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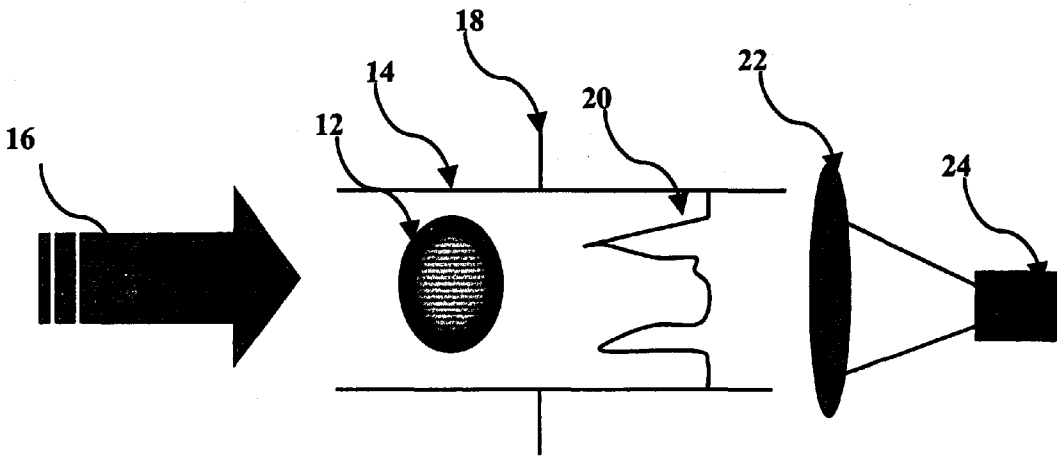

(57) Abstract: The present invention relates to an apparatus and to a method for the measurement of dimensions of biological structures. In particular, but not exclusively, the invention relates to the measurement of changes over time in the physical dimensions of tubular biological structures such as blood vessels in response to stimuli such as hormones, agonists, potential drugs. The method may be used in determining the effect of a gene.

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"Apparatus and Method for Measurement of Biological Structures"

The present invention relates to an apparatus and to a method for the measurement of dimensions of biological structures. In particular, but not exclusively, the invention relates to the measurement of changes over time in the physical dimensions of biological structures.

The measurement of the size of biological structures, such as arteries or muscles, has a number of applications in the laboratory. In particular, it is often desirable to investigate contraction or relaxation of blood vessels and the like in response to hormones, agonists, or potential drugs.

Typically, such investigations are conducted with use of a myograph, that is, a device for measurement of muscle characteristics. Wire myography involves mounting the vessel under study onto a pair of steel

wires, which are secured to supports. Mounting the vessel is a difficult, time-consuming technique which requires considerable practice and often results in damage to the vessel, mainly because of the difficulty in aligning the wires with the lumen of the vessel. One support is attached to a micrometer, allowing control of vessel circumference, while the other support is attached to a force transducer, for measurements of tension development. The whole assembly is maintained in a chamber containing physiological salt solution, to which various test substances may be added to assess the vessel response. However, the quality of the information obtained from a wire myograph is limited because the vessel is not stretched in a manner that reflects the situation *in vivo*.

An alternative technique is pressure myography, where vessels are cannulated at both ends and examined under pressure.

Pressure myography creates a situation closer to that found *in vivo*; however, mounting vessels onto cannula is a difficult, time-consuming procedure.

Diameter and length of vessels under study may also be determined visually, either by an experienced experimenter under the microscope, or automatically by means of a computing device running image analysis software. However, such software must be relatively sophisticated to accurately image and analyse the dimensions of the vessel under study and

such image analysis is difficult to apply to the internal diameter of vessels.

Perfusion myography requires substantial amounts of expensive equipment yet the number of experiments that can be conducted is extremely low.

Therefore, the use of transducers and imaging technologies are not conductive to conducting large numbers of experiments because of three fundamental disadvantages. Firstly, the tissue must be 'mounted' onto the measurement apparatus. This involves skilled technicians or scientists and can take valuable time. Secondly, a lack of automation in these systems means that they must be supervised and managed throughout the assay by a skilled technician. Thirdly, the systems are very expensive. These fundamental problems represent 'barriers to entry' that limit usage of such systems.

It is among the objects of embodiments of the present invention to obviate or alleviate these and other disadvantages of known myography techniques and systems.

According to a first aspect of the present invention, there is provided a method of detecting a change in the physical dimensions of a biological structure, the method comprising the steps of:

illuminating a biological structure with light of a first wavelength;

detecting the intensity of light transmitted by the biological structure in at least first and second physical states; and

comparing the intensity of transmitted light with a reference light intensity of the same wavelength, to determine a change in the absorption of the biological structure, said change in the absorption corresponding to a change in physical dimensions.

The present invention therefore makes use of the differing degree of absorption of light by biological structures with different diameters and thicknesses. Further, the present invention also allows detection of changes in the internal diameter of a tube structure, since this will affect the thickness and hence absorption of the structure.

Preferably, the method is used to measure a biological tube structure; for example, a blood vessel or the like.

Preferably, the detection of transmitted light comprises the step of detecting the intensity of transmitted light across substantially the whole diameter of the biological structure. It has been found that such a relatively crude measurement technique in fact provides sufficient information to

determine a change in the dimensions of the structure from a change in the absorption of the structure. There is thus no requirement to obtain a detailed optical image, nor to obtain images of many different points across the structure, in contrast to known myography techniques. This therefore provides advantages in terms of information gathering and processing.

Preferably, the method further comprises the step of repeating the illumination, detection, and comparison steps with light of a second wavelength. This may itself be performed as many times as desired, with further wavelengths. The use of additional wavelengths of light can provide additional information regarding the biological structure, due to the differing transmissivity of such structures at different wavelengths. Preferably, the wavelengths are selected such that in one of the wavelengths the biological structure is essentially opaque, whilst in another of the wavelengths the biological structure is semi-transparent. It will be readily apparent to the skilled person that with a general knowledge of the tissue being studied, it will be possible to select wavelengths such that some are highly absorbing while others provide contrast between paths of large and small optical density. The illumination, detection and comparison steps with second or subsequent wavelengths may be performed subsequently to the steps conducted with the first wavelength; alternatively, the steps may be performed

simultaneously. Separate performance of the steps ensures that the information from each wavelength is kept separate; if the steps are performed simultaneously, it is preferred that the method further includes the step of distinguishing measured intensities of each wavelength of light used. For example, separate detectors may be used, or the detected combined intensity may be subjected to post-processing to separate the various wavelength components.

Preferably, the reference light intensity corresponds to the intensity of light measured when no biological sample is illuminated; that is, a blank reference measurement. This allows account to be taken of any features of the system used for making the measurements which affect the light wavelengths used. Alternatively, the reference light intensity may correspond to the measured intensity of light transmitted by the biological sample in a different physical state. This allows measurements of the change in transmissivity of a biological structure over time to be obtained. Of course, a combination of the two reference intensities may be used, to provide both an absolute and a relative measurement of transmitted light.

According to a second aspect of the present invention, there is provided a method of measuring changes in the physical dimensions of a biological structure over time, the method comprising the steps of:

illuminating a biological structure at first and second time points with light of a first wavelength;

detecting the intensity of light transmitted by the biological structure at said first and second time points; and

comparing the intensity of transmitted light at the first time point with the intensity of transmitted light at the second time point to determine a change in the intensity of transmitted light, said intensity change being indicative of a change in physical dimensions of the biological structure.

The method may further comprise the step of altering the local environment of the biological structure between said first and second time points. For example, this may include addition or removal of agonists, hormones, potential drugs, test samples and the like to the vicinity of the biological structure, or direct physical stimulation of the structure. The method may also include the step of transfecting the biological structure with exogenous nucleic acid; this may be of use in determining the effect of a gene or similar nucleic acid structure, or the potential stability of a transfection vector or of the biological structure for transfection. Alternatively, or in addition, peptide or protein structures may be added to the biological structure, to determine the effects of such peptides or

proteins directly, without the requirement for expression of a transfected gene.

Formulae for use in the methods of the invention are described in detail with reference to the examples of the invention. The invention also provides use of these equations in the measurement of physical changes in tubular biological structure.

According to a further aspect of the present invention, there is provided an apparatus for detecting changes in physical dimensions of biological structures, the apparatus comprising a light source, a light detector, means for locating a biological structure between the source and the detector, and data processing means for recording and analysing detected light which has been transmitted by the biological structure to detect and analyse changes in characteristics of the transmitted light to determine changes in the physical dimensions of the biological structure.

The light source may generate light of a single wavelength, or of a plurality of wavelengths. A number of distinct light sources may alternatively be provided.

The light detector may comprise, for example, a CCD device, a photoresistor, a photodiode, or the like. The detector may be capable of detecting light of only a single wavelength or of a plurality of wavelengths. Where the detector can detect a

plurality of wavelengths of light, the apparatus preferably further comprises means for separating detected light of multiple wavelengths: this may comprise post-detection means, such as a data processing means; or may comprise pre-detection means, such as means for generating light of a single wavelength at a time, or filter means for allowing only light of a single wavelength to reach the detector.

The apparatus may further comprise means for aligning generated and detected light. Preferably, this comprises aligned optical fibres, at least one of which carries generated light, and at least one of which carries detected light. The fibres are connected at one end to a light source or light detector, as appropriate, with the other ends of the fibres being in opposed alignment, such that light leaving the source fibre can enter the detection fibre. The apparatus may further comprise a plastics or similar optical fibre holder, arranged to hold optical fibres in oppositely aligned relationship. The holder may conveniently comprise a bifurcated tube, the tips of each fork of which are in alignment; in use, an optical fibre may be inserted into each fork of the holder, with the free ends of the fibres being coterminous with the ends of the forks. This ensures the optical fibres are aligned correctly.

The means for locating a biological structure may comprise a wire holder, a cannula, or the like.

