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(54) Title: SERS PROBE COMPRISING A DUAL-CORE OPTICAL FIBER AND A SPACER ONTO WHICH SERS-ACTIVE NANOPARTICLES ARE ATTACHED

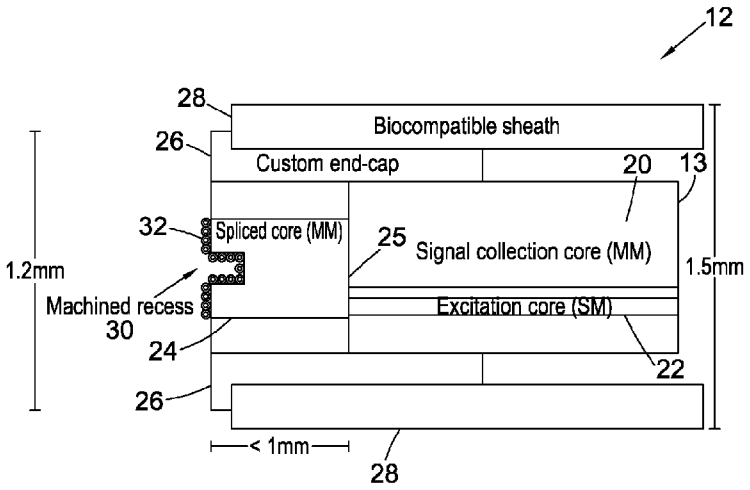


Fig. 2

(57) Abstract: A spectroscopy probe apparatus comprising an excitation path configured to transmit excitation light for exciting a response; a collection path different from the excitation path and configured to transmit signal light comprising a response signal, wherein at least one of the excitation path or collection path comprises or is formed in at least one optical fibre; and a light guide having a proximal end coupled to the excitation path and to the collection path, wherein the guide is configured to pass the excitation light from the excitation path to a target region to generate the response signal, and to pass the signal light comprising the response signal from the target region to the collection path.

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SERS PROBE COMPRISING A DUAL-CORE OPTICAL FIBER AND A SPACER ONTO WHICH SERS-ACTIVE NANOPARTICLES ARE ATTACHED

Field

The present invention relates to a fibre-optic probe, for example a fibre-optic probe configured for use in Surface Enhanced Raman Spectroscopy.

Background

The pulmonary parenchyma (the part of the lung involved in gas transfer) is responsible for ensuring adequate gas exchange. In health, the alveoli acinar units of the lung may be devoid of infiltrating inflammatory cells and pathogens. In inflammatory lung diseases such as pneumonia, a pathological hallmark may involve dense infiltration by myeloid derived peripheral blood cells in response to an injurious stimulus. These infiltrating cells have an arsenal of oxidative killing mechanisms and a proteolytic secretome, which has the potential to drastically perturb physiological homeostasis in the alveoli.

In chronic lung diseases such as chronic obstructive pulmonary disease, characterized by recurrent cycles of inflammation and infection, homeostatic baselines of physiology may be permanently off-set. Indeed, many fundamental biological processes that are modelled *in vitro* may be exquisitely sensitive to physiological aberrations. However, little is known about the physiological state of the distal lung. A lack of knowledge of the physiological state of the distal lung may be due to an inability to concurrently access the alveolar sacs and perform real-time sensing.

Current approaches to sampling the distal lung include bronchoalveolar lavage (introducing fluid into a part of the lungs, then collecting the fluid for analysis), or transbronchial lung biopsy (collecting a tissue sample from the lungs) or transbronchial needle aspiration (collecting a tissue sample from the lungs using a needle).

Current sampling approaches such as bronchoalveolar lavage, transbronchial lung biopsy or transbronchial needle aspiration may not be suitable for bedside *in situ* physiologic monitoring. Each of these methods requires a sample to be taken from the patient and for the sample to be subsequently analysed. Aspirate samples may

ordinarily be examined using *in vitro* cytologic and histochemical methods. Therefore, these methods may not provide real time information. Data may be obtained at some time after the alveolar sacs are accessed.

Moreover, the pH of post-aspirated fluid or biopsies may not be representative of the pH of the endogenous and epithelial lining fluids within the alveolar environment due to the intrusive nature of the aspiration process and uncertainties such as aspirate volume, dilution and temperature.

Summary

In a first aspect of the invention there is provided a probe apparatus, for example a spectroscopy probe apparatus, comprising: an excitation path configured to transmit excitation light for exciting a response; a collection path different from the excitation path and configured to transmit signal light comprising a response signal; and a light guide having a proximal end coupled to the excitation path and to the collection path, wherein the guide is configured to pass the excitation light from the excitation path to a target region to generate the response signal, and to pass the signal light comprising the response signal from the target region to the collection path. At least one of the excitation path or collection path may comprise or be formed in at least one optical fibre.

Thus, background signals arising from the excitation path may be reduced or eliminated in the response signal.

The collection path and the excitation path may be substantially non-overlapping over substantially all of their length. The collection path and the excitation path may be substantially decoupled.

The response signal may comprises a Raman response signal.

The excitation path may comprise material that generates at least one of a Raman background signal, a fluorescence background signal or other background response signal in response to the excitation light.

The excitation path may be configured to transmit light of a single frequency.

The excitation light may comprise laser light, optionally narrowband or broadband laser light.

The collection path may be configured to transmit light having any of a range of frequencies.

The guide may be configured to transmit light having any of a range of frequencies.

The target region may have a cross-sectional area greater than a cross-sectional area of the excitation path.

The guide may be configured to project light from the excitation path to the target region over a wider area than the cross-sectional area of the excitation path thereby to illuminate the target region.

In operation the excitation light and the signal light comprising the response signal may overlap in the guide.

The excitation path may comprise a first optical fibre core and the collection path may comprise at least one second optical fibre core or may comprise optical fibre cladding.

The excitation path may comprise a core that supports at most 21 modes, optionally at most 10 modes, optionally at most 6 modes, optionally at most 3 modes, optionally a single mode.

The collection path may comprise at least one core that supports at least 6 modes, optionally at least 15 modes, optionally at least 40 modes, optionally at least 100 modes.

The excitation path may comprise a hollow core of the hollow core optical fibre, and the collection path may comprise a cladding of the hollow core optical fibre.

The excitation path and the collection path may be formed in different optical fibres.

The collection path may comprise a plurality of fibre cores and/or the excitation path may comprise a plurality of fibre cores.

The fibre core of the excitation path or at least one of the fibre cores of the excitation path may be in the same optical fibre as the fibre core of the collection path or at least one of the fibre cores of the collection path.

The excitation path and collection path may be formed in a single optical fibre.

The light guide may comprise a tapered region of the optical fibre or at least one of the optical fibres.

The light guide may be spliced to the optical fibre or at least one of the optical fibres.

The light guide may be formed in or may comprise an end-cap configured to fit onto an end of the optical fibre or at least one of the optical fibres.

The light guide may have a cross-sectional area greater than the cross-sectional area of the optical fibre core or the optical fibre or a combination of all of the optical fibres and/or the guide may extend back around at least part of an outer diameter of the optical fibre or optical fibres.

An outer diameter of the optical fibre may be less than 2.0 mm, optionally less than 1.5 mm, optionally less than 1.0mm, optionally less than 0.8 mm, optionally less than 0.5 mm, optionally less than 0.3 mm, optionally less than 0.1 mm.

The light guide may have a length from the end of the collection path and/or excitation path to a distal end of the light guide in a range 0.5 mm to 50 mm.

The light guide may comprise at least one of a section of multi-mode fibre, an undoped section of fibre, a photonic lantern, a multi-mode waveguide, a transparent body.

A cross-sectional area of the collection path may be greater than a cross-sectional area of the excitation path, and/or a cross-sectional area of the collection path may be in a

range 2 um^2 to 10 um^2 and/or a cross-sectional area of the excitation path may be in a range 20 um^2 to 500 um^2, and/or the collection path and excitation path may be spaced apart so as to substantially prevent coupling.

The apparatus may be configured to be insertable into a, or form part of, at least one of a bronchoscope, an endoscope.

The target region may comprise material configured to cause Raman scattering of at least part of the excitation light, thereby to provide the signal light.

The target region may be located at a distal end of the light guide.

The excitation light may be for producing laser induced breakdown of tissue at the target region and/or for producing fluorescence at the target region.

The apparatus may comprise or form part of a Raman spectroscopy probe, a laser induced breakdown spectroscopy (LIBS) probe, an endoscope, or a fluorescence spectroscopy probe.

The target region may comprise a target comprising reporter molecules.

The reporter molecules may comprise SERS reporter molecules, optionally the reporter molecules may comprise para-mercaptobenzoic acid (p-MBA).

The target may comprise nanoshells coated with the reporter molecules.

A response of the reporter molecules to the excitation light may vary with a property of an environment of the target, optionally the property may comprise at least one of pH, redox potential, glucose level, oxygen tension.

The reporter molecules may be attached to the distal end of the light guide.

The apparatus may be configured to be inserted into a human or animal body such that the target region comprises or is in contact with human or animal tissue or fluid.

In a further aspect of the invention, there is provided a spectroscopy apparatus comprising a probe apparatus as claimed or described herein, and further comprising a light source configured to provide the excitation light to the excitation path, and a spectrometer configured to analyse the signal light collected by the collection path thereby to obtain a spectrum.

In another aspect of the invention, which may be provided independently, there is provided a method of performing a measurement comprising transmitting excitation light along an excitation path via a light guide to a target region for exciting a response; and receiving a response signal from the target region via the light guide and a collection path different from the excitation path, wherein at least one of the excitation path or collection path comprises or is formed in at least one optical fibre; and the light guide has a proximal end coupled to the excitation path and to the collection path.

In another aspect of the invention, which may be provided independently, there is provided a method of producing a probe apparatus comprising: forming an excitation path and a collection path, wherein the excitation path is different from the collection path and the forming comprises forming at least one of the collection path and excitation path in at least one optical fibre; and coupling a light guide at its proximal end to the excitation path and to the collection path.

Features in one aspect may be provided as features in any other aspect as appropriate. For example, features of a method may be provided as features of an apparatus and vice versa. Any feature or features in one aspect may be provided in combination with any suitable feature or features in any other aspect.

Detailed description of embodiments

Embodiments of the invention are now described, by way of non-limiting example, and are illustrated in the following figures, in which:-

Figure 1 is a schematic illustration showing bronchoscopic deployment of a probe in accordance with an embodiment;

Figure 2 is a schematic illustration of the distal-end optical configuration of a probe in accordance with an embodiment;

Figure 3 is a flow-chart illustrating in overview fabrication stages of a dual-core fibre;

Figures 4(a) to 4(c) are schematic illustration of fabrication stages of a dual core fibre in which Figure 4(a) illustrates the formation of a stack, Figure 4(b) illustrates formation of a large signal collection core and Figure 4(c) illustrates an optical micrograph of a transverse cross-section of a dual-core fibre;

Figure 5 is a photograph showing a probe in accordance with an embodiment, and also showing custom silica end-caps;

Figure 6 is a schematic illustration of the proximal-end optical configuration of a probe in accordance with an embodiment;

Figure 7a is a plot of a p-MBA SERS spectrum acquired when a multi-mode core is used for both excitation and collection (normal mode);

Figure 7b is a plot of a p-MBA SERS spectrum acquired when a single-mode core is used for excitation and a multi-mode core is used for collection (background suppressed mode);

Figure 7c is a plot of a characteristic p-MBA SERS spectrum obtained using the fibre-optic probe;

Figure 8 is a schematic illustration of the proximal-end optical configuration of a probe in accordance with a further embodiment;

Figures 9a, 9b and 9c are schematic illustrations of a probe in accordance with an embodiment;

Figures 10a, 10b and 10c are schematic illustrations of a probe in accordance with a further embodiment;

Figures 11a, 11b and 11c are schematic illustrations of a probe in accordance with a further embodiment;

Figures 12a, 12b and 12c are schematic illustrations of a probe in accordance with a further embodiment;

Figures 13a, 13b and 13c are schematic illustrations of a probe in accordance with a further embodiment;

Figures 14a, 14b and 14c are schematic illustrations of a probe in accordance with a further embodiment;

Figures 15a, 15b and 15c are schematic illustrations of a probe in accordance with a further embodiment;

Figure 16 is a plot of a p-MBA SERS spectrum from 1300 cm^{-1} to 1750 cm^{-1} showing a pH sensitive spectral response in the vicinity of 1380 cm^{-1} and 1700 cm^{-1};

Figure 17 is a plot of variation of area-under-the-curve (AUC) ratio with respect to pH in the range 4.0 to 9.0 obtained after computational data processing;

Figure 18 is a plot of variation of AUC ratio with respect to pH in the range 6.0 to 7.0;

Figure 19 is a schematic illustration representing six sub-segments of an *ex vivo* sheep lung model that are interrogated in an experiment using a fibre-optic probe;

Figure 20 is a plot of p-MBA spectrum between 1300 cm^{-1} and 1800 cm^{-1} obtained from the interrogation of the six distal sub-segments of Figure 19;

Figure 21 is a plot of pH measured at the six distal sub-segments of Figure 19 (no perfusion) using the fibre-optic probe (y-axis) versus pH measured using a commercial pH monitor (x-axis) at the incised locations indicated in Figure 19;

Figure 22 are plots of the alveolar pH measured using the fibre-optic probe and also the perfusate pH as a function of time in an *ex vivo* ovine lung model with ceased ventilation; and

Figures 23a and 23b are schematic illustrations of a probe in accordance with a further embodiment.

An apparatus in accordance with an embodiment is illustrated in Figures 1 and 2. The apparatus comprises a bronchoscope-deployable SERS-based fibre-optic pH sensing probe 12. The end of the probe 12 is coated in gold nanoshell SERS (Surface Enhanced Raman Spectroscopy) sensors.

The probe 12 may be used *in vivo* to sense the environment inside the body (for example, at the distal end of the lung) by Raman spectroscopy of the SERS sensors. One potential issue with using a fibre-optic probe for sensing the environment inside the body by Raman spectroscopy is the possibility that significant Raman background could be caused by a glass optical fibre itself. For example, it is possible that the Raman background from the fibre could cause a significant degradation in the signal-to-background of a measured SERS signal, and therefore reduce signal-to-noise, and may swamp SERS features of interest . For example, if signal light from the SERS sensors were to be received via the same fibre-optic core through which excitation light is transmitted, it is possible that a large Raman background may be present. However, as described below, the probe 12 of the present embodiment is configured such that the Raman background may be suppressed. In other embodiments, the excitation light and the material of the excitation path are such that significant other background signals may be produced in the excitation path by the excitation light, as well as or instead of Raman background signals, for example fluorescence signals. The

background signals may within or overlap with frequency ranges of interest, for example a frequency range of the response signal from the target region.

Figure 1 illustrates the bronchoscopic deployment of the probe 12 to sense physiology in the distal lung acinar units. Due to the sequential branching and ever decreasing size of the respiratory tract, it has been found that the terminal entry to alveoli may be best achieved through a transbronchial approach with a small diameter (less than 1.5 mm) sheathed fibre after bronchoscopic guidance to the 3rd or 4th order bronchi.

In use, the probe 12 is inserted into the working channel of a bronchoscope 10. The bronchoscope 10 is inserted into the patient's airway 102, and into the bronchi of the lung 104. Once the bronchoscope is positioned in the 3rd or 4th order bronchi, the sensing probe 12 is extended further into the distal gas exchanging regions of the lung (alveolar sacs 106). Although in the present embodiment the probe 12 is configured to be insertable into the working channel of the bronchoscope 10, in other embodiments the probe 12 may be deployed by a method other than by a bronchoscope 10.

Figure 2 illustrates in detail the distal end of the probe 12 (i.e. the end of the probe 12 that is configured to be inserted into the body of a human or animal subject). Figure 2 is a schematic illustration and is not drawn to scale. In the present embodiment, the probe 12 comprises a single multi-core fibre-based probe, which may also be referred to as an optrode (in this embodiment, dual-core), with an asymmetric core size geometry that may allow fibre Raman background to be greatly suppressed during data acquisition. The terms optrode and probe are used interchangeably herein.

