the millimolar range, but the levels can also be in the micromolar range for certain substances), the presence of selected substances in the body of the subject. In other embodiments, a relative assessment (two or more measurements of the subject analyzed for differences or changes) may be performed, alone, or with the use of base or reference spectra. The relative assessment can include obtaining at least two different Raman spectrum signals of the subject, over time, and comparing any shift in peaks, and, thus, physiological changes, in the subject at particular frequencies.

As shown in FIG. **5**, in one embodiment of the present invention, the system **5** can be described has having a light (excitation or illumination) signal generation portion **8** and a detection portion **9** (FIG. **10***b*) with a laser **10** and an optical system **15***x*, along with a fixation target **25**. The optical system **15***x* can be broken into an input portion **15***xi* (relays the light to the subject's eye) and an output portion **15***xo* (relays the reflected light from the subject). As shown in FIG. **10***a*, the light signal generation portion **8** includes the laser **10**, a signal processor **35***s*, the input optical system **15***xi* which is configured to direct the light signal into a selected region in the eye **12** of the subject (whether the conjunctiva vessel(s), the AH, or the vitreous humor). The light generation portion **8** shown also includes a fixation target **25** used to help focus the subject's eye to the light transmission path. The target **25** may be configured to extend adjacently above, below or about the perimeter of a light exit/entrance aperture, preferably proximate to the aperture to allow the eye to focus directly at or into the aperture during operation or data acquisition.

As shown in FIG. **10***b*, the system **5** also includes the detection portion **9** which receives the light as it exits the selected region of the eye and detects the Raman spectrum associated therewith. The detection portion **9** can include the output optical system **15***xo* (which may share some of the optical components used in the input optical system **15***xi*), and a spectrometer **32***s* with a detector **32**, and a signal processor **35***s*. The signal processor **35***s* is typically the same in the transmission and detection portions **8, 9** of the system **5**.

A schematic diagram of one embodiment of the system **5** is shown in FIG. **5**. In certain embodiments, in operation, a tunable, narrow-band laser beam from laser **10** is focused into the anterior chamber **11** of the eye **12** through an objective or ocular lens **13** via lens **15** and filter **16**, beam splitter **17**, and filter **20**. In certain embodiments, a non-fluorescing objective lens with suitable numerical aperture (e.g. 0.2–0.5) can be used such that Raman scattering from the selected region (i.e., the aqueous humor) is maximized while scattering from adjacent structures (e.g., lens, cornea, and iris) is reduced. The objective lens can be configured with respect to the eye so that it has adequate working distance to permit focusing of the laser into the selected region of the eye (for the AH, the middle of the anterior chamber of the eye) without direct contact with or touching of the cornea. An integrated fixation target projected from display screen **25** can be projected via lens **26** through the same objective lens as the laser, but is focused on the retina of the eye. Focusing this fixation target on the retina simultaneously can control direction and focusing of the laser light into the desired region of the eye (such as, in some embodiments, to the anterior chamber and the AH).

As shown in FIG. **5**, in certain embodiments, the light collected by the objective lens is directed through holographic notch filters **20, 21** to remove the undesired portion of the reflected scattered light, such as the Raleigh scattered light. The Raman scattered light passes through these filters with minimal attenuation and is focused through a confocal pinhole aperture **22** by lenses **23, 24**. The pinhole and the focal point in the eye are confocal such that light from adjacent structures in the eye can be filtered at this aperture. The pinhole **22** also serves as the entrance aperture to the spectrometer. The spectrometer shown is an imaging spectrograph with a grating **30**, lens **31** and a CCD detector array **32**.

A signal processor **35***s*, or controller which can be provided in a computer **35**, controls the laser **10**, the fixation target and readout display **25**, and receives data from the CCD detector **32**. The architecture of the spectrometer shown in the figures is merely one example of devices that are suitable for this application. Many types of spectrographs can be utilized including Fourier transform spectrographs, spectrographs using liquid crystal tunable filters or other tunable elements. Information can be transmitted to a remote source such as a computer, database, remote physician or the like via modem or other connection through a suitable communication link **36** via a wireless link or a computer network such including an intranet or a global computer network link such as an internet link (such as the world wide web), etc.

In certain embodiments, the CCD detector **32** in the spectrometer is of a red-shifted, back-thinned, thick epitaxial design such that its sensitivity is optimal in the 700–1100 nanometer spectral region. Other types of suitably sensitive detectors and spectral ranges may be suitable as well.

As noted above, a signal processor **32***s* such as a digital signal processor in a general purpose or special purpose computer processes the output of the detector **32**. The signal processor or computer or other controller can control the laser beam frequency and the power or intensity of the laser pulse from the laser source **10**.

In some embodiments, the signal processor **32***s*, computer, or controller can switch the frequency of the laser to permit subtraction of fluorescence as described previously. The signal processor or computer can also direct information to a digital display imaged onto the retina. The patient can then read the results of the analysis on this display. For certain embodiments, the results can be displayed on the exterior of the device so that a clinician or police officer (the latter being particularly directed for narcotics or blood alcohol evaluations) can easily read same. Further, the data or results can be date and time coded and digitally stored and printed as a medical or police record.

As shown in FIG. **6**, fibers **40**, **41** may be used to deliver the laser beam and collect the Raman scattered light. In certain embodiments, the confocal aperture is a circular aperture **42** placed on the end of a multimode fiber. As with the pinhole aperture shown in FIG. **5**, the end of the fiber can be placed so that it is confocal with the objective lens' focal point in the anterior chamber of the eye. A single mode fiber can be used to direct the laser beam into the anterior chamber of the eye. This single mode fiber can help facilitate a diffraction limited spot size at the focal point of the objective.

A fiber delivery and collection system like that depicted in FIG. **6** can be connected to alternative detection systems, one of which is shown in FIGS. **7***a–b*. This alternative detection system can allow for subtraction of the fluorescence spectrum as well as selected sampling of the frequencies most important for calculation of glucose concentration. Such an alternative detection system may decrease the size and cost of the instrument. The system depicted in FIG. **7** uses one or more fixed frequency or tunable lasers to illuminate the aqueous humor of the eye using the optical delivery system shown in FIG. **6**.

FIG. **7***a* illustrates that each spectral feature **50***a*, **50***b*, **50***c* of the Raman scattered light is related to the excitation wavelength by a fixed offset, usually expressed in wavenumbers. Changing the excitation wavelength causes the Raman spectra to shift in wavelength, as depicted by the different feature or portion of the signal, captured in detector range window Δ_λ **51** (a relatively narrow band of the signal such as about 10 nm) based on Raman spectra **52** for excitation frequency $L_{\lambda 1}$ as compared to Raman spectra **53** for excitation frequency $L_{\lambda 2}$.

An apparatus that takes advantage of the foregoing is schematically illustrated in FIG. **7***b*. Like components to FIGS. **5** and **6** are assigned like numbers. Laser drivers and/or tuning electronics **60** are operatively associated with a tunable laser or a plurality of fixed wavelength lasers **61**, **62**, **63**. A series of one or more bandpass filter/detector elements **65**, **66**, **67** operatively associated with amplifiers and an analog to digital converter **68** is used to sample the spectrum of the collected light. The center wavelength and bandwidth of each filter may be chosen to correspond with a different Raman spectral peak of aqueous humor important for quantification of the selected substance (such as alcohol, an illegal narcotic or banned substance, a poison, or glucose). If this laser is tunable over about a nanometer or so, subtraction of the fluorescent components of the acquired spectrum may be possible as discussed earlier. Using a laser with a wider tuning range can allow the Raman shifted spectra to be scanned or slid across a smaller number of bandpass filter/detectors. Since semiconductor lasers with extremely wide tuning ranges are not commercially readily available, one may instead use a set of narrowly tunable lasers each with a different center wavelength as the laser means in conjunction with a series of bandpass filter/detectors for this purpose. Other suitable light sources can also be used.

Advantageously, the systems described herein may be configured so that optical components need not and do not contact the cornea of the eye during use, which many patients find objectionable (e.g., by providing a suitable monocular eye cup for contacting the orbit around the eye).

The apparatus of the invention can be implemented as a spectrometer base unit attached by a fiberoptic cable to an ocular probe, or as a single integrated unit including foreoptics, spectrometer, detector, computer and display. The apparatus can be configured as a relatively lightweight and portable device. In some embodiments, the optics and laser source can all be configured to be held in a housing which can be mounted on the user as a headset, or on a portable platform device similar to vision screening or vision correction devices used during vision exams.

As noted above, a visual fixation target such as a mirror, LED, or display such as an active liquid crystal matrix display or the like can be built into the optical apparatus such that it is held in the housing in visual communication with the patient or user during operation to facilitate focusing of the excitation light into the anterior chamber and maintain stability of the eye (so that it is properly aligned with the eye of the subject). In the embodiment of FIGS. **5–7**, a display screen such as a liquid crystal display is employed. As shown in FIG. **8**, a blinking fixation target in the form of an hourglass **70** can be displayed, focused on the retina, during acquisition of the spectra by the apparatus. Other fixation target forms can also be used such as text, letters, numbers, or other recognizable shapes or objects. This can help optimize the Raman signal from the aqueous humor and reduce light exposure to other structures of the eye. The fixation target can be active during the time that the laser beam is active and illuminating the subject's eye.

As shown in FIG. **9**, in some embodiments, a visible indication of the results of the test in the form of alphanumeric indicia **71** (or other suitable form, such as a graphical display) can be displayed on the same screen immediately after the acquisition step (or to a remote or externally visible screen for other embodiments). The indicia of FIG. **9** provides a readout for all of the principal Raman-active compounds of the aqueous humor, but the display can be of a targeted compound of interest (filtered from the overall spectra) corresponding to one or more, of a desired concentration level of a selected substance (such as alcohol) as desired.

In some embodiments, a motion sensor can be incorporated into the device so that the test can be aborted upon movement of the eye away from the focal point or so that a clinician is alerted as to the movement. For example, upon transmission of the laser pulse to the selected region in the eye, a sensor can be concurrently operated and used to detect movement of the retina away from the target focal point.

The excitation laser light source can be any suitable laser beam source which can generate a suitable laser beam signal at a desired frequency output. A distributed feedback laser can be used to reduce instrument size. Tunable or multiple fixed frequency lasers can be combined with bandpass filters (Puppels et al., *Applied Spectroscopy* 47, 1256–67 (1993)) that can generate pulses for Raman scattering at wavelengths that provide optimum information for multivariate analysis (this can reduce the cost and size of the instrument compared to the use of holographic filters or gratings).

The laser beam can be pulsed from the generator or from the optical system to provide the desired length and power or energy to the tissue in the eye. The power of the excitation laser beam pulse should be sufficiently low to avoid tissue toxicity, but sufficiently high to provide a measurable Raman signal from the aqueous humor. In general, the laser beam pulse will be at a wavelength of from 400 to 900 nanometers. In some embodiments, the laser wavelength can be a wavelength of 780 to 860 nanometers to reduce fluorescence, increase tissue penetration, and reduce phototoxicity to the eye. In other embodiments, the wavelength can be generated at a lower wavelength, such as between 400–700 nanometers, so as to increase the signal to noise ratio of the signal and/or to decrease the power level of the signal in the eye.

The duration of the pulse can be from 1 to 60 seconds in length, and typically is from about 5–20 seconds long.

The total energy of the laser pulse transmitted to the eye will typically be between 70–500 millijoules, with instantaneous power preferably not exceeding about 30–50 milliwatts. The optical components of the apparatus used to carry out the method are preferably configured so that energy on the retina of the eye (as well as other areas susceptible to tissue toxicity, such as the lens and cornea) transmitted from the excitation laser beam pulse is not greater than 3000 mW/cm^2, more preferably not greater than 1000–2000 mW/cm^2, and even more preferably, below about 500 mW/cm^2.

In certain embodiments, a low energy excitation wave can be used to generate the Raman signal spectrum. "Low energy", as used herein, means power which is on the order of about 10–400 mJ or less, and typically between about 70–330 mJ. The energy exposure will depend on the power and pulse length of the excitation pulse. Longer wavelength pulses (i.e., above 700 nm) may be used, typically with energy closer to the higher end of the scale, while lower wavelengths (400–700 nm) may be able to employ lower energy exposure levels. In one embodiment, a wavelength of about 633 nm can be used for a pulse of about 5–10 seconds corresponding to about a 2 mW power exposure level and between a 10–20 mJ energy exposure to the patient's eye for each measurement or monitoring signal obtained. In other embodiments, an optical excitation pulse may have a 785 nm wavelength (or other suitable wavelength), and a pulse length of about 20 ms and a power rating of about 15 mW. In another embodiment, a 5 sec, 16 mW pulse (having a energy exposure level of about 75 mJ) can be used to obtain the in vivo reading of a substance such as a cancer treatment agent in the selected region of the eye (typically the AH). In particular embodiments, an excitation light having a wavelength of about 406.7 nm can be employed. The 400 nm excitation wavelength level or range may be particular suitable where a resonant Raman signal may be generated by a selected substance or molecule.

For multiple measurements, the devices may be able to be configured to focus on a different eye, alternating the eye to which the light is transmitted, to reduce the amount of exposure to any one eye.

FIG. **30** illustrates yet another embodiment of a device suitable for obtaining a Raman spectra of the eye of a subject. This device is compact, portable, and inexpensive, typically requiring only a diode laser, a filter, lens, and detector. The device can be directed to operate at a fixed excitation wavelength. As shown, the device includes a relatively inexpensive diode laser **10**L, an optically-formed notch filter **21**, a detector **32**, and a beam splitter **17**. In certain embodiments, the diode laser **10**L has a fixed operational wavelength. Typically the device is configured to detect a predetermined substance and, as such, the excitation wavelength of the laser is pre-selected to correspond to a wavelength that substantially corresponds to or approaches the resonant Raman ("RR") wavelength for the predetermined substance of interest (but is above 400 nm). As used herein, the term "resonant Raman" means an excitation wavelength that amplifies the (detected) Raman signal at least about 100 times over non-resonant or weakly resonant excitation wavelengths and/or relative to the normal baseline chemicals present in the eye; this term is intended to include strongly pre- or post-resonant wavelengths.

The above design may be particularly suitable to detect substances with a molecule of interest that is resonant or pre-resonant in the 400–1000 nm range. Some molecules, notably ethanol, are not resonant over this range, but have a peak that is relatively strong at high physiological concentrations in which it is used (such as recreational intake). The same instrument design therefore can be used to test for ethanol since the blood concentration can be relatively high (0.01–0.4%) (such as to test for drivers that may be impaired). Of course, this rationale applies to other analytes or substances that may be present in increased blood concentrations. Also, if a molecule is not going to be resonant or strongly pre-resonant, then the excitation wavelength can be chosen to avoid or reduce fluorescence—usually by employing an excitation wavelength in the range of about 600–900 nm.

