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(71) Applicant(s):
National University of Singapore
(Incorporated in Singapore)
21 Lower Kent Ridge Road, 119077, Singapore

(72) Inventor(s):
Zhiwei Huang
Mads Sylvest Bergholt
Wei Zheng
Khek Yu Ho
Khay Guan Yeoh
Shiyamala Durapaipandian

(74) Agent and/or Address for Service:
Fresh IP
St John's Innovation Centre, Cowley Road,
Cambridge, CB4 0WS, United Kingdom

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WO 2006/064446 A1 **WO 1998/041825 A1**
WO 1993/003341 A1 **US 6621574 B1**
US 5638172 A

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(54) Title of the Invention: **Methods related to instrument-independent measurements for quantitative analysis of fiber-optic Raman spectroscopy**
Abstract Title: **Calibration of fibre-optic Raman spectroscopy system**

(57) A method of calibrating a fibre-optic Raman spectroscope system 20 comprising a laser source 21, a spectroscope 30 and a fibre-optic probe 11 to transmit light from the laser source to a target and return scattered light to the spectroscope, comprises transmitting light from the laser source to a standard target having a known spectrum, recording a calibration spectrum of the scattered light from the standard target, comparing the known spectrum and the calibration spectrum and generating a transfer function, and storing the transfer function. The transfer function is then used on further spectral measurements using the spectroscope system. A method of estimating laser power in the system and a method of subtracting a background signal from a Raman spectrum is also disclosed.

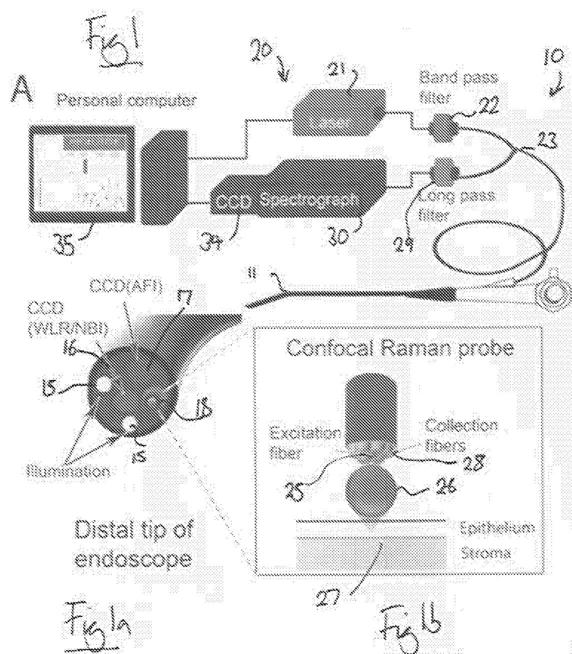

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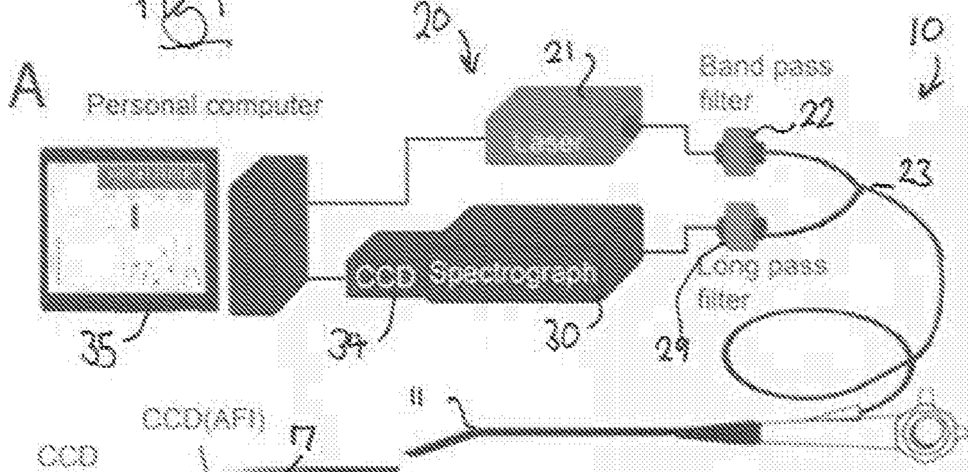
Fig 1



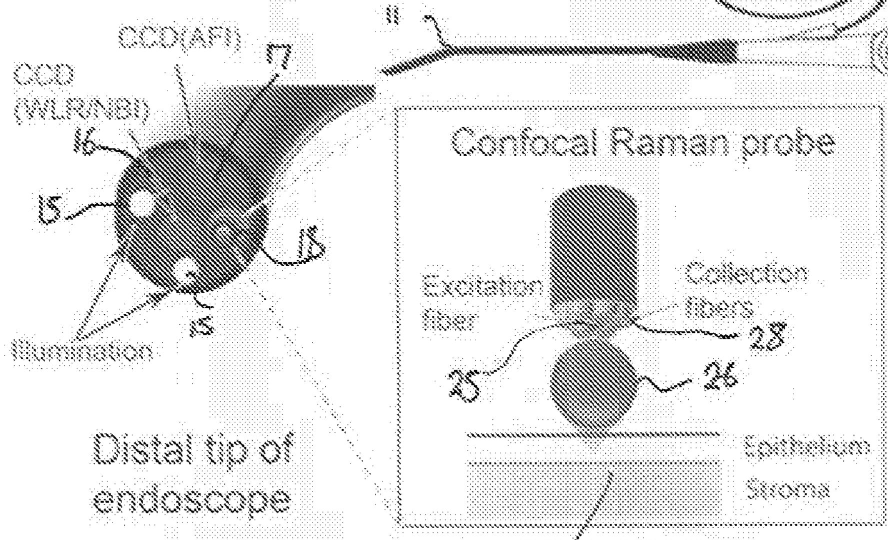


Fig 1a

Fig 1b

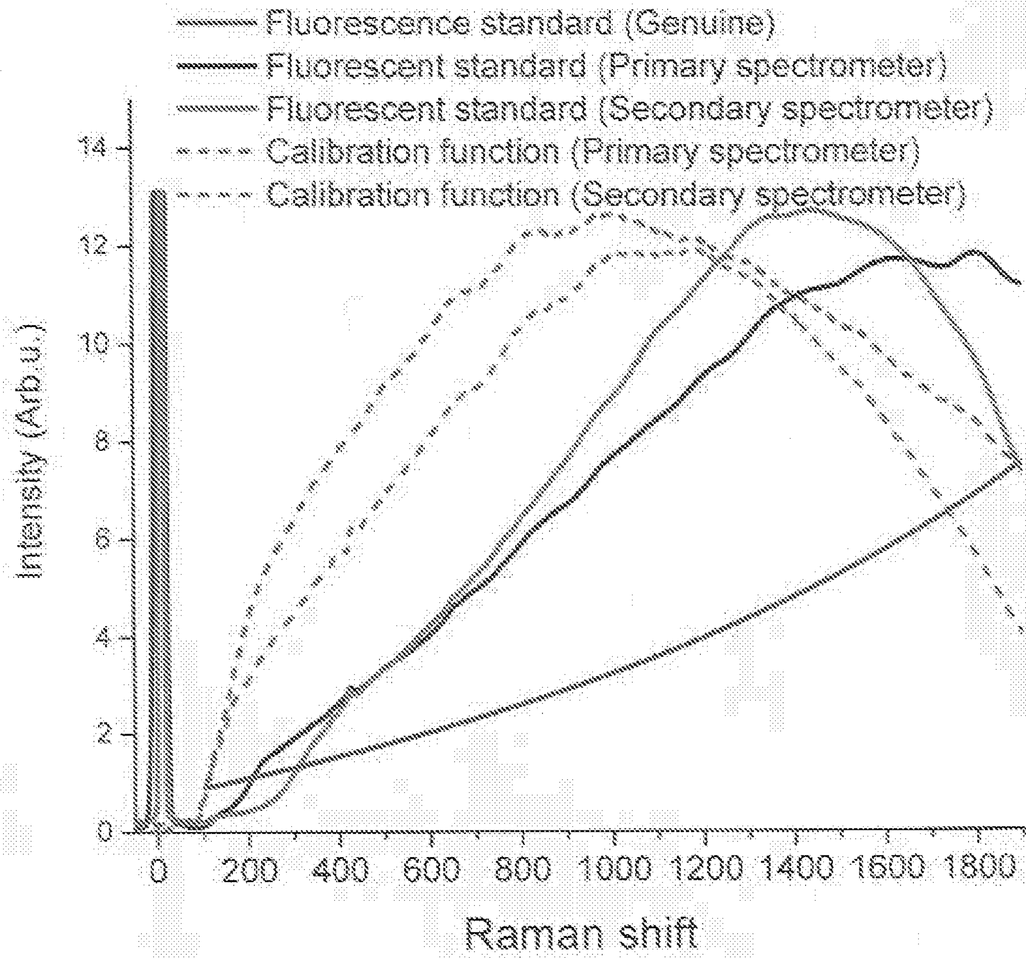
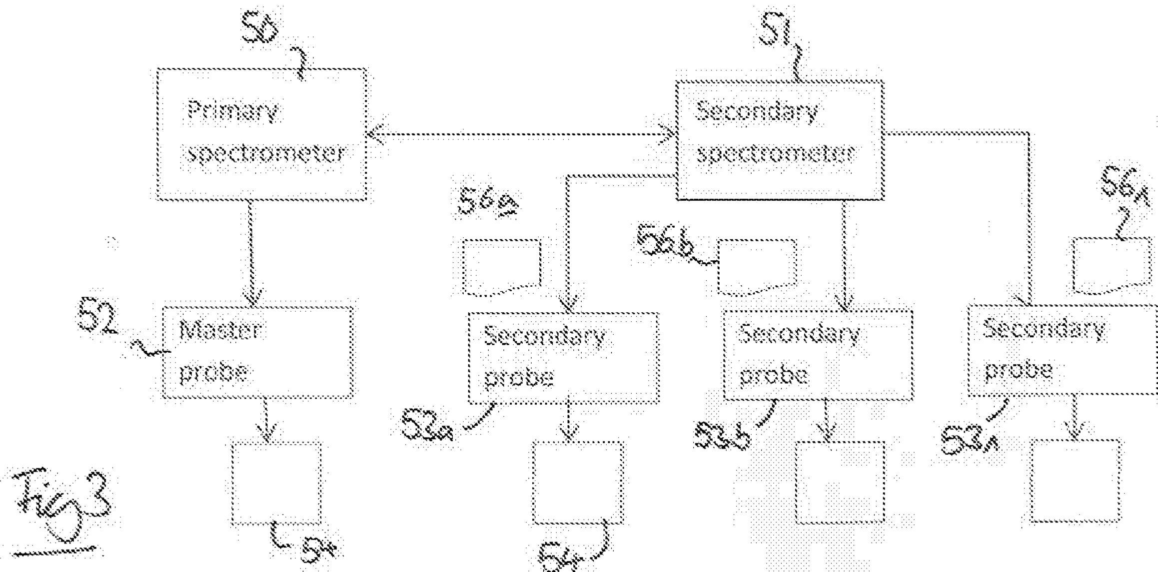
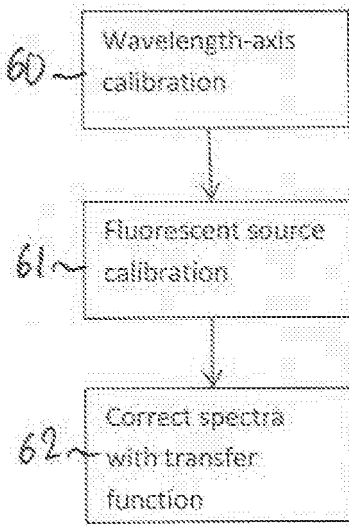
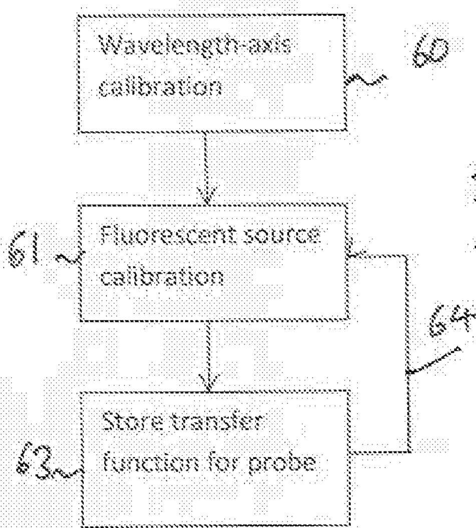
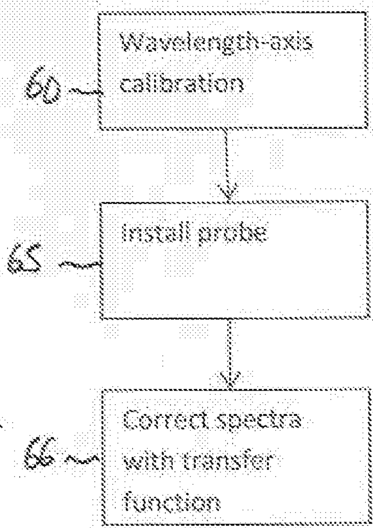