The probe 12 comprises a multi-core optical fibre (MCF) 13. In the present embodiment, the optical fibre 13 is a dual-core optical fibre comprising one excitation core 22 and one collection core 20. Excitation core 22 provides a path for excitation light and collection core 20 provides a path for signal light that is different from the path along which the excitation light is transmitted. In other embodiments, the optical fibre 13 may be any suitable multi-core optical fibre having separate excitation and collection paths. In other embodiments, the optical fibre 13 may be any suitable optical fibre having separate excitation and collection paths, such as a hollow core fibre with a suitably designed cladding structure for signal collection. The optical fibre 13 may comprise a plurality of excitation cores 22 and/or a plurality of collection cores 20.

Although in the present embodiment the probe 12 comprises a single optical fibre 13, in other embodiments the probe 12 may comprise a plurality of optical fibres 13.

Optical fibre 13 comprising collection core 20 and excitation core 22 may be considered to extend beyond the right side of the section illustrated in Figure 2, to a total length of around 3 metres. In other embodiments, any suitable length of optical fibre may be used.

In the present embodiment, the optical fibre 13 is an asymmetric dual core optical fibre with a single-mode (SM) core 22 for excitation and a multi-mode (MM) core 20 for collection. In alternative embodiments, the excitation core is not limited to being a single-mode core and may be a multi-mode core having any desired or suitable number of modes. The term "single-mode" may refer to the optical properties of the core or other waveguide at its normal wavelength of operation.

The optical fibre 13 is fabricated from pure silica with high-index Ge-doped silica regions forming the two cores 20, 22. The excitation core 22 is offset from the centre of the optical fibre 13. The excitation core 22 is 2 μm in diameter, making it single-mode at an excitation wavelength of 785 nm. The much-larger collection core 20 is 28 μm in diameter, making it multi-mode at the pump and Raman signal wavelengths. The collection core 20 is positioned in the centre of the optical fibre 13. An effective diameter encircling the excitation and collection cores is 47 μm. The outer diameter of the optical fibre 13 is 125 μm. Such an arrangement of cores may ensure that a collection path for signal light (provided by the MM collection fibre 22) is separate from an excitation path (provided by the SM excitation core 20) in which the intense Raman background may be generated.

Figure 3 is a flow-chart describing in overview the fabrication of the dual-core optical fibre 13 used in the probe 12 of the present embodiment. Figures 4(a), 4(b) and 4(c) are representative of different fabrication stages of the dual-core optical fibre 13.

The optical fibre 13 is fabricated in two stages using a modification to the stack-and-draw method developed to make photonic crystal fibres (see, for example, Knight, J.C., Photonic crystal fibres, *Nature* (2003)). In other embodiments, any suitable method for forming a multi-core fibre may be used. In the present embodiment, the optical fibre is

formed of undoped silica and Ge-doped silica. In other embodiments, the optical fibre may be formed of any suitable material, for example any suitable glass or polymer.

At stage 300 of the flow-chart of Figure 3, a stack to support the small single-mode (SM) excitation core 22 is formed by placing undoped silica rods 40, each 0.63 mm in diameter and 1.2 m in length, in a circular gap between two nested silica tubes 42, 44. Silica tube 42 has an outer diameter of 25 mm and an inner diameter of 11 mm. Silica tube 44 has an outer diameter of 9.5 mm and an inner diameter of 8.8 mm. Rods 40 and silica tubes 42, 44 are shown in cross-section in Figure 4(a).

The excitation core 22 is included by replacing one of the silica rods 40 with a silica rod 46 drawn (on a fibre drawing tower) from a commercial MM fibre preform containing a Ge-doped graded-index core with a maximum numerical aperture of 0.3.

At stage 310, the circular stack comprising tubes 42, 44 and rods 40 and 46 is drawn down to form a hollow preform 48. Figure 4(b) shows a cross-section of the hollow preform 48 drawn from the stack, having an outer diameter of 4.8 mm.

At stage 320, the large multi-mode signal collection core 20 is formed by inserting a rod 47 drawn from the same commercial preform as rod 46 (but drawn to a different size) into the void in the hollow preform 48. Figure 4(b) also shows an enlarged drawing of hollow preform 48 in cross section, in which rod 47 has been inserted into the hollow preform 48.

At stage 330, the whole assembly (comprising the hollow preform 48 and rod 47) is drawn down to form the final optical fibre 13. In each of the drawing stages 310 and 330, unwanted interstitial air gaps are evacuated to collapse them during the draw and form a solid optical fibre 13.

Figure 4(c) is a representation of an optical micrograph of a transverse cross-section of optical fibre 13. The high-index core regions formed from rods 46, 47 appear lighter than the rest of optical fibre 13 in the image of Figure 4(c). The small excitation core is visible at ~11 o'clock. The scale bar is 20 μm.

In the present embodiment, the collection core 20 in the final fibre is 28 μm in diameter.

The small excitation core 22 has a slight elliptical deformation with a long axis less than 2 μm, making the excitation core 22 single mode at the operating wavelength of 785 nm. The centre-to-centre core separation between the excitation core 22 and collection core 20 is 24 μm. In other embodiments, a multi-core fibre of any suitable dimensions may be used, or indeed any fibre with different spatial paths for signal collection and pump delivery.

The significant mismatch between the core size of the excitation core 22 and the core size of the signal collection core 20 means that less than -30 dB power is coupled from the excitation core 22 to the signal collection core 20 at the pump wavelength (785 nm) at the distal end over a length of approximately 2 m when only the excitation core is excited at the proximal end.

Turning back to Figure 2, the probe 12 further comprises a light guide, in this embodiment comprising a section of multi-mode optical fibre that is spliced to the distal end of the multicore optical fibre 13. The section of multi-mode fibre may be referred to as multi-mode section 24. Any other suitable light guide may be used in alternative embodiments, for example any suitable guide that is configured to transmit excitation light from the excitation path to the target region, and to return the signal light generated at the target region in response to the excitation signal, the response signal comprising incoherent light that includes a Raman response signal from the target region. Light guides according to further embodiments are described below. It may also be possible to use any structure that allows the pump light to overlap spatially with a region where signal light can be generated and collected by the other cores or paths.

In fabrication, a commercial multi-mode fibre having a step-index Ge-doped core that is 50 μm in diameter and has an outer diameter of 125 μm is spliced to the distal end of optical fibre 13 using a fusion splicer, the splicing occurring at splice plane 25. Loss across the splice 25 has been measured for the present embodiment. For proximal end injection of 785 nm light into the 28 μm collection core 20, the loss across the splice 25 in the proximal to distal direction is found to be less than 0.5 dB. For injection into the excitation core, 70% transmission was measured at the distal end. The measured loss in the reverse direction is consistent with that expected due to the geometric difference between the core dimensions (around 6dB at the upper limit, given the area of a 28 μm core is about 31% of the area of the spliced 50 μm core).

After being spliced to the distal end of the optical fibre 13 as described above, the spliced multi-mode fibre is cleaved at a distance of less than or equal to 1 mm from the splice plane to form multi-mode section 24 as illustrated in Figure 2. The plane at which the multi-mode fibre is cleaved becomes a distal end-facet of the multi-mode section 24.

In the present embodiment, the length of the multi-mode section 24 is 0.9 mm. The length of the multi-mode section 24 is less than 0.03% of the length of the dual-core optical fibre 13. The length of the multi-mode section 24 is deliberately kept short to attempt to minimise the Raman background generated within its core. Nonetheless, its length is chosen to ensure spatial overlap between the mode structure at the distal end-facet of the multi-mode section 24 that is generated by the excitation core 22 and collection core 20.

The distal end of the multi-mode section 24 comprises a recess 30. In the present embodiment, the recess 30 comprises a 20 μm^3 cuboidal recess. In other embodiments, any suitable size and shape of recess may be used. In further embodiments, any suitable number of recesses (or no recess) may be used. A recess may also be described as a pit. In some embodiments, a plurality of pits are chemically etched into the distal end of a probe 12, for example into the distal end of a multi-mode section 24.

After the multi-mode section 24 is spliced to the collection core 20 and excitation core 22, an intermediate assembly step is performed in which bonding of the spliced fibre to the custom end cap is performed using precision alignment tools and a vision system to ensure the end facets of both components are flush with each other and there is no glue on the end face on to which SERS sensors will be deposited. It is also ensured that the splice plane is well enclosed within the rear end of the end cap with glue encapsulation. Once bonded as above, the fibre-optic and end cap assembly is inserted into a biocompatible sheath and further bonded in place.. After the intermediate assembly step, the 20 μm^3 cuboidal recess 30 is machined into the distal facet of the 50 µm core of spliced multi-mode section 24 using high-pulse-energy 800 nm femtosecond laser pulses.

The end-preparation may be performed with the intention of reducing or preventing the loss of nanoshells during transbronchial passes (the use of nanoshells is described below) and/or reducing the Fresnel reflection component of the forward scattered Raman-background. For example, the machining process may result in a recess with a high rms value of surface roughness, which may provide a reduction of the Fresnel reflection compared to a smooth surface.

As shown in Figure 2, probe 12 further comprises an end-cap 26. In the present embodiment, the end-cap 26 is a custom end-cap which may be described as a bespoke fused silica end-cap 26. When the probe 12 is assembled, the multi-mode section 24 is accommodated in the bespoke fused silica end-cap 26. In the present embodiment, the end-cap 26 does not cover the distal facet of the multi-mode section 24. The end-cap 26 surrounds the curved outer wall of the multi-mode section 24 and also surrounds a distal portion of the curved outer wall of the optical fibre 13. In further embodiments, any suitable end-cap configuration may be used.

The end-cap 26 may ensure structural robustness and functional preservation of the probe 12 during repeated transbronchial passes performed during bronchoscopic exploration of the lung. The end-cap 26 may enable robust packaging of the optical fibre 13 and multi-mode section 24.

In the present embodiment, the end-cap 26 is fabricated using ultrafast laser inscription and selective wet etching. The fabrication comprises directly inscribing a bespoke three-dimensional support structure in a transparent fused silica substrate (Corning) using focused (with a 0.4 NA aspheric lens) femtosecond pulses from a commercial micro-Joule fibre laser system (MenloSystems, BlueCut). Any other suitable materials and NA values may be used. For example, sapphire or yttrium aluminium garnet (YAG) could be used if it was desirable for the end cap to be transparent in the mid-infrared. The substrate is translated through the focus at a speed of 4 $mm.s^{-1}$ using high-precision air-bearing translation stages (Aerotech). Using suitable irradiation conditions (in the present embodiment, pulse duration: around 350 fs, repetition rate: 0.5 MHz, pulse energy: 550 nJ), the etching selectivity of the laser modified regions is enhanced relative to the bulk material and subsequently dissolved in a dilute (5 %, aqueous) solution of hydrofluoric-acid within 2 hours. The end-cap 26, with a length of 1 mm and a maximum outer diameter of 1.2 mm, features a 150 μm diameter inner slot to

accommodate a 125 µm wide polymer-stripped section of the fibre-optrode (probe 12). Both the dual core fibre and the multimode commercial fibre include a polymer jacket that was stripped from both fibres before splicing. The diameter of both fibres in this embodiment after stripping the polymer is 125 um.

Figure 5 is a photograph showing the miniaturised fibre-optic probe 12 through the working channel of the bronchoscope 10. Custom end-caps 26 are shown alongside a one-penny coin 34.

In the present embodiment, the fibre-optrode (probe 12) and end-cap assembly is packaged inside a 2.5 m long protective biocompatible sheath 28. The dual-core optical fibre 13 is sheathed inside the biocompatible sheath, with a protruding portion of the optical fibre (for example, around 0.5m long in the described embodiment being placed into the optomechanical mount of the instrument at the proximal end, reducing or eliminating residual stress that may cause misalignment over time. The biocompatible sheath has a total outer diameter of 1.5 mm. In the present embodiment, the biocompatible sheath comprises PEEK (polyethyl ether ketone). In other embodiments, any suitable biocompatible sheath material may be used, for example polyethylene terephthalate, polyimide, or Pebax.

The outer diameter of the biocompatible sheath 28 is chosen to fit within the working channel of the bronchoscope 10. In the present embodiment, the 1.5 mm outer diameter of the biocompatible sheath 28 is chosen to be compatible with the 2.4 mm working channel of a Pentax-EPM1000 bronchoscope 10. In use, the probe 12 is inserted into the working channel of the Pentax-EPM1000 bronchoscope 10. In other embodiments, any suitable bronchoscope 10 may be used.

In the present embodiment, permanent bonding of the distal components, for example bonding the spliced fibre with the end cap, or bonding with the sheath tubing, is performed using ultraviolet epoxy (Norland NOA61). In other embodiments, any suitable bonding material may be used.

Nanoshells 32 or other sensors are introduced to the distal surface of the multi-mode section 24, including the machined recess 30. The nanoshells 32 (in this case comprising a silica core and a gold coating) are functionalised with SERS reporter

molecules. Sensors may alternatively be described as microspheres. In some embodiments, microspheres are deposited into pits that have been chemically etched into the distal end of a probe. Any method of attaching the nanoshells to the distal end-facet of the multi-mode section 24 may be used.

In general, nanoparticles functionalised with reporter molecules may be molecules that may be used as a nano-sensor to sense changes in the surrounding environment. Different molecules may be sensitive to different environmental parameters (e.g. pH, Redox potential), and different molecules may report environmental changes in different ways. In recent years, the possibility of using Surface Enhanced Raman Spectroscopy (SERS) to measure environmentally induced structural changes in reporter molecules has attracted significant attention. Reporter molecules can be attached to gold nanoshells as is the case in the present embodiment. These composite nano-sensors can be used to sense the environment via Raman spectroscopy of the attached reporter molecules. This has been used recently, for example, to measure the environment inside single cells.

In the present embodiment, the nanoshells 32 are functionalised with para-mercaptobenzoic acid (p-MBA). Commercially available (Nanospectra Biosciences, Inc.) silica encapsulated 150 nm Au nanoshells are functionalised with p-MBA as the pH sensing SERS reporter molecule. The p-MBA reporter is used since it has been characterised as being sensitive to pH changes in the physiological range. Au nanoshells with a diameter of 150 nm are used since their plasmon resonance is excited at 785 nm, which may allow the SERS effect to be observed at a wavelength at which there is minimal intrinsic fluorescence from tissue.

After applying the nanoshells 32, the end-facet of the probe 12 (which in this embodiment is the end of the multi-mode section 24) is encapsulated using a permeable sol-gel layer (not shown) to inhibit contact-induced nanoshell loss.

In the present embodiments, the SERS reporter molecules are configured for the sensing of pH. The SERS reporter molecules are reporter molecules for which a different Raman spectrum is obtained when the SERS reporter molecules are in an environment having different pH.

In other embodiments, any suitable SERS reporter molecules may be used. It may also be possible to use our invention to measure the Raman spectrum of the tissue itself in a manner that is degraded less by the Raman background from the optical fibre. The SERS reporter molecules may be configured for the sensing of any appropriate physiological parameter, for example redox state or glucose level. The SERS reporter molecules may be capable of determining physiological parameters (for example, pH or redox potential) deep within the distal lung.

In further embodiments, reporter molecules may be introduced to the distal end of the probe 12 by a method that does not use nanoshells. Any method of attaching reporter molecules to the distal end of probe 12 may be used. In other embodiments, reporter molecules are introduced into tissue that is in contact with the distal end of the probe, or measurements are performed directly on tissue itself without introduction of additional reporter molecules. The probe 12 is used for Raman spectroscopy of the tissue that is in contact with the distal end of the probe 12.

The method of forming probe 12 that is described above may provide a robust technique for embedding SERS reporter molecules on the end of the probe 12, which may enable use of the SERS reporter molecules as physiological sensors *in vivo*.

In use, excitation light travels down the excitation core 22 of the optical fibre into the multi-mode section 24 and to the SERS reporter molecules on the nanoshells 32 at the distal end-facet of the multi-mode section 24. A part of the excitation light is Raman scattered by the SERS reporter molecules. The nanoshells are incoherent sources that emit Raman scattered light in all directions, a fraction of which is collected and returned by the collection core.

The multi-mode section 24 is configured such that a path of the excitation light that issues from the proximal end of excitation core 22 overlaps with a path of the signal light emitted by the SERS sensors. The multi-mode section 24 acts as a light guide, facilitating the overlap of the path of the excitation light and the path of the signal light. In the present embodiment, the light paths overlap at the distal end-facet of the multi-mode section 24. Therefore sensing may be performed at the distal end of the probe 12.