Typically, the excitation wavelength is above 400 nm (between about 400–600 nm) and corresponds to a portion of the absorption spectrum. Absorption is non-negative since it is defined as the −log (% transmission). The excitation wavelength can be chosen to increase or maximize the signal to noise ratio. This can be done by making the signal bigger, by inducing resonant Raman by selecting an excitation close to the absorption band of the molecule (but above 400 nm) or by reducing the noise—i.e. reducing fluorescence by operating at a longer wavelength (e.g., 785 nm) for substances that cannot be made Raman resonant or strongly pre-resonant in the 400–1000 nm range. The signal enhancement for resonance is sufficiently increased that one should attempt to induce it when possible. However, it is noted that, in practice, many molecules may not be resonant or strongly pre-resonant in the 400–1000 nm wavelength range although certain drugs or other analytes of interest may be resonant or strongly pre-resonant in the 400–600 nm range. In particular embodiments, a wavelength of under about 450 nm, such as about 405–410 nm, may be employed.

The excitation range is chosen to be above 400 nm because wavelengths below 400 nm may induce the formation of cataracts. Silicon CCD detectors are typically used in Raman spectrometers. The long wavelength cut-off for these detectors is slightly over 1000 nm. Therefore, to allow for Raman shifted light, the device can be configured to operate with an excitation wavelength range from about 400–900 nm depending on the application.

The device can be configured to provide an exposure time (expose the eye to the light) of about 10–15 seconds or less with an associated power of about 1–5 mW, and typically about 1–2 mW.

As shown in FIG. **30**, the device can be configured with a sine wave generator **10S** operably associated with the diode laser **10**L and the detector **32** to modulate the signal and improve SNR. A mixer **10M** can be used to integrate the sine wave generator **10S** and the detector **32** with the low pass filter **10F** as is well known to those of skill in the art.

In certain embodiments, the device can be configured so that, in operation, the sine wave generator **10S** modulates the laser light. Generally stated, the detected light is Raman scattered off the sample and is modulated in a similar fashion. The detector measures this intensity and converts it into an electrical current. The detector also picks up noise from the ambient light and other sources. The mixer **10M** multiplies this detected signal by a reference sine wave, producing a signal. The low pass filter rejects the high frequency terms and passes the low frequency term of the Raman scattered light. Thus above-described operation may be described as coherent signal detection and is a detection technique commonly exploited by lock-in amplifiers. As is known to those of skill in the art, other designs may also be used such as without modulation of the laser beam and/or post-processing module.

In certain embodiments, the device can be configured with improved signal to noise ratio of the overall instrument by post-processing the detector **32** signal by using coherent detection, similar to methods employed by a lock-in amplifier as is well known to those of skill in the art.

FIGS. **26** and **29** illustrate a RR spectrum generated at 406.7 nm excitation wavelength for two different substances of interest, the peaks having increased intensity over spectrums generated at non-resonant or weakly resonant wavelengths. FIG. **26** illustrates the RR spectrum of a chemotherapeutic agent and FIG. **29** illustrates the RR spectrum of an anti-fungal agent. However, even for non-resonance wavelengths that approach the peak absorption wavelength of the compound, the Raman signal intensity may increase sufficiently to serve as an acceptable operable excitation wavelength. The particular excitation wavelength will depend on the drug or analyte of interest and can be selected so that, in operation, it can amplify the detected intensity of the Raman spectrum signal substantially, such as by about 100 times to 1,000 or greater (even up to about 100,000 times in certain circumstances) over that of signals generated with excitation signals that depart greater than 200 nm, away from the peak wavelength in the absorption spectrum. As such, in certain embodiments, the excitation wavelength may be chosen so that it is about 100 nm or less above the peak absorption wavelength in the absorption spectrum.

The device can be configured to detect a single peak of interest in the RR spectrum (at a predetermined wavelength shift of interest), such as the peak at 1576/cm or 1171/cm in FIG. **26** or the peak at 1152/cm or 1554/cm in FIG. **29**. Of course, as noted above in other embodiments, the device can be configured to detect multiple peaks or at multiple wavelengths (adding notch filters or a tunable laser, and the like, as is well known to those of skill in the art).

In certain embodiments, the chemical or analyte concentration in the blood can be measured within a 90–99% accuracy level as determined by extracting the aqueous humor, putting it in a test tube and measuring the Raman spectrum. The band of interest is typically in the 200–3200 cm^{-1} band.

Any suitable detector **32** can be used to detect a Raman spectrum from illuminated aqueous humor. In certain embodiments, a CCD detector or CCD camera can be used to detect reflective light as they may exhibit increased sensitivity over other types of detectors. The CCD detector preferably is configured to have high quantum efficiency in the near infrared range. The high quantum efficiency may be achieved by any suitable means, such as employing a back thinned detector, but one sufficiently thick to reduce etalon effect.

The devices of the invention can be operatively associated with a patient's medical equipment such as an insulin pump (for D-glucose) or to a dialysis machine (for urea) by wireless or wired electrical or fiber optic lines, radio frequency transmitters and receivers or the like to provide information on the appropriate analyte that can then be used to control that equipment. For example, the reading can be used to automatically activate or increase or decrease the output of the equipment such as the insulin pump in response to blood glucose levels, or to regulate the dialysis machine.

In telemedicine applications, the instrument of the invention can be conveniently operatively associated with a remote read-out terminal through a communication link such as a telephone, cable, computer, modem, intranet, internet connection or other communication line with any suitable relay or interconnection means (such as an electrical or fiber optic lines, radio frequency transmitters and receivers, etc.) to provide information on the blood or intracranial (blood-brain fluid) analyte to a remote physician or medical provider (e.g., through a telephone, cable, or wireless connection to a direct security (for in-home parole) or medical monitoring agency or to a selected destination on the intranet, internet, or world wide web).

FIG. **11** illustrates method steps for obtaining spectrographic readings of the selected region of the eye of a subject according to certain embodiments of the present invention. As shown, an excitation or illumination light pulse is transmitted to the selected region of the eye of the subject (Block **100**). In certain embodiments, this can be carried out by positioning a mask-like device over the eyes of the subject (similar to that used in eye exams) so that (a) the housing of the device aligns over the eye socket(s) to seal the eye(s) from ambient lighting, (b) the excitation pulse light is efficiently directed into the eye (without scattering into the environment), (c) and the light reflected from the eye (such as the AH) can be captured, but no physical contact with the cornea of the eye itself is required. For example, a monocular eye cup (or binocular eye cups) can be used to contact the orbit around the eye and yet space the optical components from contacting the cornea. The scattered light from the illumination light pulse is then detected (Block **120**).

During the transmission and detection steps, the eye can be focused to a desired location or target spot (Block **105**) to help direct the excitation light out of and into the eye in a repeatable and reliable manner (to define a consistent transmission path so that the reflected light is attributed to proper and reliable illumination of the VH, AH, or conjunctiva vessel(s)). In so doing, a manual or autofocusing technique can be used. For manual focusing, various methods can be used to allow the user to adjust the focus. For example, a pinwheel can be turned clockwise or counterclockwise to focus on an object or text or other visual fixation target to be displayed in the device (typically the object or text is displayed in a miniaturized display (such as an active matrix display) positioned about the opening through which the light is transmitted proximate the light transmission path). For example, LED's or active matrix displays can be used to generate text or a blinking or continuous light, which attracts the visual attention of the subject and which the subject watches or reads during the transmission and detecting steps.

Similarly, for autofocusing, the subject can look to the visual fixation target mounted in the device in visual communication with the eye. The presentation of the object can be automatically stepped through a visual protocol which can magnify or adjust the visual presentation until the subject can read or recognize the object. When this happens, the reading or recognition indicates that the focus is sufficient, and the reading can be taken (i.e., the transmission and detection of light). Autofocusing systems with corrected optic systems are well known to those of skill in the art. For example, optic systems similar to those used to assess vision and determine a suitable lens correction for a subject during vision examinations can be incorporated into the devices according to embodiments of the present invention. Although a contact lens may remain in place during the reading, it is preferred that glasses or other objects which may physically interfere with the mounting of the device over the eyes, be removed during the test.

The power or beam strength of the light pulse can be monitored so that fluctuation in the power or strength of the light beam which is transmitted to the user can be adjusted so that it the current and/or heat is regulated to be substantially constant (constant current or heat) or numerically corrected for, typically in the signal processor **35***s* (Block **110**). A Raman spectrum of the targeted region of the eye can then be generated based on the detected and corrected light spectroscopic signal (Block **130**). Typically, well-known statistical correlation or normalization techniques are used to generate the Raman spectrum from the scattered light data received at the detector. See e.g., *Standard Practices for Infrared, Multivariate, Quantitative Analysis*, ASTM E 1655–97; Beebe et al., *An Introduction to Multivariate Calibration and Analysis*, Anal. Chem. 59, 1007A–10017A (1987); Draper et al., *Applied Regression Analysis* (2d ed., John Wiley and Sons, 1981); Maliowski et al., *Factor Analysis in Chemistry* (2d ed., John Wiley and Sons, 1991); Mark, H., *Principles and Practice of Spectroscopic Calibration* (John Wiley and Sons, 1991); Martens et al., *Multivariate Calibration* (John Wiley and Sons, 1989); and McClure, G., Ed., *Computerized Quantitative Infrared Analysis*, ASTM STP 934, ASTM (Philadelphia, 1987). The contents of these references are hereby incorporated by reference as if recited in full herein.

Thus, any number of suitable computational analysis may be used including linear (e.g., algebraic or least squares/partial least squares) or nonlinear (e.g., artificial neural networks) multivariate analysis techniques, such as, multiple linear regression (MLR), classical least-squares (CLS, K-matrix), inverse-least-squares (ILS, P-matrix), principal component regression (PCR, PCA, factor analysis), and partial least-squares in latent variables (PbS). See Schulze et al., *Artificial neural network and classical least squares methods for neurotransmitter mixture analysis*, Jnl. Neuroscience Methods 56(2); 155–167 (February 1995); and Wang et al., *Analysis of metabolites in aqueous solutions by using laser Raman spectroscopy*, Applied Optics, 32(6); 925–9 (February 1993). See also Brown, C. 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FIG. **10**C illustrates that the device **5** may include a closed loop feedback system **5***f* for monitoring and regulating the strength or intensity of the light excitation beam output to the eye of the subject. The feedback system **5***f* may employ a beam splitter positioned to bleed off a portion of the beam at a location which is proximate to the aperture (the beam outlet port) and substantially continuously detect the beam power, intensity, or energy associated therewith to monitor the power of the excitation beam as it is transmitted out of the device. Other embodiments can include dynamically monitoring the current or power load of the laser **10** to adjust for temperature or other operational drift or power fluctuation during operation. The signal processor **35***s* can then adjust the input to the laser as needed and/or store the excitation beam data so that the Raman spectrum can be adjusted to reflect the actual beam energy or power transmitted to the user.

FIG. **10**D illustrates one operational embodiment of a device with power regulation according to the present invention. As shown, the laser **10** is operably associated with a power supply **10***p*. The laser **10** directs the light through a fiber optic cable **40** which includes or is coupled to a fiber optic splitter **40***s* which directs or bleeds a small portion of the light away from the primary light transmission path (and into the eye of the patient). A substantial portion of the light remains in the primary light transmission path and is directed into the Raman probe head **15** probe which can, in operation, be positioned adjacent the eye **12** of the patient. The Raman spectrometer **32** can be positioned in communication with the Raman probe so as to be able to detect the light upon its return (in the return path).

As shown, the splitter **40***s* may split about 1% of the excitation light away from the primary beam to a detection path **115** and into a photodetector **116** (or other suitable sensor). The photodetector **116** is in electrical communication with an A/D (analog to digital) converter **118**, which converts the corresponding electrical signal so that it can be digitally input into the processor **35**. Thus, the voltage or current from the photodetector **116** is measured and digitized (preferably in a substantially continuous or continuous manner during operation of the device) and the information transmitted to the processor **35** (typically a computer). The computer or processor **35** can then measure the laser power and computationally determine a new adjusted current (or voltage) to adjust the laser **10** output to the desired or target output value such that it is able to be held to within about 95% of the target output strength. In certain embodiments, the output signal strength can be held substantially constant such as within at least about 98–99%. The computer output can be converted back into analog form via a D/A converter **119** which is used to regulate the voltage power supply **10***p* which, in turn, regulates the input to the laser **10**.

The optical system **15***xi* may also be configured to transmit the excitation beam such that it presents a wider cone to focus to the retina in a manner which is able to cover a larger area of the selected region in the eye to thereby operate with a lower energy/area ratio (such as, for at least some AH applications, configuring the optical system such that the numerical apertures of the input optic system **15***xi* is substantially matched to the output optic system **15***xo* (or spectrometer). In certain embodiments, a numerical aperture of about 0.2–0.5 can be used. The graph in FIG. **20**C shows a sample power density and exposed retinal area for a numerical aperture of 0.25 and a 10× objective (Point A) and a larger numerical aperture of about a 0.4 (23.6 degrees for a 20× objective) at Point B.

FIGS. **20**A–**20**C illustrate optical power density calculations performed using a canonical model of the adult eye of a human (FIG. **20**A). Typically, optical toxicity power limitations for the eye are established by regulatory agencies.

FIG. **20**A shows a diagram of the adult eye exposed to light through an eyepiece with a corresponding objective and numerical aperture. Marginal rays from this eyepiece are shown focusing through the cornea and into the aqueous humor within the anterior chamber. The confocal optical configuration of the instrument allows it to sample Raman scattered light in the region near the focal spot (and substantially isolated to that region). Thus, in certain embodiments, the optic design can be such that scattering from the cornea or the intraocular lens can be reduced and can be confocally blocked from the instrument's fiber-optic entrance aperture within the optical probehead (FIG. **10***d*, **15** probe).

FIG. **20**A also shows the nodepoint (N) originally derived by Gullstrand. Gullstrand used special instrumentation to observe Purkinje reflections from both sides of the cornea and lens to develop a simple eye model which lumped the power of the cornea and the intraocular lens into a single equivalent lens with node point (N). Based on the knowledge of the position of node point (N) relative to the retina, one can calculate sizes of objects on the retinal surface. This information can be used to compute the surface area of the retina exposed by the numerical aperture (NA) (such as NA=0.25 or NA=0.4 according to certain embodiments of the design of the device and the associated eyepiece).