Fig.2


50
51
Primary spectrometer
Secondary spectrometer
56a
56n
56b
52
Master probe
Secondary probe
Secondary probe
Secondary probe
52a
53b
53n
54
54
Fig 3


60
Wavelength-axis calibration
61
Fluorescent source calibration
62
Correct spectra with transfer function
Fig 4a


Wavelength-axis calibration
60
Fig 4b
61
Fluorescent source calibration
64
63
Store transfer function for probe


Wavelength-axis calibration
60
Fig 4c
Install probe
65
66
Correct spectra with transfer function

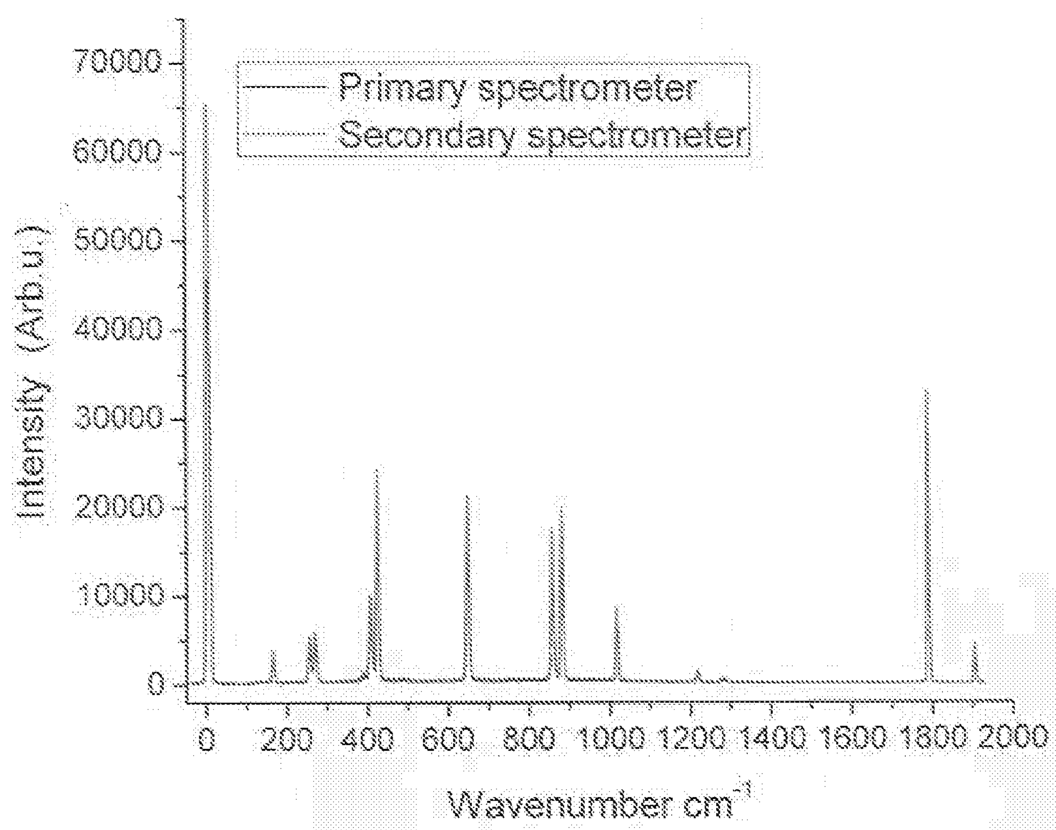


Fig. 5

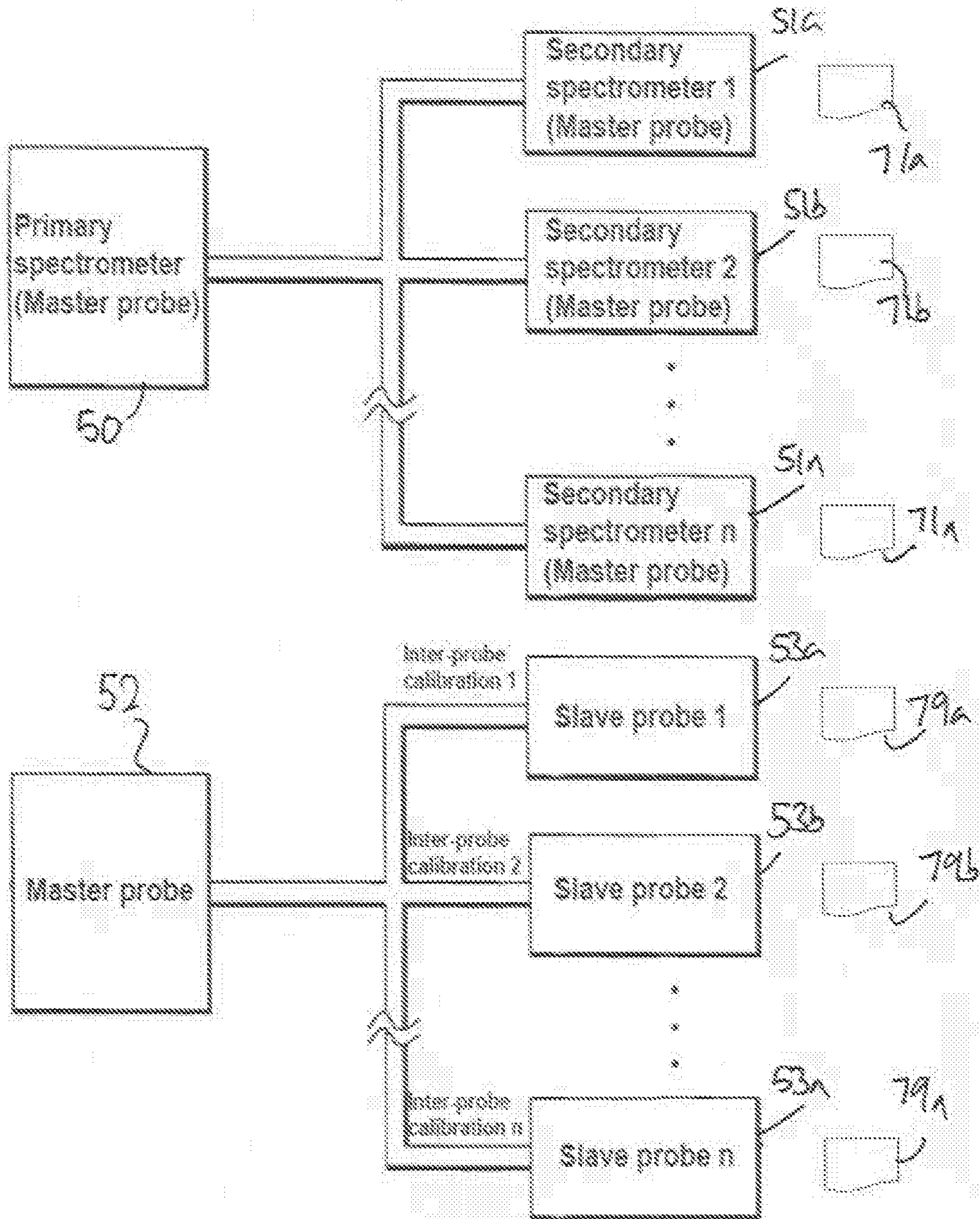