If no additional light guide such as the multi-mode section 24 were present and the SERS reporter molecules were positioned directly on the distal end of optical fibre 13, signal light from SERS reporter molecules excited by excitation light from excitation core 22 may not return through collection core 20, due to the geometric arrangement of the cores.

The use of the light guide (in this embodiment, multi-mode section 24) enables accurate relative positioning of the excitation core 22, collection core 20 and SERS reporter molecules on nanoshells 32. Pump and collection areas at the end of the probe are spatially overlapping.

A Raman spectrum may be obtained for sensors occupying a known position at the distal end of the probe. The present embodiment allows direct spectroscopy of material that is at the end of the probe, in contact with the end face of the probe. By contrast, in some existing Raman spectroscopy methods, an extended volume of tissue may be illuminated and it may not be clear where signal light is coming from. Using some known probes, it may be difficult to obtain a controlled spacing between the probe and what the probe is trying to measure, because the probe measures a spectrum at a distance from the end of the probe. With some known probes, it may be difficult to sense material that is close to a surface of the probe.

A proximal-end optical instrument is coupled to the proximal end of the optical fibre 13. The proximal-end optical instrument is configured to input couple excitation light into the excitation core 22 of the optical fibre 13 and to output couple Raman-shifted signal light from the collection core 20 to a spectrometer 18. Figure 6 is a schematic illustration of the proximal-end optical instrument of the present embodiment. Figure 6 shows various components of the proximal-end optical instrument, which are each described below.

The proximal-end optical instrument comprises a laser source 15 that is configured to provide 785 nm excitation light to the excitation core 22. In the present embodiment, a continuous-wave beam from a commercial 785 nm laser source (Thorlabs) with linewidth of less than 0.1 nm is used. In other embodiments, a different laser source may be used. In the present embodiment, an illumination power of 1 mW is used. In other embodiments, a different illumination power may be used.

In the present embodiment, the laser source 15 provides 785 nm light to an optical fibre 14 (which is different from the optical fibre 13 that forms part of the probe 12). Optical fibre 14 is a single mode fibre (Thorlabs, 780-HP). The mode at 785 nm from optical fibre 14 is imaged at unit magnification using aspheric lenses L1 and L2 onto the proximal end of the excitation core 22 of the optical fibre 13.

The 785 nm light may be referred to as excitation light or pump light. The wavelength of the excitation light is chosen to be a wavelength capable of exciting the SERS reporter molecules on the nanoshells 32 at the distal end of the multi-mode section 24.

Considering the light path of the excitation light from the laser source 15 in greater detail, the excitation light provided from the laser source 15 passes through optical fibre 14 via lens L1 onto fold mirror FM 1. The excitation light then passes through a short pass filter SP. The short-pass filter SP is placed in the input beam path to prevent the SERS signal from being contaminated by long wavelength amplified spontaneous emission from the laser source 15. After passing through the short pass filter SP, the light passes to dichroic mirror DM, at which it is reflected to lens L2. The dichroic mirror is configured to reflect a narrow range of frequencies around 785 nm. The light is imaged at unit magnification onto the excitation core 22 by lens L2.

The excitation light travels from the proximal end of excitation core 22 to the distal end of excitation core 22 and then passes through multi-mode section 24 to the distal end-facet of multi-mode section 24, to which the nanoshells 32 are attached. A part of the excitation light is Raman scattered by the SERS reporter molecules on the nanoshells 32. At least part of the Raman-scattered light (signal light) passes from the distal end-facet of multi-mode section 24 through the multi-mode section 24 to the spliced distal end of collection core 20.

Signal light is returned from the collection core 20 of optical fibre 13 to the proximal-end optical instrument. Signal light from the collection core 20 of optical fibre 13 is output coupled using lens L2 and is imaged at unit magnification using lens L3 and a mirror FM 2 mounted on a kinematic adjuster mount onto a step-index 50 µm core of a multi-mode patch-cable 16 after passing through dichroic mirror DM. The output of the patch-cable 16 is directly coupled to a spectrometer 18 (Ocean Optics, QE Pro) through a 50

μm slit. In the present embodiment, spectral resolution is limited by the spectrometer to around 0.4 nm, narrower than the observed SERS spectral features (the observed SERS spectral features are shown in Figure 7(c), which is described below).

Considering the path of the signal light in the proximal-end optical instrument in greater detail, the signal light is output coupled from the collection core 20 at unit magnification using lens L2. It passes through dichroic mirror DM and through long-pass filter LP to mirror FM 2. The long-pass filter LP is placed in the output beam path to attempt to prevent 785 nm light from being acquired by the spectrometer. Acquisition of 785 nm light by the spectrometer may cause detrimental noise effects in the acquired Raman-shifted spectrum. After passing through the long-pass filter LP, the signal light is imaged at unit magnification onto the step-index 50 μm core of multi-mode patch-cable 16 by lens L3.

The signal light is analysed by the spectrometer 18. The Raman shifts of the light received by the spectrometer 18 are used to determine the pH of the region of the distal lung that is being sensed by the probe. In the present embodiment, the raw spectra received from the spectrometer 18 are processed using algorithms devised to adaptively learn pH driven changes and remove the contribution of any residual background from the spectra. In other embodiments, different processing may be performed, or no processing may be performed.

By adjusting the kinematic adjuster of FM 2, the instrument may be switched between operating in background suppressed mode and in a mode of operation that may be called a normal mode. In background suppressed mode, the proximal-end optical instrument supplies excitation light to the excitation core 22 and receives signal light from the collection core 20 as described above with reference to Figure 6. In background-suppressed mode, light in the single mode excitation core 22 is explicitly excluded from being collected and routed to the spectrometer 18. The SERS reporter molecules are excited using one path in a fibre and signal light is collected using a different path in the same fibre. Our method also allows any material, such as tissue, placed at the distal end to be excited using one path in a fibre and the signal light to be collected using a different path in the same fibre.

In the normal mode, the same multi-mode core (in this case, collection core 20) is used

for excitation as is used for collection. The proximal-end optical instrument supplies excitation light to the collection core 20 (and does not supply excitation light to the excitation core 22) and receives signal light from the collection core 20. In normal mode, the excitation light may generate a Raman background in the optical fibre 13, which may affect the signal-to-background of the received signal light.

Switching between background suppressed mode and normal mode may allow comparative results to be obtained. Figures 7a and 7b show examples of such comparative results, with Figure 7a showing results in normal mode and Figure 7b showing results in background-suppressed mode.

The results of Figures 7a and 7b are obtained using the embodiment of the packaged fibre-optic probe 12 described above with reference to Figures 1 and 2. The results of Figures 7a and 7b are obtained using identical excitation power (0.2 mW) and acquisition time (60 s). The number of counts in 60 seconds (in arbitrary units) is plotted against Raman shift (in cm^{-1}).

Figure 7a is a plot of a p-MBA SERS spectrum acquired between 800 cm^{-1} and 2000 cm^{-1} when the MM collection core 20 is used for both excitation and collection (normal mode) and with the probe being in air. In normal mode, the probe 12 acts as a bidirectional probe. Bidirectional probes may be optrodes in which the same path, for example the same fibre core, is used for excitation light as for collection of signal light.

It has previously been found that in the low wavenumber region (less than or equal to 2000 cm^{-1}) SERS spectra acquired using bidirectional optrodes may be accompanied by an intense broad continuum. The intense broad continuum may originate due to inelastic scattering from the Raman-active material of the fibre core (which may typically comprise doped silica) along which a guided mode or modes of monochromatic excitation light propagate. The fibre-generated background may impose a limitation on optrode sensing schemes in which bidirectional optrodes are used.

Figure 7a shows such an intense broad continuum of Raman background. It may be seen that a large background is present for normal mode operation, especially at low

Raman shift. In practice, an even larger Raman background may be present at Raman shifts below those shown in Figure 7a.

Figure 7b is a plot of a p-MBA SERS spectrum acquired between 800 cm^{-1} and 2000 cm^{-1} when the SM excitation core 22 is used for excitation and the MM collection core 20 is used for collection (background suppressed mode). Figure 7b does not exhibit the intense broad continuum of Raman background that is present in the plot of Figure 7a. The probe 12 may facilitate the acquisition of a SERS signal where the background spectrum from the fibre itself is greatly reduced. In some circumstances, the probe 12 may be considered to acquire a signal that is substantially fibre-background-free.

Figure 7c shows a characteristic p-MBA SERS spectrum acquired using the fibre-optic probe 12. Figure 7c shows the same results as Figure 7b, but shows them at a different scale so that more detail may be seen.

In background suppressed mode, shown in Figure 7b, suppression of the fibre Raman background by around 100-fold is achieved in comparison to the normal mode spectrum shown in Figure 7a. The use of separate cores (co-located in one fibre) to deliver the pump laser input and collect the return signal may remove the unwanted background signal. The use of separate paths, or cores, for excitation and collection may ensure that a large amount of silica background Raman signal that is generated in the excitation core 22 by the pump light from the (laser source 15) is not collected and transmitted to the spectrometer 18 by the collection core 20.

In the results of Figures 7a and 7b, desired SERS spectral features may be distinguishable even in the presence of the Raman background. See, for example, the peak at around 1580 cm^{-1} in Figures 7a, 7b and 7c which may be distinguished even in the presence of the Raman background in Figure 7a. However, the SERS signal acquired in background-suppressed mode may have better signal-to-background than that acquired in normal mode.

In other embodiments, the SERS signal level may be lower. In some embodiments, it may not be possible to observe the SERS signal in the presence of the background. The removal of Raman background may be highly relevant for SERS reporter molecules having signal features at lower Raman shift, at which fibre background may

in some circumstances be orders of magnitude larger. In some embodiments, using different SERS reporter molecules may put the SERS emissions into a region of the spectrum where fibre Raman background is more significant or is dominant and where improved signal-to-background may be important.

In some embodiments, there are no SERS reporter molecules on the end of the probe. Instead, a part of the excitation light undergoes Raman scattering in tissue that is in contact with the distal end of the probe. The signal level of a Raman signal from the tissue may be low. For example, the signal level of the Raman signal from the tissue may be orders of magnitude lower than that of a Raman signal obtained from SERS reporter molecules. If the signal level is low, the removal of the Raman background resulting from using separate excitation and collection paths may be particularly beneficial.

The removal of fibre background may be particularly relevant if the fibre optic spectroscopic sensor is to be deployed within the healthcare scenario, such as intensive care units, where the real-time detection of minute changes in physiological conditions within the patient's body may be performed.

Probe 12 may be a robust probe that may enable advanced sensing measurements. The probe 12 may be a simple optical instrument that may in some circumstances generate background suppressed data using existing spectroscopic sensors without the need for complex signal processing. By using a different core, or path, to deliver the coherent light than is used to collect the Raman-shifted light, the fibre Raman background may be suppressed. The probe 12 may be configured so as to substantially prevent, or greatly reduce, the fibre-background from contaminating the SERS signal of interest. The simple and robust configuration of the distal spliced optrode may enable augmented SERS signal collection with 100-fold or greater improvement of signal to background.

The use of an end-coupled (optrode) sensing scheme may be well-suited for *in vivo* application as it may enable the use of a single fibre (in this embodiment, optical fibre 13) to deliver coherent light to excite SERS sensors coated on the distal end-facet, collect the incoherent Raman-shifted light that encodes the physiologic information, and guide the Raman-shifted light back to the proximal end for processing.

The use of a single fibre 13 may make the probe 12 compact. Devices using multiple fibres may require additional packaging to make them more rugged. Such extra packaging may increase the size of the probe.

The probe 12 in the embodiment of Figures 1 and 2 has been miniaturised to less than 1.4 mm lateral diameter with potential for further reduction. The probe 12 is a miniaturised, flexible single fibre-based instrument. Its small lateral diameter may allow its use in the distal pulmonary tree. Instruments with larger lateral dimensions (for example, greater than 5 mm lateral dimension) may be unsuitable for use in the distal pulmonary tree and/or in some other anatomical sites. The miniaturisation of the robust optrode based physiological sensor (probe 12) when compared with some existing systems may have widespread application in other endoscopically accessible organs.

The technical methodology described with reference to Figures 1, 2, and 6 may enable the *in situ* physiological sensing of the respiratory acinar unit. The methodology may permit minimally invasive, real-time *in vivo* monitoring of physiology. The probe 12 may be used to achieve non-surgical dynamic physiological sensing in multiple sites in the distal lung.

Being able to determine physiological parameters deep within the distal lung may provide valuable information on tissue function. The information provided may increase understanding of respiratory physiology in health and disease. In some circumstances, it may be possible to obtain measurements of distal lung physiology that were previously unobtainable, or difficult to obtain.

The use of the probe 12 to sense the distal lung may provide more accurate and/or less invasive monitoring of the distal lung than some current methods, for example that sampling by bronchoalveolar lavage, transbronchial lung biopsy or transbronchial needle aspiration.

A benefit of using SERS as an analytical technique may be that spectral signatures from Raman-active analytes may be detected with exceptional sensitivity and specificity, which may be due to the plasmonic enhancement of the Raman signal and an inherent multiplexing ability. The SERS reporter molecules on the end of probe 12

are capable of being used to determine physiological parameters (for example, pH or redox potential) directly in the lung.

In other embodiments, the probe may be configured to determine other physiological parameters and/or to determine physiological parameters in a different anatomical region.

The miniaturized fibre-optic pH sensing probe 12 may be considered to be a generic platform that may be multiplexed with other SERS reporters to enable the concurrent *in vivo* and *in situ* monitoring of additional physiologically relevant parameters in the alveolar space or in other anatomical regions. For example, other SERS reporters may be added which report redox state, oxygen tension and/or glucose levels.

In some embodiments, at least molecules of at least two different SERS reporters are embedded at the distal end of probe 12. The different SERS reporter molecules are used to sense different physiological parameters.

In the present embodiment, the optical fibre 13 comprises a single excitation core 22 transmitting a single frequency of excitation light. In other embodiments, the optical fibre may comprise any number of excitation cores and/or collection cores. In some embodiments, a plurality of optical fibres may be used. For example, one optical fibre may be used for excitation and another optical fibre for collection.

In some embodiments, an optical fibre comprises a plurality of excitation cores, each of which is used to transmit a different frequency of excitation light. In some such embodiments, at least two different types of SERS reporter molecules are present on the distal end of the probe, and the different types of SERS reporter molecules may be excited by different or the same frequencies of excitation light. Different reporter molecules may be multiplexed on different individual cores. The probe 12 may provide a sensing fibre with integrated reporters for multiplex analysis of physiological parameters.

In some embodiments, the optical fibre comprises a plurality of excitation cores and a matched plurality of collection cores. The excitation cores and collection cores are

arranged in pairs such that, in each pair, a signal induced by the excitation light delivered via the excitation core of the pair is collected by the collection core of the pair.

In some embodiments, the probe may be integrated into an instrument that also provides additional functionality, for example imaging. In one embodiment, the probe 12 is packaged as part of a composite fibre with a small capillary to allow local delivery of substances to the lung, for example delivery of Smartprobes to facilitate imaging. The probe 12 may be packaged as part of a composite fibre with a fibre comprising a plurality of imaging cores. This capillary could also form part of a single fibre used for sensing and imaging.

Figure 8 is a schematic illustration of the proximal end of an optical instrument according to a further embodiment. In the embodiment of Figure 8, the optical fibre 13 is a multi-core fibre (MCF) having 19 cores. One of the 19 cores is used as an excitation core. The other 18 cores are used as collection cores.

In order to measure the substantially background-free spectrum that is transmitted by an MCF to its proximal end, an optical instrument is designed that enables pump and signal paths to be spatially separated.

In the system of Figure 8, pump light is generated by a 785 nm laser diode 15. The pump light is delivered down a single-mode fibre (SMF) 14. The single mode fibre 14 supplying light from the laser diode 15 has a mode field diameter (MFD) of around 5 μm. The end of SMF 14 is imaged at the same plane as pick-off mirror 19. The end of the SMF 14 is imaged to the plane of the pick-off mirror using lens L1. The light from the SMF also passes through a shortpass filter F1, which is a shortpass filter at 800 nm.

This plane is then re-imaged to the proximal end of MCF 13, where the pump light is coupled into one of the MCF cores (the one core that acts as an excitation core). The plane is re-imaged using lens L2. An inset in Figure 8 shows the distal end of the 19 multi-core fibre 13. A 105 μm (core diameter of the spliced fibre) multi-mode section 24 is spliced to the end of the MCF 13.