In this model, rays passing through (N) can be treated as if they are unrefracted as they travel through the eye and onto the retina. One can construct rays that pass through (N) but are also collinear with the eyepieces marginal rays as shown. A pair of collinear rays entering the lens equivalent model should focus on the retina. Going through this exercise with both pairs of marginal rays of the eyepiece shows that one can treat the incident beam as a point source which emanates from (N) at an angle θi, the same angle as the marginal rays make with the optical axis.

As shown in FIG. **20**B, simple calculus can be employed to derive an expression for the surface area of a sphere over a solid angle delineated by θc, the angle of a ray from normal that extends to the margins of the surface area exposed on the sphere. The expression for surface area is given by equation 1.

$$A=2\pi R^2(1-\cos\theta_c) \qquad \text{Equation 1}$$

θi is given (calculated as $\sin^{-1}$ of NA of the eyepiece) and not θc, an expression can be derived which relates these as a function of the radius of the globe and the relative position of N given by x_0. As shown by equation 2 in FIG. **20**B, trigonometry can be used to derive this relationship. For any θi, equation 2 can be solved numerically for θc, using the Newton-Rapson method.

$$R \sin \theta_c = (R \cos\theta_c + x_o)\tan \theta, \qquad \text{Equation 2}$$

FIG. **20**C is a graph which plots exposed retinal surface area (right vertical axis) and power density assuming a 30 mW illumination (on the left vertical axis) on the retina as a function of various eyepiece designs defined by the angle of incidence "θi". The line indicated at the angle of incidence just below 15 degrees (Point A) corresponds to the numerical aperture 0.25 used for obtaining certain of the experimental data shown or described herein. The angle of incidence associated with a numerical aperture of 0.4 is at about 23.6 degrees (larger exposed retina area and lower power density), shown at Point B, used to obtain the data illustrated in FIGS. **21**A–C. This angle can also be expressed in terms of NA or "f"-number (the focal length divided of the fully illuminated diameter of the limiting aperture). The numerical aperture of the entire system places an upper bound on the effective numerical aperture. For example, the numerical aperture of the embodiment of the device **5** of the spectrometer used to determine certain of the described results is f/1.8 (NA=0.267). However, the fiber optic cable between the spectrometer and the probe head, limits the numerical aperture to f/2.0 (NA=0.24). Thus using an objective at a higher f number than the fiber is counterproductive since Raman scattered light at the outer angles is not captured by the fiber. Using the instrument with the same incident power but with an objective with an f number smaller than f/2 will generally result in higher power densities on the retina with no increase in Raman signal gain. This is because maximum collection efficiency is achieved at a particular by matching the f number of the eyepiece to that of the system. The present invention can provide designs which can be considered close to optimal, by configuring the system with an f number which is approximately equal to the eyepiece numerical aperture. Using 30 mW of illumination, one embodiment can provide a lens configuration which exposes about 0.58 cm^2 of the retina to a power density of about 51.5 mW/cm^2.

FIG. **18** illustrates that the illumination target in the eye can be the vitreous humor, a substantially clear gel-like region positioned beyond the aqueous humor. The devices described herein can be configured to operate similarly to that for the AH target, i.e., by transmitting the excitation beam to a deeper depth into the eye and into the VH (through the lens and/or the portion of the eye accessible when the eye is open), illuminating same and then detecting the transmitted light returned therefrom. The energy levels are preferably as noted above for the AH. As the Raman signal from VH may exhibit increased fluorescence, signal correction (subtraction, differentiation, etc.) may be used to obtain/detect the desired signal data.

FIG. **19** illustrates that the illumination target in the eye can be one or more blood vessels located on the conjunctiva at the outer surface of the eye. Using the conjunctiva vessel(s) can allow a direct blood level measurement to be obtained as this embodiment can focus to the blood flowing in the vessel (i.e., the blood itself to directly determine the targeted blood level measurement). In operation, the excitation beam is focused to at least one blood vessel on the externally accessible surface of the eye to illuminate the blood flowing in the blood vessel (because the blood vessel is located on the outer surface of the eye, there is no need to illuminate through skin). The targeted blood vessel can be any of the blood vessels located on or adjacent the conjunctiva of the eye (typically these are visually apparent), such as the long ciliary arteries or veins. One suitable target location may be one of the blood vessels located outside the iris away form the pupil. The detected Raman spectrum signal can be analyzed and a direct blood level measurement of the analyte of interest obtained. In certain embodiments, at least two readings can be taken to obtain data samples at different points of time to watch the spectra change. For example, one can attempt to include data associated with the vessel at a point in time when the blood cells are absent in the illuminated region in the vessel. Further, the Raman signal can be processed (discarded or filtered) to remove the data corresponding to red blood cells to enhance the desired signal of the analyte of interest (such as glucose). In some embodiments, the beam can be altered to disregard the Raman signal of the blood portion and to pursue the Raman signal of plasma (such as by shuttering the beam to discard the blood portion or to delay upon detection of signal associated with blood).

In certain embodiments, the fluorescence spectrum for the aqueous humor can be subtracted from the Raman spectrum by stimulating the aqueous humor with a second excitation laser light pulse at a wavelength slightly different from that of the first pulse (e.g., up to two nanometers from the first pulse), and then subtracting one spectrum from the other in a processor in accordance with conventional techniques. Such techniques are known. See, e.g., Funfschilling and Williams, *Applied Spectroscopy* 30, 443 (1976); Baraga et al., *Applied Spectroscopy* 46, 187 (1992); Wicksted et al., *Applied Spectroscopy* 49, 987 (1995)). In the alternative, the fluorescence spectrum can be subtracted out through the use of software or other processing techniques. Thus, the term "subtraction" as used herein is intended to include techniques such as filtering as well as taking the derivatives of the signal. While not essential, other potentially interfering spectra or signals such as Raman scattering from the lens, iris, or cornea can also be filtered or subtracted through a hardware and/or software processor. Water is typically not an issue for Raman spectroscopy, but a water spectrum can be subtracted if desired.

For the difference spectrum adjustment embodiments, the blood level of the selected substance (or the cerebral spinal fluid level) for the subject can be determined from the difference spectrum by correlating the difference spectrum to predetermined spectrums or data representing the concentration of a particular substance.

The computational analysis can use the differential spectrum or the non-differential spectrum depending on the desired application (typically associated with the degree of fluorescence of the selected region of the eye to determine the Raman spectrum and identify the presence of any selected spectroscopic profile or "signatures" having peaks in a spatial region of the spectrum, which, in turn, allows for the identification of the presence or concentration of a selected substance in the selected region of the eye. The correlation can be based on an empirically based model, formula, or matrix of test data garnered from an actual subject or from in vitro samples. The data can be correlated and the end results computed in a hardware and/or software processor. The model can be obtained through linear (e.g., partial least squares) or nonlinear (e.g., artificial neural networks) multivariate analysis.

In some embodiments, it may be important that a reference set or reference standards of known substances and concentrations be predetermined and stored for computational reference. For example, a training set of Raman spectra can be used. The training set can include samples from (or representative of) the selected region of the eye such as the aqueous humor with a broad range of concentrations of the Raman scattering metabolites. In the training set, the principal Raman-scattering metabolites (or "Raman active compounds") should be provided such that they (for at least the AH this may include glucose, lactate, urea, ascorbate, and any exogeneous compounds or drugs present) preferably do not vary co-linearly with one another among the samples in the training set. For direct blood measurements (i.e., the conjunctiva vessel measurements), the Raman active compounds will be those typically present in the blood. The model can be produced with spectra samples obtained from one or more prior subjects, with spectra samples obtained from the subject from whom the current blood level of the analyte of interest is being determined (in which case that subject would be required to provide a blood sample for determination of the blood concentration of the analyte of interest), or from test vials or artificial AH's with known concentrations of selected metabolites and/or the substance of interest. Thus, for an AH-based measurement, a training set can be used, with vials having a plurality of constituents, those normally present in the eye (including lactate, urea, and ascorbate) and in the substance of interest.

In some embodiments, to provide an atypical training sample, an artificial cover (such as a contact lens) may be intentionally positioned over the aqueous humor being used to generate a sample in a training set. This cover or lens can operate to cause the actual AH to have abnormal levels of lactic acid (a lactic acid build up) as it is inhibited from normal operation.

Typically, the training set uses at least 10, and more preferably 20, 25, 30 or more Raman spectra samples (and samples of the corresponding blood levels of each of the selected analytes of interest) with substantial intersample variability in the levels of the major Raman scattering metabolites (e.g., glucose, ascorbate, lactate, urea, and any drugs, analytes, or exogenous compounds present). The concentration of the analyte of interest (in either the blood or aqueous humor) preferably varies by at least a factor of 2, 5, 10 or 20 or more from the sample with the lowest concentration to the sample with the highest concentration. This may include providing concentrations of constituents less than and greatly above their normal levels such as 0.5 times to 15 times the normal levels (for the AH this can include urea, ascorbate, lactate, and, can also include glucose or other substances of interest, in saline). The mixtures can be prepared such that they provide a statistically uniform distribution with no bias/co-linearity (i.e., 100 different mixtures of four random substances in the test tube). It is anticipated that the calibration curve can be biased to cluster a training set biased within the normal or low range (i.e., to be skewed, depending on the application).

The concentration ratios or correlations may require development of the model using subjects with multiple different diseases (e.g., renal failure, diabetes, seizures, mitochondrial myopathics, sickle cell disease, heart failure, blood clots, etc.) and/or other parameters such as gender, age, weight, national origin, etc. For human applications, the model may even be determined with spectra samples obtained from animals, particularly primates. The sample of the selected region in the eye may be a natural sample (human or animal), or may be a man-made or surrogate aqueous humor sample created to mimic natural samples, where the blood level is calculated from a priori knowledge of the relationship between blood levels and aqueous humor levels for the analyte(s) of interest (for the blood level measurements). Similarly, for the cerebral spinal or intracranial fluid, the aqueous humor sample may be a natural sample (human or animal), or may be a man-made or surrogate aqueous humor sample created to mimic natural samples, where the level is calculated from a priori knowledge of the relationship between cerebral spinal or intracranial levels and aqueous humor levels for the analyte(s) of interest.

For example, when the analyte of interest is glucose, the training set of samples for the empirical model can comprise at least 20, 25 or 30 aqueous humor spectra samples. The samples can include varying levels of constituents which correspond to measured blood glucose levels, where the principal Raman active compounds in the aqueous humor samples (glucose, ascorbate, lactate, urea, and preferably any exogenous compounds such as drugs) vary substantially non-colinearly among the samples. The physiological levels of the blood and/or corresponding aqueous humor glucose concentration in the test samples can range from 0 or 50 mg/dL to 800 or 1,000 mg/dL, typically including at least a 100, 200, 300 or 400 mg/dL difference in concentration between the sample with the lowest concentration and the sample with the highest concentration.

Turning now to FIG. **12**, one embodiment of the invention illustrated therein is particularly suited for the non-invasive detection, identification and/or measurement of poison in the blood. A plurality of reference standard spectroscopic spectrum signals are generated (Block **150**). The reference signals can be converted into data defining peaks and frequencies and features of interest and the standards can correspond to in vitro samples of a plurality of different selected substances, and in some embodiments, each presented at different concentration levels (Block **155**). The data associated with the reference standards can be programmed into a computer or signal processor and made available for subsequent use. As noted above, the reference standards can correspond to a plurality of different Raman spectrum data corresponding to a plurality of different drugs, or typical or common poisonous substances. For example, Raman signatures of the most prevalent chemicals associated with pediatric poison control calls or emergency room visits can be analyzed in various concentrations to define an associated Raman signature. These can include common over-the-counter household drugs or medicinal supplies such as the drugs identified by brand names acetaminophen, ibuprofen, isopropyl alcohol, methanol, ethylene glycol and the like, or prescription drugs such as blood pressure medicines (such as HYTRIN), steroids, antibiotics, birth control pills, and the like, as well as household cleaning chemicals, pesticides, herbicides, and petroleum products.

An in situ Raman spectroscopic reading can be obtained of the subject (Block **160**). The subject may have been exposed to an unknown poison or substance. In some embodiments, the term "unknown poison or substance" can also include an unknown quantity of a known substance. For example, a guardian acknowledges that a TYLENOL bottle was found empty next to a young child, but it is unknown how many pills may have been in the bottle. The present invention may be able to determine whether a toxic amount was consumed.

In any event, the presence (or absence) of at least one selected substance in the subject can be identified (Block **170**). The amount of the identified substance in the selected region of the eye of the subject may also be determined. Based on the amount in the eye, an amount (a) in the blood and/or (b) in the cerebral spinal fluid or intracranial fluid can be established (anticipating that the amount in the AH can be correlated to either the amount in the blood or the amount in the cerebral spinal or intracranial fluid), thus allowing the presence and concentration to be indirectly established or estimated based on the detected presence and/or concentration in the selected region of the eye (such as the AH) (Block **180**). Physiological differences among different population segments may require determination of different correlation factors for each population segment (segmented by selected parameters of interest such as by age, gender, national origin, etc.)

In one embodiment, a Raman spectra/spectrum poison control substance identification database can be generated and the in situ reading then compared thereto so that a screening and assessment of the identity of which poison or poisons that the subject has been exposed can be readily identified by matching the in situ reading to one or more of the pre-determined and digitally stored Raman spectra. The database of selected substances may be held on a central database which can be remotely located or located at regional or selected locations (via the internet, intranet, or other communication means) and the localized reading can be uplinked to the larger database for a more comprehensive screening. Alternatively, the signal processor or computer associated with the Raman excitation/detection device or system may be configured with data corresponding to the most common types of substances and if a localized scan fails to match the in situ reading, a more comprehensive computational screening can be performed off-line or at the remote or larger database location.

As shown in FIG. **13**, another embodiment of the present invention is directed to an in vivo method for monitoring the administration of a targeted therapy such as a drug, chemical, gene, or other therapy, given to a subject for treatment of a condition in the brain (such as various cancers or neurologie conditions). For example, the present invention may be used during a treatment session using delivery of a cytotoxic agent to treat a cancerous tumor in the brain (or to other patients for specialized drug treatments targeted to the brain such as for treating neurological impairments in the brain). The method includes administering a dose of a selected treatment agent (typically chemotherapy) to a subject (Block **200**). The dynamics of the blood brain barrier are deliberately or intentionally altered from the normal state (Block **210**). The dynamics or function/operation of the blood brain barrier are monitored by non-invasively obtaining the Raman spectrum of the aqueous humor (Block **220**). A physiologically suitable non-specific marker, selected for its normal reluctance to cross the blood brain barrier, can be administered to the subject (Block **225**), and the monitoring step can be carried out by identifying its presence in the selected region of the eye (typically in the AH, but the vitreous humor or "VH" may also be suitable in certain embodiments). The non-specific marker can be synthetic or natural as noted (such as an antibiotic or conjugated billirubin or other suitable analyte).