Fig 6

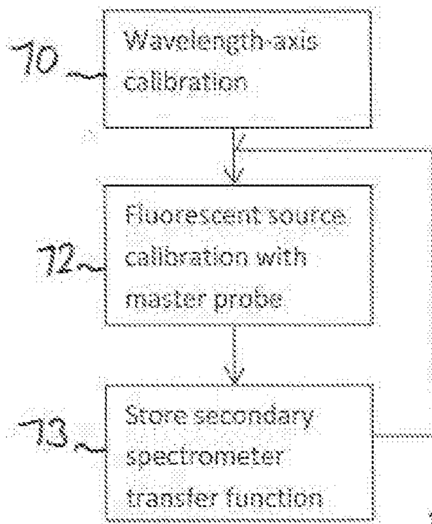


Fig 7a

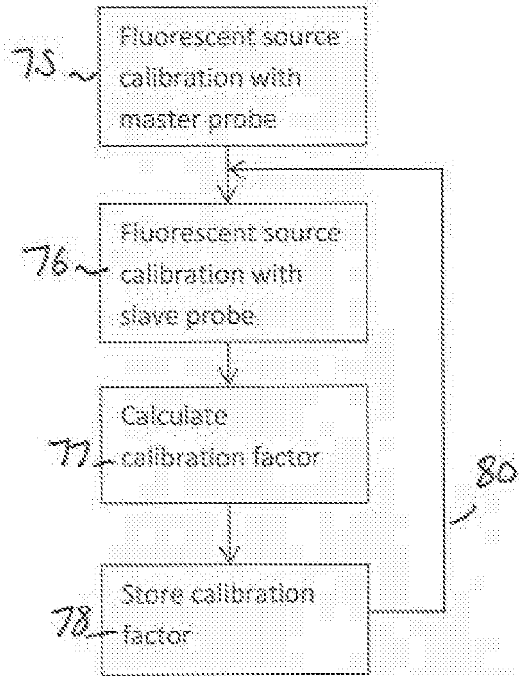


Fig 7b

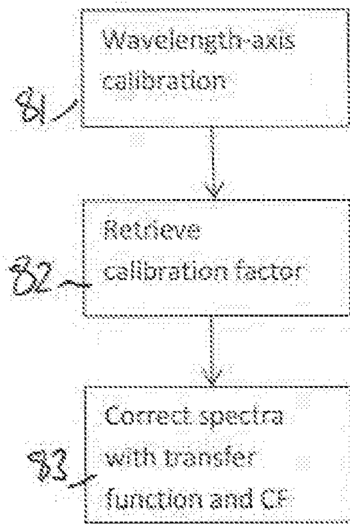


Fig 7c

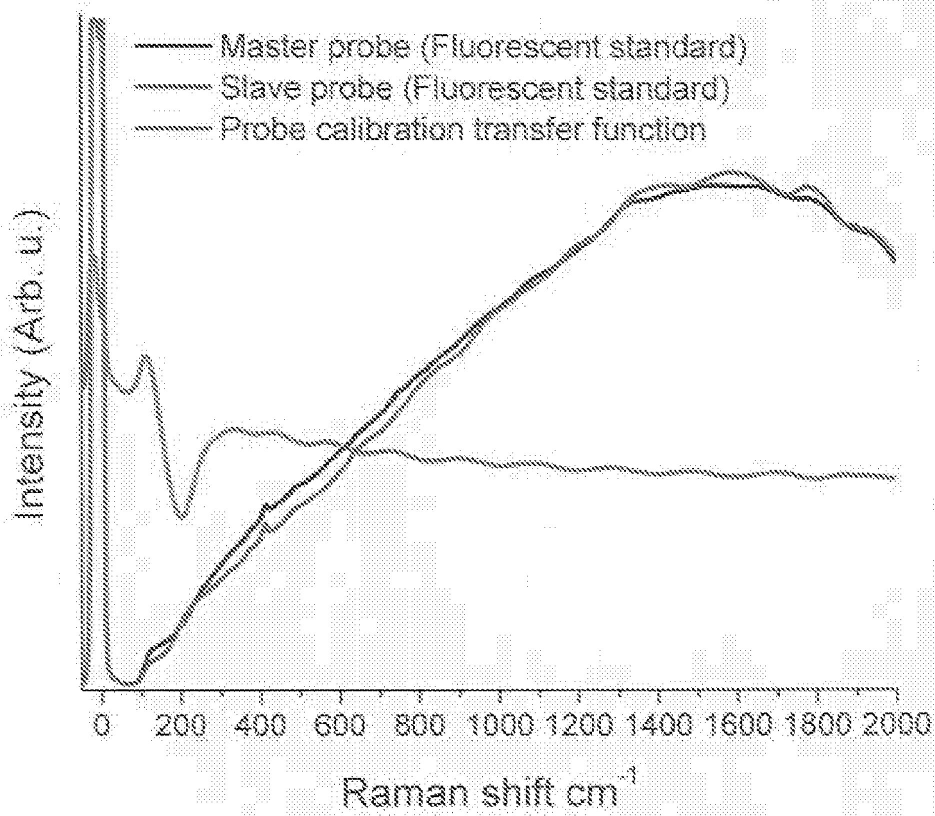


Fig. 8

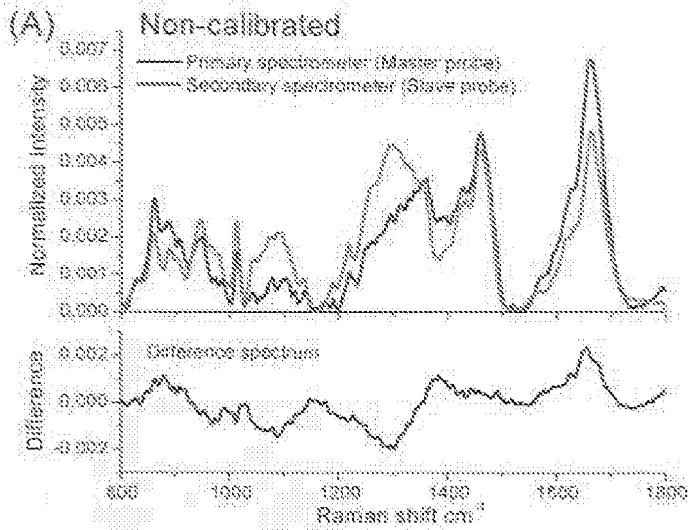


Fig. 9a

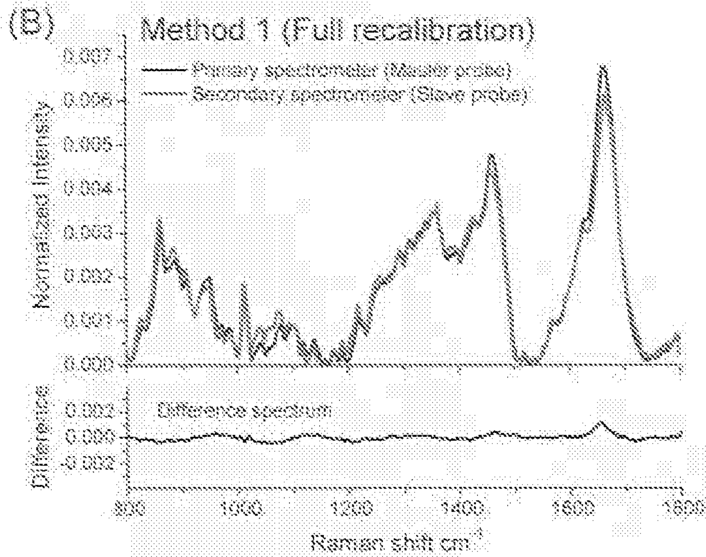


Fig. 9b

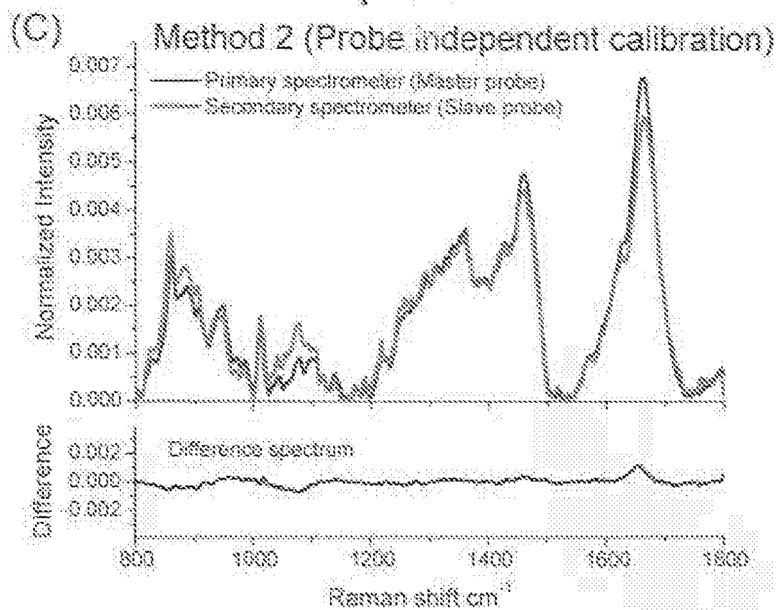


Fig. 9c

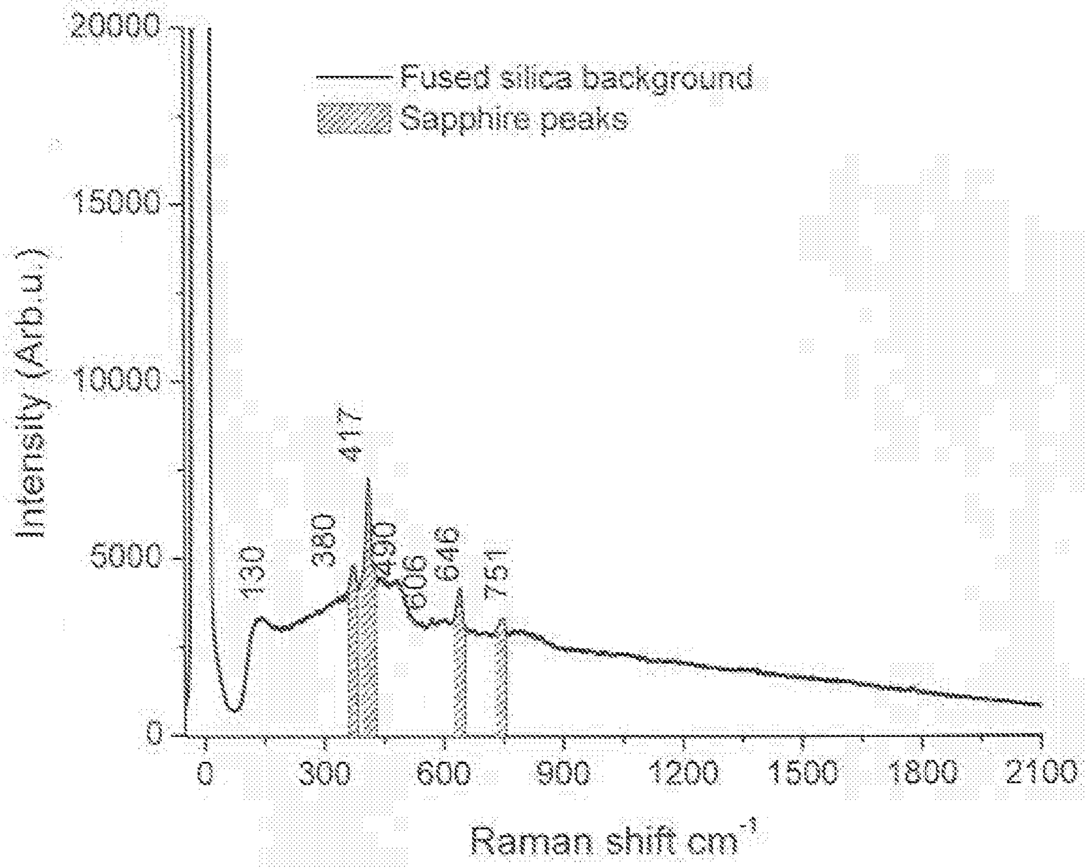


Fig. 10

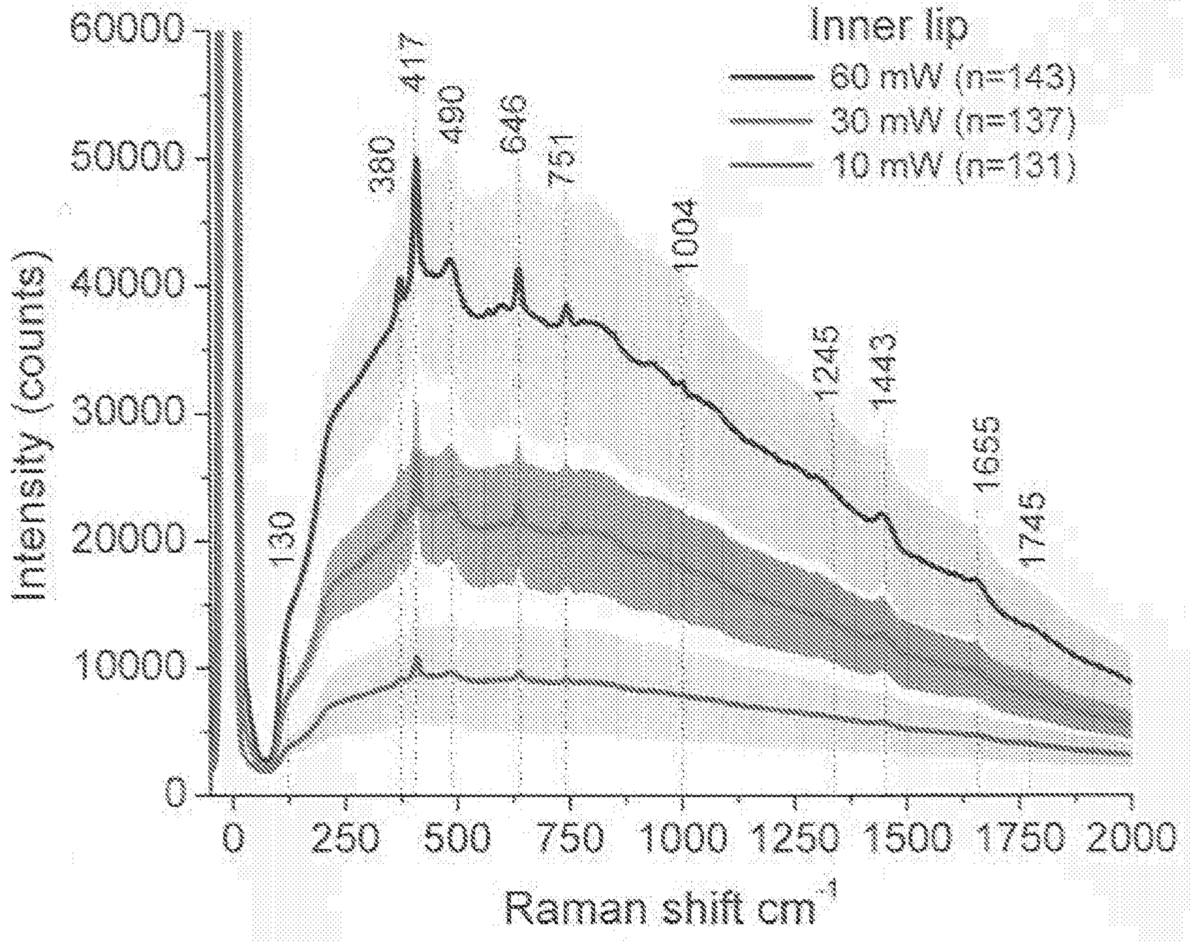


Fig. 11

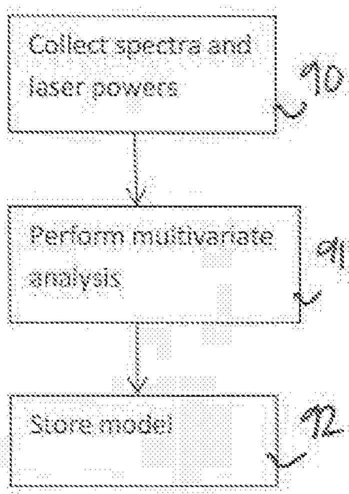


Fig 12a