Pump light travels down the MCF 13 where it then excites SERS sensors deposited on the distal-end of the fibre. The excitation of the SERS sensors by the pump light generates the SERS signal of interest, a proportion of which is collected by other cores of the MCF (the collection cores). The signal is transported down the MCF 13 and focused to the pick-off mirror plane by lens L2. The pick-off mirror 19 is used to reflect the SERS signal towards lens L3 and is eventually coupled into a multi-mode fibre 16 for transmission to a spectrometer (not shown) for analysis. The multi-mode fibre has a diameter of around 50 µm. In the present embodiment, the spectrometer is a QE Pro spectrometer with a slit width of 50 µm.

The signal, after reflection from the pick-off mirror 19, passes through filter F2 which is a longpass filter at 800 nm, and is focused by lens L3. In the embodiment of Figure 8, each of lenses L1, L2 and L3 is a matched achromatic doublet with magnification of around 1:3.33.

Since the pump and signal paths of the instrument of Figure 8 are spatially separate, the acquired signal spectrum may be substantially free of, or may at least exhibit a greatly reduced contribution from, the fibre Raman spectrum.

A highly simplified view of the probe 12 described above with reference to Figures 1 and 2 is illustrated schematically in Figures 9a, 9b and 9c, for comparison with further probe embodiments that are described below with reference to Figures 10a to 15c. Each of the probes of Figures 9a to 15c comprises a light guide, which in the embodiment of Figures 9a to 9c is multi-mode section 24, but may be a different type of guide in different embodiments.

Figure 9a shows an end view of the proximal end of probe 12. Figure 9b shows a perspective view of probe 12. Figure 9c shows an end view of the distal end of probe 12. The features of Figures 9a to 9c (and of Figures 10a to 15c below) are not shown to scale.

Figures 9a and 9b show optical fibre 13 comprising excitation core 22 and collection core 20. The two cores 20, 22 are of different sizes. In other embodiments, a different layout of cores may be used. Any appropriate core sizes may be used. The cores 20,

22 have a higher refractive index (represented by shading) than the rest of the optical fibre 13.

Figures 9b and 9c show multi-mode section 24, which is spliced onto the distal end of optical fibre 13. Figure 9c displays the 50 μm multi-mode core 24a of multi-mode section 24. The cross-sectional area of multi-mode core 24a overlaps the cross-sectional areas of excitation core 22 and collection core 20 of the multi-core optical fibre 13.

In the present embodiment, the excitation path along which the excitation light travels comprises the small, single-mode excitation core 22. The collection path along which the signal light travels comprises the larger, multi-mode collection core 20. The excitation core 22 and collection core 20 are formed in a single optical fibre 13.

The multi-mode section 24 is spliced to the distal end of the optical fibre 13 and SERS reporter molecules are embedded in the distal end-facet of the multi-mode section 24. The SERS reporter molecules form a target for the excitation light.

In use, the multi-mode section 24 acts as a guide that guides excitation light from the distal end of the excitation core 22 to the SERS reporter molecules on the distal end-facet of the multi-mode section 24, and guides signal light from the SERS reporter molecules to the distal end of the collection core 20. In the present embodiment, light is guided within the multi-mode core 24a of multi-mode section 24.

The length of the multi-mode section 24 is kept short enough to minimise Raman background generated within its core, while being long enough that the mode structure of the excitation light and signal light overlap. The length of the multi-mode section 24 is such that light from the excitation core 22 can excite SERS molecules which then provide signal light to the collection core 20, without the use of additional optical elements such as lenses to guide the signal light into the collection core 20.

The probe 12 may be used to sense the environment at the distal end of the probe. Since the sensing is performed using sensors on the distal end-facet of the probe, it is possible to know where the signal originates from (in contrast to other systems that may sense the environment some distance into the tissue, in which it may be difficult to

determine that distance).

Figures 10a, 10b and 10c are schematic illustrations of a further embodiment of a probe 50. As shown in Figures 10a and 10b, probe 50 comprises an optical fibre 51 comprising an excitation core 52 and collection core 53, which may be similar to those of probe 12 of the previous embodiment. However, instead of a multi-mode section of optical fibre, an undoped section of solid glass 54 is spliced to the distal end of optical fibre 51. SERS reporter molecules are introduced to the distal end-facet of the solid glass section 54. The solid glass section 54 is configured to guide excitation light from the distal end of the excitation core 51 to the SERS reporter molecules on the distal end-facet of the solid glass section 54, and to guide signal light from the SERS reporter molecules to the distal end of the collection core 52. In other embodiment, a section of polymer instead of a section of glass may be used.

Figures 11a, 11b and 11c are schematic illustrations of a further embodiment of a probe 60. As shown in Figures 11a and 11b, probe 60 comprises an optical fibre 61 comprising an excitation core 62 and collection core 63, which may be similar to those of probes 12 and/or 50. A glass end-cap 64 is configured to be fitted to the distal end of the optical fibre 61. The end-cap 64 comprises a multi-mode core 65, which in this embodiment is a laser-written multi-mode core. The cross-sectional area of the multi-mode core 65 overlaps the cross-sectional area of the excitation core 62 and of the collection core 63.

SERS reporter molecules are deposited on a distal end-facet of the glass end-cap 64. The multi-mode core 65 of the end-cap 64 is configured to guide excitation light from the distal end of the excitation core 62 to the SERS reporter molecules on the distal end-facet of the end-cap 64, and to guide signal light from the SERS reporter molecules to the distal end of the collection core 63.

In each of the embodiments of Figures 9a to 11c, the optical fibre 13, 51, 61 is a dual-core optical fibre comprising one excitation core 22, 52, 62 and one collection core 20, 53, 63. In other embodiments, a different optical fibre is used. In some embodiments, the optical fibre comprises a single excitation core configured to deliver excitation light, and a plurality of collection cores configured to collect signal light.

Each of the embodiments described above with reference to Figures 9a to 11c comprises a guide configured to guide excitation light from an excitation core to SERS reporter molecules on the distal end of the guide, and to guide signal light from the SERS reporter molecules to a collection core. In the embodiments of Figures 9a to 11c, the guide comprises respectively a spliced multi-mode section, an undoped glass section, and an end-cap comprising a multi-mode core.

In other embodiments, a different approach is taken to forming the guide. In some embodiments, the guide is formed by tapering a distal section of the optical fibre such that a path followed by the excitation light and a path followed by the signal light overlap.

Figure 12a, 12b and 12c are schematic illustrations of a further embodiment of a probe 70. As shown in Figures 12a and 12b, probe 70 comprises an optical fibre 71 comprising seven equal size cores. The cores have a high index compared with the rest of the fibre, the high index being shown shaded. The seven cores comprise a single excitation core 72 and six collection cores 73.

Figure 12b shows that a distal section 74 of optical fibre 71 is tapered so that all cores 72, 73 merge within the distal section 74. The tapered distal section 74 of the optical fibre 71 may be considered to form a photonic lantern. The photonic lantern brings the separate cores together to form a large multi-mode waveguide and hence to spatially overlap the modes from the different cores. The excitation core 72 and collection cores 73 may each be considered to terminate at a point at which they cease to be distinct from other cores.

Figure 12c shows an end view of the tapered distal section 74. SERS reporter molecules are introduced to the distal end-facet of the tapered distal section 74 (i.e. to the distal end-facet of the photonic lantern). The tapered distal section 74 is configured to guide excitation light from the excitation core 72 to the SERS reporter molecules on the distal end-facet of the tapered distal section 74, and to guide signal light from the SERS reporter molecules to the collection cores 73.

The tapering of the optical fibre 71 to form a photonic lantern may provide a smoother transition than that provided, for example, by splicing a multi-mode section onto an

optical fibre. In some circumstances, the smooth transition resulting from the tapering may allow more efficient excitation and collection.

In a further embodiment (not shown), a probe comprises an optical fibre comprising one excitation core and six collection cores, which may be similar to the optical fibre 71 shown in Figures 12a and 12b. The probe further comprises a multi-mode section, which may be similar to the multi-mode section 24 of the embodiment of Figures 9a to 9c. The proximal end of the multi-mode section is spliced to the distal end of the optical fibre.

SERS reporter molecules are introduced to the distal end-facet of the multi-mode section. The multi-mode section comprises a multi-mode core that has a cross-sectional area configured to overlap the cross sectional area of the excitation core and of all six of the collection cores. The multi-mode core of the multi-mode section is configured to guide excitation light from the distal end of the excitation core to the SERS reporter molecules on the distal end of the multi-mode section, and to guide signal light from the SERS reporter molecules to the distal end of each of the six collection cores. In other embodiments, an optical fibre comprising any suitable number of excitation cores and any suitable number of collection cores may be used in combination with a photonic lantern, a spliced multi-mode section, a solid glass section, an end-cap comprising a multi-mode core, or any other suitable guide.

Figures 13a, 13b and 13c are schematic illustrations of a further embodiment of a probe 80. Probe 80 comprises an all solid bandgap fibre 81. An end view of the bandgap fibre 81 is shown in Figure 13a. High index regions 83 of the bandgap fibre are shaded. The high index regions may be referred to as high index inclusions.

Figure 13b provides a further view of probe 80 comprising bandgap fibre 81, which comprises central region 82 and high index regions 83. A distal section 84 of bandgap fibre 81 is tapered. The tapering stops excitation light from being guided in the low index central region 82. The tapered region 84 is shown in Figure 13c.

In use, excitation light is guided in a central region 82 of the bandgap fibre in which there is no high index region. The central region provides a path for excitation light. The guidance bandwidth of the central region may be small and may be changed by

tapering. Signal light returns through the high index regions 83 which act as a plurality of collection paths.

SERS reporter molecules are introduced to the distal end-facet of the tapered distal section 84. The distal section 84 is configured to guide excitation light from a point at which the low index region ceases to guide the excitation light to the SERS reporter molecules on the distal end-facet of the distal section 84. The distal section 84 is further configured to guide signal light from the SERS reporter molecules to the high index regions 83.

In further embodiments, a bandgap fibre, for example a bandgap fibre 81 as described above with reference to Figures 13a to 13c, may be combined with a different guide instead of or in addition to the tapered distal section. For example, the bandgap fibre may be combined with a spliced multi-mode section, a solid glass section, an end-cap comprising a multi-mode core, or any other appropriate guide.

Figures 14a, 14b and 14c schematically illustrate a further embodiment of a probe 90. Probe 90 comprises a hollow core optical fibre 91. In Figures 14a, 14b and 14c, low index regions are shaded (a different convention to that of Figures 9a to 13c in which high index regions are shaded). Hollow core fibre 91 comprises a hollow core 92 and cladding 93. The hollow core 92 is an air core. The cladding 93 comprises a low index material, for example a low index glass or polymer material. Figure 14a shows an end view of the proximal end of the hollow core fibre 91.

Figure 14b shows a perspective view of the probe 90. In use, hollow core 92 provides an excitation path for excitation light. Cladding 93 provides a collection path for signal light. The hollow core 92 may have a narrow guidance bandwidth when compared with a guidance bandwidth of the cladding.

In some embodiments, the cladding 93 is surrounded by an additional cladding. For example, the cladding 93 may be formed of silica glass and may be surrounded by an additional cladding of a lower index. In some embodiments, the additional cladding comprises glass. In some embodiments, the additional cladding comprises a low-index polymer. The additional cladding may be surrounded by a polymer coating.

Hollow core fibre 91 comprises a distal section 94 which is tapered. Figure 14 shows a view of the tapered distal section 94. Within the tapered section, the air holes of the hollow core are collapsed, leaving the low index core outer ring. SERS reporter molecules are introduced to the distal end-facet of the tapered section 94.

In some embodiments, the hollow core fibre 91 can be used without tapering, such that laser light with the desired properties can be delivered to tissue, and any optical signal generated by the tissue can be collected using a different path or core within the fibre/s and transported to proximal instrumentation for analysis.

The distal section 94 is configured to guide excitation light from a point at which the hollow core 92 ceases to guide the excitation light to the SERS reporter molecules on the distal end of the distal section 94. The distal section 94 is further configured to guide signal light from the SERS reporter molecules through the cladding 91.

In some circumstances, using the hollow core of a hollow core fibre as an excitation path may not generate much Raman background, because the excitation path may mostly be not in glass. However, using the probe of Figures 14a to 14c (or of Figures 15a to 15c below) in which excitation path and collection path are separated may remove any remaining Raman background.

Figures 15a, 15b and 15c schematically illustrate a further embodiment of a probe 100. As shown in Figures 15a and 15b, probe 100 comprises a hollow core fibre 101 which may be similar to the hollow core fibre 91 described above in relation to Figures 14a to 14c. Hollow core fibre 101 comprises a hollow core 102, which provides a path for excitation light, and cladding 103, which provides a path for signal light. A section of multi-mode fibre 104 is spliced to the distal end of hollow core fibre 101. The multi-mode section 104 is shown in Figure 15c using the convention in which high-index material is shaded (in contrast to Figures 15a and 15b, in which low-index material is shaded). Multi-mode section 104 comprises a multi-mode core 105.

SERS reporter molecules are introduced to the distal end-facet of the multi-mode section 104. The multi-mode core 105 of the multi-mode section 104 provides a guide configured to guide excitation light from the distal end of the hollow core 102 to the SERS reporter molecules on the distal end-facet of the multi-mode section 104. The

multi-mode core 105 of multi-mode section 104 is further configured to guide signal light from the SERS reporter molecules to the distal end of the cladding 103.

In other embodiments, any suitable guide may be used in combination with a hollow core fibre. In further embodiments, any suitable guide may be used in combination with any suitable optical fibre.

The guide may comprise, for example, a tapered region (which may be a photonic lantern), a multi-mode section or a solid glass section. The guide may comprise an end-cap. The end-cap may have a multi-mode waveguide or a photonic lantern fabricated inside it. The end of the guide may be coated with sensors, for example with gold nanoshell sensors. The length of the guide is such that the paths of excitation light and signal light overlap at the distal end of the probe. Signal light produced by the sensors in response to excitation light from the excitation core is provided to the collection core or cores.

The optical fibre may comprise any number of excitation cores and/or collection cores. In further embodiments, a plurality of optical fibres may be used. For example, one optical fibre may be used for excitation and another optical fibre for collection.

Figures 23a and 23b are schematic illustrations of part of a further embodiment of a probe 70. As illustrated in Figures 23a and 23b, a multimode collection fibre 112 and a separate single-mode excitation fibre 114 are threaded into a fluorine-doped (ie, reduced refractive index) silica capillary then tapered down together to form a type of "photonic lantern" 116. In the tapering process the two fibres 112, 114 fuse together and the capillary shrinks onto them to form a new multimode guide 118 at the boundary with the fluorine-doped glass. Because the single-mode core is reduced in size, the excitation light spreads out from it to fill the new guide, thus overlapping with the (reduced) core of the collection fibre 112 where the sensing particles are deposited. It is straightforward to physically separate the excitation and collection light at the proximal end.

Experimental results

Experimental results were obtained using a bespoke sensing optrode utilising an asymmetric dual core optical fibre and SERS with 150 nm gold nanoshells embedded in a protective end cap with 785 nm coherent excitation. The nanosensors were deposited at the distal end of the multimode section, which is flush with the end face of the end cap. The large combined surface area of the fibre plus endcap made deposition easier than just on the fibre end face. The nanosensors were encapsulated using a sol gel layer. Experimental results are described below with reference to Figures 7a to 7c, and Figures 16 to 22. Multiple site pH sensing was demonstrated in the respiratory acinar units of a whole *ex vivo* ovine lung model.

Experimental results detailed below indicate a ~100-fold improvement in signal to background ratio when compared to a bidirectional sensing methodology. The ~100-fold improvement in signal to background ratio may imply that measurement may no longer be limited by the fibre Raman contribution.

Testing of the illumination intensity determined that, in the probe 12 under test, illumination powers greater than 1 mW appeared to cause degradation of the SERS signal over extended periods. The observed degradation of the SERS signal may be due to heating. It was observed that around 0.4 mW power exited the probe 12 after SERS absorption. For the experiments, the power or power to area ratio was set below a suitable threshold, for example at less than half the observed threshold for spectral degradation.

Raman spectra obtained for the probe 12 in background suppressed mode and in normal mode have already been described above and are shown in Figures 7a, 7b and 7c.