The dose of the (chemotherapeutic) agent delivered to the brain can be estimated by determining the concentration in the AH (Block **230**). The monitoring step can optionally be repeated a plurality of times during the administering step (Block **235**). The blood brain barrier can be returned substantially to its normal state after a sufficient quantity of (chemotherapeutic) agent has been delivered to the brain (Block **240**). The altering step can be carried out by administering a chemical which can be flushed from the system or the dosing terminated to allow the normal function to return. In some embodiments, the non-specific marker can be the therapeutic agent itself.

In one embodiment, the altering step is carried out by introducing an osmotic agent or chemical (such as a drug identified by a trade name of MANNITOL) to the subject to temporarily open the blood brain barrier to allow larger molecules to pass therethrough. In another embodiment, the altering step can be carried out by increasing the intracranial pressure of the subject (such as by positioning the subject in a pressurized chamber).

Another embodiment is shown in FIG. **14**. This embodiment is directed to a method of non-invasively monitoring the operation/function of the blood brain barrier (which can include detecting any change from a normal state). The method comprises the steps of: (a) generating an excitation beam at a wavelength of from 400 to 900 nanometers (Block **250**); (b) focusing the excitation beam of said generating step into the anterior chamber of an eye of the subject so that aqueous humor in the anterior chamber is illuminated (Block **260**); (c) detecting a Raman spectrum corresponding to the illuminated aqueous humor (Block **270**); and (d) monitoring the blood brain barrier dynamics based on the detecting step (Block **280**). The detected Raman spectrum can indicate when or if a particular drug or analyte penetrates the blood brain barrier (increases in quantity or becomes detectable) when it normally travels in the vessels and/or either does not penetrate the barrier or exists in a stable or predictable amount in the eye (such as in the AH, VH, or vessel in the eye). The method may also include the step of exposing the subject to selected conditions during the focusing and detecting steps (Block **275**).

In certain embodiments, the monitoring step can be used to assess whether the dynamics thereof alter sufficiently to allow selected analytes, which would normally be inhibited from traveling through the blood brain barrier, to pass into the intracranial spinal fluid through the blood brain barrier. This can be useful for clinical trials or assessments of new drugs or treatment therapies. In other embodiments, the monitoring step can be carried out when a person is under or exposed to extreme conditions such as when diving, flying, or mountain climbing, or when suffering from a traumatic head or brain injury, high stress, or the like.

The method can also include the steps of comparing the Raman spectrum from the detecting step to reference spectrums corresponding to at least one selected analyte of interest, and indirectly identifying the presence of the least one analyte of interest in the subject's cerebral spinal fluid based on the detecting and comparing steps.

FIG. **15** illustrates yet another embodiment of the present invention. This method for identifying an alteration in the blood brain barrier of a biological subject comprises the steps of: (a) non-invasively obtaining a first in vivo Raman spectrum of the aqueous humor of the subject (Block **300**); (b) non-invasively obtaining a second in vivo Raman spectrum of the aqueous humor of the subject (Block **310**); (c) comparing the first and second readings (Block **320**); and (d) detecting an alteration in the blood brain barrier (or the penetration or a particular drug or analyte) by comparing the first and second Raman spectrums (Block **330**).

In another embodiment, shown in FIG. **16**, a method for identifying an alteration in the blood brain barrier of a biological subject comprises the steps of: (a) non-invasively obtaining a first in vivo Raman spectrum of the AH of the subject (Block **350**); (b) obtaining a reference spectrum of an in vitro sample representing the aqueous humor and comprising at least one selected analyte (Block **360**); and (c) comparing the in vivo Raman spectrum to the reference spectrum to identify an abnormality in the blood brain barrier (Block **370**). The abnormality may be indicated by detecting in the AH the presence of at least one selected analyte (typically one which is normally inhibited from passing through the blood brain barrier because of its molecular size or permeability and, as such, is not normally present in the intracranial fluid) thereby its presence in the AH also indicates its presence in the intracranial fluid of the subject.

The at least one selected analyte can be one which typically does not cross the blood brain barrier so that a determination of its presence in the sample is indicative of an abnormality or impairment or successful intentional alteration of the blood brain barrier dynamics. In one embodiment, the at least one selected analyte can be a bacteria.

FIG. **17** illustrates an additional embodiment of the present invention. In this embodiment, a reference library of the profiles of the chemical peaks (chemical peak profiles can also include the chemical valleys or line shapes) of the Raman spectra signals of a plurality of substances can be established or generated (Block **400**). This can include providing the chemical peak profiles for each of the plurality of the substances at different concentrations. A Raman spectrum signal can be obtained in vivo of the AH (or VH) of the eye of a subject (Block **410**). The chemical peak profile of the in vivo obtained Raman signal can be determined (Block **420**). Next, the profile of the in vivo data is compared to the reference library to see if there is a statistical correlation (or best fit match) between one or more of the library profiles with the in vivo obtained profile (Block **430**). If the answer is yes, then a list of one or more substances can be generated with a percent correlation of the likelihood of the match (Block **440**). The method may also rule out potential suspects (Block **441**). The list is likely to include one or more substances, as many will contain chemicals with overlapping peaks, but the presence or absence of other peaks can help statistically fit the data to identify the closest matches. If there is no match, or the correlation percentage is below a certain threshold (such as below 20–50%), then the test can be identified as inconclusive (Block **450**). The method may also allow a physician to query whether as suspect drug or substance is indicated, which may for example, consider whether a particular "suspect" substance which is believed to be the ingested or consumed poison may be in the subject. In turn, the method can respond to the inquiry to exclude the substance as a suspect with an identified statistical percentage (i.e., 95% confidence level that substance is not methanol) or to otherwise note the probability of this as the poison (i.e., 5% probability that the substance is methanol).

As will be appreciated by one of skill in the art, the present invention may be embodied as a device, method, or system comprising a digital processing system, and/or computer program product. Accordingly, the present invention may take the form of an entirely hardware embodiment or an embodiment combining software and hardware aspects. Furthermore, the present invention may include a computer program product on a computer-usable storage medium having computer-usable program code means embodied in the medium. Any suitable computer readable medium may be utilized including hard disks, CD-ROMs, optical storage devices, or magnetic storage devices.

The computer-usable or computer-readable medium may be or include, for example, but not limited to, an electronic, magnetic, optical, electromagnetic, infrared, or semiconductor system, apparatus, device, or propagation medium. More specific examples (a non-exhaustive list) of the computer-readable medium would include the following: an electrical connection having one or more wires, a portable computer diskette, a random access memory (RAM), a read-only memory (ROM), an erasable programmable read-only memory (EPROM or Flash memory), an optical fiber, and a portable compact disc read-only memory (CD-ROM), a CD ROM, a DVD (digital video disk), or other electronic storage medium. Note that the computer-usable or computer-readable medium could even be paper or another suitable medium upon which the program is printed, as the program can be electronically captured, via, for instance, optical scanning of the paper or other medium, then compiled, interpreted or otherwise processed in a suitable manner if necessary, and then stored in a computer memory.

Computer program code for carrying out operations of the present invention may be written in an object oriented programming language such as Java®, Smalltalk or C++. However, the computer program code for carrying out operations of the present invention may also be written in conventional procedural programming languages, such as the "C" or FORTRAN programming language or even assembly language. The program code may execute entirely on the user's computer, partly on the user's computer, as a stand-alone software package, partly on the user's computer and partly on a remote computer or entirely on the remote computer. In the latter scenario, the remote computer may be connected to the user's computer through a local area network (LAN) or a wide area network (WAN), or the connection may be made to an external computer (for example, through the Internet using an Internet Service Provider).

The present invention is described herein with reference to flowchart illustrations and/or block diagrams of methods, apparatus (systems) and computer program products according to embodiments of the invention. It will be understood that each block of the flowchart illustrations and/or block diagrams, and combinations of blocks in the flowchart illustrations and/or block diagrams, can be implemented by computer program instructions. These computer program instructions may be provided to a processor of a general purpose computer, special purpose computer, embedded processor or other programmable data processing apparatus to produce a machine, such that the instructions, which execute via the processor of the computer or other programmable data processing apparatus, create means for implementing the functions specified in the flowchart and/or block diagram block or blocks.

These computer program instructions may also be stored in a computer-readable memory that can direct a computer or other programmable data processing apparatus to function in a particular manner, such that the instructions stored in the computer-readable memory produce an article of manufacture or output a test result including instruction means which implement the function specified in the flowchart and/or block diagram block or blocks.

The computer program instructions may also be loaded onto a computer or other programmable data processing apparatus to cause a series of operational steps to be performed on the computer or other programmable apparatus to produce a computer implemented process such that the instructions which execute on the computer or other programmable apparatus provide steps for implementing the functions specified in the flowchart and/or block diagram block or blocks.

As is seen in FIGS. **10***a, b, c*, FIGS. **5** and **6**, the system **5** includes a signal processor or controller **32***s*. The signal processor or controller can be a general purpose microprocessor, a digital signal processor or a specific purpose processor. The signal processor can be an electronic device, a software or program-implemented device running on a general-purpose computer, or combinations of such. The present invention should not be construed as limited to the particular configurations illustrated in the figures but is intended to encompass other configurations capable of carrying out the operations and/or functions described herein.

The signal processor **32***s* can be any commercially available or custom microprocessor. The computer **32** or signal processor or controller may include a data processing system with I/O data port(s) or other such devices that also communicate with the processor. The I/O data port can be used to transfer information between the data processing system or signal processor **32***s* and another computer system or a network (e.g., the Internet) or to other devices controlled by the processor. These components may be conventional components such as those used in many conventional data processing systems which may be configured in accordance with the present invention to operate as described herein.

The devices or the present invention may include memory devices containing the software and data used to implement the functionality of the data processing system or signal processor **32***s*. The memory can include both programmable and read-only memory. Typical programmable memory includes, but is not limited to static RAM (SRAM), dynamic RAM (DRAM), flash RAM, EEPROM or other such programmable memory devices whose contents may be modified. The read only memory may be of a type which may not be modified after its initial programming, such as ROM or PROM but may also be programmable in certain embodiments of the present invention.

The memory may contain several categories of software and data used in the data processing system or system processor: the operating system; the input/output (I/O) device drivers; and the image (and/or in the case of dual stimulus, the auditory) data. As will be appreciated by those of skill in the art, the operating system may be any operating system suitable for use with a data processing system, such as OS/2, AIX or OS/390 from International Business Machines Corporation, Armonk, N.Y., WindowsCE, WindowsNT, Windows95, Windows98 or Windows2000 from Microsoft Corporation, Redmond, Wash., PalmOS from Palm, Inc., MacOS from Apple Computer, UNIX or Linux, proprietary operating systems or dedicated operating systems, for example, for embedded data processing systems.

The I/O device drivers typically include software routines accessed through the operating system to communicate with devices such as the input devices, the display **25** (FIG. **7**), the I/O data port(s), and certain components of the memory. The data can be static and dynamic data used by the operating system, I/O device drivers, the detector, and other software programs that may reside in the memory.

While the present invention is described, for example, with reference to particular divisions of programs, functions and memories, the present invention should not be construed as limited to such logical divisions. Thus, the present invention should not be construed as limited thereto as it is intended to encompass any configuration capable of carrying out the operations described herein.

The present invention is explained in greater detail in the following non-limiting Examples, in which "μL" means microliters; "dL" means deciliters, "mW" means milliwatts, "nm" means nanometers, "Kg" means kilograms, "J" means Joules, "cm^2" means square centimeters, and temperatures are given in degrees Centigrade.

EXAMPLE 1

Measurement of Aqueous Humor Glucose in Vitro with Raman Spectroscopy

Aqueous humor was obtained from sixteen New Zealand white rabbits within one minute of sacrifice by other investigators. These animals had experienced experimental myocardial infarction **48** hours prior to euthanasia. They were sacrificed by rapid exsanguination under ketamine and xylazine anesthesia. Aqueous humor samples were kept frozen until glucose levels could be measured and Raman spectroscopy performed. Glucose concentration was measured with a commercial glucometer (Glucometer Elite, Bayer, Elkhart, Ind., USA) and confirmed against concentration standards. Each measurement was repeated, and the average measurement was considered actual glucose concentration.

Samples were placed in conical quartz cuvettes designed to hold a volume of 80 μL and permit direct optical access to the solution by the spectrometer without traversing glass walls or coverslips.

Raman spectroscopy was performed with a f/1.8 holographic imaging spectrograph (Kaiser Optical Systems, Ann Arbor, Mich., USA) attached to an Olympus BX60 microscope with 10× objective. Data were collected using a Princeton Instruments (Trenton, N.J., USA) camera with a 1024×256 CCD array (EEV, United Kingdom) cooled to −80° C. with liquid nitrogen. Illumination of the sample through the microscope objective was achieved with a Ti:Sapphire laser (Spectra Physics 3900S, Mountain View, Calif., USA) pumped by an argon laser (Spectra Physics 2010E). Spectrographic data was integrated while the sample was illuminated at a wavelength of 785.0 nm (30 mW) for 10 seconds. This illumination was then repeated at a wavelength of 787.2 nm.

The integrated spectra at the two slightly different wavelengths were then subtracted from one another. This effectively eliminates the broadband fluorescence, which does not shift relative to the excitation wavelength. It leaves the Raman-shifted spectra appearing as a bipolar pattern (FIG. **1**).

Multivariate analysis of the spectra was accomplished using Holograms (Princeton Instruments, Trenton, N.J.) and Grams (Galactic Industries, Salem, N.H. USA) software packages. Thirty-two aqueous humor samples (from sixteen rabbits) were evaluated using a "round robin" approach to iteratively group all but one of the samples into a training set. Hence, the system trains on all but one of the samples, estimates the glucose level in that sample, then rotates the test sample into the training set. This cycle is repeated until all samples have served as an unknown test sample.