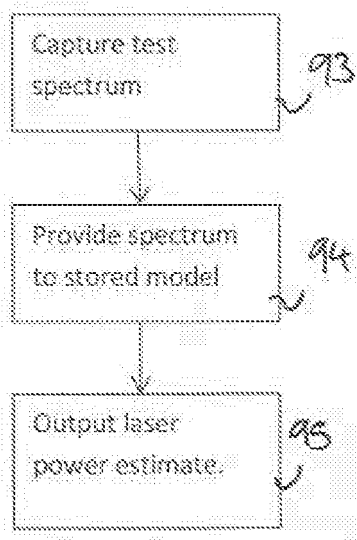


Fig 12b

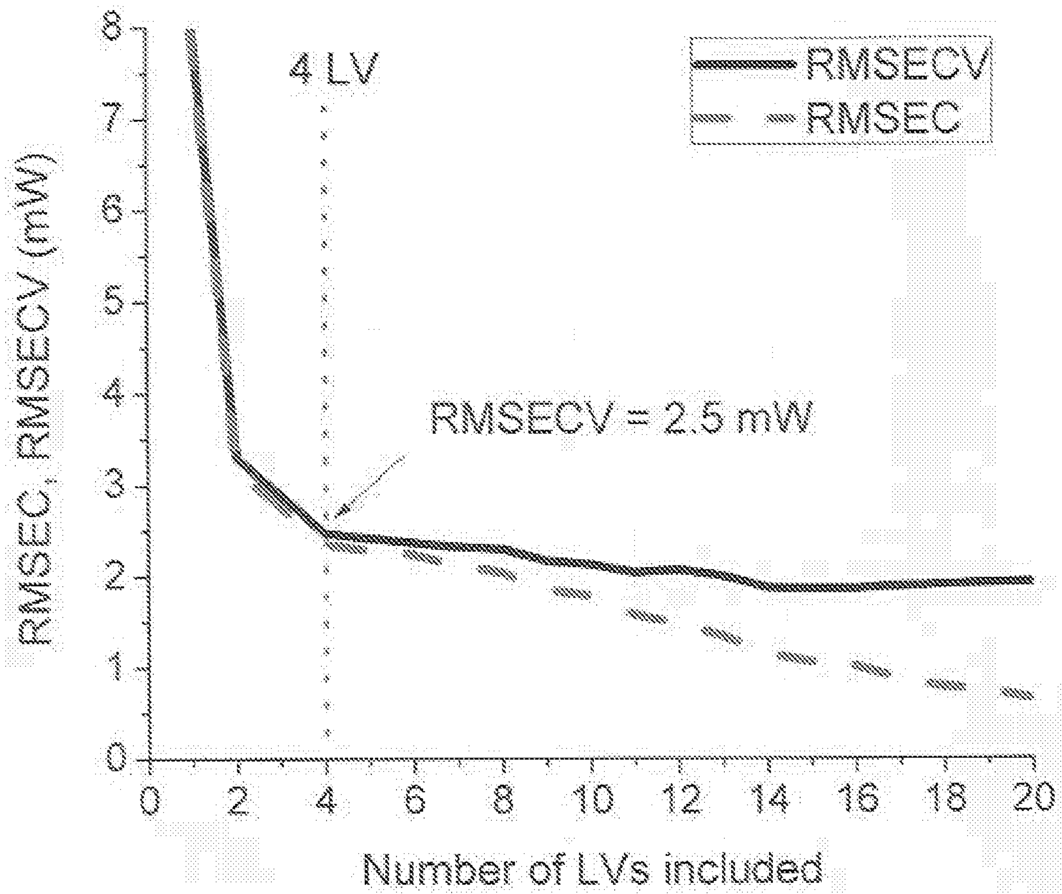


Fig. 13a

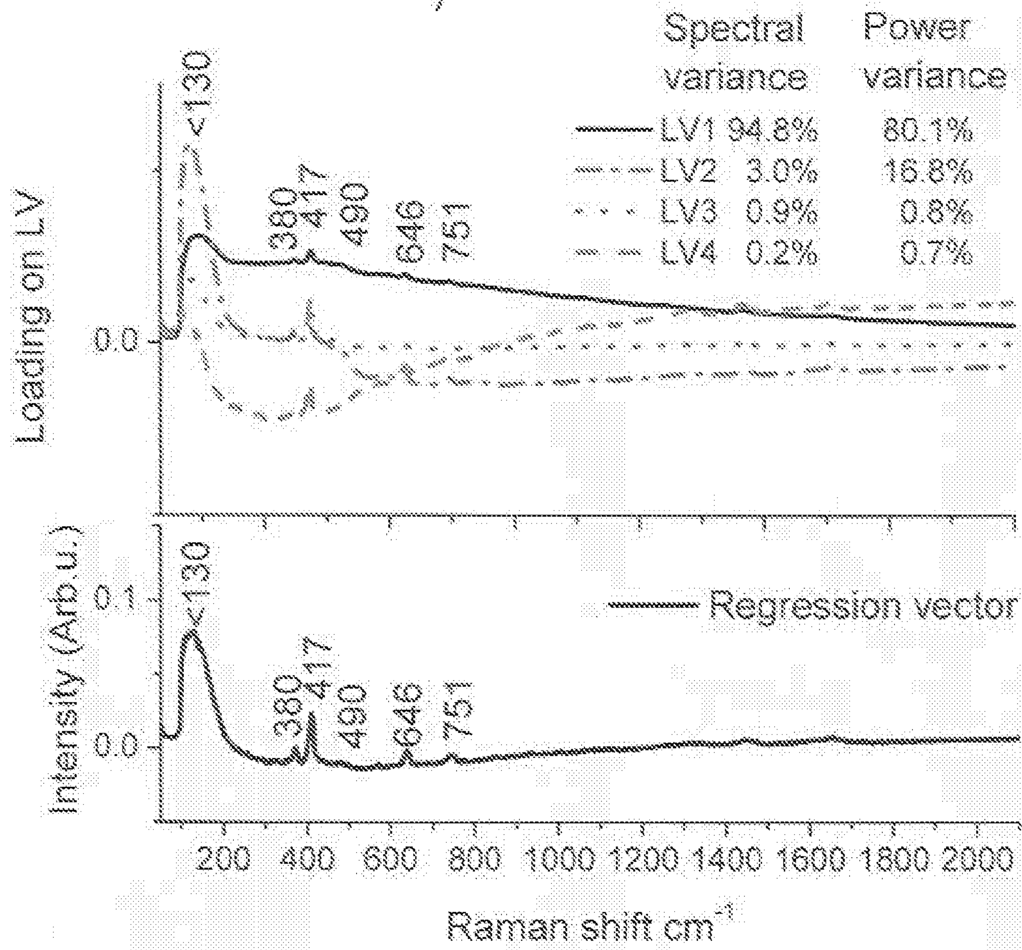


Fig. 13b

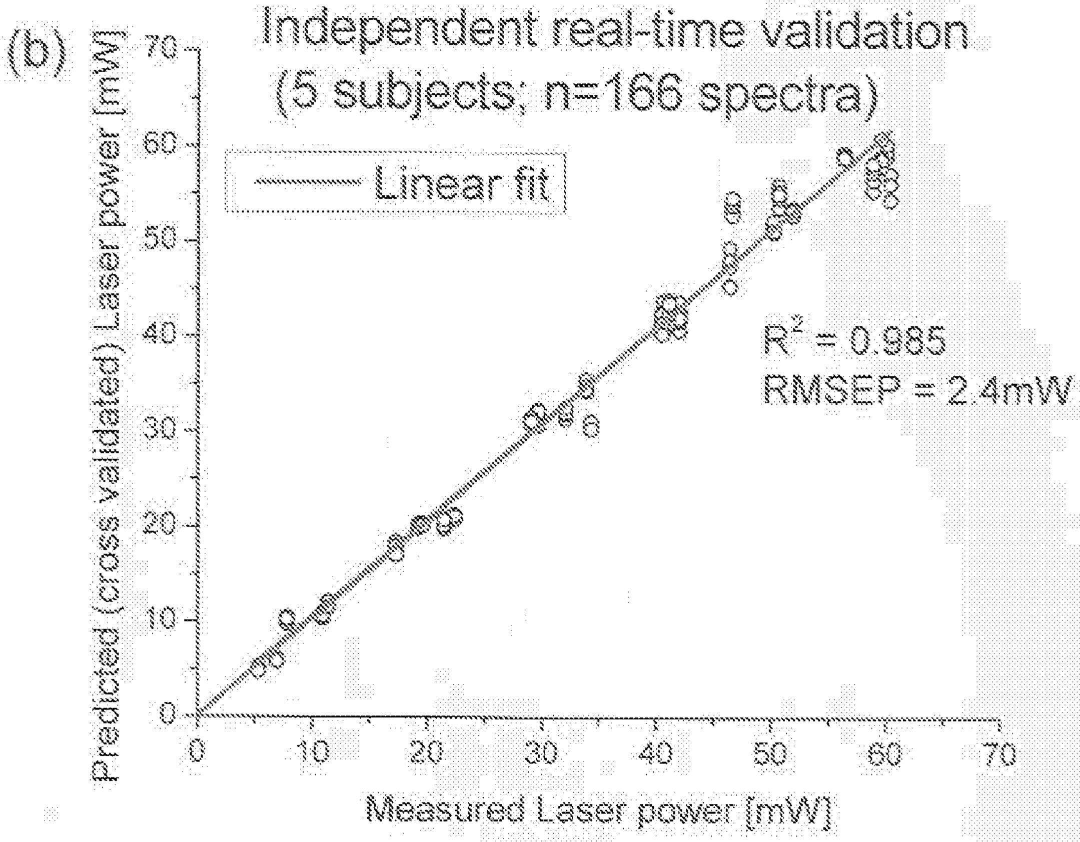


Fig. 14

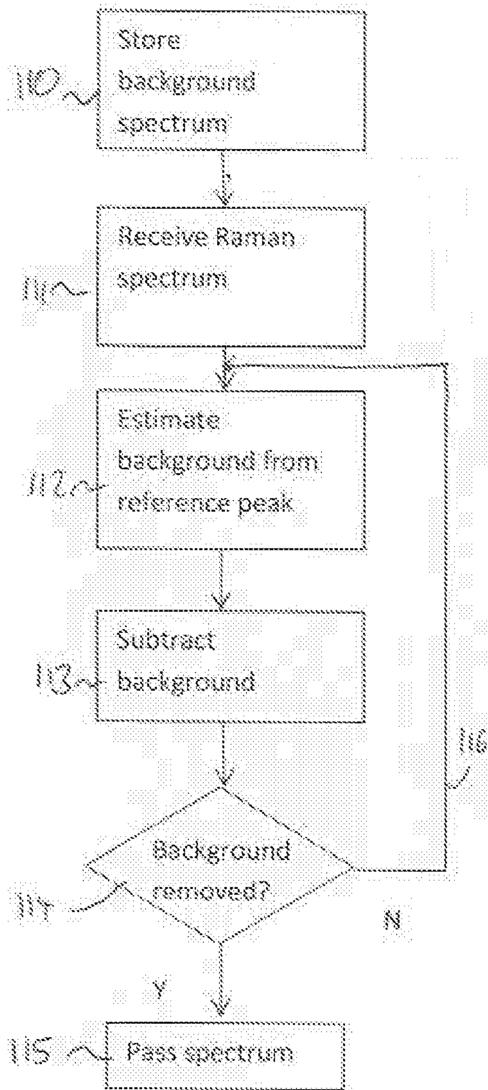


Fig 15

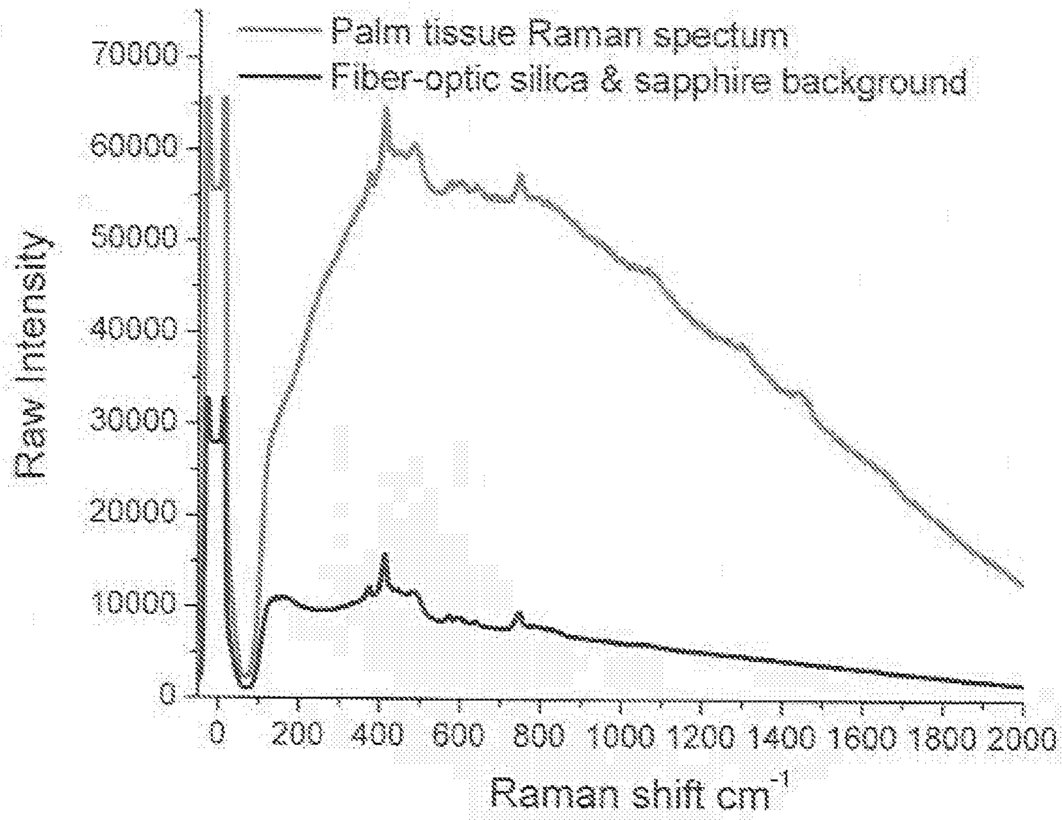


Fig. 16

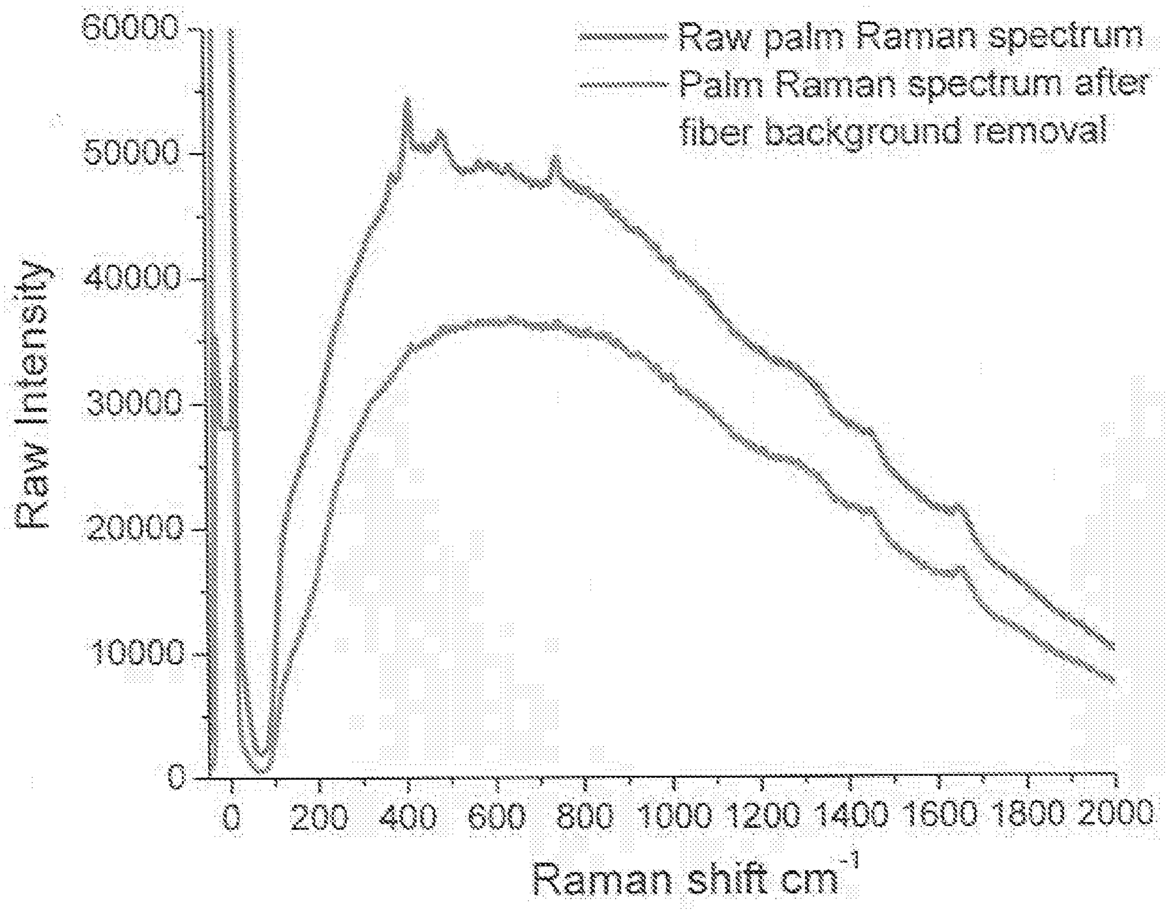


Fig. 17

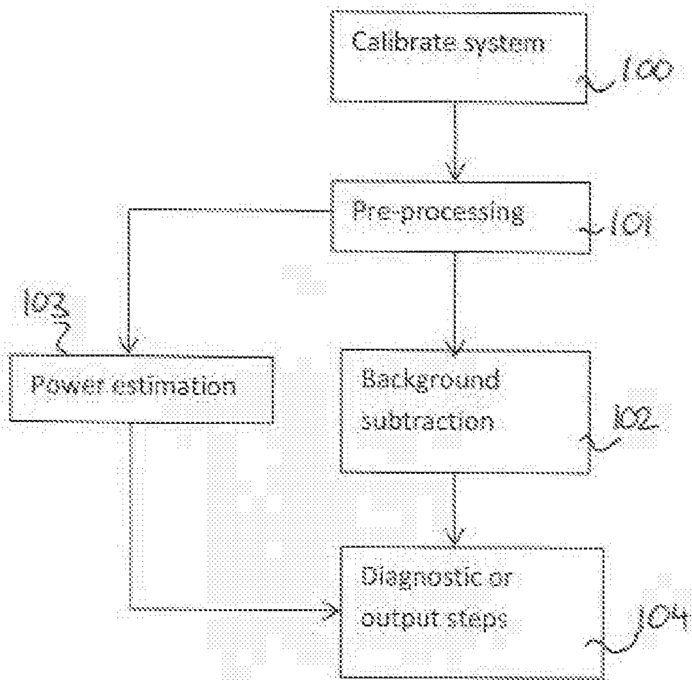


Fig 18

Methods related to instrument-independent measurements for quantitative analysis of fiber-optic Raman spectroscopy

[1] The present invention relates to methods for instrument-independent measurements for quantitative analysis in fiber-optic Raman spectroscopy. a method of calibrating multiple fiber-optic Raman spectroscopy systems, a method of calibrating multiple fiber-optic Raman spectroscopy systems independent of fiber-optic probes, a fiber-optic Raman spectroscopy system, a method of fiber probe background subtraction, a method of estimating laser power in a fiber-optic Raman spectroscopy system *in situ*, and a method of subtracting a background signal, particularly but not exclusively for biomedical measurements.