It was found that the characteristic SERS spectrum of the p-MBA molecule in this embodiment, which is shown in Figure 7c, is almost completely identical to that recorded for the p-MBA molecule on gold-coated slides. The similarity of the measured SERS spectrum to that on gold-coated slides may allow the use of the SERS reporter molecules of the present embodiment for pH analysis.

The pH-sensitive variation in the p-MBA SERS spectrum obtained using the packaged miniaturised fibre-optic probe 12 was assessed using buffers with pH ranging from 4.0

to 9.0. 21 buffer solutions were prepared within the physiologically relevant range of pH 6.0 to 8.0.

165 SERS spectra were acquired and averaged in 5 non-sequential replicate measurements, with the order of measurement randomised within each replicate. The raw spectra were computationally processed using algorithms devised to adaptively learn pH driven spectral changes and remove the background.

Figure 16 is a plot of a p-MBA SERS spectrum from 1300 cm^{-1} to 1800 cm^{-1} showing a pH sensitive response in the vicinity of 1380 cm^{-1} and 1700 cm^{-1}. In the vicinity of 1380 cm^{-1} and 1700 cm^{-1}, the spectrum obtained is found to be different for different values of pH.

In a further approach, the spectral shape of a known fibre background was subtracted from the raw spectra. The spectral shape of the known fibre background was normalised to a reference Raman-shift position (1070 cm^{-1}) that is invariant with pH. In experiments using the probe 12 of the embodiment of Figures 1 and 2, the method of subtracting the spectral shape of the known fibre background was found to provide an inferior pH estimate to the method using the algorithms that were devised to adaptively learn pH driven spectral changes and remove the background. However, in other embodiments, any suitable method for subtracting fibre background may be used.

The post-processed spectra were subsequently evaluated for changes in the ratio between the area under the curve (AUC) within a 50 cm^{-1} window centred at Raman shifts of 1380 cm^{-1} and 1700 cm^{-1}, which may be the spectral features most sensitive to pH variation. The post-processed spectra demonstrated a clear and consistent variation within the physiologically relevant pH range, in particular between 6.0 and 7.0.

Figure 17 is a plot of variation of the AUC ratio with respect to pH in the range 4.0 to 9.0 obtained after computational data processing. The error bars represent the standard error of the mean over five technical replicate measurements, acquired over measurement intervals up to 9 hours. The extended time intervals between replicate measurements may increase the extent of the error bars. Figure 18 shows a result

obtained from a separate experiment performed within a narrower pH range (pH 6.0 to pH 7.0).

An *ex vivo* ventilated ovine lung model was used to test the feasibility of performing serial alveolar pH measurements in a human-size lung model in disparate bronchopulmonary segments using the probe 12 described above with reference to Figures 1 and 2.

A set-up for *ex vivo* ovine lung perfusion and ventilation comprised an incubator, a physiology monitor, a bronchoscopy screen, a ventilator and closed breathing circuit, a ventilated ovine lung, a water bath and perfusate circuit, and a roller pump.

Ovine lungs were from ewes destined for cull and were euthanized under Schedule 1 of Animals (Scientific Procedures) Act 1986. Heart and lungs were excised from the culled donor sheep and immediately flushed with 0.9 % sterile NaCl (Baxter) (with the addition of Heparin sodium (LEO Laboratories Limited, Berkshire UK) at 500 $U.L^{-1}$ for perfusion models).

Lungs were held on ice until commencement of ventilation, whereby they were placed inside a neonatal incubator (Druger Isolette C2000) and maintained at 35 ℃, 50 % humidity. Lungs were prepared for ventilation using a soft tracheal tube (Rusch) and hand ventilation.

Once recruitment was observed for the whole lung, automated ventilation was initiated (Breas PV 403 PEEP) by gradually increasing respiratory rate to 12 breaths per minute and peak airway pressure approximately 30 cmH_2O.

Six distal sub-segments in the lung were instilled with 10 mL of buffer solutions with pH ranging from 2.0 to 12.0. 10 mL of each pH buffer (pH 2, 4, 6, 8, 10, 12) was instilled into anatomically distinct regions of the ovine lung through a Flexible APC Probe (ERBE USA Incorporated) inserted down the working channel of the bronchoscope 10 (Pentax EPM-1000) to the distal lung.

The lung was not perfused during this experiment, in order to prevent the instilled solutions from accessing perfused vasculature and affecting other sub-segments. This enabled 6 independent pH measurements to be acquired.

After 30 minutes of incubation (to allow buffer dispersal), the fibre-optic probe 12 was inserted into the lung through the working channel of the bronchoscope 10 to measure the tissue pH at each of the buffer instillation sites.

After the 30 minute interval to permit self-buffering, the sub-segments were sequentially interrogated using the probe 12 and spectra recorded with an integration time of 60 s using 0.2 mW of 785 nm coherent excitation light. The working channel of a standard flexible bronchoscope was used to navigate the packaged fibre-optic probe through the bronchial tree prior to a transbronchial pass into the alveolar space of each sub-segment. Alveolar sensing was ensured through proximally wedging the bronchoscope in 3rd order bronchi and extending the sheath a defined distance to reach the subpleural alveolar regions.

Following the internal tissue measurements with the optical probe 12, perfusion was stopped. For each sub-segment, the location of the probe 12 at the distal end was marked on the exterior of the lung and spectral measurements were performed in three sequential repetitions. Each optical probe measurement site was measured by commercial tissue pH probe (Mettler Toledo) via small incisions made vertically through the exterior of the lung.

Three incisions were made within the vicinity (within around 5 mm) of each marked location (each probe location marked on the exterior of the lung 104) and a commercial large-bore tissue pH monitor (Mettler Toledo) was used to measure the pH in the respective sub-segments. The decision to objectively validate the optical measurements using multiple closely-spaced incisions was primarily based on the large difference in bore diameter between the two probes (probe 12 and the commercial tissue pH probe) and the consequent impracticality of precisely determining (within millimetres) the exact location in the alveoli where the fibre-optic interrogation was performed.

For each of the marked locations on the lung, measurements using the commercial tissue pH probe were taken from the central site (i.e. from the marked location) and from 5 mm either side of the central site, in order to best sample the region into which the optical probe 12 was inserted.

Figure 19 shows the location of the six measured sub-segments (1, 2, 3, 4, 5, 6) on the ovine lungs. The numbers 1 to 6 indicate the order in which the instilled sub-segments were interrogated using the fibre-optic probe 12.

Figure 20 is a plot of p-MBA spectrum between 1300 cm^{-1} and 1800 cm^{-1} obtained from the sequential interrogation of the six distal sub-segments shown in Figure 19.

Figure 21 is a plot of alveolar pH measured using the fibre-optic probe 12 for the six sub-segments of Figure 19. The data-points are labelled 1 to 6, corresponding with the sub-segments 1 to 6 as shown in Figure 19. The y-axis of the plot of Figure 21 represents pH measured using the probe 12 for each of the six measured sub-segments 1 to 6 (labelled 1 to 6 on the plot). The x-axis of the plot of Figure 21 represents the pH measured using the commercial pH monitor at the incised locations in each sub-segment 1 to 6.

For the six sub-segments interrogated using the fibre-optic probe 12, the alveolar pH evaluated from the spectral AUC (area under the curve) ratio was found to be in good agreement with the pH measured using the commercial probe. Each sub-segment showed an expected disparity in pH from that of the pre-instilled fluid due to self-buffering. In 3 sub-segments (1, 2 and 4), the pH measured using the commercial probe revealed notable variations (greater than 0.8 pH units) across a short spatial measurement range (less than 1 cm). This resulted in an apparent lowering in correlation between the respective validation measurements. For these sub-segments, the tip of the large-bore commercial probe could not be precisely co-located with the miniature optical probe 12.

An experiment to demonstrate the sensitivity of the fibre-optic probe to temporal variations in the distal lung pH was performed using an *ex vivo* lung perfusion (EVLP) and ventilated model. A comparison of tissue pH and blood pH was also measured in the EVLP model.

To perform perfusion, a complete circuit was formed with a Peristaltic Pump (504 Du IP55 Washdown, Watson-Marlow) tube feeding into the pulmonary artery and out of the left ventricle to the collection reservoir. Any open blood vessels were clamped or sutured closed to prevent leakage of perfusate. The circuit was perfused with Hank's Buffered Salt Solution (HBSS, Gibco) supplemented with 5 % foetal bovine serum (FBS, Gibco) and 250 ml freshly harvested human blood. A total of 5000 U of heparin was added to the circuit in a final volume of 2 L.

Ventilation was performed as described above for 3 hours, with 500 µl blood samples removed from the circuit hourly. Ventilation was subsequently stopped, with continuing perfusion.

A single sub-segment in the lung was selected and the fibre-optic probe 12 was positioned at the distal end through the working channel of the bronchoscope 10. Over a duration of 60 minutes, samples of perfused blood were extracted at 10-minute intervals. All samples were probed with the commercial pH meter at the time of collection. Thereby, the pH was measured with the commercial pH probe while alveolar SERS spectra were concurrently or near-concurrently acquired using the fibre-optic probe 12. The temporal variation in alveolar pH was found to be consistent with that of perfused blood, with both measurements showing the expected correlated reduction in pH when ventilation was stopped.

Figure 22 shows alveolar pH variation as a function of time in an *ex vivo* ovine lung model with ceased ventilation (t = 0) measured using the fibre-optic probe 12. The variation of perfusate pH with time measured using a commercial pH probe is also shown.

The experimental implementation of a bronchoscope-deployable SERS based fibre-optic pH sensing probe and validation in a whole *ex vivo* ovine lung model has been described above. A ~100-fold enhancement in SERS signal to fibre background ratio is demonstrated.

One embodiment of the probe 12 has been described above with reference to Figures 1 and 2, and experimental results for that probe are shown in Figures 7a to 7c, and

Figure 16 to 22. However, numerous embodiments of the probe may be conceived, some of which were described above with reference to Figures 10a to 15c.

In some embodiments, a stronger SERS signal may be generated by altering the chemistry of the sensor itself or by synthesising an entirely different chemical compound. In some embodiments, signal-processing protocols may be applied to the acquired data in order to extract the desired information.

It is believed that the remaining limit to sensitivity in the measurement of pH using a probe with SERS reporter molecules as described above may in some cases be due to dark noise inherent in the spectrometer 18. It is possible that the dark noise inherent in the spectrometer may be further improved with evolving spectrometers.

With further development and ongoing improvements in SERS sensitivity and optical performance, a technology platform in accordance with embodiments described above may have the potential to complement endoscopic procedures and to generate signatures of distal lung physiology to improve our understanding of pulmonary biology.

The embodiments described above have described the deployment of a probe 12 into the lung 104 of a subject. In other embodiments, the optical fibre probe 12 may be used to sense the environment inside any suitable region of any human or animal subject, for example any part of the body that may be accessed using an endoscope. For example, the probe may be used to sense the environment inside the gastrointestinal tract or urinary tract or indeed other fibre-optic accessible organs.

Any suitable SERS sensors may be used to obtain any appropriate data, for example relating to pH, redox state, oxygen tension, or glucose levels.

Although embodiments have been described in relation to the measurement of Raman spectra, in other embodiments the apparatus may be used or configured for any suitable application. For example, in some embodiments the apparatus is a laser induced breakdown spectroscopy (LIBS) apparatus and the excitation light transmitted along the excitation path is such as to produce breakdown, for example atomisation, of tissue at the target region. In some embodiments, the apparatus is a fluorescence spectroscopy apparatus and the excitation light is such as to produce fluorescence in

material at the target region. The apparatus in some embodiments may comprise or form part of an endoscope or other apparatus for insertion into the human or animal body.

Although some embodiments above are described with reference to specific materials (for example, silica or glass), in other embodiments any suitable materials may be used, for example any suitable glass or polymer materials.

It may be understood that the present invention has been described above purely by way of example, and that modifications of detail can be made within the scope of the invention.

Each feature disclosed in the description and (where appropriate) the claims and drawings may be provided independently or in any appropriate combination.

CLAIMS:

1. A spectroscopy probe apparatus comprising:

an excitation path configured to transmit excitation light for exciting a response;

a collection path different from the excitation path and configured to transmit signal light comprising a response signal, wherein at least one of the excitation path or collection path comprises or is formed in at least one optical fibre;

and a light guide having a proximal end coupled to the excitation path and to the collection path, wherein the guide is configured to pass the excitation light from the excitation path to a target region to generate the response signal, and to pass the signal light comprising the response signal from the target region to the collection path.

2. An apparatus according to Claim 1, wherein the response comprises a Raman response.

3. An apparatus according to Claim 1 or 2, wherein the excitation path comprises material that generates at least one of a Raman background signal, a fluorescence background signal or other background response signal in response to the excitation light.

4. An apparatus according to any preceding claim, wherein the target region has a cross-sectional area greater than a cross-sectional area of the excitation path.

5. An apparatus according to Claim 4, wherein the guide is configured to project light from the excitation path to the target region over a wider area than the cross-sectional area of the excitation path thereby to illuminate the target region.

6. An apparatus according to any preceding claim, wherein in operation the excitation light and the signal light comprising the response signal overlap in the guide.

7. An apparatus according to any preceding claim, wherein the excitation path and collection path are formed in a single optical fibre.

8. An apparatus according to any preceding claim, wherein the excitation path and the collection path are formed in different optical fibres.

9. An apparatus according to any preceding claim, wherein the collection path comprises a fibre core or a plurality of fibre cores and/or the excitation path comprises a fibre core or a plurality of fibre cores.

10. An apparatus according to Claim 9, wherein the fibre core of the excitation path or at least one of the fibre cores of the excitation path is in the same optical fibre as the fibre core of the collection path or at least one of the fibre cores of the collection path.

11. An apparatus according to any preceding claim, wherein the light guide comprises a tapered region of the optical fibre or at least one of the optical fibres.

12. An apparatus according to any preceding claim, wherein the light guide is spliced to the optical fibre or at least one of the optical fibres.

13. An apparatus according to any preceding claim, wherein the light guide is formed in an end-cap configured to fit onto an end of the optical fibre or at least one of the optical fibres.

14. An apparatus according to any preceding claim, wherein the excitation path comprises a first optical fibre core and the collection path comprises at least one second optical fibre core or comprises optical fibre cladding.

15. An apparatus according to any preceding claim, wherein the excitation path comprises a core and/or supports at most 21 modes, optionally at most 10 modes, optionally at most 6 modes, optionally at most 3 modes, optionally a single mode.

16. An apparatus according to any preceding claim, wherein the collection path comprises at least one core and/or supports at least 6 modes, optionally at least 15 modes, optionally at least 40 modes, optionally at least 100 modes.

17. An apparatus according to any preceding claim, wherein the excitation path comprises a hollow core of the hollow core optical fibre, and the collection path comprises a cladding of the hollow core optical fibre.

18. An apparatus according to any of Claims 14 to 17, wherein the light guide has a cross-sectional area greater than the cross-sectional area of the optical fibre core or the optical fibre or a combination of all of the optical fibres and/or the guide extends back around at least part of an outer diameter of the optical fibre or optical fibres.

19 An apparatus according to any preceding claim, wherein the excitation path is configured to transmit light of a single frequency.

20. An apparatus according to any preceding claim, wherein the excitation light comprises laser light, optionally narrowband or broadband laser light.

21. An apparatus according to any preceding claim, wherein the collection path is configured to transmit light having any of a range of frequencies.

22. An apparatus according to any preceding claim, wherein the guide is configured to transmit light having any of a range of frequencies.

23. An apparatus according to any preceding claim, wherein an outer diameter of the or each optical fibre is less than 2.0 mm, optionally less than 1.5 mm, optionally less than 1.0mm, optionally less than 0.8 mm, optionally less than 0.5 mm, optionally less than 0.3 mm, optionally less than 0.1 mm.

24. An apparatus according to any preceding claim, wherein the light guide has a length from the end of the collection path and/or excitation path to a distal end of the light guide in a range 0.5 mm to 50 mm.

25. An apparatus according to any preceding claim, wherein the light guide comprises at least one of a section of multi-mode fibre, an undoped section of fibre, a photonic lantern, a multi-mode waveguide, a transparent body.

26 An apparatus according to any preceding claim, wherein a cross-sectional area of the collection path is greater than a cross-sectional area of the excitation path, and/or wherein a cross-sectional area of the collection path is in a range 2 um^2 to ~ 10 um^2 and/or a cross-sectional area of the excitation path is in a range 20 um^2 to ~ 500 um^2, and/or wherein the collection path and excitation path are spaced apart so as to substantially prevent coupling.