A back propagation neural network (D. Rumelhart et al., *Nature* 323, 533 (1986)) was employed to determine if a nonlinear regression method would better predict the glucose concentration from the Raman spectra of aqueous humor. Such a model may compensate for as yet unknown interactions between analytes in aqueous humor. A two-layer back propagation neural network (NeuralWare, Inc., Pittsburgh, Pa., USA) was employed using a sigmoidal function as the nonlinear element. Factors derived from the partial least squares algorithm served as the inputs to the neural network. Raw spectral data could have been used but would have required a prohibitively large training set. As in the linear regression preformed earlier, round robin training and testing was utilized. During training, the neural network's weights were adjusted to minimize the total squared error between the actual glucose concentration and the predicted glucose concentrations. Each sample was tested using a neural network trained on the remaining 31 samples.

EXAMPLE 2

Correlation of Aqueous Humor Glucose with Rising Blood Glucose

Nine female New Zealand white rabbits were used for this part of the study. They were anesthetized with ketamine (50 mg/kg) and xylazine (7.5 mg/kg) given as a single intramuscular injection. Xylazine blocks release of insulin from the pancreas and causes blood glucose to rise (K. Chalabi et al., *Ophthalmic Res.* 19, 289 (1987); J. Arnbjerg et al., *Ophthalmic Res.* 22, 265 (1990)).

Blood samples were taken from the central ear arteries at various times following the injection of anesthetic once the animals were adequately anesthetized. Whole blood was immediately measured for glucose concentration with a commercial glucometer (Glucometer Elite, Bayer). In most instances two measurements were made. The average measurements are reported. If the measurements differed by more than 20%, a third measurement was made. If one measurement differed by more than 20% from the mean, it was discarded. In instances when blood glucose concentration appeared stable, occasionally only one measurement was made.

At various times after induction of anesthesia, samples of aqueous humor were taken. This was done by paracentesis with a 25-gauge needle through clear cornea near the limbus after administration of proparacaine eye drops. Aqueous humor glucose concentration was measured with the glucometer in a manner similar to the blood.

In rabbits, there is massive breakdown of the blood-aqueous barrier after a single paracentesis of the anterior chamber of the eye (W. Unger et al., *Exp. Eye Res.* 20, 255 (1975)). The aqueous humor becomes too viscous for repeat paracentesis for at least 30 minutes. Consequently, the results are reported for the first paracentesis of an eye. In a few instances, however, a second paracentesis was made an hour later to assess how the breakdown of the blood-aqueous barrier may have affected the correlation of aqueous glucose with plasma glucose.

Average blood glucose concentrations for each animal were plotted against time after anesthetic injection. Since it was not possible to obtain blood samples simultaneous with aqueous humor samples, a best-fit second-order polynomial was calculated for each animal. This was used to calculate the blood glucose concentration at the time that the aqueous humor sample was taken. Aqueous humor glucose concentration was then plotted against the calculated simultaneous blood glucose concentration for all animals. DeltaGraph software (Delta Point, Inc., Monterey, Calif. USA) was used for all statistical calculations.

EXAMPLE 3

Results for Measurement of Aqueous Humor Glucose in Vitro with Raman Spectroscopy

The raw spectra of rabbit aqueous humor show broad fluorescence peaks that obscure the underlying Raman signature (FIG. **1**). When the spectra from the two slightly different wavelengths are subtracted, one from the other, the Raman shifted spectra become apparent as bipolar peaks.

Actual aqueous humor glucose concentration, measured by the glucometer, ranged from 37 to 323 mg/dL in the thirty-two samples. Multivariate analysis of the raw spectra from these samples with the partial least squares algorithm revealed fair correlation ($r^2=0.76$) between the predicted aqueous humor glucose concentration and the actual concentration. Multivariate analysis of the subtracted spectra from these samples with the partial least squares algorithm resulted in improved correlation ($r^2=0.90$) between the predicted aqueous humor glucose concentration and the actual concentration. When back propagation with an artificial neural network is further applied to the data, correlation is excellent ($r^2=0.98$) (FIG. **2**).

EXAMPLE 4

Results for Correlation of Aqueous Humor Glucose with Rising Blood Glucose

The rate of rise of blood glucose after xylazine injection is highly variable from animal to animal (FIG. **3**). In fact, in one rabbit there was little change in the blood glucose concentration over time.

If only samples taken in the first fifteen minutes after anesthetic injection are considered, aqueous humor glucose concentration is higher than blood glucose concentration (207±28 mg/dL for aqueous humor; 135±27 mg/dL for blood). When calculated blood glucose rises above 200 mg/dL, simultaneous aqueous humor glucose parallels blood glucose nearly linearly [aqueous humor glucose=1.18 (blood glucose)−72.7; $r^2=0.88$] (FIG. **4**).

The number of aqueous humor samples from second paracenteses was insufficient to draw conclusions about their significance. They did not appear, however, to correlate well with aqueous humor glucose levels from initial paracenteses (FIG. **4**).

These data indicate that the baseline ratio of aqueous humor glucose to blood glucose in rabbits is approximately 1.5. (It may actually be higher than this as a true baseline was not obtained, and the blood glucose levels were likely elevated by the time the first samples were obtained, even in the first 15 minutes after injection.) This is much higher than in humans and higher than in previous reports on rabbits. All previous reports on rabbits, however, were prior to recognition of the hyperglycemic effect of xylazine anesthesia, yet many of them used xylazine anesthesia. In addition, most previous reports assumed that the rabbits were at steady-state euglycemia, without actually confirming that this was the case. These facts may account for some of the highly variable results in previous reports.

These data also demonstrate that aqueous humor glucose in the rabbit responds almost immediately once blood glucose exceeds 200 mg/dL. The relationship of aqueous humor glucose to blood glucose is nearly linear while blood glucose is rising above 200 mg/dL. Below that level, aqueous humor glucose appears stable. What happens to aqueous humor glucose when blood glucose is clamped at a hyperglycemic level, or when blood glucose concentration drops, has yet to be determined. Nonetheless, equilibration of aqueous humor glucose with blood glucose probably occurs within minutes in rabbits. If rapid equilibration of aqueous humor glucose also occurs in humans, it could serve as an excellent substrate for non-invasive glucose monitoring.

Previous investigators have found no breakdown in the blood-aqueous barrier of albino rabbits exposed to infrared radiation with energy densities up to 106 J/cm (D. Reddy, supra; G. Peyman et al., *Exp. Eye Res.* 42, 249 (1986); T. Kurnik et al., *Inv. Ophthalmol. Vis. Sci.* 30, 717 (1989)). Infrared energy densities as low as 44 J/cm^2 may be sufficient to breakdown the blood-aqueous barrier in pigmented rabbits, however. This is still substantially higher than the energy density that would need be applied with the Raman technique disclosed herein.

FIGS. **21**A–C illustrate experimental results obtained for (a) the spectrum for an artificial in vitro AH in a model anterior chamber (FIG. **21**A); (b) the spectrum for an actual in vitro measured (extracted real AH) in a model anterior chamber (FIG. **21**B); and (c) the spectrum for an actual in vivo AH measurements (FIG. **21**C). A 20× objective with a numerical aperture of about 0.4 was used to obtain the data. The “ensemble” spectra (of the constituents of the AH) correspond well between the three figures. As may be expected, the in vivo and in vitro intensity of the Raman spectra was less than the in vitro artificial sample, but the spectra peaks corresponded well between the measurements (the device able to detect the physiological levels). The in vitro measurements of the AH were obtained using extracted rabbit eyes placed under a contact lens.

EXAMPLE 5

Measurement of the Integrity of Blood-Aqueous and Blood-Brain Barriers

The blood-brain and blood-aqueous barriers block passage of large molecules into the cerebrospinal fluid or aqueous humor. Many drugs and disease processes result in disruption of both the blood-aqueous barrier and the blood-brain barrier. In the case of such drugs or disease processes, disruption of the blood-brain barrier can be inferred from breakdown of the blood-aqueous barrier. Breakdown of the blood-aqueous barrier can be measured by measuring the protein content of the aqueous humor or by measuring the concentration of other substances (e.g., drugs) within the aqueous humor. Such substances may be quantifiable by Raman spectroscopy and the protein can be calculated from the fluorescence spectrum since the fluorescence spectrum is largely generated by protein. Consequently, the devices disclosed herein for measuring glucose and other Raman scattering metabolites in the eye can be used for measuring the integrity of the blood-aqueous and blood-brain barriers.

Raman scattering molecules of different sizes that do not normally cross the blood-aqueous or blood-brain barrier could be administered to a patient intravenously. The presence of these molecules is then identified and/or quantified by Raman spectroscopy of the anterior chamber of the eye. This can be used to determine the size of molecules which pass through the blood aqueous barrier. The signature of the Raman spectroscopy signal can then digitally be compared to reference signals (without requiring the use of a difference methodology which would subtract the amount of fluorescence to reveal the Raman spectra of these substances) with the device disclosed herein would reflect the passage of natural proteins through the blood-aqueous and blood brain barriers.

Therapeutic drugs can also be measured to determine their effectiveness in crossing the blood-aqueous or blood-brain barrier or to determine if a drug has undesirable passed therethrough.

EXAMPLE 6

Monitoring the Blood Brain Barrier During a Treatment Session

A patient undergoing treatment for a cancer located in the brain or on the other side of the blood brain barrier is administered a non-specific marker which is configured such that it does not normally cross the blood brain barrier (or is reluctant to do so) and which is relatively easily detectable when in the AH by the use of Raman spectroscopic analysis. The blood brain barrier can be altered by administering an osmotic agent such as a chemical substance (such as a drug identified by the trade name "MANNITOL" or a similar substance) which acts to physiologically alter the blood brain barrier. In so doing, the blood-aqueous barrier is also altered. In turn, the non-specific marker travels to the AH. An optical measurement can be obtained to confirm that the non-specific marker is indeed in the AH (in a sufficient or elevated quantity to indicate that the blood-aqueous barrier has been altered). The present invention, recognizing that the mechanics of the blood-aqueous barrier and the blood-brain barrier are similar, then presumes that the blood brain barrier has also been altered sufficiently (scale correlation factors may be applied to assure that the blood brain barrier is opened/altered a sufficient amount).

A therapeutic drug (which may be a chemotherapy or cytotoxic drug) can then be administered (injected or otherwise delivered) to the subject. Administering the therapeutic drug after the barrier has been altered can help ensure that the therapeutic drug is not introduced until the blood-brain barrier is altered sufficiently and can allow an elevated amount of the therapeutic drug to pass through the blood-brain barrier over a non-altered state. This can facilitate that more of the drug goes where it is targeted and can reduce the amount in the system of the subject which can often be exposed to undesirable amounts of cytotoxic drugs in conventional treatments in order to ensure that a sufficient amount actually reaches the tumor.

Thus, the present invention now allows one to intentionally break down the barrier and non-invasively confirm the alteration before delivering medicines, which do not normally readily cross the blood brain barrier, to tumors and the like positioned in the blood aqueous or blood brain barrier. Correlations of the amount of the drug administered to the subject, to the amount of the marker administered and detected in the AH, may be established to allow an estimate of the amount of the drug in the spinal fluid or in the blood. Thus, the present invention may also be able to titrate the dose so that the levels in the spinal fluid are sufficiently high but not toxic to the patient in non-targeted regions of the body.

EXAMPLE 7

Measurement of Brain Alcohol Level

A portable device can be used to hold the optical generation and measurement system. The device can be readily carried by police enforcement agencies and conveniently used when a suspect is evaluated for potential DUI infractions. Thus, the device can be positioned over the eye(s) of the subject and the subject can be instructed to read or identify the text or object encoded or displayed as the fixation target in the headset over the eye or eyes. When the subject verbally affirms the proper identification of the fixation target, the device can be operated to obtain a reading. The device may include an auto or manual focus to correct the vision in the device and to allow the subject to focus to the target as needed. Almost instantaneously, the police officer can have quantitative results of the brain alcohol or blood alcohol level of the suspect. The amount or concentration of the alcohol determined to be in the AH (or conjunctiva vessel) can be correlated to either a blood alcohol and/or brain alcohol level. Many states presently regulate blood alcohol levels; however, it is anticipated that an impairment can be assessed also based on either or both blood or brain alcohol levels. As such, the device may generate a plurality of numbers, such as the concentration in the eye, and, the indirectly measured or correlated concentration in the blood and/or brain. If above the legal limit, which can be programmed for each state or locality, a documentary arrest record can be generated with a date and time stamp encoded onto the measurement record that can be automatically generated and electronically stored or printed.

In some embodiments, the Raman spectrum of the subject may include many wavelengths and the determination of the concentration or presence of alcohol may include determining the significant peaks associated with alcohol and subtracting the AH constituents from the signal. It is anticipated that the method and devices will be able to identify blood alcohol and brain alcohol levels between about 0.001–1%, and typically between 0.01–0.8% (noting that concentrations above 1% may be lethal) and that medical assistance may be indicated for certain levels.

EXAMPLE 8

Non-invasive in Vivo Identification of the Presence of Illegal Narcotics

A portable device can be used to hold the optical generation and measurement system. The device can be readily carried by state or federal police enforcement agencies (or hospitals including medical examiners for autopsy) and conveniently used when a suspect is evaluated for potential illegal possession of drugs. In operation, the device can be configured as a headset with the display and light transmission path positioned in front of the eye(s) of the subject. The subject can be instructed to read or identify the text or object encoded or displayed in the device as the fixation target in the headset over the eye or eyes. The device may include an auto or manual focus to correct the vision in the device and to allow the subject to focus to the target as needed. When the subject verbally affirms the proper identification of the fixation target, the device can be operated to obtain a reading. Almost instantaneously, the police officer can have quantitative results of whether the suspect has inhaled, ingested, injected, or otherwise consumed an illegal narcotic. It is anticipated that concentrations in the range of between about 0.001–1% (in the AH) or higher in the conjunctiva vessel (i.e., in the blood volume) may be quantified. Drugs present in small quantities (such as the picomolar range) may be able to be identified (or the bodies' reaction thereto identified thereby identifying the drug) but may not be able to be reliably quantified.

It is noted that illegal narcotics may vary jurisdiction to jurisdiction and the device can be programmed according to the laws of the jurisdiction of use. Examples of illegal narcotics include opiate-based drugs such as cocaine in several forms such as crack, powder, and the like, and other drugs such as LSD, pcp, angel dust, marijuana, and others. It is anticipated that the most commonly used or street available illegal drugs or abused prescription drugs can be analyzed to predetermine the Raman signature as discussed above and these reference signature standards for these substances stored for reference to the in situ reading obtained on the suspect.

In certain embodiments, the device and method can include assessing both, the brain alcohol (or blood alcohol) level of the suspect, and the presence of an illegal narcotic with one reading. Further, the present invention can be used to develop legal limits for other non-alcohol products so as to ascertain whether an individual is impaired for improved safety on the roadways.