Preferably, the locating means comprises a plastics tube bearing an external circumferential groove; in use, a biological tube structure may be located over the plastic tube, and secured thereto by means of tying a fastener around the groove. Conveniently, the tube is of polypropylene.

Preferably, the apparatus further comprises means for subjecting the biological structure to fluids and the like. Preferably, this comprises a chamber having a fluid inlet and a fluid outlet, in which the biological structure is located. The apparatus may further comprise one or more fluid reservoirs, for storing circulatory fluid. Conveniently, the chamber comprises a fluid pump, for circulating fluid within the chamber, or into and out of the chamber. Conveniently also, the pump may be arranged to maintain the chamber at substantially physiological fluid pressure. The apparatus may still further comprise means for introducing substances to the biological structure; for example, a syringe may be used to introduce a test substance to the circulating fluid. Preferably, a plurality of chambers are provided, enabling a plurality of biological structure samples to be studied simultaneously.

Preferably, the data processing means comprises a computer.

Following dissection, isolated tissue can be difficult to manipulate as it is flaccid in nature.

This is especially true with respect to the accurate positioning of the tissue with regard to the locating means, which may comprise a wire holder, a cannula or the like. To obviate this disadvantage and according to a further aspect of the invention there is provided a method of immobilising and positioning the tissue that ensures alignment with the locating device, the method comprising placing the tissue upon a layer comprising one or more proteins selected from the following: collagen, elastin, gelatin, fibronectin, fibrinogen, vitronectin.

The method may further comprise the step of first placing the protein layer upon a polypropylene or polyethylene surface. Preferably, the plastic surface comprises the inner aspect of half of a conical funnel, ensuring a concave surface upon which to place the tissue. Two such funnels are then placed in opposition upon a rigid bath, with the mouths of the funnels facing each other. One funnel is fixed as part of the bath, the second funnel is placed on the bath, preferably attached to a rail that allows movement towards or away from the first funnel in the horizontal plane.

Further, the accurate positioning of the tissue with respect to the chosen locating device is ensured by placing one end of the tissue in the neck of the fixed conical funnel at a point that equals the external diameter of the tissue. The second funnel is moved along the horizontal plane until the end of

the tissue in the neck of the conical funnel again lies above the point in the funnel that equals the external diameter of the tissue. The tissue is then placed on the protein layer of the moveable second funnel.

The locating device can be guided firstly through the narrow portion of the funnel and then through the immobilised tissue.

The means for locating the biological structure in the apparatus of the invention may include a layer of protein to immobilise the structure.

Typically the protein may be or may be derived from collagen, elastin, gelatin, fibronectin, fibrinogen, vitronectin or any combination thereof.

The invention also provides the use of at least one protein chosen from collagen, elastin, gelatin, fibronectin, fibrinogen and vitronectin to support biological tissue in testing apparatus.

The apparatus may further comprise temperature regulation means, for regulating the temperature of a biological sample.

These and other aspects of the present invention will now be described by way of example only and with reference to the accompanying drawings, in which:

Fig. 1 shows a schematic diagram of an implementation of the method of measuring biological structures of one aspect of the present invention;

Fig. 2 shows a hypothetical example of the intensity of light of two different wavelengths transmitted through a hypothetical biological structure;

Fig. 3 shows a schematic diagram of an apparatus for measurement of biological structures in accordance with a further embodiment of the present invention;

Fig. 4 shows a fibre optic holder as may be used with an apparatus for measurement of biological structures in accordance with an embodiment of the present invention;

Fig. 5 shows a biological structure mounting means as may be used with an apparatus in accordance with an embodiment of the present invention;

Fig. 6 shows an apparatus for measurement of biological structures in accordance with an embodiment of the present invention;

Fig. 7 shows a graph of the percentage change in lumen diameter of a blood vessel in response to differing concentrations of an agonist, as determined with the apparatus of Fig. 6;

Fig. 8 shows the geometry of a vessel under tension in a myograph, as described in more detail with reference to Experiment 2 below;

Fig. 9 is a schematic diagram of the experimental apparatus for absorption spectroscopy using the Mulvany myograph, as described in Experiment 2 below;

Fig. 10 shows transmission spectra obtained using the adapted Mulvany myograph for normal (N1, N2: BP=135.4 mmHg, media thickness=25.32μm) and hypertensive (H1, H2: BP=231 mmHg, media thickness=46.7μm) vessels under tension produced by a linear stretch of 250 μm (N1, H1) and 300 μm (N2, H2). Spectra are obtained from paired specimens;

Fig. 11 shows the fitted averaged normalised transmission spectrum of a control vessel under a linear stretch of 350 μm. Data points are represented by circles and the continuous line is given by;

$$T = \frac{0.39}{1+\exp(-0.057[\lambda - 412])} + \frac{0.098}{1+\exp(-0.179[\lambda - 753])}$$

Fig. 12 is a table showing blood pressures (tail-cuff) and morphological characteristics of mesentery arteries (mean ± sem);

Fig. 13 is a matrix of correlation coefficients. Data derived from all groups (4 week,

8 week clipped and control.) Total number of spectra analysed = 659 (*$p<0.001$, normotensive vs. hypertensive); and

Fig. 14 represents a locating means to position tissue for testing in apparatus of the invention.

Referring first of all to Fig. 1, this shows a schematic arrangement for measuring biological structures, in accordance with one embodiment of the present invention. A biological tube structure 12, such as a blood vessel, is suspended in an enclosure 14, such that the blood vessel 12 may be illuminated by a light source 16. An aperture 18 allows only a portion of the light to be transmitted. The light source 16 is a combination of a number of spectral components; each spectral component will generate an intensity profile 20 of light transmitted by the blood vessel 12, whose characteristics will be a function of the blood vessel geometry and optical properties. A focussing lens 22 and a detector 24 are used to obtain a single point intensity measurement of the transmitted light for each spectral component. This single measurement is essentially the integrated intensity profile for the spectral component.

A set of measurements consists of extinction values (integrated intensity profiles) of the tissue-transmitted intensity for each distinct spectral component, these are labelled ε_i. A reference set of measurements ε_{0i} are taken without tissue present.

The spectral components are chosen so that in one (or some) of the components the tissue is essentially opaque whilst in another (others) the tissue is semi-transparent. The transition between these conditions is actually quite narrow, because the light absorption increases (essentially) exponentially with optical density. Thus with a general knowledge of the tissue being studied it is always possible to select spectral components that are highly absorbing and ones that provide contrast between paths of large and small optical density. This property is used in the standard perfusion myograph to provide imaging contrast between the vessel lumen and vessel wall.

Figure 2 shows a series of hypothetical representative intensity measurements for a blood vessel model with two spectral components, i and j. For each spectral component there is shown a reference measurement ε_{0i}, ε_{0j}, denoted by reference numerals 26, 28, and a pair of extinction measurements ε_i (t_1), ε_i (t_2) (reference numerals 30, 32) taken at successive times. It will be seen that the characteristics of the vessel alter from time t_1 to time t_2 (for example, a hormone is added to the system causing the vessel to contract), and that the vessel is essentially opaque for spectral component j, so that no information can be derived concerning the vessel lumen from this component alone. Because these measurements are all derived from a fixed aperture, a simple model of the relationship

between the extinction value and the profile may be written as:

$$\varepsilon_{0i} = MI_i$$

$$\varepsilon_i(t) = (M - V(t))I_i + (V(t) - L(t))I_i\alpha_{Vi} + L(t)I_i\alpha_{Li}$$

where we have assumed that the illumination pattern is uniform with intensity per unit length I_i and M is a measure of the aperture size. The variable vessel and lumen sizes are represented by (V(t),L(t)) respectively and the light absorption coefficients in the vessel and lumen are represented by (α_{Vi},α_{Li}). It is convenient to form the normalised extinction coefficients as follows, where the normalised values are denoted by a hat over the variable:

$$\hat{\varepsilon}_i(t) = \frac{\varepsilon_i(t)}{\varepsilon_{0i}} = 1 - \hat{V}(t) + (\hat{V}(t) - \hat{L}(t))\alpha_{Vi} + \hat{L}(t)\alpha_{Li}$$

Two instances of this equation (i.e. for two spectral components) can be solved for the vessel and lumen sizes, providing the tissue optical proprieties are known (or can be calibrated). Further simplification can be achieved when the vessel wall is essentially opaque for certain selected spectral components, such as spectral

component j, in which case the normalised extinction coefficient reduces to:

$$\hat{\varepsilon}_j(t) = 1 - \hat{V}(t)$$

and hence the lumen size may be estimated from:

$$\hat{L}(t) = \frac{\hat{\varepsilon}_j(t)(1-\alpha_{Vi}) + \alpha_{Vi} - \hat{\varepsilon}_i(t)}{\alpha_{Vi} - \alpha_{Li}}$$

With careful choice of spectral components, as selected by a person of skill in the art, the following condition may apply

$$\alpha_{Vi} \gg \alpha_{Li}$$

And hence

$$\hat{L}(t) \cong 1 + \hat{\varepsilon}_j(t)\left(\frac{1}{\alpha_{Vi}} - 1\right) - \frac{1}{\alpha_{Vi}}\hat{\varepsilon}_i(t)$$

Many more models of the tissue geometry and associated extinction profiles could be envisaged. The one considered here is only presented as an example. It is intended to illustrate how a relationship exists between the vessel lumen and the measured normalised extinction coefficients of at least two contrasting spectral components. Although the relationship derived here is a linear one, in

principle the relationship can be non-linear. In a real system this relationship can be explored by means of calibration with respect to tissue phantoms (that is, a material constructed to mimic the geometrical and optical properties of a real tissue sample; for example, a cylinder constructed of optical resin embedded with fine sand) that mimic the geometry and scattering properties of a range of vessels. Such phantoms are straightforward to construct.