Background to the Invention

[2] Raman spectroscopy is a technique which uses inelastic or Raman scattering of monochromatic light. Conventionally, the monochromatic light source is a laser in the visible or near infrared ("NIR") range. The energy of the scattered photons is shifted up or down in response to interaction with vibrational modes or excitations in the illuminated material, varying the wavelength of the scattered photons. Accordingly, the spectra from the scattered light can provide information about the scattering material.

[3] It is known to use NIR Raman spectroscopy as a potential technique for characterisation and diagnosis of precancerous and cancerous cells in vivo in a number of organs. The technique is desirable as it can be non-invasive or minimally invasive, not requiring biopsies or the other removal of tissue. It is known to use NIR Raman spectroscopy in two wavelength ranges. The first is the so-called fingerprint ("FP") range, with wave numbers from 800 to 1800 cm-1 , owing to the wealth of highly specific bimolecular information, for example from protein, DNA and lipid contents, contained in this spectral region for tissue characterisation and diagnosis. The disadvantage of this wavelength range is, that when used with a commonly used 785 nm laser source, the illuminated tissue autofluorescences, generating a strong background signal. Further, where the probe uses optical fiber links, a Raman signal is scattered from the fused silica in the optical fibers. In

particular, where a charge-coupled device ("CCD") is used to measure the scattered spectra, the autofluorescent signal can saturate the CCD and interfere with the detection of the inherently very weak Raman signals in this wavelength area.

[4] Another problem with Raman spectroscopy as a technique is that of standardization of instruments. The Raman spectroscopy technique has mainly been limited to single systems and no attempts have been made to transfer into multi-centre clinical trials or routine medical diagnostics. This is mainly because no Raman spectrometer instruments are similar (i.e., optics, response function, alignment, throughput etc.) and in general produce very different Raman spectra. As a consequence, multivariate diagnostic algorithms developed on a primary clinical platform cannot be applied to secondary clinical platforms. In particular, the quantitative measurement of tissue Raman intensity is one of the most challenging issues in fiber optic biomedical Raman applications. The instrument/fiber probe-independent intensity calibration and standardization with real-time excitation light power monitoring is essential to the realization of global use and quantitative analysis of fiber optic Raman spectroscopy in biomedicine. Moreover, fiber optic Raman probes have limited lifetimes and must be replaced or interchanged periodically. Unfortunately, Raman data acquired using different fiber optic probes cannot be compared, because each fiber optic probe has its own unique background as well as being associated with different transmissive spectral properties. The different transmissive characteristics significantly distort the spectral intensities making the tissue Raman spectra obtained with different fiber optic probes incomparable. For this reason, an existing multivariate statistical diagnostic model constructed using a 'master' probe cannot be applied to spectra measured with a 'slave' probe. In order for Raman technique to become a widespread tool for cancer screening on a global scale, there is a necessity to standardize both Raman spectrometers and fiber optic probes especially for biomedical applications. Most of the reported studies have focused on inter-Raman spectrometer standardization for measurements of simple chemical mixtures without fiber optic probes. In general Raman spectroscopy of simple chemical mixtures cannot be compared with the fiber optic Raman spectroscopy of heterogeneous biological tissue samples.

[5] A further problem with standardizing results across instruments is that of spectral variation associated with the laser excitation power. Conventionally, Raman spectra are

normalized which preserves the general spectrum shape, but this removes the absolute quantitative spectral characteristics. It has been known to attempt to monitor delivered laser power in fibre-optic Raman probes by, for example, embedding a diamond in the fibre tip or locating a polymer cap in the laser light path as a reference. However, these solutions are not satisfactory and may cause errors in the required spectral regions.

Summary of the Invention

[6] According to a first aspect of the invention there is provided a method of calibrating a fiber-optic Raman spectroscope system, the system comprising a laser source, a spectroscope and a fiber optic probe to transmit light from the laser source to a target and return scattered light to the spectroscope, the method comprising transmitting light from the laser source to a standard target having a known spectrum, recording a calibration spectrum of the scattered light from the standard target, comparing the known spectrum and the calibration system and generating a transfer function, and storing the transfer function.

[7] The method may further comprise the steps of subsequently illuminating a test subject, recording a spectrum and correcting the spectrum in accordance with the stored transfer function.

[8] The method may comprise recording calibration spectra for each of a plurality of fiber optic probes, calculating a transfer function for the system including each of said probes, and associating the transfer function with the corresponding probe.

[9] The spectrometer has an associated spectrometer transfer function and the probe may have an associated probe transfer function, and the transfer function may be a function of the spectrometer transfer function and the probe transfer function.

[10] The method may comprise, on a primary spectrometer system, calculating a first transfer function with a primary fiber optic probe, and a second transfer function with a secondary fiber optic probe, and calculating a (inter-probe) calibration function based on the first transfer function and second transfer function.

[11] The method may comprise associating the calibration function with the secondary fiber optic probe.

[12] The method may comprise, on a secondary spectrometer system, using the primary fiber optic probe and generating a secondary system transfer function and storing the secondary system transfer function.

[13] The method may comprise using the secondary fiber optic probe with the secondary spectrometer system and modifying the stored secondary system transfer function in accordance with the calibration function.

[14] The method may comprise the initial step of performing a wavelength-axis calibration of the secondary spectrometer system in accordance with the primary spectrometer system.

[15] According to a second aspect of the invention there is provided a method of operating a Raman spectroscope system, the system comprising a laser source, a spectroscope and a fiber optic probe to transmit light from the laser source to a target and return scattered light to the spectroscope, the method comprising transmitting light from the laser source to a target having a known spectrum, recording a spectrum of the scattered light from the target, and modifying the recorded spectrum in accordance with a stored transfer function.

[16] The stored transfer function may be associated with the spectrometer and the fiber optic probe.

[17] The stored transfer function may be associated with the spectrometer and a primary fiber optic probe and the method may further comprise modifying the stored transfer function in accordance with a stored calibration function associated with the fiber optic probe.

[18] According to a third aspect of the invention there is provided a Raman spectroscope system comprising a laser source, a spectroscope and a fiber optic probe to transmit light from the laser source to a target and return scattered light to the spectroscope, and a stored transfer function, the system being operable to transmit light from the laser source to a target having a known spectrum, record a spectrum of the scattered light from the target, and modify the recorded spectrum in accordance with the stored transfer function.

[19] The stored transfer function may be associated with the spectrometer and the fiber optic probe.

[20] The stored transfer function may be associated with the spectrometer and a primary fiber optic probe and the method may further comprise modifying the stored transfer function in accordance with a stored calibration function associated with the fiber optic probe.

[21] According to a fourth aspect of the invention there is provided a method of estimating the laser power transmitted in a Raman spectrometer system, the system comprising a laser source, a spectroscope and a fiber optic probe to transmit light from the laser source to a target and return scattered light to the spectroscope, the method comprising transmitting light from the laser source to a plurality of targets, for each target, measuring the transmitted power of the light from the laser source and the spectrum of the scattered light at the spectroscope, performing a multivariate analysis of the captured spectra with the measured transmitted power as a dependent variable, and storing a resulting model.

[22] The method may comprise the step of transmitting laser light to a test target, supplying a captured spectrum to the model, and calculating an estimate of the transmitted power.

[23] According to a fifth aspect of the invention there is provided a method of subtracting a background signal from a fiber-optic Raman spectroscope system having a fiber-optic probe, the method comprising the steps of;

a) storing a background spectrum,

b) receiving a test spectrum,

c) estimating a background contribution using one or more reference peaks,

d) multiplying the background spectrum by a correction factor based on the estimated background contribution and subtracting it from the test spectrum,

e) checking the test spectrum for a remaining background contribution, and

f) if the background contribution is negligible, outputting the test spectrum, otherwise repeating steps (c) to (e).

[24] The one or more reference peaks may comprise one or more peaks corresponding to silica or sapphire in the fiber-optic probe.

Brief Description of the Drawings

[25] Embodiments of the invention are described by way of example only with reference to the accompanying drawings wherein

[26] Fig. 1 is a diagrammatic illustration of a Raman spectroscopic system embodying the present invention,

[27] Fig. 1a is a view of the end of the endoscope of Fig. 1 on a larger scale,

[28] Fig. 1b is a view of the Raman probe of the endoscope of Fig.1a in more detail,

[29] Fig. 2 is a graph illustrating a comparison of measured spectra to a reference target,

[30] Fig. 3 is a diagrammatic illustration of a first calibration method,

[31] Fig. 4a is a flow chart showing a first process for use with the first calibration method,

[32] Fig. 4b is a flow chart showing a first part of a second process for use with the first calibration method,

[33] Fig.4c is a flow chart showing a second part of a second process for use with the first calibration method,

[34] Fig. 5 is a diagrammatic illustration of a second calibration method,

[35] Fig. 6 is a graph showing the spectral calibration of a primary spectrometer and a secondary spectrometer,

[36] Fig. 7a is a flow chart showing a first process for use with the first calibration method,

[37] Fig. 7b is a flow chart showing a first part of a second process for use with the second calibration method,

[38] Fig.7c is a flow chart showing a second part of a second process for use with the second calibration method,

[39] Fig. 8 is a graph showing a probe calibration function,

[40] Fig. 9a is a graph comparing uncalibrated primary and secondary spectrometers,

[41] Fig. 9b is a graph showing spectra from primary and secondary spectrometers after recalibration using a first method,

[42] Fig. 9c is a graph showing spectra from primary and secondary spectrometers after recalibration using a second method,

[43] Fig. 10 is a graph showing spectral peaks due to the fibre probe in a Raman spectrum,

[44] Fig. 11 is a graph showing variation of the Raman spectra with excitation laser power,

[45] Fig. 12a is a flow chart illustrating a method of generating a model for estimating laser power,

[46] Fig. 12b is a flow chart illustrating a method of estimating laser power,

[47] Fig. 13a is a graph indicating the root being square error for any number of included latent variables,

[48] Fig. 13b shows the loading and regression factor for the latent variables of the method of Fig. 10,

[49] Fig. 14 is a graph showing measured laser power against predicted laser power in *in vivo* test subjects,

[50] Fig. 15 is a flow chart showing a method of subtracting a background signal,

[51] Fig. 16 is a graph showing a spectrum received from a palm and the fibre-optical silica and sapphire background,

[52] Fig. 17 is a graph comparing the Raman spectrum of Fig. 12 and the spectrum after background removal, and

[53] Fig. 18 is a flow chart showing a combination of the methods.

Detailed Description of the Preferred Embodiments

[54] With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the

preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

[55] Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings. The invention is applicable to other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

[56] Referring now to Fig. 1, a diagnostic instrument comprising an endoscope system generally embodying the invention is shown at 10. The endoscope itself is shown at 11 and an instrument head 12 of the endoscope 11 is generally illustrated in Fig. 1a. To provide for guidance and visual viewing of the area being tested, the endoscope 11 is provided with a suitable video system in general shown at 13. Light from a xenon light source is transmitted to illumination windows 15 in the end of the endoscope 12. CCDs 16 and 17, responsive to white light reflection imaging, narrowband imaging or autofluorescence imaging, receive the reflected light and transmit video data to allow for visual inspection of the tested tissues and for guidance of the endoscope to a desired position. The confocal Raman probe head is showing at 18, and in more detail in figure 1b.