If an illegal narcotic is identified or if the alcohol level is above the legal limit, (the legal limit can be programmed for each state or locality), a documentary arrest record can be generated with a date and time stamp encoded onto the measurement record which can be automatically generated and electronically stored or printed.

EXAMPLE 9

Determination and/or Identification of Drug or Steroid Use

In lieu of conventional urine tests, the present invention can be used to screen potential employees for drug use, or to assure employees are drug or alcohol free prior to allowing them to operate equipment (such as airplanes, trains, buses, subways, or other mass transit systems or heavy equipment and the like). This convenient and non-invasive screening may be a suitable testing system to help assure that the operators entrusted with the lives of others are not operating in an impaired state.

The present invention can also be used to screen athletes for banned substances prior to competitions or at periodic or audit intervals, and/or to screen parolees for violation of parole. Indeed, the readings can be obtained locally and then electronically relayed to a remote monitoring station where the results can be analyzed and proper authorities alerted of a positive test.

EXAMPLE 10

Identification of Ingested Poison(s)

Similar to Example 8, an in situ reading can be taken on an initial evaluation of a poison suspect, either by a paramedic at a home or in route to a hospital, or by a clinician, such as at an Emergency Room, to allow a relatively fast triage assessment of whether one or more poisons are present in the body. One reading may be able to identify one or more of several different signature peaks associated with several different poisons. The poisons may be common household products such as different medications, or a pesticide/herbicide, a cleaning agent, or antifreeze. By pre-evaluating the Raman signature of a plurality of common substances and electronically storing the data associated therewith in an electronic poison control reference library, the methods and devices of the present invention can deconvolute the in situ obtained Raman spectrum signal to build or match a signal pattern with the Raman reference standards. Based upon the substances with signatures, which substantially match the in situ Raman signal, probable or possible substances can be identified (or to exclude other suspect substances). Knowing which of the chemicals or substances have the profile signature closest to the in situ obtained signature, the Raman signal(s) allows for the identification of which poisons may be in the body. The match can be based on a computational comparison of peaks or a graphical comparison. For example, the match can be carried out by matching the peaks or line shape profile across the spectrum of interest via best-fit curves or statistical correlation methods can identify products which align or match that of the data from the subject. A list of substances with associated probability ratings can be provided to the clinician.

In addition, a quantitative determination of the amount of the identified substance or substances in the body may be able to be made depending on the strength of the signal (stronger signals typically indicate higher concentrations).

In one embodiment, the Raman signature of a selected metabolite can be reviewed to see if the level is carcinogenic in the subject. For example, antifreeze (ethylene glycol) can be toxic to the liver. If it is identified that the either ethylene glycol was ingested or that the liver is emitting toxins or products, a treatment such as alcohol can be administered to offset the toxicity of the poison. Similarly, for other embodiments, the substance or drug itself may not be detectable in minute quantities in the body (typically in the pico or micro molar range). However, it may be possible to detect either a constituent of the substance of interest, or a physiologic reaction to the substance, to see if it is in an elevated level in the body (due to the body breaking down or reacting to the substance).

EXAMPLE 11

Identifying a Pre-determined Substance Using Resonant or Strongly Pre-resonant Raman Spectra and/or Double Bond Peaks in the Raman Spectrum

In certain embodiments, the excitation wavelength of the substance of interest is selected so that it amplifies the detectable RR response signal or spectra. The excitation wavelength is selected so that it lies within or proximate to the absorption spectrum of the selected substance. The RR spectra is typically generated by a wavelength that resides within the absorption spectrum (and above 400 nm). As noted above, the term "resonant Raman" includes non-resonant excitation wavelengths approaching the absorption spectrum that can be used to generate spectra with increased signal strength (such as signal strengths having 100–1000 times or more signal intensity strength relative to excitation wavelengths that substantially depart from the wavelengths associated with the RR spectra). One or more peaks of interest in the RR spectra can be monitored to identify the presence and/or quantity of the substance in the subject. In particular embodiments, a chemical shift peak associated with a double carbon bond may be monitored.

In certain embodiments, one or a plurality of selected segments (typically relatively narrow regions of less than about 200 cm^{-1}, and more typically less than about 100 cm^{-1} in width) in the Raman spectrum can be used to determine the concentration levels in a subject. The regions used to assess concentration can be selected so as to exclude regions that are associated with spectral shift that may occur at increased concentration levels to thereby provide reliable concentration data over a broader in vivo range of probable bio-concentrations.

FIG. **22**A illustrates the chemical structure of a chemotherapeutic agent known as fenretinide (4-HPR), and shows a conjugated chain structure portion of the structure that permits resonance enhancement. Note that even at 10-micromolar concentration the solution has a yellow color. This can be important because the concentration may be such that the drug can block the penetration of the excitation light and limits the Raman scattering (e.g., see FIG. **26**). Typically, at lower concentrations this is not a problem.

FIG. **23** is a graph of the absorption spectrum of fenretinide, illustrating that the absorption peaks at 365 nm. Exciting the molecule at 406.7 nm enhances the signal such that most of Raman shifted scattered light is not absorbed by the molecule itself, and so that undesirable excitation or exposure wavelengths are avoided (such as those below <400 nm).

FIG. **24** is graph of the Raman spectrum of intensity verses wavelength shift of a non-resonant Raman spectroscopy (using an excitation wavelength of 785 nm) of fenretinide (concentration 10 mM, dissolved in ethanol). The spectrum shows key peaks of fenretinide as distinct from those of ethanol (ETOH Peak at 881 cm^{-1}). Particular peaks of interest for the fenretinide one or more of which may be monitored (or the entire profile) include the peak at 1171 cm^{-1} (corresponding to the carbon-carbon bond) in the molecular structure and the peak at 1576 cm^{-1} (corresponding to the carbon to carbon (double bond)) in the molecular structure.

FIG. **25**A is a graph of the absorption spectrum (absorption versus wavelength) of a solution of 10 mM of fenretinide. FIG. **25**A is inset within FIG. **25**B, which is a graph of pre-resonant (excitation wavelengths above the resonant wavelength) spectra of a 10 mM fenretinide corresponding to the substance with the absorption spectrum shown in FIG. **25**A. As shown, the intensity is normalized to the ETOH signal (arbitrary units), but the data need not be adjusted in this manner. Three different excitation wavelengths are shown, the signal strength increasing for a decreasing wavelength. That is, the spectra corresponding to the 514 nm excitation wavelength has a larger signal intensity than the spectra corresponding to either the 633 nm or 785 nm wavelengths. As shown, the intensity values of the peaks at 1576 cm^{-1} and 1171 cm^{-1} are increased relative to the peaks at these positions in the Raman spectra for the 785 nm or 633 nm excitation wavelengths. Accordingly, FIG. **25**B demonstrates that Raman sensitivity increases as the excitation wavelength approaches the absorption spectrum of a particular molecule or substance (shown for illustrative purposes as fenretinide, even at pre-resonance wavelengths). This may be useful in that devices can be configured to operate above resonance wavelengths (outside the absorption spectrum or curve) although approaching absorption spectrum values and may be particularly suitable for detection of the peaks due to carbon double bonds that occur even for chemicals that do not resonate or for which resonant Raman spectroscopy would be difficult.

FIG. **26** is a graph of resonant Raman (RR) spectra of fenretinide generated at an excitation wavelength of about 406.7 nm. The peaks for fenretinide at 1171/cm and 1576/cm can be detected at concentrations as low as 0.1 micromoles. The peaks (S) from the solvent, ethanol, are diminished at the higher fenretinide concentrations due to the absorption of excitation light by the fenretinide itself.

FIG. **27** is a drawing of the chemical conjugated structure of amphotericin B (C_{47}, H_{73}, NO_{17}), an exemplary antifungal agent. This agent is a broad-spectrum antifungal agent used to treat potentially fatal fungal infections of the CNS. It has a molecular weight of 924.1.

FIG. **28** is a graph of the absorption spectrum of amphotericin B at a concentration of 5 μg/ml.

FIG. **29** is a graph of the RR spectrum of (generated at an excitation wavelength of 406.7 nm) of amphotericin at three different concentrations (mixed in MeOH): 5.0 μg/ml, 0.5 μg/ml, and 0.05 μg/ml. Characteristic peaks of amphotericin can be detected by Resonant Raman (RR) spectroscopy (406 nm) at concentrations as low as 0.05 micrograms/ml (and potentially much lower). The therapeutic level of amphotericin is about 0.5–5.0 micrograms/ml.

EXAMPLE 12

Non-Invasive Ethanol Monitoring Using Raman Spectroscopy

Raman-based instruments and information processing capability can be used to non-invasively measure ethanol and/or its metabolites in the human aqueous humor.

It is anticipated that the ability to measure ethanol non-invasively may revolutionize the treatment and study of alcoholism. Conventional techniques that measure ethanol by electrochemical, enzymatic, or colorimetric techniques in the breath, urine, or excreted body fluids are generally considered intrusive, unreliable, or subject to user non-compliance. Spectroscopic techniques have been proposed in the past, but the techniques remain elusive. See, e.g., Marose S, Lindemann C, Ulber R, Scheper T. *Optical sensor systems for bioprocess monitoring*, Trends in Biotechnology 17: 30–34, 1999.

In pilot studies undertaken in connection with the present invention, a correlation of ethanol concentration predicted by Raman spectroscopy with actual ethanol concentration in aqueous solution at levels typically found in blood following alcohol consumption has been demonstrated. Metabolites of ethanol, such as acetaldehyde and acetate, may be measured non-invasively, allowing for the added benefit of studying alcohol metabolism.

In the past, non-invasive spectroscopic detection of alcohol has been problematic because blood, tissue, and most excreted fluids contain numerous substances that confound ethanol spectral signatures. Appropriate selection of sources or techniques for spectroscopic analysis can inhibit or suppress these confounding signatures. One suitable monitoring site/source is the aqueous humor (AH) filling the anterior chamber of the eye (between the lens and cornea). The AH contains relatively few molecules (such as proteins) capable of interfering with the spectroscopic detection of ethanol. The primary constituents of AH are glucose, lactate, ascorbate, and urea. Accurate and reliable measurement of these metabolites at physiologic levels is possible with Raman spectroscopy using reasonably low power levels as described in the specification above in more detail.

Furthermore, as with cerebrospinal fluid, AH can be substantially freely permeable to ethanol. This fact and its optically accessible location make AH a suitable choice as a site for non-invasive analysis of ethanol. In addition, Raman Ethanol Assay (REA) of the aqueous humor can be obtained by or linked to an identify-verification device that identifies individuals based on an image analysis of retinal or iris architecture. Examples of biometric evaluations of the eye using retinal or iris structures include U.S. Pat. Nos. 5,291,560; 6,119,096; 5,615,277; 5,613,012; 5,572,596; 5,471,203; 5,336,970; the contents of these patents are hereby incorporated by reference as if recited in full herein. Such dual-purpose devices can be used for public safety or security purposes (such as analysis of drivers, pilots, workers in certain industries or for verifying the identify of an athlete, and the like). One manufacturer of iris-based biometric devices is IriScan, Inc., located in Mt. Laurel, N.J.

The present invention can be embodied in portable devices that can obtain the signals from the eye(s) of a subject (for AH) sampling at desired field locations. For example, the image or signal acquisition can be carried out with a miniaturized device such as one that can be attached to headgear or face gear such as spectacles, goggles, face-mask, or a contact lens device. The signal acquisition device that generates the REA of the aqueous humor can be operated in an intermittent, semi-continuous, or even potentially in a low power continuous manner. REA of the aqueous humor has an advantage of being operable in a completely non-invasive manner. As such, it may be operable for intermittent rapid determination of ethanol alcohol levels at a particular point or points in time or as a longer-term monitoring device that collects data over a desired monitoring period of hours, days, or, even months. The data can be collected and analyzed by the (preferably portable) device itself or stored and telemetrically or periodically downloaded to a remote analysis system.

In certain embodiments, one or a plurality of selected segments (typically relatively narrow regions of less than about 200 cm^{-1}, and in certain embodiments less than about 100 cm^{-1} in width) in the Raman spectrum can be used to determine the ethanol concentration levels in a subject. The regions used to assess concentration can be selected so as to exclude regions that are associated with spectral shift that may occur at increased concentration levels to thereby provide reliable concentration data over a broader in vivo range of probable bio-concentrations.

Exemplary Raman Instrument for Spectral Measurements of AH

The REA of aqueous humor can utilize an existing f/1.8 Kaiser Optical Systems holographic imaging spectrograph which is fiber-optically coupled to a probe head that is configured to detect in a continuous or intermittent manner. The probe head may be handheld, or mounted on a translation stage for studying sedated animals. In the past, a 20× objective was used to acquire spectra of 50–100 μL samples of rabbit aqueous humor with excellent results in measurement of glucose, lactate, and urea. See Borchert et al, *A non-invasive glucose monitor: Preliminary results in rabbits*, Diabetes Tech. and Therapeutics. 2:141–151, 1999. An existing external cavity diode laser manufactured by SDL can be utilized as the excitation source. An excitation wavelength of 785 nm may be selected because it is barely visible to the subject and provides a comfortable fixation target as well as a source which may be used in a minimally or non-intrusive continuous monitoring device. Near-infrared excitation also greatly reduces the background fluorescence from the eye as well as from minor constituents within the aqueous humor. However, Raman scattering efficiency decreases inversely with wavelength to the fourth power. This decrease in Raman signal associated with near-infrared excitation can be compensated by using a liquid nitrogen cooled, thick-epitaxial, deep depletion CCD detector with high or extremely high quantum efficiency in the 700–1000 nm wavelength range (typically about 785 nm). Since the silicon CCD becomes increasing transparent in the NIR, a thick-epitaxial design can reduce the effects from etaloning. The REA system can employ a confocal configuration for the eye, which allows for selective collection of spectra from the aqueous humor of the eye as well as for monitoring of centration of the beam.

A diagram of examplary probe head architecture is shown in FIG. **6**. As shown, the probe-head design incorporates a single-mode excitation fiber and a step-index multi-mode collection fiber. The probe-head objective images the focal point to the multi-mode collection fiber. A 50 micron chrome aperture was evaporated onto the end of collection fiber to adjust the confocality of the probe head to collect as much Raman scattered light from within the anterior chamber as possible while rejecting light from the cornea or lens of the eye. A system configured in this manner was tested for its ability to collect the spectra of saline by placing this solution under a contact lens resting on a quartz plate. The signature of the contact lens, saline, and quartz plate could each be isolated by focusing on each element of this physical model of the anterior chamber. This experiment was repeated on the eye of a living rabbit and similar results were obtained. See Lambert et al., *Measurement of Aqueous Glucose in a Model Anterior Chamber Using Raman Spectroscopy*, Jnl. Of Raman Spectroscopy (In Press, 2002). Spectra for both experiments are shown in FIGS. **31**A and **31**B.