Referring now to Figure 3, this shows in broad outline an apparatus 40 for measurement of biological structures such as a blood vessel 42. The apparatus 40 includes a chamber 44 in which the blood vessel 42 is mounted and supplied with suitable media for maintaining the condition of the vessel 42. A light source 46 generates a range of spectral components 48, which components 48 are passed through a lens 50 which collimates and aligns the distinct components into a single light field 52. The light field 52 is then directed by means of a fibre optic arrangement 54 to illuminate a suitable area of the chamber 44 and vessel 42. Light which is transmitted 56 by the vessel 42 is received and directed by means of a further optical fibre arrangement 58 to a suitable light detector 60 which provides an integrated intensity profile of the transmitted light. The intensity data is passed to a data processor 62, such as a personal computer, which can also be arranged to receive data from and pass data to the other components of the apparatus

40, and to control the operation of the apparatus. The PC 62 is also connected to an output device 64, such as a monitor or a printer, for generating a record of experimental data.

Various components of the apparatus 40 will now be described in more detail. Figure 4 shows an optical fibre holder 70 which may be used with the apparatus 40 to ensure appropriate direction of illuminating and transmitted light (by means of optical fibre arrangements 54 and 58 on Figure 3). The holder 70 comprises tubular moulded plastic 72 forming an inverted cup shape with a bifurcation point 74 resulting in two channel portions 76, 78, the free ends of which are opposed. The holder 70 is mounted on a circular swivel mount 80, allowing the holder to be easily moved into and out of position. In use, two optical fibres, one serving as a transmitter, and one as a receiver, are inserted into the tube 72 from the swivel mount 80. The transmitter fibre is fed along the tube to the left channel portion 76, while the receiving fibre is fed to the right channel portion 78. The ends of the fibre are adjusted to sit flush with the ends of the channel portions 76, 78, to ensure that light leaving the transmitter fibre can be detected by the receiving fibre. The other ends of the fibres are connected to the appropriate transmitter or detector parts of the apparatus. In use, the holder 70 is placed so that each channel portion 76, 78 is on either side of a biological structure to be measured.

The biological structure, if it is a tube structure, may be supported between the portions of the holder by means of a tissue mount as shown in Figure 5. The tissue mount 82 comprises a polypropylene tube 84 with parallel walls and an external diameter roughly one tenth of the length, and an internal diameter approximately four-fifths of the external diameter. At a distance from one end generally equal to the external diameter of the tube, a 20mm groove 86 is present. To manufacture the tissue mount 82, standard polypropylene tubing (Camlab, Cambridge, UK) is cut to appropriate lengths and mounted on a microlathe. A micromanipulator holding a heated surgical needle (28 gauge) is slowly advanced towards the turning plastic tube, cutting a 20mm circumferential groove in the tubing. To mount a vessel on the mount 82, a section of vessel under study is pulled onto the mount until the edge of the vessel passes the groove 86. A small plastic tie is applied to the vessel and tightened gently over the groove to secure the vessel on the mount 82.

Figure 6 illustrates a further apparatus 90 for measurement of biological structures according to an embodiment of the invention. The apparatus 90 includes four chambers 92 in which an experimental sample can be located, each of which is provided with an optical fibre holder 70 as described.

The apparatus includes an oscilloscope 94, for monitoring the intensity of transmitted light, and is connected to a personal computer 96 with monitor

98 for analysing and displaying experimental data. The apparatus 90 also has multiple fluid reservoirs 100 for storing and dispensing media or other substances for use in experimental procedures, and a multi-channel cassette pump (not shown in the interest of clarity) for fluid pumping.

To prepare the apparatus 90 for experimental procedures, tissue mounts as described are attached to each of two stainless steel pipes in each chamber 92. A section of isolated blood vessel (length range 8-16mm; diameter range 100-1000μm) is pulled onto the proximal cannula until 2mm of vessel surrounds the tissue mount. The vessel is secured by means of a plastic tie, as described.

Prior to the study of the tissue, the optical fibre holder 70 is swivelled across and positioned across the tissue so that the end faces of the optical fibres are in apposition and the light emitted is directly perpendicular to the longitudinal axis of the tissue (as shown in chain dotted outline 79 in Figure 4).

The fluid reservoirs 100, connected to perfusion loops by a system of tubing and controlled by solenoid valves are filled with the appropriate solutions prior to commencing the experimental protocol. Generally, reservoirs will contain rinsing solution and drugs. Filling of the reservoir is done manually via filling hole prior to each study.

Figure 14 allows accurate positioning of tissue with regard to the locating means.

Following dissection, isolated tissue can be difficult to manipulate as it is flaccid in nature. This is especially true with respect to the accurate positioning of the tissue with regard to the locating means, which may comprise a wire holder, a cannula or the like. To obviate this disadvantage and as shown in Figure 14 immobilising and positioning the tissue can help alignment with the locating device. By placing the tissue structure gently using forceps upon a layer comprising one or more proteins selected from the following: collagen, elastin, gelatin, fibronectin, fibrinogen, vitronectin the tissue can be held in place.

The protein layer can be placed upon a polypropylene or polyethylene surface. Preferably, the plastic surface comprises the inner aspect of half of a conical funnel, ensuring a concave surface upon which to place the tissue. As can be seen in Figure 14, two such funnels are then placed in opposition upon a rigid bath, with the mouths of the funnels facing each other. One funnel (A) is fixed as part of the bath, the second funnel (B) is placed on the bath, preferably attached to a rail that allows movement towards or away from the first funnel in the horizontal plane.

Further, the accurate positioning of the tissue with respect to the chosen locating device is ensured by

gently placing one end of the tissue in the neck of the fixed conical funnel (A) at a point that equals the external diameter of the tissue. The second funnel (B) is moved along the horizontal plane until the end of the tissue in the neck of the conical funnel again lies above the point in the funnel that equals the external diameter of the tissue. The tissue is then gently placed on the protein layer of the moveable second funnel (B).

The locating device can be guided firstly through the narrow portion of the funnel and then through the immobilised tissue.

Various examples of experiments will now be described. The experiments were all performed with a modified pressure myograph (P110, Danish MyoTech, Aarhus, Denmark) with four chambers in each module and multi-reservoir capabilities. A Type 205U/CA multi-channel cassette pump (Watson-Marlow, Camlab, Cambridge, UK) was also used.

The suppliers' instructions were used for operation and preparation of the myograph and pump, modified where appropriate for implementation of the present measurement protocol.

Examples

Example 1. Absorption spectroscopy of agonist-induced vasoconstriction and vasodilation in rat mesenteric arteries.

3rd order mesenteric arteries from normotensive male Wistar rats were set up in two perfusion chambers in normal physiological salt solution (PSS), at 37^{0}C and gassed with 95%O_2/5%CO_2. The vessels were pressurised to 60 mmHg and allowed to equilibrate for 30 minutes. Thereafter, one vessel acted as a control and the other vessel received increasing concentrations of the thromboxane A2 mimetic, U46619. Transmission spectra were recorded at one second intervals and analysed according to the methods described above.

U46619-induced vasoconstriction was detected via shifts in the transmission spectra due to changes in lumen diameter. The optical extinction model described above was used to accurately and continuously measure lumen diameter. This data can then be expressed as graph of concentration (of agonist) versus response (% change in lumen diameter) (figure 7).

Experiment 2. Absorption spectroscopy of rat arteries.

Third order mesenteric resistance arteries were obtained from adult female normotensive Wistar and hypertensive Goldblatt (one kidney-one clip) rats 8 weeks following the induction of hypertension. Twenty arterial samples (10 normotensive; 10 hypertensive) were studied from 16 animals (6 eight-week normotensive, 5 eight-week hypertensive, 3 four-week normotensive, and 2 four-week

hypertensive) . Dissected arterial segments were stored in cold saline solution prior to mounting in the myograph.

Ring preparations were mounted on two stainless steel wires (40μm in diameter) in physiological salt solution gassed in 95%O_2/5%CO_2 and maintained at 37°C to a pH of 7.4. The wires were secured onto the myograph mounting heads to enable the internal diameter of the vessel to be controlled directly via an automated linear stage attached to one mounting head. Morphological measurements of media thickness (w), wire diameter (d) and wire separation (s) were made using light microscopy and a filar calibrated eyepiece with the vessels under conditions of minimum tension (Figure 8). The average of three values taken at locations equidistant along each vessel was used. Assuming a circular cross-section under physiological conditions, effective internal circumference (C), lumen diameter (L) and cross-sectional area of the vessel wall (A) were calculated using the following equations:

$$C = d\ (2 + P) + 2s$$

$$L = C\ ,\ P$$

$$A = Pw\ (L + w)$$

Spectroscopy measurements were achieved by a simple optic fibre-coupled spectrometer integrated into the Mulvany myograph via a probe terminating above the vessel. Light from a tungsten halogen source (150W) was channelled via an optical fibre and collimating

lens to the myograph (Figure 9). The fibre optic probe (tip: 1mm diameter; glass fibre: 50 µm core diameter) was positioned above the vessel by means of a probe assembly clamped to horizontal (resolution 0.01mm) and vertical (resolution 0.05mm) linear stages to enable accurate alignment of the transmission system. A diode array spectrometer coupled to the probe recorded at 2-3 seconds intervals as the lumen diameter was stretched in 50 µm steps from 100 µm to 350 µm. Transmission spectra were obtained from two probe positions: one collected with a stationary probe head and the other with the probe head moved 20 µm for each 50 µm horizontal stretch. A total of 659 spectra were used in the subsequent analysis. A standard normalisation method was used to transform the raw spectra into transmission spectra, defined as the ratio between the background subtracted raw arterial spectrum and the white light spectrum. Analysis of transmission spectra Assuming that observed features distinguishing normal and hypertensive vessels reflect an underlying compositional change in the vessel wall, a quantitative analysis of the spectra could in principle have been performed by multivariate analysis of the constituents. However, since at this stage the existence and nature of such compositional changes has not been established an empirical approach was preferred in order to establish the extent and significance of the main spectral features. Given the overall shape of the transmission spectra (T) under examination, a

compact representation was possible using the logistic functional:

$$T = \sum_i \frac{a_i}{1 + \exp(-b_i[\lambda - \lambda_i])}$$

A quasi-Newton algorithm was used to fit this non-linear function to the observed spectra. Sufficiently accurate data fits were obtained (RMS error = 3%) using only two terms from this equation which were adopted for the analysis reported here.