27. An apparatus according to any preceding claim, wherein the apparatus is configured to be insertable into a, or forms part of, at least one of a bronchoscope, an endoscope.

28. An apparatus according to any preceding claim, wherein the target region comprises material configured to cause Raman scattering of at least part of the excitation light, thereby to provide the signal light.

29. An apparatus according to any preceding claim, wherein the target region is located at a distal end of the light guide.

30. An apparatus according to any preceding claim, wherein the excitation light is for producing laser induced breakdown of tissue at the target region and/or for producing fluorescence at the target region.

31. An apparatus according to any preceding claim, wherein the apparatus comprises or forms part of a Raman spectroscopy probe, a laser induced breakdown spectroscopy (LIBS) probe, an endoscope, or a fluorescence spectroscopy probe.

32. An apparatus according to any preceding claim, wherein the target region comprises a target comprising reporter molecules.

33. An apparatus according to Claim 32, wherein the reporter molecules comprise SERS reporter molecules, optionally wherein the reporter molecules comprise para-mercaptobenzoic acid (p-MBA).

34. An apparatus according to Claim 32 or 33, wherein the target comprises nanoshells coated with the reporter molecules.

35. An apparatus according to any of Claims 32 to 34, wherein a response of the reporter molecules to the excitation light varies with a property of an environment of the target, optionally wherein the property comprises at least one of pH, redox potential, glucose level, oxygen tension.

36. An apparatus according to any of Claims 32 to 35, wherein the reporter molecules are attached to the distal end of the light guide.

37. An apparatus according to any preceding claim, configured to be inserted into a human or animal body such that the target region comprises or is in contact with human or animal tissue or fluid.

38. A spectroscopy apparatus comprising the probe apparatus of any of Claims 1 to 37, and further comprising a light source configured to provide the excitation light to the excitation path, and a spectrometer configured to analyse the signal light collected by the collection path thereby to obtain a spectrum.

39. A method of performing a measurement comprising transmitting excitation light along an excitation path via a light guide to a target region for exciting a response; and

receiving a response signal from the target region via the light guide and a collection path different from the excitation path, wherein

at least one of the excitation path or collection path comprises or is formed in at least one optical fibre;

and the light guide has a proximal end coupled to the excitation path and to the collection path.

40. A method of producing a probe apparatus comprising:

forming an excitation path and a collection path, wherein the excitation path is different from the collection path and the forming comprises forming at least one of the collection path and excitation path in at least one optical fibre; and

coupling a light guide at its proximal end to the excitation path and to the collection path.

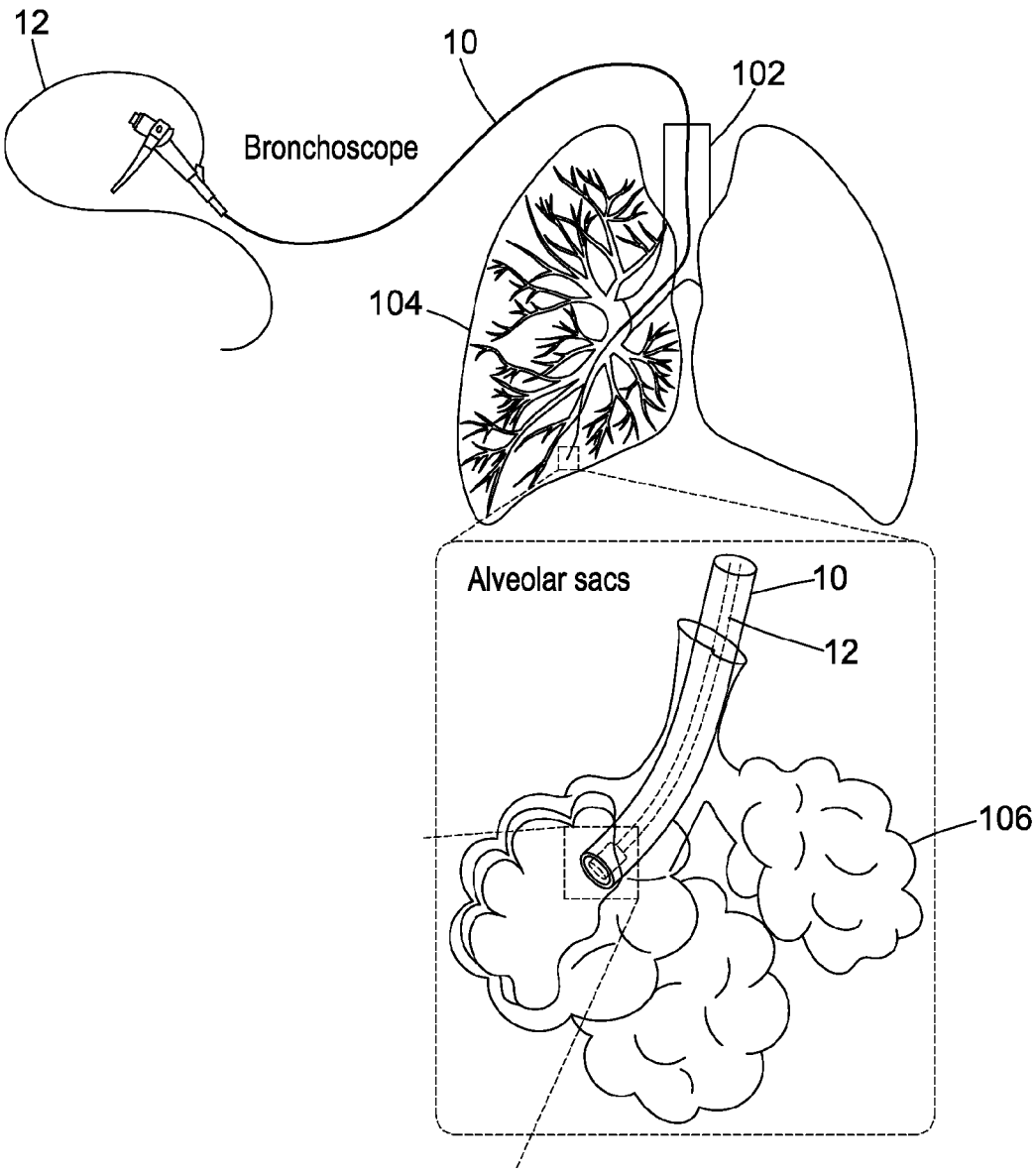


Fig. 1

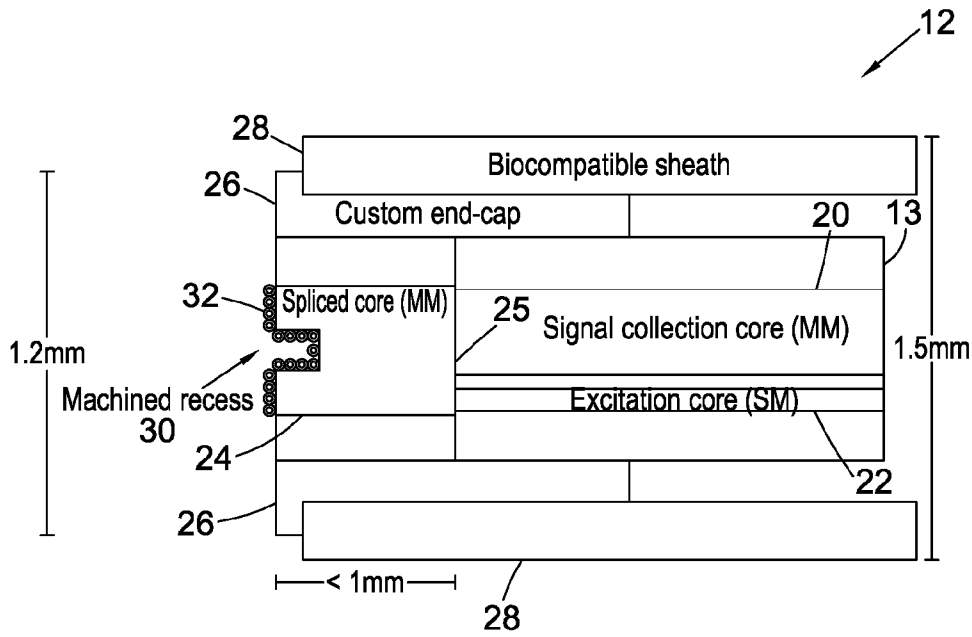

12
28
Biocompatible sheath
26
Custom end-cap
20
13
32
Spliced core (MM)
25
Signal collection core (MM)
1.2mm
1.5mm
Machined recess
30
Excitation core (SM)
24
22
26
< 1mm
28

Fig. 2

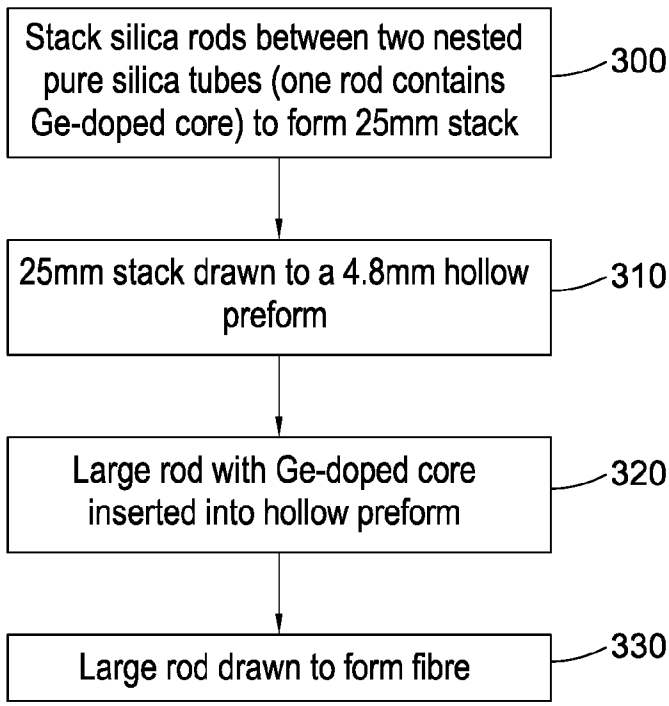

Stack silica rods between two nested pure silica tubes (one rod contains Ge-doped core) to form 25mm stack
300
25mm stack drawn to a 4.8mm hollow preform
310
Large rod with Ge-doped core inserted into hollow preform
320
Large rod drawn to form fibre
330

Fig. 3

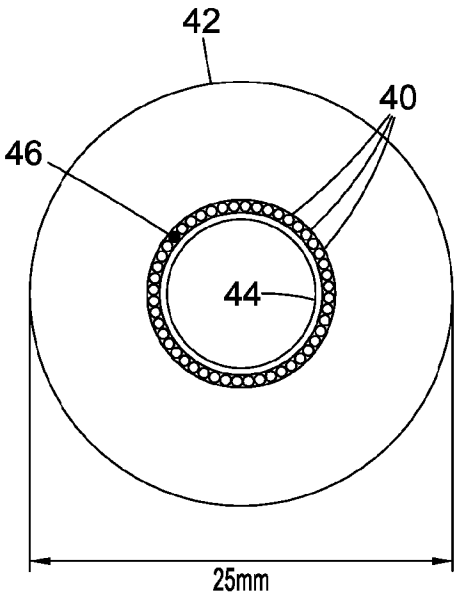


Fig. 4(a)

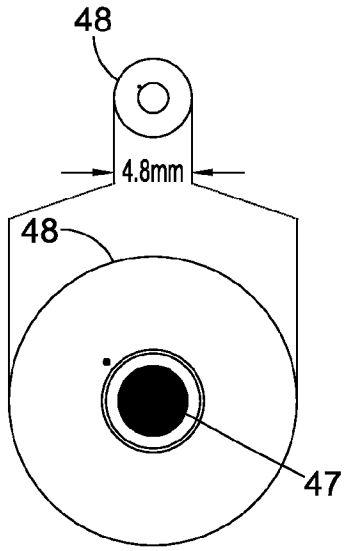


Fig. 4(b)

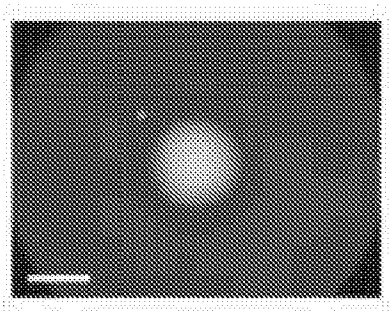


Fig. 4(c)

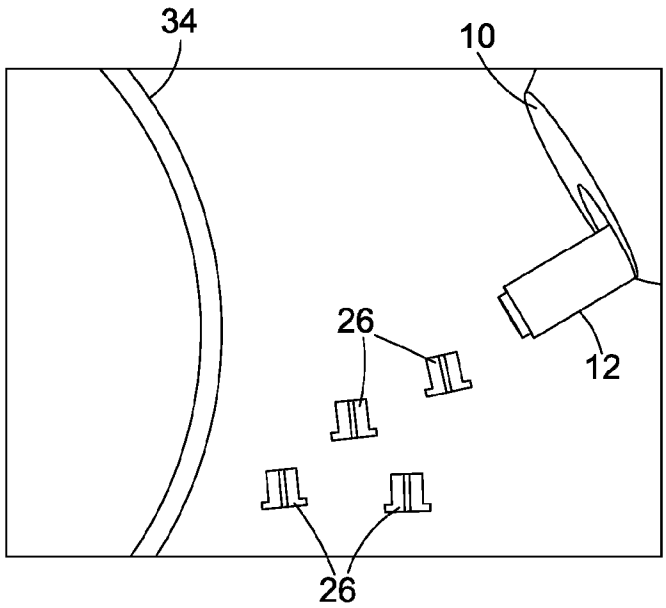

34
10
26
12
26

Fig. 5

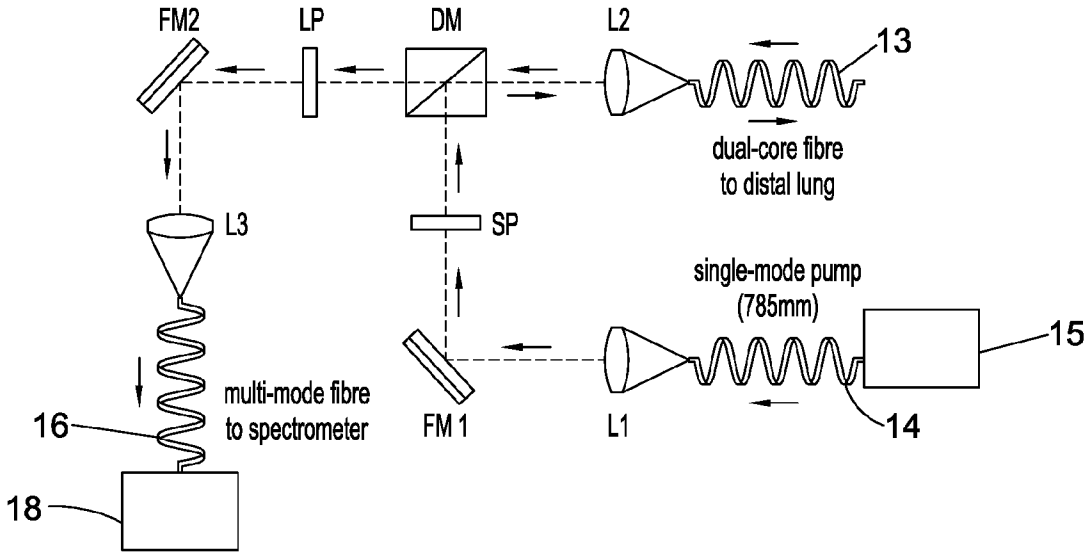

FM2
LP
DM
L2
13
dual-core fibre
to distal lung
L3
SP
single-mode pump
(785mm)
15
multi-mode fibre
to spectrometer
16
FM 1
L1
14
18