FIG. **31**A illustrates the Raman spectra of artificial aqueous humor (AAH) and components of a physical model of the anterior chamber collected using confocal optics. FIG. **31**B illustrates the in vivo Raman spectra of structures of the anterior chamber of a rabbit. Raman spectra of AH can clearly be distinguished from those of nearby cornea and lens.

Mathematical Models for Calculating Analyte Concentration from Measured Spectra

A variety of chemometric methods have been developed to derive quantitative information from the spectrum of an aqueous test sample having a mixture of constituents. In general, chemometric models are developed using Raman spectra acquired from a set of training samples where the concentration of one or more of the constituents in each sample is known. Calibration models are developed and subsequently used to predict the concentrations of the constituents within sample unknowns for which these models were developed. A summary of the commonly used linear methods is presented below. Review articles are available which discuss these methods in detail. See, e.g., Geladi et al., *Partial least squares regression: A tutorial*, Analytica Chimica Acta. 1986, 185: 1–17; and Haaland et al., *Partial least squares methods for spectral analysis. Relation to other quantitative calibration methods and the extraction of qualitative information*, Anal. Chem. 1988: 60; 1193–1210.

The vast calibration models developed to date are linear and therefore implicitly assume that the Beer-Lambert law is valid for the samples being utilized on the training or test set. The Beer-Lambert law states that there must be a linear relationship between the concentrations of the constituents and the amount of Raman light scattered at a given wavelength from each constituent. For any given sample the observed Raman signature is a linear combination of the spectra of its constituents. The Beer-Lambert law may not be valid when constituents react with each other or when they interact with the solvent. For example, some of the Raman peaks for ethanol in aqueous solution shift as the concentration of ethanol is increased. However, at concentrations in the range of interest (5–500 mg/dL), this effect is smaller than the resolution of most Raman instruments (5 cm^{-1}). Even for high concentrations of ethanol, selected regions of the spectra in which the spectral shifts occur may be excluded from analysis to maintain the validity of the linear model.

Alternatively, a piecewise linear calibration model can be developed over several concentration ranges, where the Beer-Lambert law remains valid over each range. Generally stated, linear chemometric methods can be divided into two types of algorithms: explicit and implicit methods. Explicit methods are mathematical models that employ knowledge of the spectra of every constituent. Explicit methods include linear regression, classical least squares (CLS) and ordinary least squares (OLS). These methods are generally capable of producing superior calibration models. However, significant performance degradation can occur if a constituent is omitted from the calibration model or in the presence of spectral artifacts such as baseline shifts. Employing explicit methods in complex biological fluids such as aqueous humor can be difficult in general, since many constituents may be involved. To generate a reliable calibration model using these techniques, a chemical assay of each and every constituent in the training set should be used. If the constituents in the training set are not completely known, errors in the solution of the model may result.

Implicit methods utilize information only from the constituent of interest. These methods do not deduce any of the pure spectra present within a test sample. They can compute the concentration of the constituent of interest within the test sample directly from information derived from a training set of mixture spectra with a known value of concentration for the constituent of interest. Care should be taken in developing implicit calibration models to ensure that other constituents in the training set are not collinearly related to the constituent of interest. Partial least squares (PLS) and principal component analysis (PCA) followed by principal component regression (PCR) are well known implicit methods that can provide good performance in the assessment of ethanol and/or its metabolites or other analyte of interest in applications evaluating a selected region of the eye. PLS and PCR calibration models employ a set of mixture spectra from typical samples of the fluid of interest along with the concentration of the constituent of interest.

Knowledge of the pure spectra of the constituent(s) of interest, particularly ethanol and/or its metabolites, is relatively easily obtained. However, the implicit methods discussed above may not be able to incorporate this information in their calibration models. The explicit models can incorporate this information, but spectral information from a plethora of other possible contaminants may make implementation difficult. Andrew Berger recently developed a method known as hybrid linear analysis (HLA), which has been shown to provide better accuracy than PLS with a mixture of glucose, lactic acid, and creatinine ranging in concentration from 0 to 66 mM. See Berger et al., *An enhanced algorithm for linear multivariate calibration*, Anal. Chem. 1998; 70: 623–627, the contents of which are hereby incorporated by reference as if recited in full herein.

PLS and HLA were used to predict glucose levels in whole blood and serum with reasonable success with a standard error of prediction (SEP)=21 mg/dL for PLS and SEP=17 mg/dL for HLA. See Berger et al., *Multi-component Blood Analysis by Near-Infrared Raman Spectroscopy*, Applied Optics, 1999: 38: 2916–2926, the contents of which are hereby incorporated by reference as if recited in full herein.

PLS and/or HLA can be used as linear models to solve for the concentration of ethanol in a selected region in the eye, such as in the aqueous humor. Both PLS and HLA calibration models are likely to perform well in predicting certain substances, such as, but not limited to, ethanol, in physiological fluids. PLS can be extended to solve for additional analytes by applying it separately for each analyte of interest. HLA may be extendable algorithmically to include multiple pure spectra (e.g. ethanol, acetaldehyde, and acetate) within an enhanced calibration model for the analysis of complex mixtures.

Notwithstanding the above, as before, in certain embodiments, the model can analyze one, and typically a plurality of selected segments (typically relatively narrow regions of less than about 200 cm^{-1}, and more typically less than about 100 cm^{-1} in width) in the Raman spectrum can to determine the concentration levels in a subject. The regions used to assess concentration can be selected so as to exclude regions that are associated with spectral shift that may occur at increased concentration levels to thereby provide reliable concentration data over a broader in vivo range of probable bio-concentrations.

In Vitro Raman Ethanol Assay of "Artificial" Aqueous Humor

Fifteen samples of artificial aqueous humor were prepared with physiologic levels of glucose (97 mg/dl), ascorbate (16 mg/dl), lactate (84 mg/dl), and urea (36 mg.dl) in normal saline. Ethanol was added to these samples to achieve a final ethanol concentration ranging from 0 to 0.5%. Raman spectra were acquired for each of the 15 samples in quartz cuvettes. Eleven Raman spectra of each sample were collected using the Kaiser 785 nm spectrometer. The incident power of 15 mW for 20 seconds was utilized. The spectrum of each sample was normalized by dividing each spectra by the area of the hydrogen bonding region in the 400–600 cm^{-1} range. Each collected spectrum was then subtracted from a spectrum of artificial aqueous humor with 0% ethanol (FIG. **32**).

A PLS training model was developed using 10 Raman spectra collected for each of 15 samples. The eleventh spectra collected for each sample was treated as an "unknown" and its concentration was predicted using the PLS training model. Using only two factors in this model, the standard error of prediction (SEP) was 23 mg/dL, and the correlation coefficient (R2) was 0.99935. Additionally, all samples were incorporated into a PLS model and tested using cross-validation by using a leave-one-out round robin approach. The Standard error of cross-validation (SECV) and correlation coefficient were 0.019 mg/dL and 0.9991, respectively. Predicted (Raman measured) vs. actual ethanol concentrations are shown in FIG. **33**.

FIG. **32** illustrates normalized spectra for various levels of ethanol in artificial aqueous humor at wave numbers ranging from 700–1700 cm^{-1} and amplitude (in arbitrary units). FIG.

32 shows the normalized mixture spectrum and pure spectra of ethanol and its metabolites, acetaldehyde and acetate, mixed in high equimolar quantities so the spectral features and relative Raman cross section of each could be easily compared (measured concentration versus actual concentration of % ethanol). FIG. **33** is a graph of PLS-measured ethanol concentration plotted with respect to actual ethanol concentration in aqueous solution.

FIG. **34** illustrates the Raman spectra for 0.333M aqueous solutions of ethanol, acetaldehyde, acetic acid, and mixed metabolites across a range of wave numbers between 700–1700 with amplitude in arbitrary units. FIG. **34** shows that the Raman spectra for 0.333M aqueous solutions of ethanol, acetaldehyde, and acetic acid each have similar Raman cross-sections. The spectrum of a mixture of the three metabolites is a sum of the individual spectra, demonstrating adherence to the Beer-Lambert law.

Mathematical addition of the spectra of the pure constituents results in a curve that is virtually identical to the measured spectra of the mixed sample. Therefore, the mixture of these constituents obeys the Beer-Lambert law in aqueous solution. Thus, as contemplated by embodiments of the present invention, PLS and/or HLA models can measure the concentrations of ethanol and/or its metabolites in the spectral sample from a signal of mixed constituents (to determine blood and/or brain (or cerebral spinal fluid) concentration).

FIG. **35** is a block diagram of exemplary embodiments of data processing systems that illustrates systems, methods, and computer program products in accordance with embodiments of the present invention. The processor **310** communicates with the memory **314** via an address/data bus **348**. The processor **310** can be any commercially available or custom microprocessor. The memory **314** is representative of the overall hierarchy of memory devices containing the software and data used to implement the functionality of a data processing system **305**. The memory **314** can include, but is not limited to, the following types of devices: cache, ROM, PROM, EPROM, EEPROM, flash memory, SRAM, and DRAM.

As shown in FIG. **35**, the memory **314** may include several categories of software and data used in the data processing system: the operating system **352**; the application programs **354**; the input/output (I/O) device drivers **358**; module of a Raman spectral analyzer configured to analyze at least one selected analyte of interest by evaluating the spectra of a selected region in the eye **350**; and the data **356**. The data **356** can include reference Raman spectrum signal data of the selected analyte(s) at a variety of concentrations as well as the Raman spectrum data being interrogated **362** which may be obtained in substantially real-time from a Raman spectrometer or signal acquisition system **320**. As will be appreciated by those of skill in the art, the operating system **352** may be any operating system suitable for use with a data processing system.

The I/O device drivers **358** typically include software routines accessed through the operating system **352** by the application programs **354** to communicate with devices such as I/O data port(s), data storage **356** and certain memory **314** components and/or the image acquisition system **320**. The application programs **354** are illustrative of the programs that implement the various features of the data processing system and preferably include at least one application that supports operations according to embodiments of the present invention. Finally, the data **356** represents the static and dynamic data used by the application programs **354**, the operating system **352**, the I/O device drivers **358**, and other software programs that may reside in the memory **314**.

While the present invention is illustrated, for example, with reference to the Raman spectral anaylzer module **350** being an application program in FIG. **35**, as will be appreciated by those of skill in the art, other configurations may also be utilized while still benefiting from the teachings of the present invention. For example, the module **350** may also be incorporated into the operating system **352**, the I/O device drivers **358** or other such logical division of the data processing system **305**. Thus, the present invention should not be construed as limited to the configuration of FIG. **35**, which is intended to encompass any configuration capable of carrying out the operations described herein.

In certain embodiments, the module **350** includes computer program code for confirming the presence of and/or quantifying the concentration of at least one selected analyte of interest based on a predetermined statistical calibration model that identifies and analyzes certain particular wave numbers of interest. The module **350** may comprise at least one of: (a) partial least squares model; and (b) a hybridized linear model of the Raman spectra of the analyte of interest. The statistical model can be configured so that the module **350** evaluates a subset of the wave numbers (of pre-selected wave numbers) chosen so that they provide sufficient data about the amount of analyte present in the sample without requiring sampling of the entire wave number spectrum. In particular embodiments, the wave numbers can be in the range of between about 700–1700 cm^{-1}.

The module **350** can be configured to detect chemical signatures, or quantify levels in the blood and/or brain of selected analytes, including, but not limited to, ethanol and/or its metabolites, glucose, or other desired analyte. In particular embodiments, the module **350** includes a mathematical model for obtaining spectra and analyzing the spectra to determine the concentration (blood and/or brain) of ethanol, acetaldehyde, and acetic acid. The model may analyze a plurality of predetermined narrow (less than about 200 cm^{-1}, and typically less than about 100 cm^{-1}) segments in the Raman signal (selectively excluding regions that may shift due to increase or decrease in vivo concentration). The module **350** can include computer program code that can normalize the data by dividing the spectra of the ethanol, acetaldehyde and acetic acid by the area under the curve of a selected portion of the spectrum. In certain embodiments, the normalization is carried out using spectra data associated with the hydrogen-bonding region in the 400–600 cm^{-1} range of the collected Raman response signal. The module **350** can also include computer program code for evaluating the retina or iris for identity purposes as discussed above (the identification program code and equipment may be also held in a separate module).

The data/processor structure or devices shown and described with respect to FIG. **35** may also be used to implement other computer program products to carry out certain operations of any of the embodiments described herein.

An I/O data port can be used to transfer information between the data processing system **310** and the Raman spectrometer or signal acquisition system **320** or another computer system or a network (e.g., the Internet) or to other devices controlled by the processor. The computer program code with the module **350** may be held in a device proximate the spectrometer **320** or remotely. For remote configurations, the signal data may be wirelessly relayed for evaluation. These components may be conventional components such as those used in many conventional data processing systems, which may be configured in accordance with the present invention to operate as described herein.

While the present invention is illustrated, for example, with reference to particular divisions of programs, functions and memories, the present invention should not be construed as limited to such logical divisions. Thus, the present invention should not be construed as limited to the configuration of FIG. **35** but is intended to encompass any configuration capable of carrying out the operations described herein.

The flowcharts and block diagrams of certain of the figures herein illustrate the architecture, functionality, and operation of possible implementations of probe cell estimation means according to the present invention. In this regard, each block in the flow charts or block diagrams represents a module, segment, or portion of code, which comprises one or more executable instructions for implementing the specified logical function(s). It should also be noted that in some alternative implementations, the functions noted in the blocks may occur out of the order noted in the figures. For example, two blocks shown in succession may in fact be executed substantially concurrently or the blocks may sometimes be executed in the reverse order, depending upon the functionality involved.

In summary, embodiments of the present invention are directed to intermittent, semi-continuous, or continuous monitoring of systemic levels of analytes of interest, such as ethanol and its metabolites, with Raman spectroscopy. This can be with techniques that are non-invasive or minimally invasive. In addition, the devices used to carry out the interrogation of the biosample (i.e, AH) can be miniaturized for portability. These devices can have the capability of real-time data recording and telemetry for communication with integrated data analysis systems. Such systems, with remote data analysis, can allow research on human behavior, physiology, evaluation of the efficacy of drug and social therapies with regard to inhibiting or preventing alcohol consumption and the like.