Results

The mean dimensions of the arterial specimens measured by microscopy are given in Figure 12.

The presence of vascular growth was apparent in both hypertensive age groups. The 4-week hypertensive animals showed a marked increase of 98% in media cross-sectional area (65% in media:lumen ratio) compared to their paired normotensive controls. The vessels from the 8-week hypertensive group also showed increases in these parameters (20% in media cross-sectional area; 20% in media:lumen ratio). The data for the 4-week group was skewed since one single animal developed an unusually high pressure (231mmHg) and marked structural growth. Since the purpose of this study was to ascertain the usefulness of spectroscopy, and not to investigate aspects of this particular model of hypertension, these data were subsequently extremely useful.

Transmission spectra (resolution = 1 nm) derived from the vessels of 4-week animals show a clear separation between normotensive and hypertensive vessels since changes in vessel wall thickness are reflected in vertical shifts of the spectra (Figure 10). The difference observed between the 8-week groups were not as marked due to the thicker walls exhibited by the 4-week group. Differential increase of the arterial lumen (N1 to N2 and H1 to H2) affected the observed absorption spectra in the vertical plane confirming the correlation between wall thickness and absorption spectra. More subtle features distinguishing hypertensive and normotensive vessels were observed including a high absorption characteristic in the blue-green region of the spectrum (400nm - 550nm, Figure 10). Given the consistency of these observations, a future detailed quantitative analysis is appropriate to ascertain the source of these data.

A typical transmission spectrum and its empirical model are shown in Figure 11 for which the model parameters are: $a_1 = 0.39$; $a_2 = 0.098$; $b_1 = 0.057nm^{-1}$; $b_2 = 0.179nm^{-1}$; λ_1=412nm; λ_2= 753nm. Each of these parameters exhibits a different sensitivity with respect to the data fitting procedure and therefore the precision of the parameter estimates was individually assessed. This assessment has been performed elsewhere and the conclusion of that analysis leads to the following worst case estimated errors on the determination of model parameters: a_1 ($\pm$ 0.02) ; a_2 ($\pm$0.03); b_1 ($\pm$ 0.006); b_2 ($\pm$ 1.5); λ_1

(± 4); λ_2 (± 44). These errors are much larger than those of the majority of the spectra examined and are quoted to allow a conservative interpretation of the results. It should also be stressed that these errors are derived from the limited data sets provided and should only be used as a guide in the consideration of absolute errors.

Comparing spectral and morphological results.

Both hypertensive age groups showed media growth trends. The linear correlation coefficients obtained from a comparison between the transmission spectral features (a_1, a_2, b_1, b_2, λ_1, λ_2) and the parameters which are conventionally used in the study and clinical definition of hypertension (media:lumen ratio (R), media cross sectional area (A) and blood pressure (BP)) are detailed in Figure 13. A number of statistically significant correlations exist between the features extracted from spectroscopy arid those conventionally used to define hypertension, with those of greater significance and closer approach to linearity being found in the 4 week group. The media:lumen ratio is expected to reflect changes in vessel size associated with anatomical variability and media growth, and this should in turn be reflected in a rescaling of the transmission spectra. These effects are indeed implied by the significant correlation between R and the scaling factors for the 4-week group. In the 8-week group, with a less marked difference in structure, the number of

correlations is reduced however significant changes can still be detected in two scaling factors. Indeed, the most significant correlations occur between b_1, λ_1 and BP, R. Since BP and R are themselves strongly correlated in this (correlation coefficient = 0.98 for 4 week group) and many other studies their relationship to the observed spectral features is the most important result of this present study. Since these spectral features relate to the logistic function (equation above) which models the average spectral shape in the 350-550nm range, a connection is established between the presence of hypertension and the existence of differential absorption in the blue-green region of the spectrum.

The following examples relate to hypothetical measurements which could be made using the apparatus and method of the present invention.

Example 1. Absorption spectroscopy of proximal and distal human subcutaneous arteries from critical limb ischaemia patients.

Human subcutaneous arteries from critical limb ischaemia patients are set up in the perfusion unit and perfused with normal PSS. Vessels proximal to the site of limb ischaemia are compared to vessels distal to the site of limb ischaemia. The absorption spectra from each type of vessel is used to examine differences in media:lumen ratio, cross-

sectional area of the vessel wall, and wall thickness.

The results demonstrate whether ischaemic (I) arteries have markedly different absorption spectra from proximal non-ischaemic (N) vessels, reflecting any differences in their wall thickness, media:lumen ratio and lumen diameters.

The apparatus may also be used for transfection experiments. During this process the blood vessel will be exposed to any drug or vector chosen by the investigator. If this is a vector for transfecting genetic material then the material will be carried into the vascular tissue if the pressure and flow settings are chosen appropriately. Once there, the cells of the blood vessel will start to produce the specific protein coded by the genetic material. These proteins will make their way to the cell membrane and lodge there. The time required for this process depends on the vector and particular system being encouraged. Following a suitable transfection period (determined by the investigator and programmed at the beginning of the test) the perfusion reservoir is changed to a reservoir containing an agonist of choice, selected to be appropriate to stimulate the protein induced during transfection. On contact with the proteins in the tissue, the agonist will induce a functional response in the tissue (usually vasoconstriction or vasodilation, possibly changes in wall structure).

Any such change will be reflected in an alteration in the optical properties of the vascular tissue.

Example 2. Transient transfection of NOS synthase gene in NOS-knockout mice using LipofectAMINE reagent and functional detection of transfection by absorption spectroscopy.

Mouse 1st order mesenteric arteries are dissected out and maintained in Optimem I supplemented with 10% foetal bovine serum. cDNAs (for NOS) inserted into appropriate plasmids are mixed with LipofectAMINE according to the manufacturers instructions. Two arteries are set up in separate perfusion chambers; the first tissue (A) acts as a control (receives LipofectAMINE and plasmids without the cDNA insert), the second tissue (B) receives LipofectAMINE with plasmids containing the NOS cDNA insert.
The arteries are exposed to the appropriate transfection solutions for 8 hours.

Determination of NOS cDNA transfection efficiency.

After 8 hours, the unit switches automatically and perfuses the vessels with normal PSS. Analysis of the lumen diameter via absorption spectroscopy begins, providing continual information on the contractile state of the artery.

Functional detection of NOS activity is achieved by way of a two-stage protocol. Firstly, concentration-response curves to ACh are conducted

in both vessels. During the concentration-response curve, changes in the state of the blood vessels are continuously monitored by absorption spectroscopy. ACh selectively activates expressed NOS synthase and causes release of the vasodilator nitric oxide (NO). Therefore, the presence of NOS is demonstrated by vasodilation of the blood vessel in response to ACh. The control vessel should display little vasodilation to ACh, as NOS cDNA was not transfected in this tissue.

Both vessels are then perfused with normal PSS for thirty minutes. To ensure that ACh-induced vasodilation is due to NOS activation and not other unidentified factors, the second stage of the protocol is initiated. The concentration response curve to ACh is repeated in the presence of the selective inhibitor of NOS, L-NAME. If ACh produced vasodilation via NOS activation in the first stage of the protocol then L-NAME should abolish further responses to ACh.

Example 5. Transfection of gastrointestinal tract from ORL-1 knockout mice with ORL cDNA using adenoviral vectors.

Two 3cm segments of mouse gastrointestinal tract are set up in perfusion chambers and perfused with normal PSS. cDNAs for the ORL-1 receptor are ligated into an adenovirus transfer plasmid with the cytomegalovirus promotor. The virus is diluted in Optimem I and then perfused through one segment of

gastrointestinal tract for up to 2 hours, the other segment acts as a control and receives the virus without the ORL-1 cDNA.

After the transfection period, functional detection of the expressed ORL-1 receptor is achieved by spectroscopic detection of contractions upon perfusion of the selective ORL-1 agonist, nociceptin. The control tissue should display no response. To ensure that the actions of nociceptin are through the ORL-1 receptor, the addition of nociceptin is repeated in the presence of a selective ORL-1 antagonist, such as naloxone. Therefore, this antagonist blocks responses involving the ORL-1 receptor, and nociceptin should have no effect.

CLAIMS

1. A method of detecting a change in the physical dimensions of a biological structure, the method comprising the steps of:

illuminating a biological structure with light of a first wavelength;

detecting the intensity of light transmitted by the biological structure in at least first and second physical states; and

comparing the intensity of transmitted light with a reference light intensity of the same wavelength, to determine a change in the absorption of the biological structure, said change in the absorption corresponding to a change in physical dimensions.

2. A method as claimed in Claim 1 wherein the step of detecting transmitted light comprises the step of detecting the intensity of transmitted light across substantially the whole diameter of the biological structure.

3. A method as claimed in Claim 1 or Claim 2 which comprises the step of repeating the illumination, detection, and comparison steps at least once with light of at least one other wavelength.

4. A method as claimed in Claim 3 wherein wavelengths are selected such that in one of the wavelengths the biological structure is essentially opaque, whilst in another of the wavelengths the biological structure is semi-transparent.

5. A method as claimed in Claim 3 or Claim 4 wherein the illumination, detection and comparison steps with second or subsequent wavelengths are performed subsequently to the steps conducted with the first wavelength.