[57] The Raman spectroscopy system is generally shown at 20. A monochromatic laser source is shown at 21, in the present example a diode laser with an output wavelength of about 785 nm. Light from the laser diode 21 is passed through a proximal band pass filter 22, comprising a narrowband pass filter being centred at 785 nm with a full width half max of ±2.5 nm. The light is passed through a coupling 23 into an excitation optical fiber 25 provided as part of a fiber bundle. The excitation fiber 25 has a diameter of 200 µm and a numerical aperture ('NA') 0f 0.22. Light transmitted by the excitation fiber 25 enters a ball lens 26 at the end of the endoscope 11, in the present example comprising a sapphire ball

lens with a diameter of about 1.0 mm and a refractive index n=1.77. As illustrated in Fig. 1b, transmitted light from the excitation optical fiber 25 is internally reflected within the ball lens 26. Where the ball lens is in contact with the tissue to be tested, as shown here at 27, the transmitted light from the excitation fiber 25 at least in part undergoes Raman scattering within the tissue 27, to a depth of ~ 140μm. The scattered light is again internally reflected in the ball lens 26 and received in a plurality of collection fibers 28, also provided as part of the fiber bundle. In the present example twenty-six 100μm collection fibers are used, with an NA of 0.22. The collection fibres 28 may be arranged in any suitable configuration, for example in a circular arrangement surrounding the excitation fiber 25.

[58] Collected scattered light returned by collection fibers 28 is passed through a long pass inline collection filter 29 which similarly has a cutoff at ~ 800nm. The configuration of sapphire ball lens 26, excitation and collection fibers 25, 28, band-pass filters 22, and long-pass filter 29 provides a good system for selectively collecting backscattered Raman photons from the tissue 27.

[59] The scattered returned light is then separated at spectrograph 30 and the resulting spectrum is imaged at a light-sensing array 34, in the present example a charge-couple device ('CCD'). A computer shown at 35 controls the operation of the system, processes and stores the spectra and control data, and provides results and data to a user,

[60] The computer 35 also performs preprocessing the spectral data. As the measured tissue Raman spectra are substantially obscured by the tissue autofluorescence background, preprocessing of in vivo tissue Raman spectra is necessary to extract the weak Raman signals. The raw Raman spectra measured from in vivo tissue represent a combination of the weak Raman signal, intense autofluorescence background, and noise. The spectra are first normalized to the integration time and laser power. The spectra are then smoothed using a first-order Savitzky – Golay smoothing filter (window width of 3 pixels) to reduce the noise. A fifth-order polynomial was found to be optimal for fitting the autofluorescence background in the noise-smoothed spectrum, and this polynomial is then subtracted from the raw spectrum to yield the tissue Raman spectrum alone. The computer 35 can also including diagnostic algorithms for precancer and cancer detection.

<u>Spectrometer and Fibre-Optic Probe Calibration</u>

[61] It is known that different spectrometers will have different transfer functions, i.e. will show differing intensity variations within spectra even when illuminated using the same source. As illustrated in Fig. 2, the spectrum from a standard source is shown. The standard source in this example is a fluorescent standard target that emits a known fluorescent spectrum when excited by a laser such as laser source 21. The fluorescent standard target must be consistent and stable and emit a broad fluorescence spectrum under a laser excitation (e.g., 785 nm). The fluorescence spectrum must be stable over time and efficiently characterize the spectral transmissive properties over the entire spectral region of interest (e.g., 400-1800 cm^{-1}, 2000-3800 cm^{-1}). An example is kopp2412 filter glass. The resulting spectra from two spectrometers are shown, which are clearly different. To compensate for the spectrometer response, or transfer function, it is known to apply a calibration function which will correct the spectrum received from the spectrometer. Examples and calibration functions are shown in Fig. 2 which, when applied to the corresponding spectrum of the spectrometer, will bring the spectrum into line with the known standard spectrum.

[62] Using a fluorescent standard source, the transfer function, i.e. the wavelength-dependent response of the spectrometer, can be given by

$$F(\lambda) = \frac{S(\lambda)}{T(\lambda)}$$

(eqn. 1) where F(λ) is the correct fluorescent standard spectrum, S(λ) is the measured spectrum of the fluorescent standard source and T(λ) is the transfer function of the spectrometer. Accordingly, as T(λ) is known, a correctly calibrated Raman spectrum of a new sample R(λ) can be calculated by

$$R(\lambda) = \frac{S(\lambda)}{T(\lambda)}$$

(eqn. 2) where S(λ) is the measured sample spectrum.

[63] The transfer function T(λ) is a function both of the spectrometer transfer function $T_S(\lambda)$ and a probe transfer function $T_P(\lambda)$. Equation 2 can therefore be written as

$$R(\lambda) = \frac{S(\lambda)}{T_S(\lambda)T_P(\lambda)}$$

(eqn. 3). As fibre-optic probes are replaceable and may be consumables, it will be apparent

that when a new probe with a new probe transfer function T_P is inserted, the overall transfer function of the system will change.

[64] Referring now to Fig. 3, a primary or master spectrometer is shown at 50 and a secondary or slave spectrometer is shown at 51. The spectrometers 50, 51 each have a configuration similar to that shown in Fig. 1, but may have different fibre probes and spectrograph characteristics. Ideally, the personal computer 35 controlling each spectrograph uses a common library of programs to provide control of the system and data processing, and it is therefore desirable that characteristics of the primary and secondary spectrometers 50, 51 are consistent. In this example, the primary spectrometer 50 is associated with the primary or master probe 52, and the secondary spectrometer 51 is associated with a plurality of secondary or slave probes shown at 53a, 53a, 53b. In each case, the calibration is performed with reference to a standard fluorescent source diagrammatically illustrated at 54.

[65] A first method of calibration is shown in Fig. 4a. At step 60, the secondary spectrometer is calibrated in accordance with the primary spectrometer 50 as shown by arrow 55 in figure 3. In this case, wavelength-axis calibration of the secondary spectrometer 51 is performed, for example using an argon-mercury spectral lamp with defined spectral lines, and pixel resolution matching using linear interpolation is then performed to ensure that the size of the axis of the second spectrometer matches that of the primary spectrometer. The results of this calibration are shown in Fig. 5, where the spectra from the primary and secondary spectrometers 50, 51 show the spectral lines from the lamp precisely aligned. At step 61, calibration is performed for the second spectrometer and the probe 53a using a fluorescent source 54. In a similar manner to the graph of Fig. 2, a spectrum will be recorded from the fluorescent source, and a transfer function can then be calculated to bring the measured spectrum into line with the known spectrum, and stored, for example by the personal computer 35. At step 62, the spectrometer 51 may then be used for in vivo Raman testing or otherwise, and the measured Raman spectra can be corrected using the calibration function recorded at step 61.

[66] When probe 53 is discarded and it is desired to carry out tests on a new subject, a replacement probe 53b may be substituted, in which case the method of Fig. 4a is repeated.

[67] In an alternative process as illustrated in figures 4b and 4c, a plurality of calibration functions may first be recorded for the secondary spectrometer and a plurality of secondary probes. At step 60 in figure 4b, as in Fig. 4a, the secondary spectrometer 51 is calibrated for consistency with primary spectrometer 60. At step 61, a calibration function for secondary probe 53a is measured, and at step 63 this calibration function is stored and associated with probe 53a in some way, for example by saving the calibration function as a computer file 56a tagged with a reference number corresponding to the secondary probe 53a. As shown by arrow 64, this process is then repeated for any number of probes 53b, … ,53n to provide a stock or reserve of probes. As shown in figure 4c, when it is desired to carry out testing using the spectrometer 51, at step 60 the spectrometer is calibrated in accordance with the primary spectrometer 50 as above. At step 65 probe 53n is installed on the system and a corresponding stored transfer function 56n retrieved. At step 66, tests using the secondary spectrometer 51 may be performed and calibrated using the retrieved calibration function 56n.

[68] An alternative approach is illustrated with reference to Fig. 6, in which the slave or secondary probes 53a,…,53n are calibrated on the primary or master 50. In accordance with equation 2, where the primary spectrometer is tested with a primary or master probe with transfer function $T_{PP}(\lambda)$ and a secondary or slave probe with transfer function $T_{SP}(\lambda)$, the spectrum from the fluorescent source $F(\lambda)$ will result in a spectrum $S_{pp}(\lambda)$ for the primary probe, where

$$F(\lambda) = \frac{S_{PP}(\lambda)}{T_S(\lambda) T_{PP}(\lambda)}$$

(eqn. 4) and a spectrum $S_{SP}(\lambda)$ using the secondary probe, where

$$F(\lambda) = \frac{S_{SP}(\lambda)}{T_S(\lambda) T_{SP}(\lambda)}$$

(eqn. 5). Equations 4 and 5 can be divided to relate the two probe transfer values through a probe calibration function T_{CF}, where

$$T_{CF} = \frac{T_{SP}(\lambda)}{T_{PP}(\lambda)} = \frac{S_{SP}(\lambda)}{S_{PP}(\lambda)}$$

(eqn. 6). Consequently, from equations 2 and 6, when the secondary spectrometer is used with the secondary probe, the measured spectrum $S(\lambda)$ and Raman spectrum $R(\lambda)$ are related by

$$R(\lambda) = \frac{S(\lambda)}{T_S(\lambda)T_{SP}(\lambda)} = \frac{S(\lambda)}{T(\lambda)T_{CF}}$$

(eqn. 7) where $T(\lambda) = T_S(\lambda)T_{PP}(\lambda)$ is the stored system transfer function measured for the secondary spectrometer using the master probe.

[69] As illustrated in figures 6 to 7c, this allows any number of secondary or slave probes 53a, 53b, 53n to be matched to any number of secondary spectrometers 51a, 51b, 51n. As shown in Fig. 7a, at a first step 70 the secondary spectrometer 51a is calibrated in accordance with primary spectrometer 50 in similar manner to step 60, using the master probe 52. The system transfer function 71a is found at step 72 by testing the secondary spectrometer against a fluorescent standard source 54 in like manner to the method of figures 3 to 4c. The system transfer function 71a is associated with the corresponding spectrometer 51a in any appropriate manner, for example in the control software or otherwise at step 73. As shown by arrow 74, this may be repeated for any number of secondary spectrometer systems 51b,…51n, to generate appropriate system transfer functions 71b,…71n..

[70] As shown in figure 7b, the secondary or slave probes 53a, 53b,…, 53n are calibrated against the master probe 52. At step 75, the primary spectrometer system 50 is suitably calibrated with the master probe against a fluorescent source 54, although this may be omitted if this step has already been performed and the transfer function associated with the master probe already stored. At step 76, the master probe is replaced by probe 53a, and the combination of the primary spectrometer system and corresponding slave probe then tested against a fluorescent standard 54. At step 77, a calibration function T_{CF} is calculated from the ratio of the primary and secondary probe spectra and at 78 this is recorded and stored associated with the secondary probe as shown at 79a. As shown by arrow 80, this can be repeated for any number of secondary probes 53b,…53n and the corresponding calibration function T_{CF} stored as shown at 79b,…79b.

[71] As illustrated in figure 7c, one of the secondary spectrometer systems 51n may be used with any one of the secondary probes 53n, as the system transfer function 71a using the master probe 52 is known and the calibration function T_{CF} relating the secondary probe 53n to the master probe 52 is known. As shown at step 81, the secondary spectrometer system 51n is calibrated in accordance with the primary spectrometer system 50. At step 82,

the secondary probe calibration function T_{CF} is retrieved and the store system transfer function 71n modified in accordance with the stored calibration function T_{CF}. At step 83, in vivo Raman tests or otherwise can then be performed and the captured Raman spectra corrected.