Fig. 6

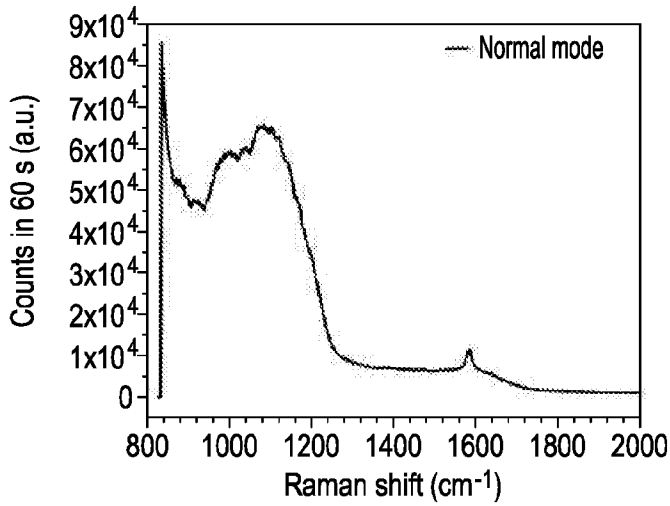


Fig. 7(a)

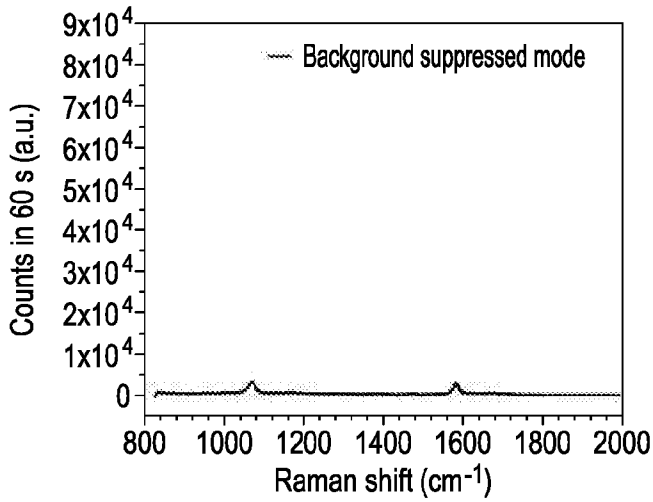


Fig. 7(b)

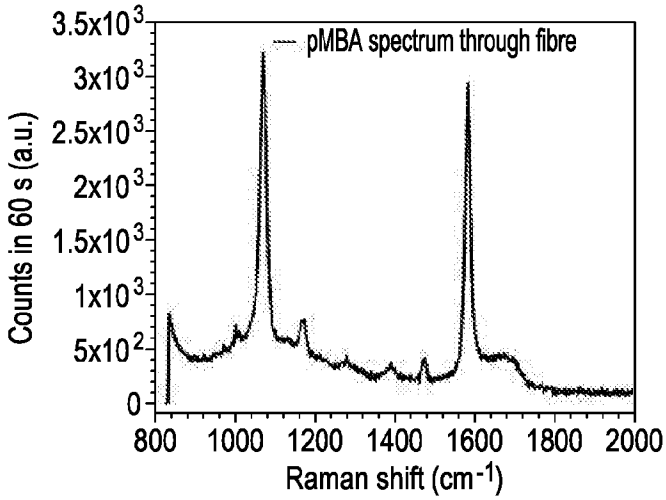


Fig. 7(c)

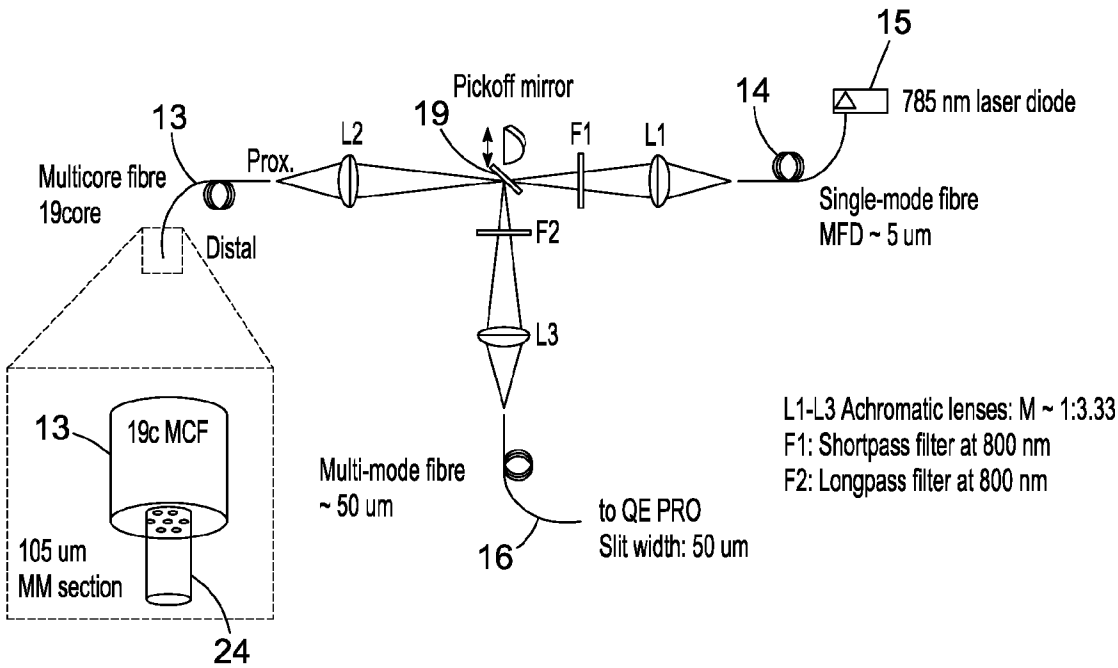

15
785 nm laser diode
14
Pickoff mirror
19
13
L2
F1
L1
Prox.
Multicore fibre
19core
Distal
Single-mode fibre
MFD ~ 5 um
F2
L3
13
19c MCF
105 um
MM section
24
Multi-mode fibre
~ 50 um
to QE PRO
Slit width: 50 um
16
L1-L3 Achromatic lenses: M ~ 1:3.33
F1: Shortpass filter at 800 nm
F2: Longpass filter at 800 nm

Fig. 8

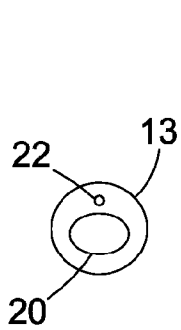


Fig. 9(a)

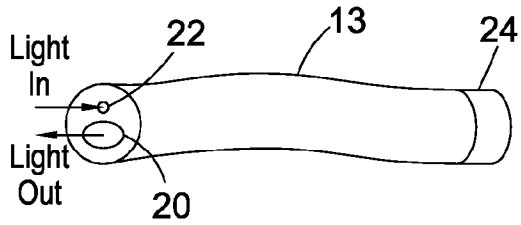


Fig. 9(b)

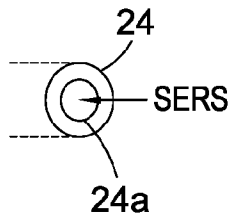


Fig. 9(c)

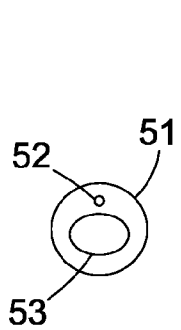


Fig. 10(a)

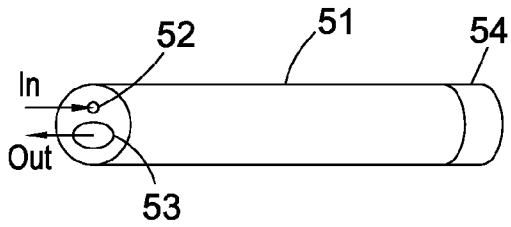


Fig. 10(b)

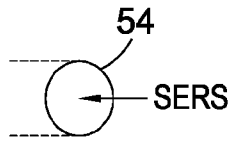


Fig. 10(c)

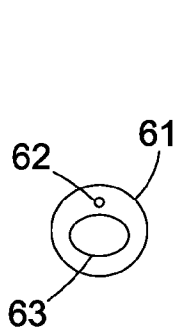


Fig. 11(a)

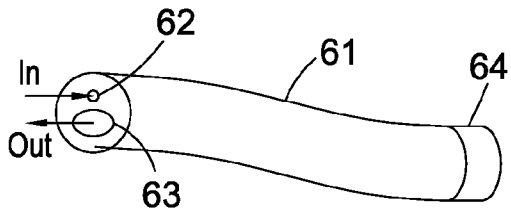


Fig. 11(b)

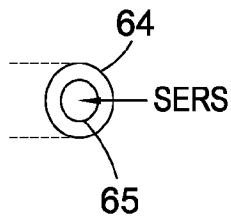


Fig. 11(c)

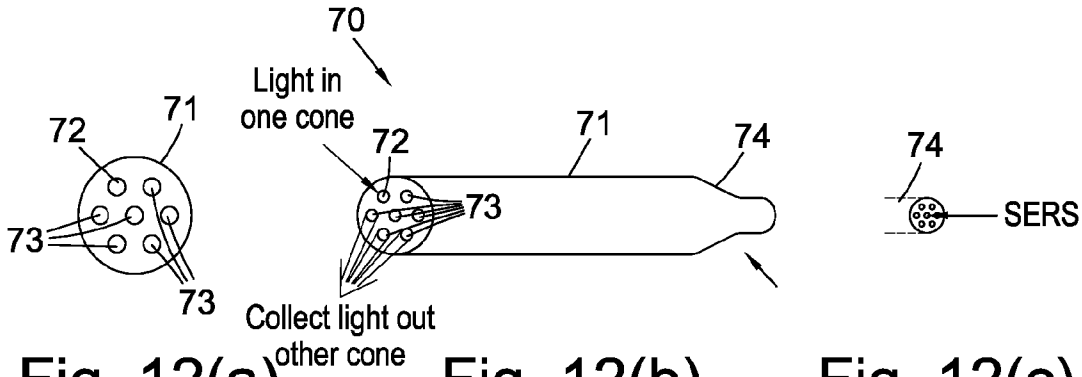


Fig. 12(a) Fig. 12(b) Fig. 12(c)

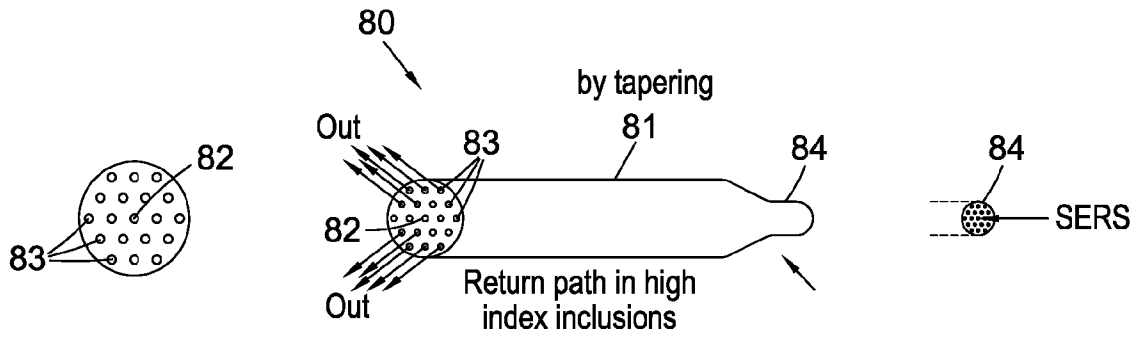


Fig. 13(a) Fig. 13(b) Fig. 13(c)

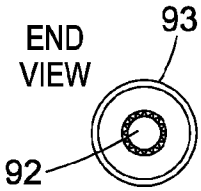


Fig. 14(a)

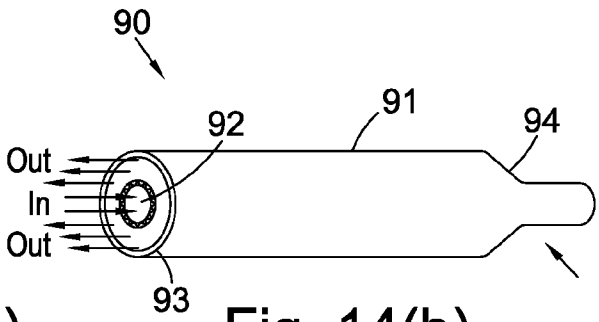


Fig. 14(b)

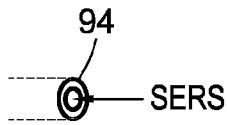


Fig. 14(c)

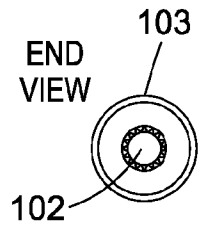


Fig. 15(a)

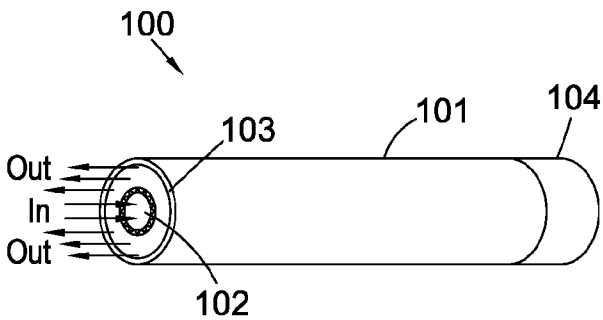


Fig. 15(b)

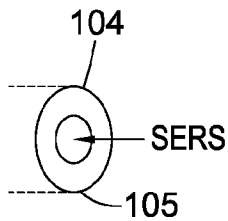


Fig. 15(c)

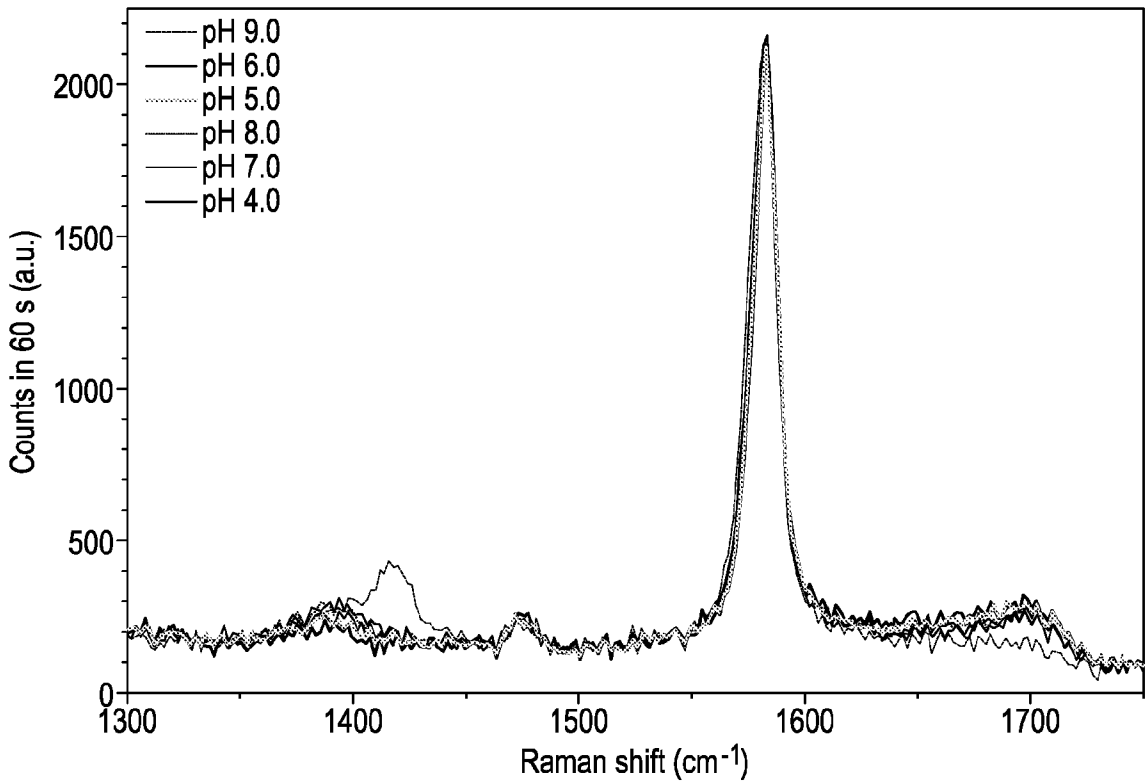

pH 9.0
pH 6.0
pH 5.0
pH 8.0
pH 7.0
pH 4.0
Counts in 60 s (a.u.)
0
500
1000
1500
2000
1300
1400
1500
1600
1700
Raman shift (cm-1)