6. A method as claimed in Claim 3 or Claim 4 wherein the illumination, detection and comparison steps with second or subsequent wavelengths are performed simultaneously with the first wavelength.

7. A method as claimed in any of Claims 3 to 6 which includes the step of distinguishing measured intensities of each wavelength of light used.

8. A method as claimed in Claim 7 wherein separate detectors are used.

9. A method as claimed in Claim 7 or Claim 8 wherein the detected combined intensity is subjected to post-processing to separate the various wavelength components.

10. A method as claimed in any preceding Claim wherein the reference light intensity corresponds to

the intensity of light measured when no biological sample is illuminated.

11. A method as claimed in any preceding Claim wherein the reference light intensity corresponds to the measured intensity of light transmitted by the biological sample in a different physical state.

12. A method as claimed in any of the preceding Claims wherein the changes in the physical dimensions of a biological structure are measured over time, the method comprising the steps of:

illuminating a biological structure at first and second time points with light of a first wavelength;

detecting the intensity of light transmitted by the biological structure at first and second time points; and

comparing the intensity of transmitted light at the first time point with the intensity of transmitted light at the second time point to determine a change in the intensity of transmitted light, said intensity change being indicative of a change in physical dimensions of the biological structure.

13. A method as claimed in Claim 12 further comprising the step of altering the local

environment of the biological structure between said first and second time points.

14. A method as claimed in Claim 13 wherein the step of altering the local environment includes addition or removal of agonists, hormones, potential drugs and/or test samples to the vicinity of the biological structure, and/or direct physical stimulation of the structure and/or transfecting the biological structure with exogenous nucleic acid.

15. Use of a method as claimed in Claim 14 to determine the effect of a gene or similar nucleic acid structure, or the potential stability of a transfection vector or of the biological structure for transfection.

16. A method as claimed in any of Claims 1 to 14 wherein peptide or protein structures are added to the biological structure, to determine the effects of such peptides or proteins directly, without the requirement for expression of a transfected gene.

17. An apparatus for detecting changes in physical dimensions of biological structures, the apparatus comprising at least one light source, a light detector, means for locating a biological structure between the source and the detector, and data processing means for recording and analysing detected light which has been transmitted by the biological structure to detect and analyse changes in characteristics of the transmitted light to

determine changes in the physical dimensions of the biological structure.

18. An apparatus as claimed in Claim 17 wherein the at least one light source generates light of a single wavelength.

19. An apparatus as claimed in Claim 18 wherein the at least one light source generates light of a plurality of wavelengths.

20. An apparatus as claimed in any of Claims 17 to 19 wherein the light detector comprises a CCD device, a photoresistor or a photodiode.

21. An apparatus as claimed in any of Claims 17 to 20 wherein the detector is capable of detecting light of plurality of wavelengths.

22. An apparatus as claimed in Claim 21 which comprises means for separating detected light of multiple wavelengths, the means chosen from post-detection means or pre-detection means, or filter means for allowing only light of a single wavelength to reach the detector.

23. An apparatus as claimed in any of Claims 17 to 22 which further comprises means for aligning generated and detected light.

24. An apparatus as claimed in Claim 23 wherein the means comprises aligned optical fibres, at least one

of which carries generated light, and at least one of which carries detected light.

25. An apparatus as claimed in Claim 24 wherein the fibres are connected at one end to a light source or light detector with the other ends of the fibres being in opposed alignment, such that light leaving the source fibre can enter the detection fibre.

26. An apparatus as claimed in Claim 25 which further comprises a plastics or similar optical fibre holder, arranged to hold optical fibres in oppositely aligned relationship.

27. An apparatus as claimed in Claim 26 wherein the holder comprises a bifurcated tube, the tips of each fork of which are in alignment and wherein in use, an optical fibre is insertable into each fork of the holder, with the free ends of the fibres being coterminous with the ends of the forks to ensure the optical fibres are aligned correctly.

28. An apparatus as claimed in any of Claims 17 to 27 wherein the means for locating a biological structure comprises a wire holder, a cannula, or a plastics tube bearing an external circumferential groove; wherein in use, a biological tube structure may be located over the plastic tube, and secured thereto by means of tying a fastener around the groove.

29. An apparatus as claimed in any of Claims 17 to 28 which further comprises means for subjecting the biological structure to fluids and the like.

30. An apparatus as claimed in Claim 29 wherein the means comprises at least one chamber having a fluid inlet and a fluid outlet, in which the biological structure is located.

31. An apparatus as claimed in Claim 30 which comprises one or more fluid reservoirs, for storing circulatory fluid.

32. An apparatus as claimed in Claim 30 or Claim 31 wherein the chamber comprises a fluid pump, for circulating fluid within the chamber, or into and out of the chamber.

33. An apparatus as claimed in Claim 32 wherein the pump is arranged to maintain the chamber at substantially physiological fluid pressure.

34. An apparatus as claimed in any of Claims 17 to 33 which further comprises means for introducing substances to the biologicai structure.

35. An apparatus as claimed in Claim 30 in which a plurality of chambers are provided, enabling a plurality of biological structure samples to be studied simultaneously.

36. An apparatus as claimed in any of Claims 17 to 35 wherein the data processing means comprises a computer.

37. An apparatus as claimed in any of Claims 17 to 36 which comprises temperature regulation means, for regulating the temperature of a biological sample.

38. An apparatus as claimed in any of Claims 17 to 36 wherein the means for locating the biological structure comprises or includes a layer of protein which can immobilise the structure.

39. An apparatus as claimed in Claim 38 wherein the protein is or is derived from one or more of the group consisting of collagen, elastin, gelatin, fibronectin, fibrinogen and vitronectin.

40. Use of a method as claimed in any of Claims 1 to 16 to detect changes in the internal diameter of a tube structure.

41. Use of a method as claimed in any of Claims 1 to 16 to measure a biological tube structure such as those chosen from the group including blood vessel, lymphetic vessel, airway, gut, weter, wethra, seminiferons, tubule, vas deferens and the like.

42. Use of a protein chosen from collagen, elastin, gelatin, fibronectin, fibrinogen and vitronectin to support biological tissue in testing apparatus.