[72] In any of the methods therefore, by matching the secondary spectrometer characteristics back to the primary spectrometer characteristics, and storing either the transfer function for the spectrometer and probe combination or a transfer function for the system incorporating a master probe and a calibration function for use with a secondary probe, spectra captured using different spectrometer and probe combinations will nevertheless be consistent and comparable.

[73] This is apparent from figure 8 and figures 9a to 9c. Fig. 8 shows different responses between the master probe 52 and a secondary or slave probe 53n. The intensity response varies over the spectrum, and the calibration function as shown would map the spectrum of the secondary probe to that of the main or master probe. Uncalibrated spectra from a primary spectrometer 50 and a secondary spectrometer 51 are shown in figure 89 and the differences between them are apparent. Figures 9b and 9c show the results of calibration using each of the methods shown above and the spectra from the primary and secondary spectrometer are substantially in agreement.

Monitoring Laser Power

[74] Fig. 10 is a graph showing a background spectrum from a fibre probe, i.e. in the absence of a tissue signal. Peaks corresponding to Raman scattering or fluorescence within the silica of the fibre and peaks corresponding to the sapphire of the distal ball lens are apparent. Fig. 11 shows a graph of Raman spectra received from in vivo tissue with different levels of transmitted power. The peaks from Fig. 10 are apparent in the different lines of Fig. 11, but it will be apparent that the relative heights of the peaks and the continuum background vary with the transmitted power.

[75] Advantageously, it has been found that the spectral characteristics of the fibre probe and sapphire ball lens in captured Raman spectra can be used to derive the transmitted laser power without requiring the provision of any additional components in the optical train. As shown in the method of Fig. 12A, at step 90, a suitably large number of spectra, in

the present example 352, are collected and the transmitted laser power measured. At step 91, a suitable multi-variate statistical analysis is performed, in the present example partial least squares ("PLS") regression. PLS regression reduces the dimension of spectral data to a number of latent variables ("LV"). In this case, the variance between the spectral variation and the dependent variable, the laser power, is maximised so that the latent variables give a higher weight to spectral peaks that correlate well with the laser power. By selecting an appropriate number of latent variables, a model of the laser power as a function of the spectral characteristics can be derived, and is stored as shown at step 92. Accordingly, in operation as shown in figure 12b at step 93 a test spectrum is captured, for example from an in vivo subject or otherwise, and at step 94 the spectral values are provided to the stored model. At step 95, the laser power is derived and displayed to the operator, for example on the personal computer 35.

[76] In the present example, a graph of the number of latent variables included against the root mean square error is shown in figure 13a, and four variables are selected as giving the best balance between error and complexity. The relative loading of the four latent variables and the regression vector are shown in figure 13b. In Fig. 14, example data from real-time measurements of 166 spectra in five subjects are shown, with the measured laser power plotted against the power estimated by the model. It will be apparent that the substantially linear fit shows that the estimated power is a good indicator of the power actually delivered.

<u>Iterative Background Subtraction</u>

[77] A method of subtracting the background Raman spectrum resulting from fluorescence, Raman scattering in the silica of the probe and the sapphire of the lens is shown with reference to figures 15 to 17. This background signal is unique to each specific fiber probe. It is desirable to remove the background from the tissue Raman spectra without over- or under-subtracting the background.

[78] As shown at step 110 in figure 15, the background spectrum is captured and stored, for example by transmitting light from the laser source through the probe in the absence of a target. At step 111, the Raman spectrum from a test subject is received, for example from tissue. At step 112, the amount of fiber background signal in the test subject Raman

spectrum is estimated using the intensity of one or more distinct reference peaks. In the present example, the peaks may be due to silica and/or sapphire (e.g., 417 or 490 cm^{-1}). Using the estimated amount of background signal, the stored background signal may be multiplied by a suitable, possibly wavelength-dependent, correction factor and subtracted from the test spectrum (step 113).

[79] At step 114, the spectrum is checked for the presence of remaining background. If the background has been fully removed, (i.e., when the silica and sapphire signal contributes negligible to the tissue Raman spectrum), the spectrum is passed for output or further analysis as shown at step 115. If a background signal is still present, then steps 112 to 114 are repeated as shown by arrow 116.

[80] The method need not be limited to single silica/sapphire peaks. Multivariate analysis (e.g., partial least squares and curve resolution methods etc.) can also be used for this purpose.

By way of example, figure 16 is a graph showing a Raman spectrum received from palm tissue and a background spectrum from the probe. The peaks from fluorescence, Raman scattering in the silica of the probe and the sapphire of the lens are apparent, superposed on the Raman spectrum from the palm. This background signal is unique to each specific fiber probe

[81] As shown in figure 17, after the iterative process of figure 15 has been performed, the smooth Raman spectra is shown without the distinctive peaks of the background signal but retaining the essential Raman spectroscopic information required.

Combined System

[82] The various aspects of the invention can be combined as illustrated in Fig. 17. At step 100 the calibration method can be performed such that the system transfer function is known in accordance with a master or primary system 50 and subsequent spectra can be appropriately corrected. At step 101, pre-processing of the signal can be performed, including smoothing and tissue background subtraction. At step 102, power monitoring can be performed on the spectrum as discussed above and, in parallel, at step 103 probe background subtraction can be performed. As shown at step 104, the information of steps

102 and 103 is provided to a suitable program on the personal computer to perform other diagnostic or output steps.

[83] In the above description, an embodiment is an example or implementation of the invention. The various appearances of “one embodiment”, “an embodiment” or “some embodiments” do not necessarily all refer to the same embodiments.

[84] Although various features of `the invention may be described in the context of a single embodiment, the features may also be provided separately or in any suitable combination. Conversely, although the invention may be described herein in the context of separate embodiments for clarity, the invention may also be implemented in a single embodiment.

[85] Furthermore, it is to be understood that the invention can be carried out or practiced in various ways and that the invention can be implemented in embodiments other than the ones outlined in the description above.

[86] Meanings of technical and scientific terms used herein are to be commonly understood as by one of ordinary skill in the art to which the invention belong, unless otherwise defined.

CLAIMS

1. A method of calibrating a fiber-optic Raman spectroscope system,

the system comprising a laser source, a spectroscope and a fiber optic probe to transmit light from the laser source to a target and return scattered light to the spectroscope,

the method comprising transmitting light from the laser source to a standard target having a known spectrum,

recording a calibration spectrum of the scattered light from the standard target,

comparing the known spectrum and the calibration system and generating a transfer function, and

storing the transfer function.

2. A method according to claim 1 comprising recording calibration spectra for each of a plurality of fiber optic probes, calculating a transfer function for the system including each of said probes, and associating the transfer function with the corresponding probe.

3. A method according to claim 1 or claim 2 comprising the steps of subsequently illuminating a test subject, recording a spectrum and correcting the spectrum in accordance with the stored transfer function.

4. A method according to any one of the preceding claims wherein the spectrometer has an associated spectrometer transfer function and the probe has an associated probe transfer function, and the transfer function is a function of the spectrometer transfer function and the probe transfer function.

5. A method according to any one of the preceding claims comprising, on a primary spectrometer system, calculating a first transfer function with a primary fiber optic probe,

and a second transfer function with a secondary fiber optic probe, and calculating a calibration function based on the first transfer function and second transfer function.

6. A method according to claim 5 comprising associating the calibration function with the secondary fiber optic probe.

7. A method according to claim 5 or claim 6 comprising, on a secondary spectrometer system, using the primary fiber optic probe and generating a secondary system transfer function and storing the secondary system transfer function.

8. A method according to claim 7 comprising using the secondary fiber optic probe with the secondary spectrometer system and modifying the stored secondary system transfer function in accordance with the calibration function.

9. A method according to any one of claims 5 to 8 comprising the initial step of performing a wavelength-axis calibration of the secondary spectrometer system in accordance with the primary spectrometer system.

10. A method of operating a Raman spectroscope system,

the system comprising a laser source, a spectroscope and a fiber optic probe to transmit light from the laser source to a target and return scattered light to the spectroscope,

the method comprising transmitting light from the laser source to a target having a known spectrum,

recording a spectrum of the scattered light from the target, and

modifying the recorded spectrum in accordance with a stored transfer function.

11. A method according to claim 10 wherein the stored transfer function corresponds to the spectrometer and the fiber optic probe.

12. A method according to claim 10 wherein the stored transfer function corresponds to the spectrometer and a primary fiber optic probe and the method further comprises modifying the stored transfer function in accordance with a stored calibration function associated with the fiber optic probe.

13. A Raman spectroscope system comprising a laser source, a spectroscope and a fiber optic probe to transmit light from the laser source to a target and return scattered light to the spectroscope, and a stored transfer function,

the system being operable to transmit light from the laser source to a target having a known spectrum,

record a spectrum of the scattered light from the target, and

modify the recorded spectrum in accordance with the stored transfer function.

14. A system according to claim 13 wherein the stored transfer function corresponds to the spectrometer and the fiber optic probe.

15. A system according to claim 13 wherein the stored transfer function corresponds to the spectrometer and a primary fiber optic probe and the method further comprises modifying the stored transfer function in accordance with a stored calibration function associated with the fiber optic probe.

16 A method of estimating the laser power transmitted in a fiber-optic Raman spectrometer system,

the system comprising a laser source, a spectroscope and a fiber optic probe to transmit light from the laser source to a target and return scattered light to the spectroscope,

the method comprising transmitting light from the laser source to a plurality of targets,

for each test target, measuring the transmitted power of the light from the laser source and the spectrum of the scattered light at the spectroscope,

performing a multivariate analysis of the captured spectra with the measured transmitted power as a dependent variable,

and storing a resulting model.

17. A method according to claim 16 comprising the step of transmitting laser light to a test target,

supplying a captured spectrum to the model, and

calculating an estimate of the transmitted power.

18, A method of subtracting a background signal from a fiber-optic Raman spectroscope system having a fiber-optic probe, the method comprising the steps of;

a) storing a background spectrum,

b) receiving a test spectrum,

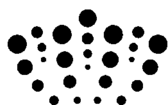
c) estimating a background contribution using one or more reference peaks,

d) multiplying the background spectrum by a correction factor based on the estimated background contribution and subtracting it from the test spectrum,

e) checking the test spectrum for a remaining background contribution, and

f) if the background contribution is negligible, outputting the test spectrum, otherwise repeating steps (c) to (e).

19, A method according to claim 18 where the one or more reference peaks comprise one or more peaks corresponding to silica or sapphire in the fiber-optic probe.



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Application No: GB1307338.2 **Examiner:** Simon Colcombe
Claims searched: 1-15 **Date of search:** 6 September 2013

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	1, 10, 13 at least	US6621574 B1 (FORNEY) Whole document
X	1, 10, 13 at least	WO98/41825 A1 (EASTMAN CHEMICAL CO) Summary, Claim 1, for example
X	1, 10, 13 at least	US5638172 A (ALSMEYER) Whole document
A	-	WO2006/064446 A1 (KONINKL PHILIPS ELECTRONICS)
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Field of Search:

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G01J; G01N

The following online and other databases have been used in the preparation of this search report

WPI, EPODOC

International Classification:

Subclass	Subgroup	Valid From
G01N	0021/65	01/01/2006
G01J	0003/44	01/01/2006