Fig. 16

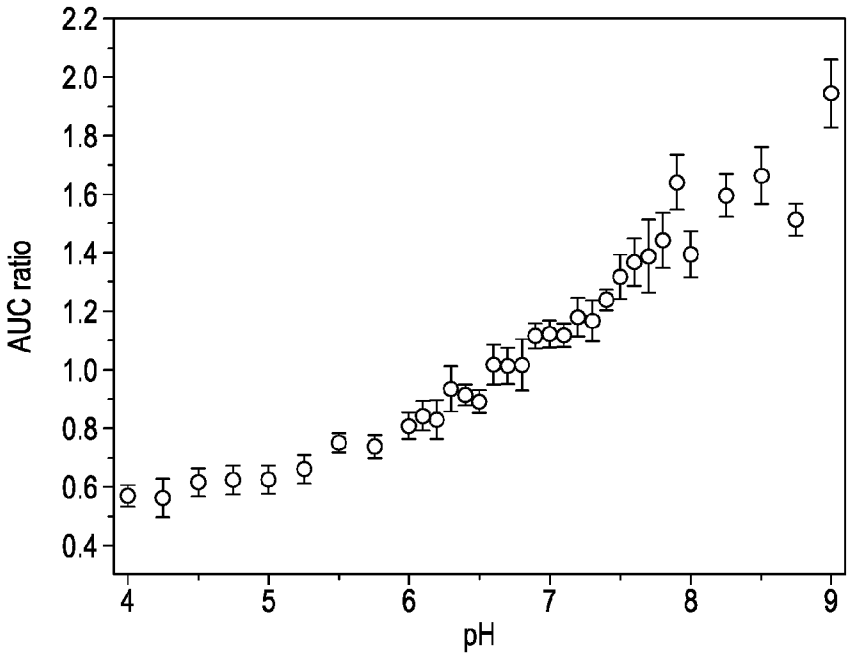

AUC ratio
pH
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
4
5
6
7
8
9

Fig. 17

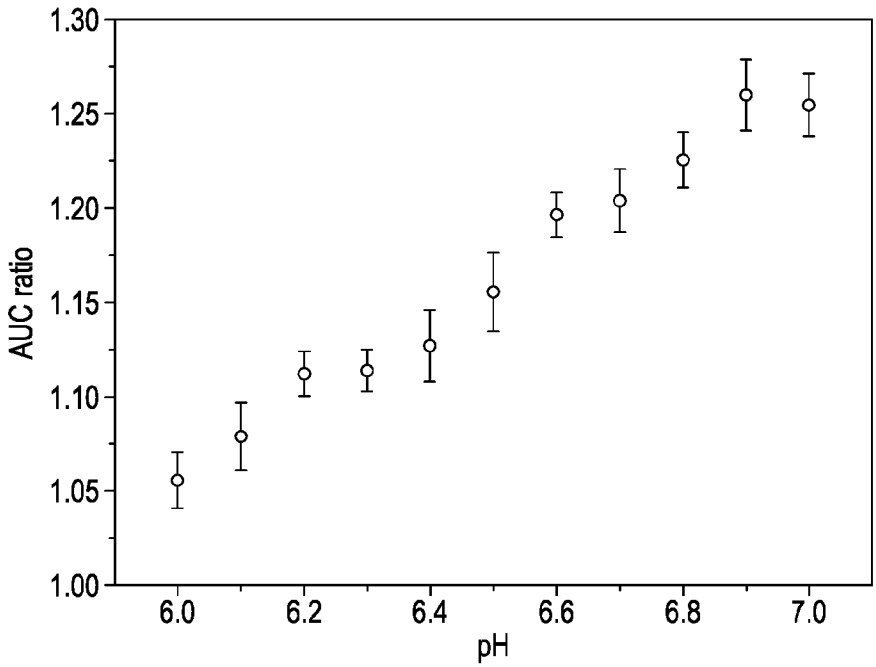

AUC ratio
pH
1.30
1.25
1.20
1.15
1.10
1.05
1.00
6.0
6.2
6.4
6.6
6.8
7.0

Fig. 18

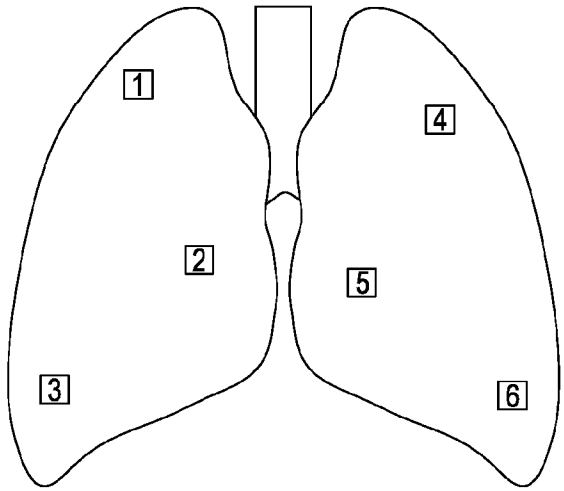

1
2
3
4
5
6

Fig. 19

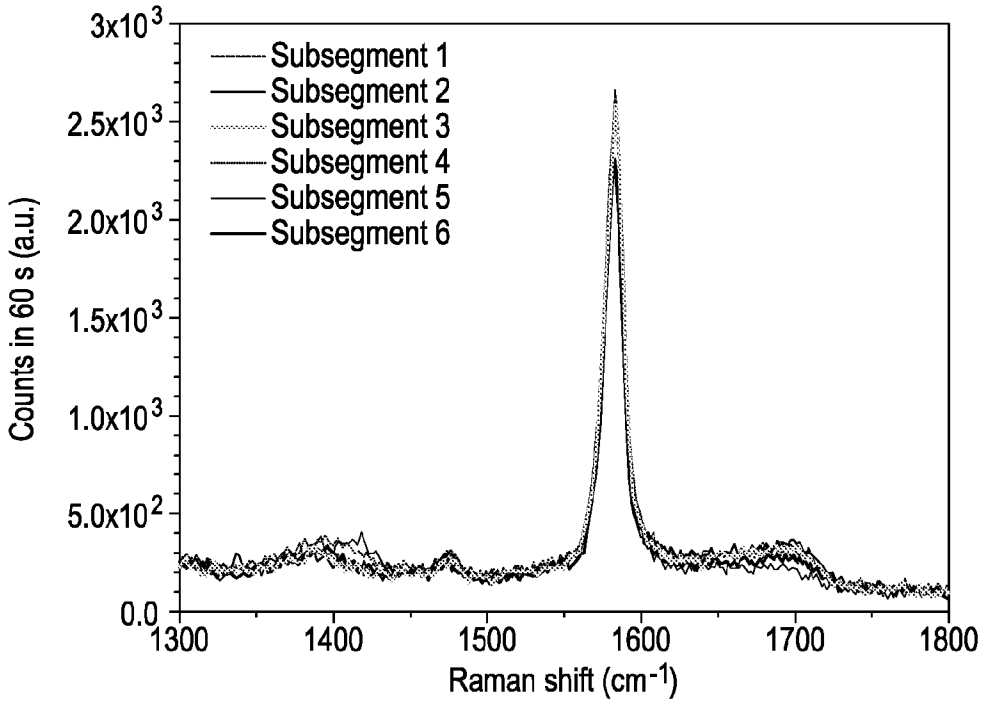

Subsegment 1
Subsegment 2
Subsegment 3
Subsegment 4
Subsegment 5
Subsegment 6
Counts in 60 s (a.u.)
3x10^3
2.5x10^3
2.0x10^3
1.5x10^3
1.0x10^3
5.0x10^2
0.0
1300
1400
1500
1600
1700
1800
Raman shift (cm^-1)

Fig. 20

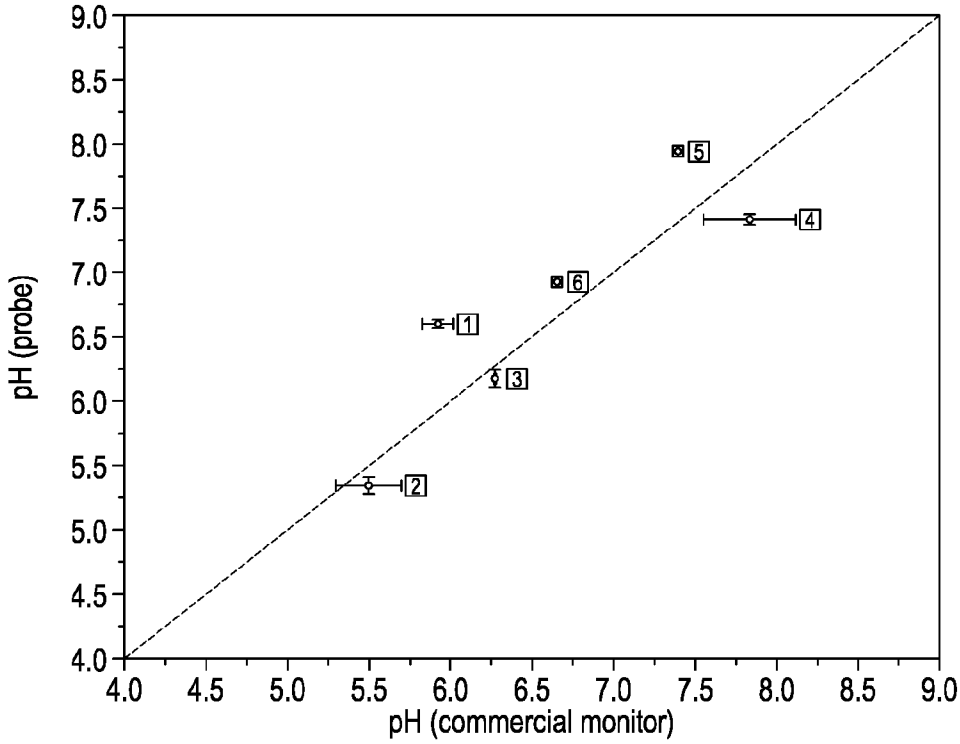

pH (probe)
pH (commercial monitor)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
1
2
3
4
5
6

Fig. 21

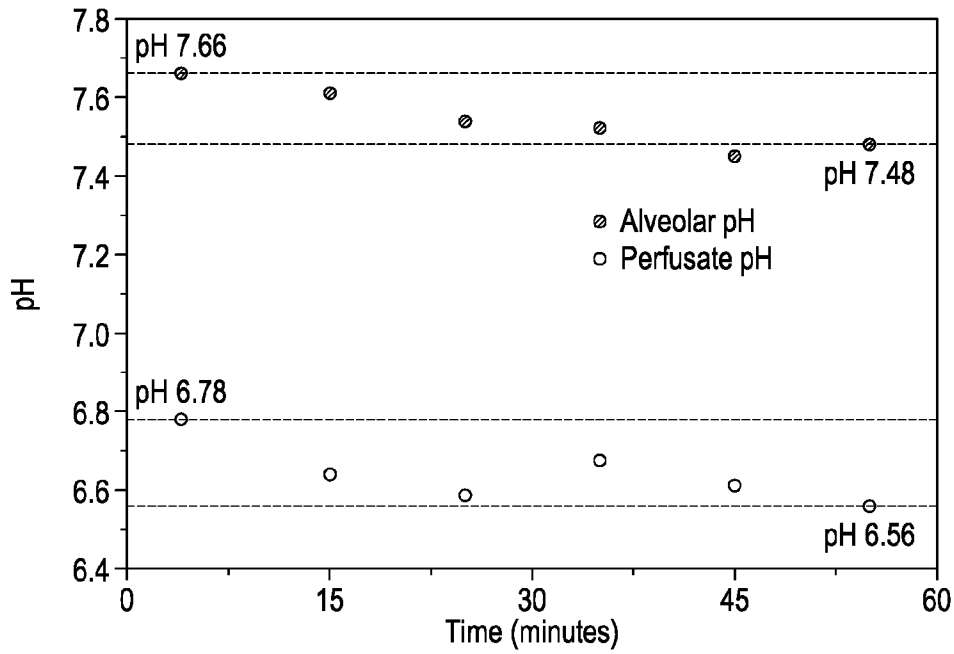

pH
Time (minutes)
pH 7.66
pH 7.48
pH 6.78
pH 6.56
Alveolar pH
Perfusate pH
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
0
15
30
45
60

Fig. 22

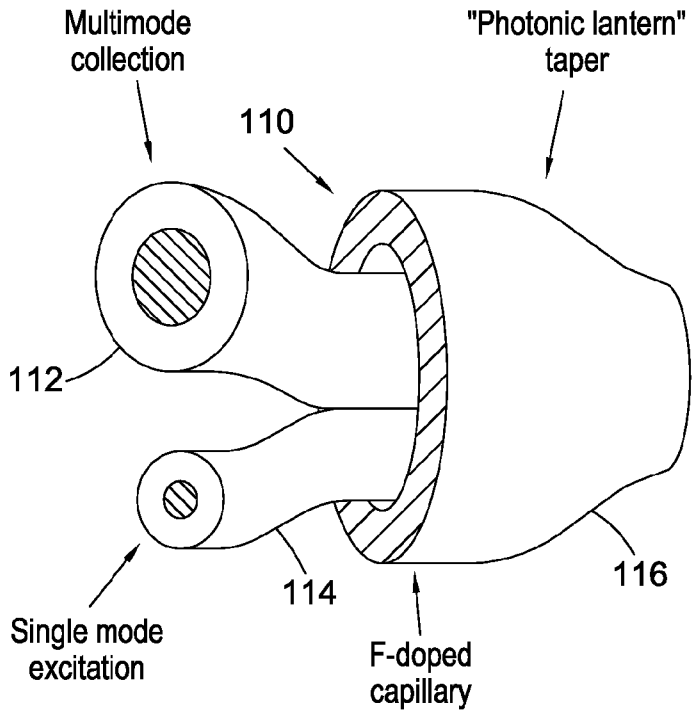


Fig. 23(a)

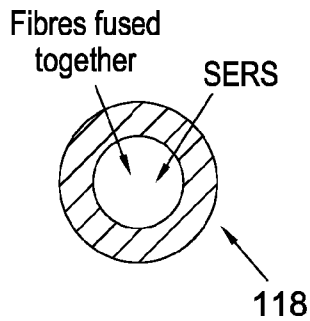


Fig. 23(b)

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2017/050677

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N21/65 G02B6/02
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N G02B

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2013/067996 A1 (INST PHOTONISCHE TECHNOLOGIEN E V) 16 May 2013 (2013-05-16) page 11 - page 13, paragraph 1; figure 9 -----	1-7,9, 10,12, 14-16, 18-23, 26-40
X	WO 2014/063257 A1 (BRITISH COLUMBIA CANCER AGENCY [CA]) 1 May 2014 (2014-05-01) paragraph [00121] paragraph [0019] paragraph [0066] paragraphs [0106] - [0119] ----- -/--	1-9,11, 13-31, 37-40

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 May 2017	24/05/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Huenges, Alexandra

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	C. L. ZAVALETA ET AL: "A Raman-based endoscopic strategy for multiplexed molecular imaging", PROCEEDINGS NATIONAL ACADEMY OF SCIENCES PNAS, vol. 110, no. 25, 23 May 2013 (2013-05-23), pages E2288-E2297, XP055372556, US ISSN: 0027-8424, DOI: 10.1073/pnas.1211309110 figure 1	1-6,8,9, 15,16, 18-29, 31-40
X	UTZINGER U ET AL: "Fiber optic probes for biomedical optical spectroscopy", INTERNATIONAL SOCIETY FOR OPTICAL ENGINEERING, SPIE, PO BOX 10 BELLINGHAM WA 98227-0010 USA, vol. 8, no. 1, 1 January 2003 (2003-01-01), pages 121-147, XP002555309, ISSN: 1083-3668, DOI: 10.1117/1.1528207 page 129, right-hand column; figures 4,6	1,3-6,8, 9,13, 18-26, 37-40
Y	US 7 236 673 B2 (YE JING Y [US] ET AL) 26 June 2007 (2007-06-26) column 3, lines 4-37	1-7,9, 10, 14-16, 18-23, 26-31, 37-40
Y	US 2012/281209 A1 (BAI SHUANG [US]) 8 November 2012 (2012-11-08) paragraphs [0031], [0036]; figure 12	1,12, 32-40

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2017/050677

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013067996 A1	16-05-2013	DE 112012004726 A5	31-07-2014
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