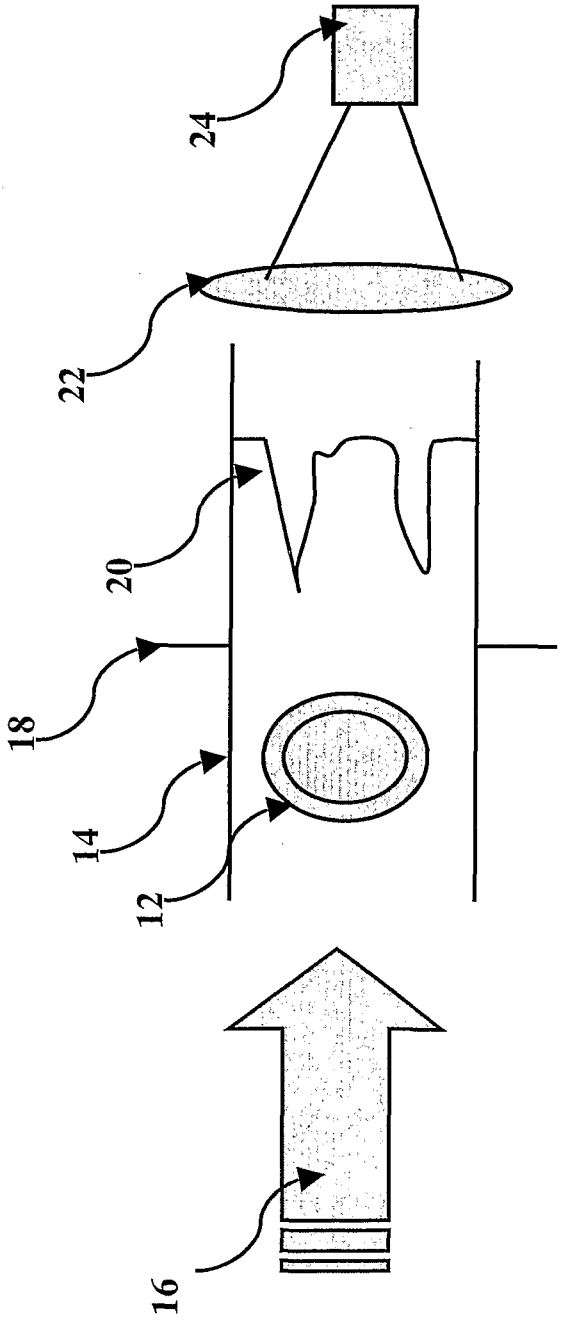

Figure 1

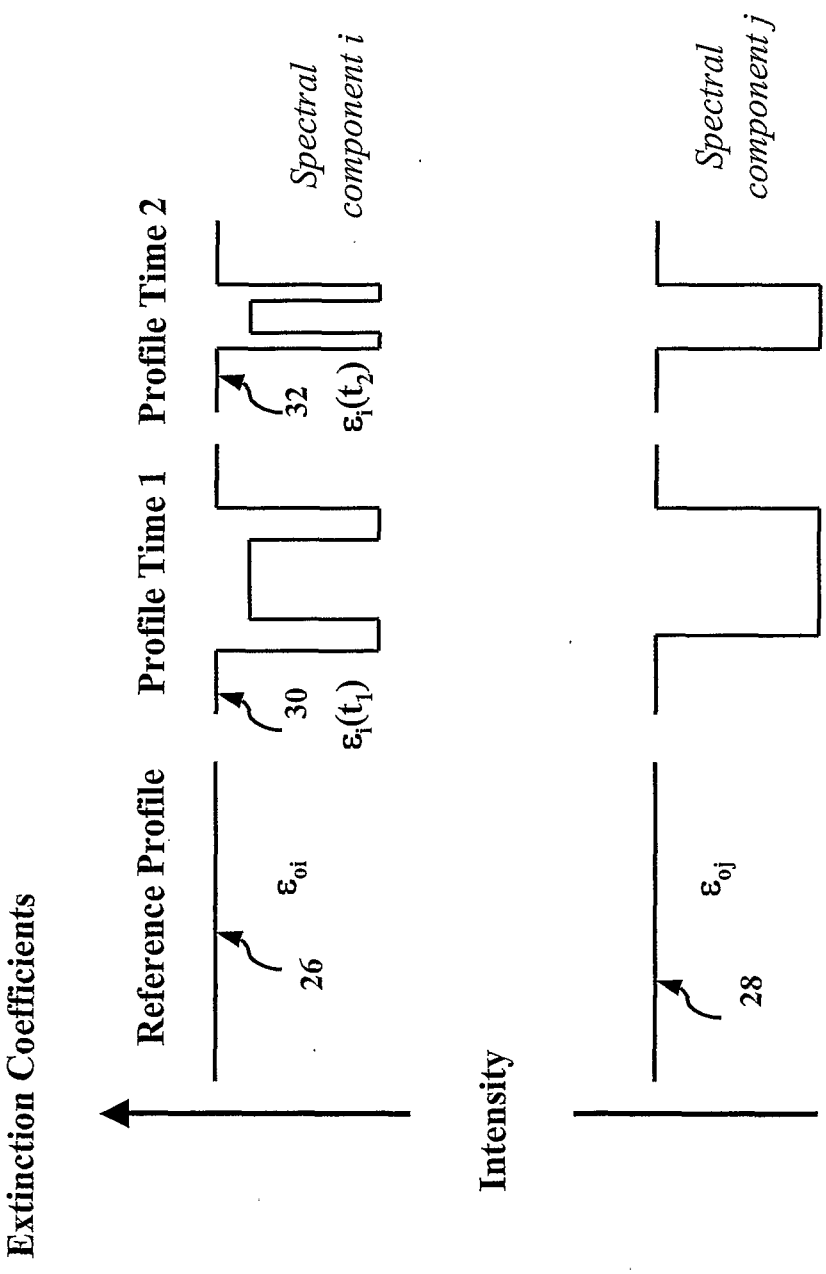
Extinction Coefficients
Reference Profile
Profile Time 1
Profile Time 2
Spectral component i
ε_{oi}
26
30
$\varepsilon_i(t_1)$
32
$\varepsilon_i(t_2)$
Intensity
ε_{oj}
28
Spectral component j