The foregoing is illustrative of the present invention and is not to be construed as limiting thereof. Although a few exemplary embodiments of this invention have been described, those skilled in the art will readily appreciate that many modifications are possible in the exemplary embodiments without materially departing from the novel teachings and advantages of this invention. Accordingly, all such modifications are intended to be included within the scope of this invention as defined in the claims. In the claims, means-plus-function clauses, where used, are intended to cover the structures described herein as performing the recited function and not only structural equivalents but also equivalent structures. Therefore, it is to be understood that the foregoing is illustrative of the present invention and is not to be construed as limited to the specific embodiments disclosed, and that modifications to the disclosed embodiments, as well as other embodiments, are intended to be included within the scope of the appended claims.

The statements characterizing one or more of the priority applications as a "continuation-in-part" application of a prior application listed under the "Related Applications" section above is used to indicate that additional subject matter was added to the specification of the prior application but does not necessarily mean that the entire invention described and claimed in the present application is not supported in full by the prior application(s).

The invention is defined by the following claims, with equivalents of the claims to be included therein.

We claim:

1. A non-invasive method for identifying the presence of and/or the concentration of a selected analyte in a subject, comprising:

illuminating an aqueous humor of a subject with an optical excitation beam, wherein the excitation beam wavelength is selected to generate a resonant Raman spectrum of the selected analyte;

detecting a resonant Raman spectrum corresponding to the illuminated aqueous humor; and

identifying the presence, absence and/or the concentration of the selected analyte in the subject based on said detecting step.

2. A method according to claim **1**, wherein the wavelength is between about 400 nm–600 nm.

3. A method according to claim **1**, wherein the selected analyte is a chemotherapeutic agent.

4. A method according to claim **1**, wherein the selected analyte is an antifungal agent.

5. A method according to claim **1**, wherein the selected analyte has a chemical structure having at least one of a carbon-carbon bond and/or a carbon double bond, the method further comprising monitoring at least one peak of interest in the detected Raman spectrum associated with the carbon-carbon bond and/or the double carbon bond of the selected analyte chemical structure.

6. A method according to claim **1**, wherein the selected analyte has a chemical structure having a carbon double bond, the method further comprising monitoring the detected Raman spectrum associated with the carbon double bond of the selected analyte chemical Structure, and wherein the excitation wavelength is between about 400–514 nm.

7. A method according to claim **6**, wherein the selected analyte comprises amphotercin B or a substantial chemical equivalent, and/or metabolites, constituents or derivatives thereof.

8. A method according to claim **6**, wherein the selected analyte comprises fenretinide or a substantial chemical equivalent, and/or metabolites, constituents or derivatives thereof.

9. A method according to claim **1**, wherein the excitation wavelength is between about 400–408 nm.

10. A method according to claim **1**, further comprising:

comparing the Raman spectrum from said detecting step to a priori reference spectra corresponding to different concentrations of a plurality of different concentrations of the selected analyte.

11. A method according to claim **1**, wherein the selected analyte comprises at least one selected drug, pharmaceutical agent, metabolite, derivative or constituent thereof, and wherein the step of identifying comprises determining a concentration level in the micromolar range.

12. A method according to claim **1**, wherein the selected analyte comprises at least one selected drug, pharmaceutical agent, metabolite, derivative or constituent thereof, and wherein the step of identifying comprises determining a concentration level in the nanomolar range.

13. A method according to claim **1**, wherein the identifying step comprises analyzing selected Raman spectra residing within 700–1700 cm^{-1} of the resonant Raman spectrum to determine the presence and/or concentration of the selected analyte.

14. A method according to claim **13**, wherein a plurality of selected narrow regions in the resonant Raman spectrum are analyzed to determine the concentration of the analyte in the subject.

15. A method according to claim **1**, wherein the identifying step comprises determining a blood level concentration of the selected analyte in the subject.

16. A method according to claim **1**, wherein the identifying step comprises determining a brain or cerebral spinal fluid concentration of the selected analyte in the subject.

17. A non-invasive method for identifying the presence of and/or the concentration of a selected analyte in a subject, comprising:

illuminating a selected region in the eye of a subject with an optical excitation beam, wherein the excitation beam wavelength is selected to generate a resonant Raman spectrum of the selected analyte;

detecting a resonant Raman spectrum corresponding to the selected illuminated region of the eye; and

identifying the presence, absence and/or the concentration of the selected analyte in the subject based on said detecting step,

wherein the detecting step is carried out to obtain a first resonant Raman spectrum of the subject before administering a selected treatment and then to obtain a second resonant spectrum after administration of the selected treatment, and wherein the identifying step comprises subtracting baseline signatures of data from the second resonant Raman spectrum using data from the first resonant Raman spectrum.

18. An evaluation apparatus for identifying the presence and/or concentration of a selected analyte in a subject, comprising:

means for illuminating an aqueous humor with an optical excitation beam, wherein the optical excitation beam has a wavelength that is at least about 400 nm, resides within the absorption spectrum, and that generates a resonant Raman spectrum of a selected analyte in the aqueous humor of a subject;

means for focusing the excitation beam into the subject so that the aqueous humor is illuminated;

means for detecting a resonant Raman spectrum of the selected analyte from the illuminated aqueous humor; and

means for identifying the presence and/or concentration of the selected analyte in the subject based on data obtained from the detecting means.

19. An apparatus according to claim **18**, wherein the means for identifying includes computer program code that determines the concentration level of the selected analyte using statistical analytical partial least squares.

20. An apparatus according to claim **18**, wherein the means for identifying includes computer program code that determines the concentration level of the selected analyte using statistical analytical hybrid linear analysis.

21. An apparatus according to claim **18**, wherein the apparatus is configured to detect micromolar ranges of concentrations of the selected analyte in the aqueous humor.

22. An apparatus according to claim **18**, wherein the apparatus is configured to detect nanomolar ranges of concentrations of the selected analyte in the aqueous humor.

23. An apparatus according to claim **18**, wherein the apparatus is configured to determine blood concentrations of the selected analyte.

24. An apparatus according to claim **18**, wherein the apparatus is configured to determine brain, intracranial, and/or cerebral spinal fluid level concentrations.

25. An apparatus according to claim **18**, wherein the selected analyte is a pharmaceutical agent.

26. An apparatus according to claim **18**, wherein the means for illuminating is configured to generate an excitation signal wavelength that is between about 400 nm–600 nm.

27. An apparatus according to claim **26**, wherein the excitation wavelength is between about 400–514 nm.

28. An apparatus according to claim **27**, wherein the excitation wavelength is between about 400–408 nm.

29. An apparatus according to claim **18**, wherein the selected analyte is a chemotherapeutic agent.

30. An apparatus according to claim **18**, wherein the selected analyte is an antifungal agent.

31. An apparatus according to claim **18**, wherein the selected analyte has a chemical structure having at least one of a carbon-carbon bond and/or a carbon double bond, the apparatus comprising computer program code for analyzing at least one peak of interest in the detected resonant Raman spectrum associated with the carbon-carbon bond and/or the carbon double bond of the selected analyte chemical structure.

32. An apparatus according to claim **18**, wherein the selected analyte comprises amphotercin B or a substantial chemical equivalent and/or metabolites, constituents, or derivatives thereof.

33. An apparatus according to claim **18**, wherein the selected analyte comprises fenretinide or a substantial chemical equivalent and/or metabolites, constituents, or derivatives thereof.

34. An apparatus according to claim **18**, wherein the means for identifying includes computer program code for comparing the resonant Raman spectrum to a priori reference spectra corresponding to different concentrations of a plurality of different concentrations of the selected analyte.

35. An apparatus according to claim **34**, wherein the reference spectra comprise a plurality of different resonant Raman spectra generated for the selected analyte at different concentrations based on in vitro samples thereof.

36. An evaluation apparatus for identifying the presence and/or concentration of a selected analyte in a subject, comprising:

means for illuminating a selected region of the eye with an optical excitation beam, wherein the optical excitation beam has a wavelength that is at least about 400 nm, resides within the absorption spectrum, and that generates a resonant Raman spectrum of a selected analyte in a subject;

means for focusing the excitation beam into a selected region in the eve of the subject so that the selected region is illuminated;

means for detecting a resonant Raman spectrum corresponding to the illuminated selected region of the eye; and

means for identifying the presence and/or concentration of the selected analyte in the subject responsive to the detecting means

wherein the means for identifying comprises computer program code that competes a first resonant Raman spectrum of the subject taken before administering a selected treatment to a second resonant Raman spectrum taken after administration of the selected treatment, and subtracts baseline signature data from the second resonant Raman spectrum using data from the first resonant Raman spectrum.

37. An apparatus according to claim **18**, wherein the means for identifying comprises computer program code for analyzing selected Raman spectra residing within 700–1700 cm^{-1} of the resonant Raman spectrum to determine the presence and/or concentration of the selected analyte.

38. An apparatus according to claim **18**, wherein the means for identifying comprises computer program code in a computer readable medium that analyzes a plurality of selected narrow regions in the resonant Raman spectrum to determine the concentration of the analyte in the subject.

39. An apparatus according to claim **18**, wherein the means for illuminating is configured to confocally deliver the excitation beam to the aqueous humor of the eye of the subject.

40. An apparatus according to claim **18**, wherein said means for identifying comprises computer program code for sampling selected wave numbers of the resonant Raman spectrum using a partial least squares statistical model.

41. An apparatus according to claim **18**, wherein said means for identifying comprises computer program code for sampling selected wave numbers of the resonant Raman spectrum using a hybrid linear analysis statistical model.

42. An apparatus according to claim **18**, wherein said excitation beam has a power rating of about 2 mW that illuminates the aqueous humor for about 10 seconds.

43. An apparatus according to claim **18**, wherein the means for identifying comprises computer program code that subtracts a fluorescence spectrum from the resonant Raman spectrum to produce a difference spectrum.

44. An apparatus according to claim **18**, wherein the apparatus is configured to detect a plurality of different selected analytes of interest.

45. An apparatus according to claim **44**, further comprising computer program code of reference data of spectra for each analyte of interest comprising spectra generated from a Raman spectra training set based on at least 10 in vitro aqueous humor samples for each analyte of interest with each of the at least 10 samples including different amounts of the analyte therein, and an analyte level corresponding to each of the samples.

46. An apparatus according to claim **45**, wherein the computer program reference data are generated from a training set of Raman spectra corresponding to at least 20 aqueous humor samples of varying concentrations of analytes therein far each of a plurality of different selected analytes and an identified blood level, and brain or cerebral spinal fluid level of said analyte of interest for each of said samples.

47. An apparatus according to claim **18**, wherein said apparatus comprises a portable housing that holds the components therein, and wherein the housing includes a user interface that is configured and sized to fit onto and/or rest against the head of the subject during operation.

48. An apparatus according to claim **47**, wherein said housing is configured and sized to be a band held device.

49. A non-invasive method for identifying the presence and/or the concentration of a selected analyte in a subject and identifying the subject comprising:

- illuminating an aqueous humor of a subject with an optical excitation beam;
- detecting a resonant Raman spectrum corresponding to the aqueous humor;
- determining the presence, absence and/or the concentration of the selected analyte in the subject based on said detecting step;
- obtaining a biometric reading of the iris or retinal structure of at least one of the subject's eyes; and
- identifying the subject based on the biometric reading.

50. A non-invasive method according to claim **49**, wherein the obtaining step is carried out by obtaining an image of the iris and comparing the image to predetermined iris characteristics.

51. An apparatus for determining the presence of and/or the concentration of a selected analyte in a subject and confirming the identity the subject comprising:

- means for illuminating an aqueous humor of a subject with an optical excitation beam;
- means for detecting a resonant Raman spectrum corresponding to the aqueous humor;
- means for determining the presence and/or the concentration of the selected analyte in the subject responsive to said means for detecting; and
- means for obtaining a biometric reading of the iris or retinal structure to identify the subject.

52. A portable apparatus for determining the presence of and/or the concentration of a selected analyte in a subject and confirming the identity the subject comprising:

- a portable housing;
- an illumination source in the housing configured to generate an optical excitation beam into an aqueous humor of a subject;
- a detector in the housing configured to obtain a resonant Raman spectrum from the aqueous humor and determine the presence and/or the concentration of a selected analyte in the subject; and
- a biometric reader in the housing configured to interrogate an iris or retinal structure of the eye of the subject to identify the subject.

53. An apparatus according to claim **52**, wherein the biometric reader is configured to obtaining an image of the iris and comparing the image to predetermined iris characteristics.

54. A non-invasive method for identifying the presence of and/or the concentration of a selected analyte in a subject, comprising:

- illuminating a vitreous humor of a subject with an optical excitation beam, wherein the excitation beam wavelength is selected to generate a resonant Raman spectrum of the selected analyte;
- detecting a resonant Raman spectrum corresponding to the illuminated vitreous humor; and
- identifying the presence, absence and/or the concentration of the selected analyte in the subject based on said detecting step.

55. A method according to claim **54**, wherein die selected analyte is a chemotherapeutic agent.

56. A method according to claim **54**, wherein the selected analyte is an antifungal agent.

57. A method according to claim **54**, wherein the selected analyte has a chemical structure having at least one of a carbon-carbon bond and/or a carbon double bond, the method further comprising monitoring at least one peak of interest in the detected Raman spectrum associated with the carbon-carbon bond and/or the double carbon bond of the selected analyte chemical structure.

58. A method according to claim **54**, wherein the selected analyte comprises amphotercin B or a substantial chemical equivalent, and/or metabolites, constituents or derivatives thereof.

59. A method according to claim **54**, wherein the selected analyte comprises fenretinide or a substantial chemical equivalent, and/or metabolites, constituents or derivatives thereof.

60. A method according to claim **54**, wherein the detecting step is carried out to obtain a first resonant Raman spectrum of the subject before administering a selected treatment and then to obtain a second resonant spectrum after administration of the selected treatment, and wherein the identifying step comprises subtracting baseline signatures of data from the second resonant Raman spectrum using data from the first resonant Raman spectrum.

61. A method according to claim **54**, wherein a plurality of selected narrow regions in the resonant Raman spectrum are analyzed to determine the concentration of the analyte in the subject.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,961,599 B2
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INVENTOR(S) : Lambert et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 57,
Line 39, should read -- housing is configured and sized to be a hand held device. --.

Column 58,
Line 35, should read -- **55**. A method according to claim **54**, wherein the selected --.

Signed and Sealed this

Twenty-seventh Day of June, 2006

Jon W. Dudas

JON W. DUDAS
Director of the United States Patent and Trademark Office