Figure 2

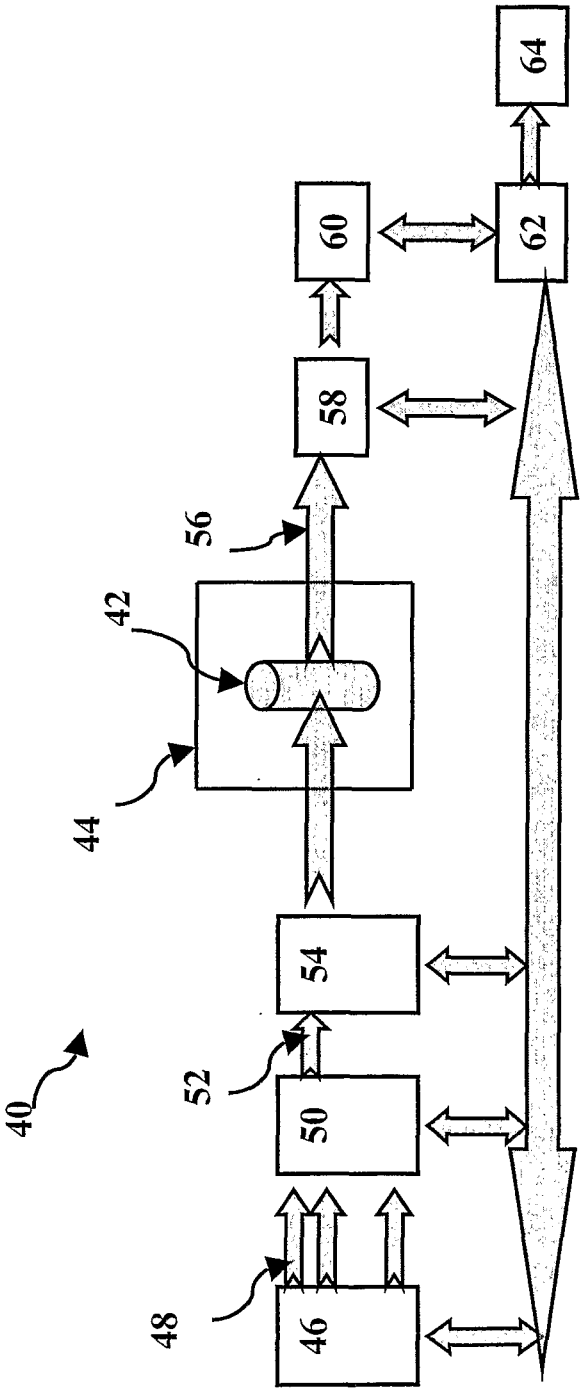

Figure 3

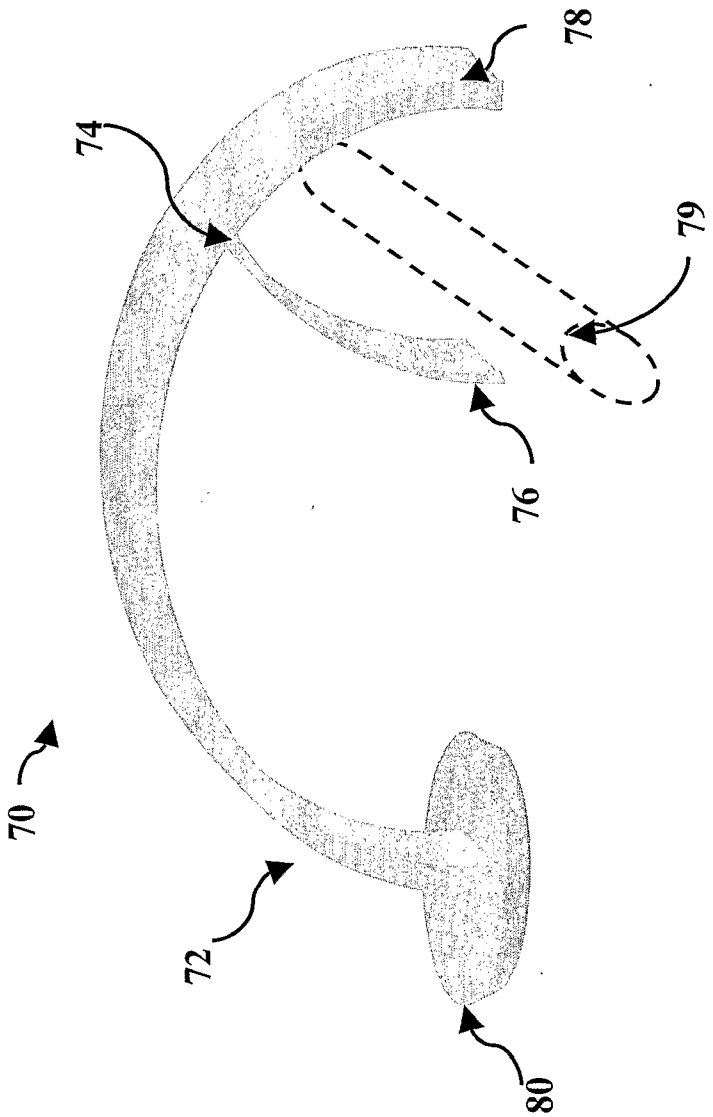

Figure 4

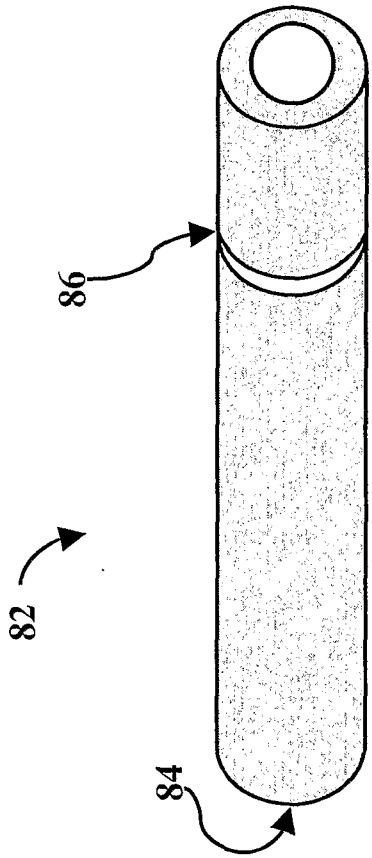

Figure 5

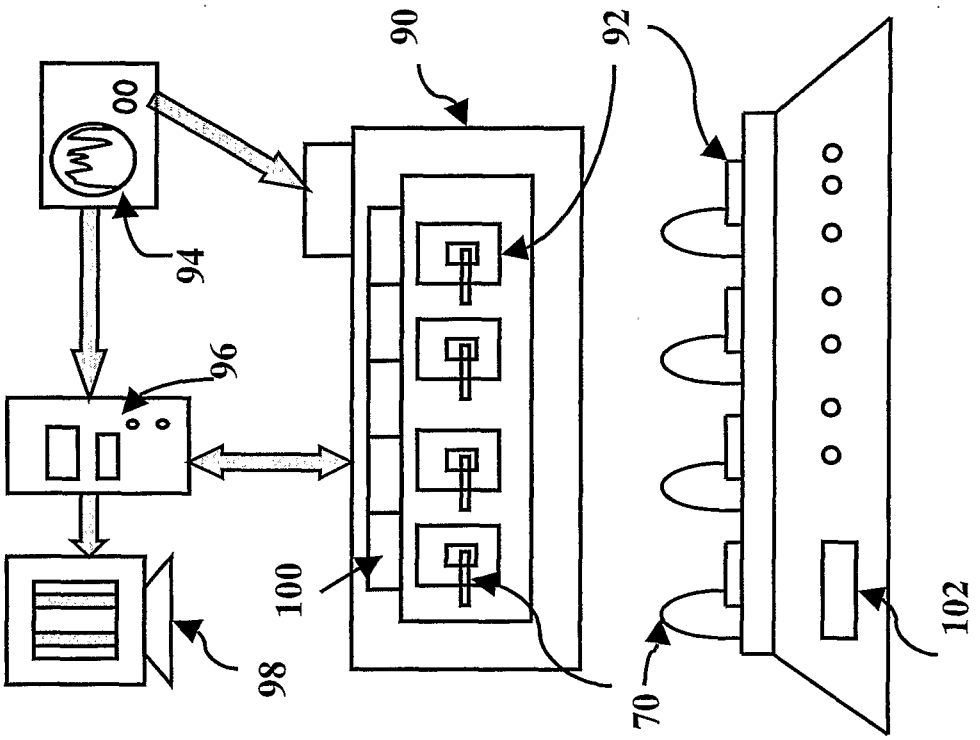

Figure 6

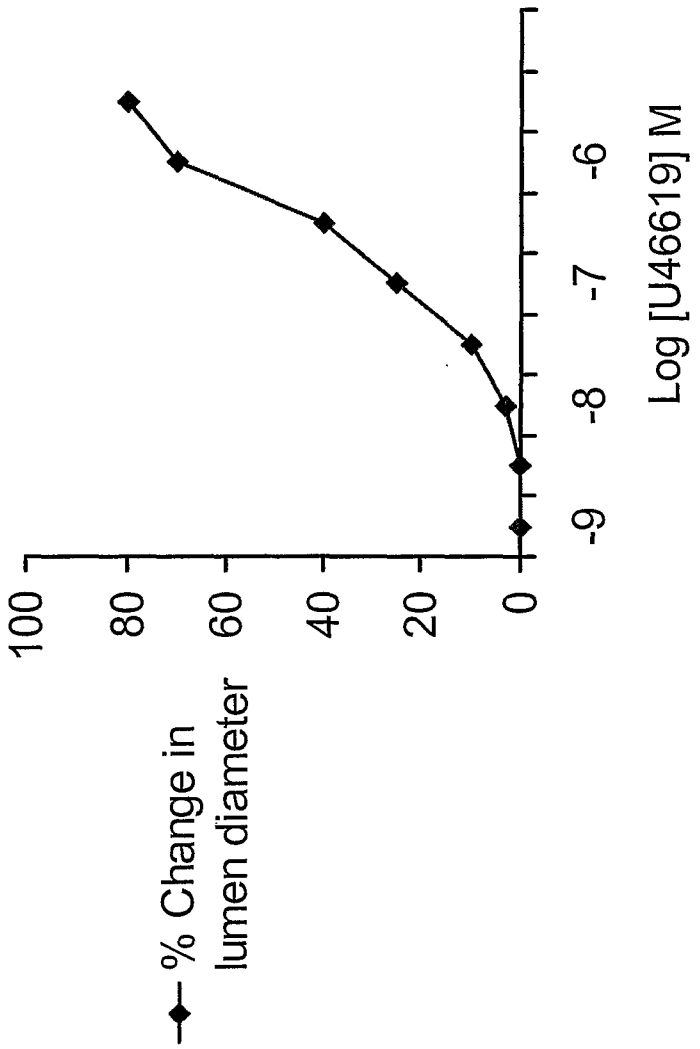

Figure 7

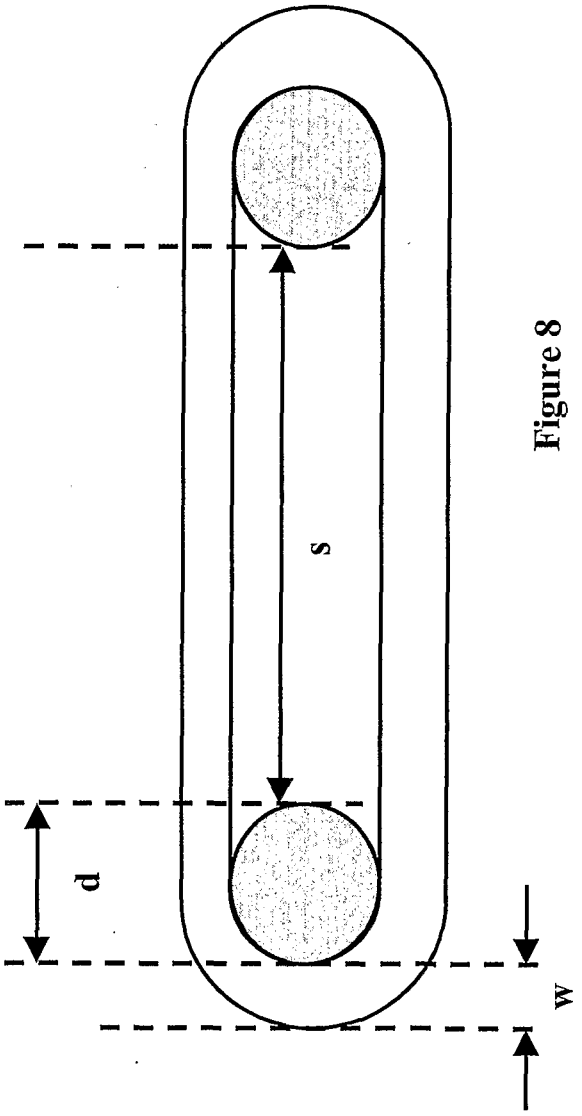

Figure 8

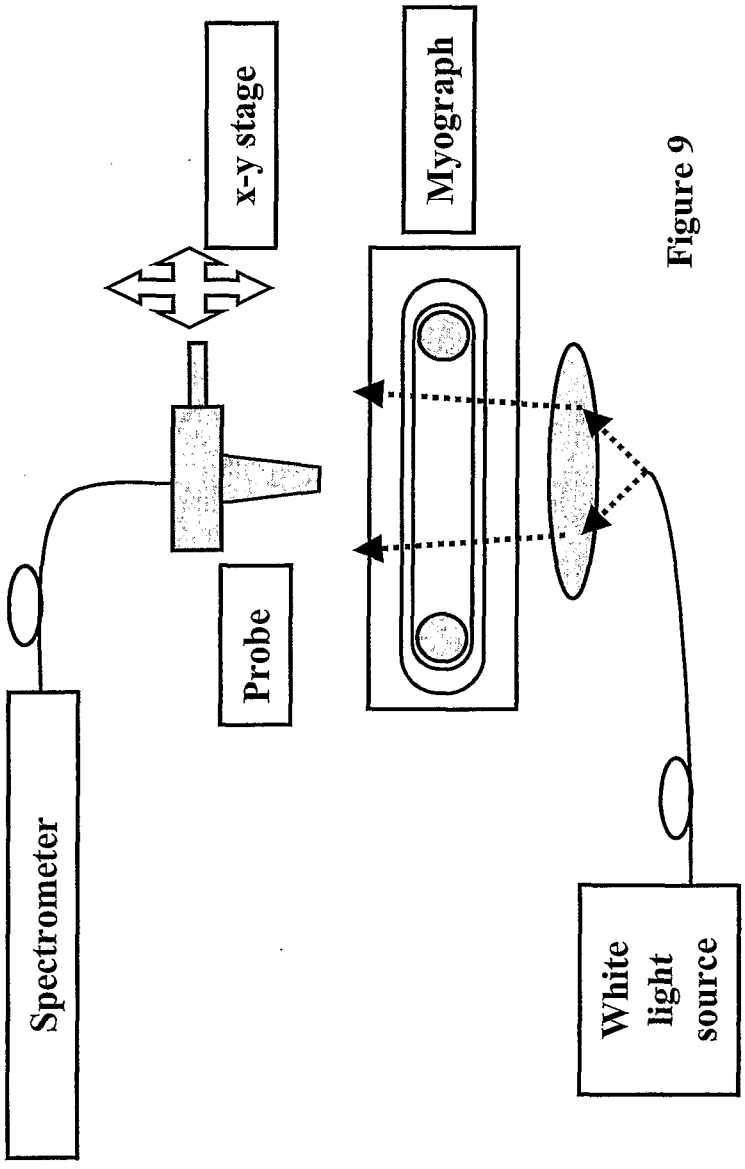

Figure 9

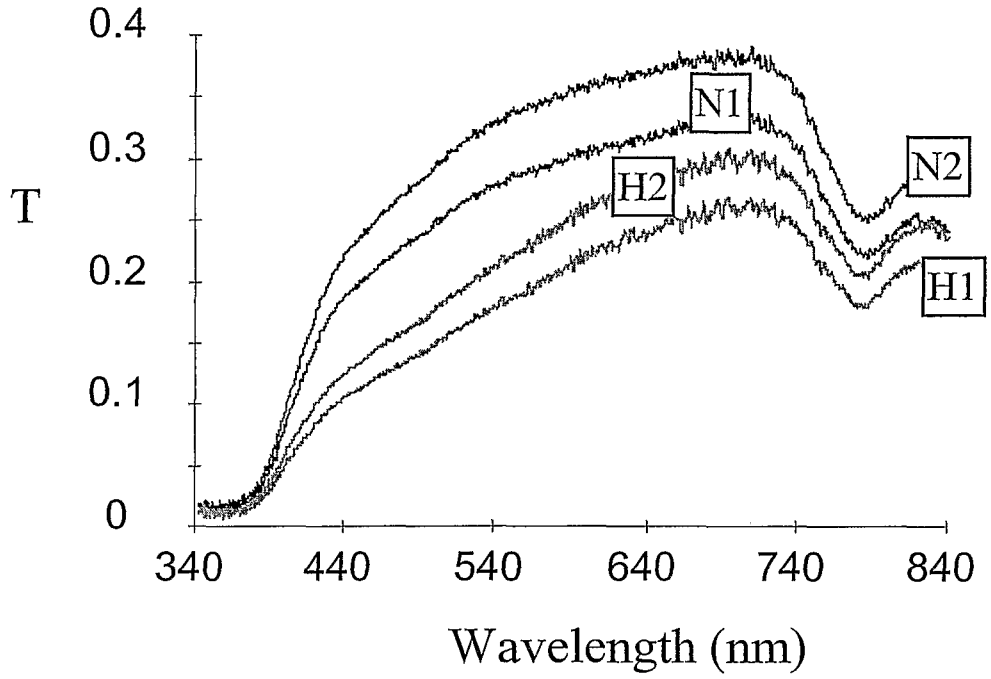

Figure 10

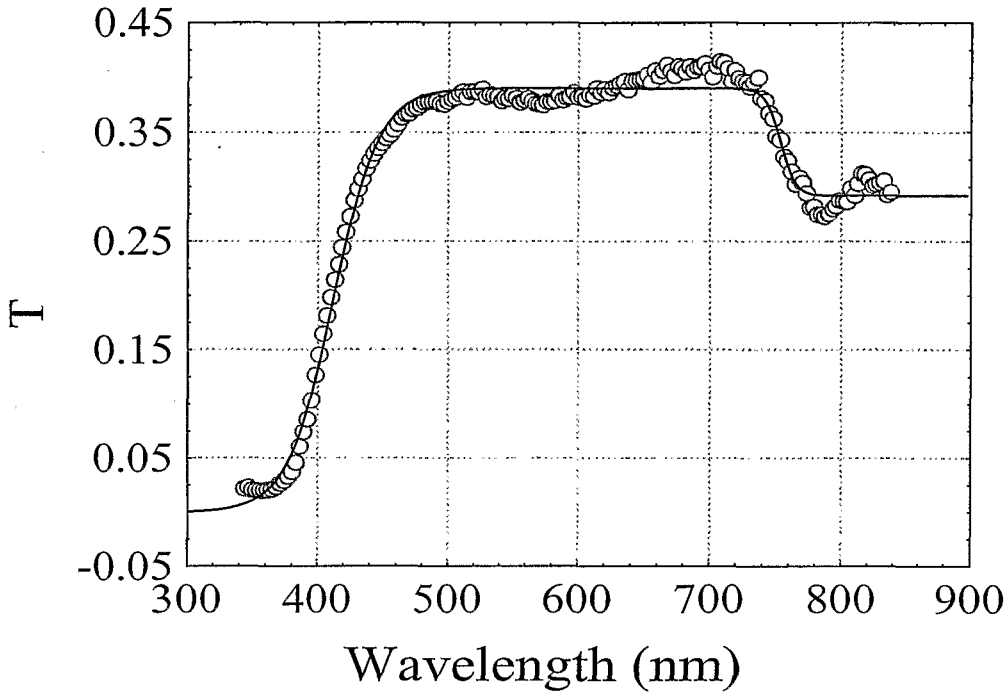
0.45
0.35
0.25
0.15
0.05
-0.05
T
300
400
500
600
700
800
900
Wavelength (nm)

Figure 11

	BP (mmHg)	Media **w** (μm)	Lumen **L** (μm)	Media area **A** (μm^2)
4 week	Normotensive (125 ± 5)	25.6 ± 0.7	135 ± 21	12901 ± 1828
	Hypertensive (206 ± 25)	44.8 ± 3.3	143 ± 13	25556 ± 2698
8 week	Normotensive (120 ± 5)	24.6 ± 3.9	139 ± 42	12478 ± 3681
	Hypertensive (192 ± 9)	29.4 ± 4.3	137 ± 37	15206 ± 2961

Figure 12

	Model parameter	Blood Pressure BP	Media Area A (μm^2)	Media:lumen R
4 week group	a_1	-0.44*	-0.19	-0.48*
	b_1	-0.67*	-0.38*	-0.75*
	λ_1	0.78*	0.47*	0.84*
	a_2	-0.50*	-0.28*	-0.55*
	b_2	0.31*	0.07	0.46*
	λ_2	0.29*	0.14	0.44*
8 week group	a_1	0.08	0.12	0.07
	b_1	-0.02	-0.35*	0.22*
	λ_1	0.11	0.53*	-0.26*
	a_2	0.28*	0.29*	0.03
	b_2	-0.30*	-0.29*	-0.07
	λ_2	-0.31*	-0.35*	0.05

Figure 13

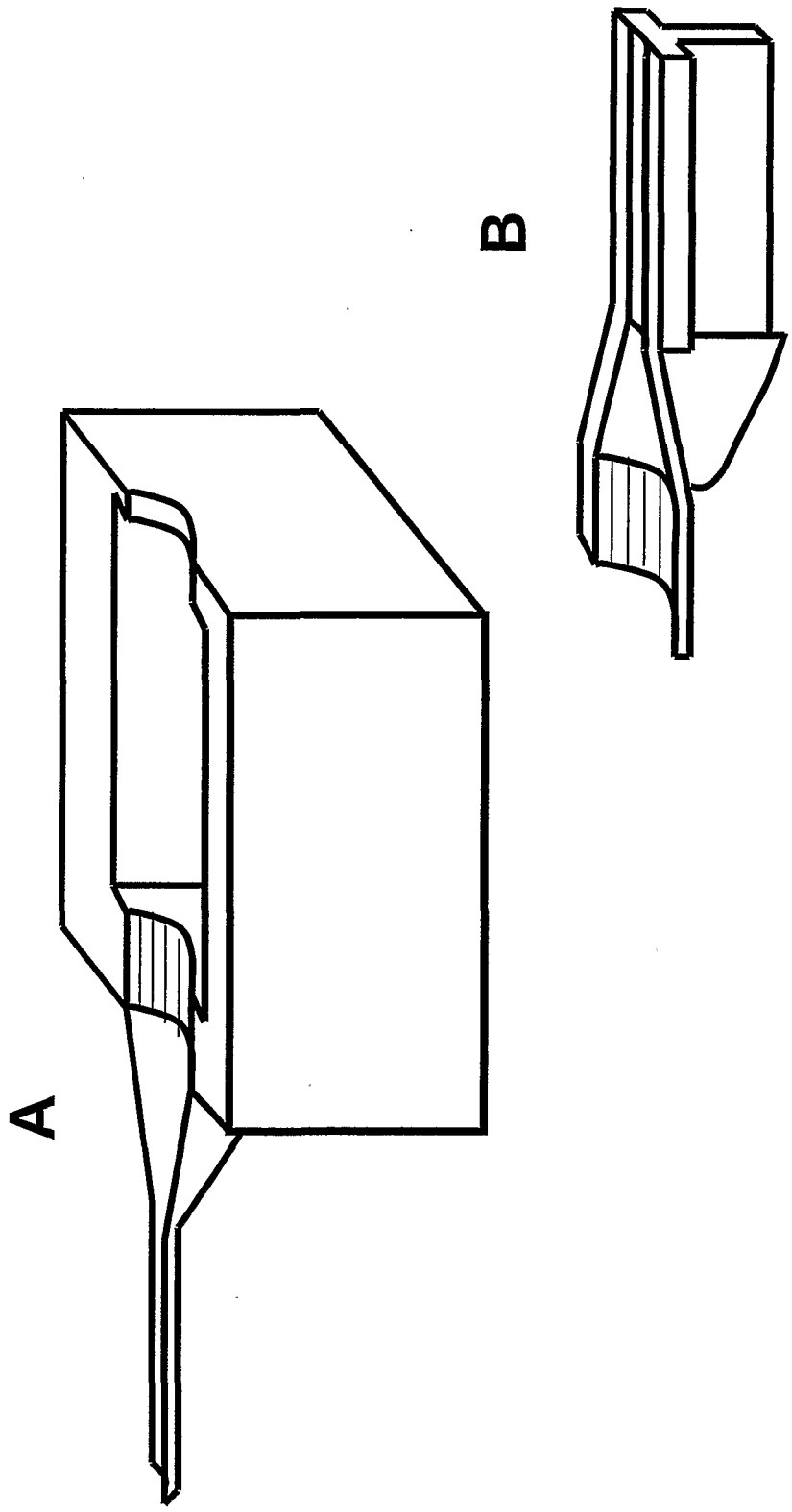

Figure 14

INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/GB 01/04438

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61B5/00

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)
IPC 7 A61B

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 practical, 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.
X	US 5 846 188 A (PALTI YORAM) 8 December 1998 (1998-12-08)	1,2,10, 11,13, 14,17, 18,20, 29,30,34
Y	column 3, line 33 - line 44 column 6, line 3 - line 17 column 6, line 62 -column 7, line 4 column 7, line 45 - line 65; tables 3,4 ---	3,5-8, 12,19,21
Y	US 5 158 090 A (WALDMAN JUERGEN ET AL) 27 October 1992 (1992-10-27) column 4, line 28 - line 62 column 7, line 13 - line 41 column 11, line 12 -column 12, line 26; tables 1-4 --- -/--	3,5-8, 12,19,21

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

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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

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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
8 February 2002	18/02/2002

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, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Weihs, J

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 986 770 A (ESSENPREIS MATTHIAS ET AL) 16 November 1999 (1999-11-16) column 1, line 8 - line 33 column 1, line 49 -column 4, line 58	1,3,5-8, 12, 17-22,36
A	WO 98 44841 A (OEBERG AAKE ;UGNELL HAAKAN (SE); FRIDOLIN IVO (SE); LINDBERG LARS) 15 October 1998 (1998-10-15) page 1, line 34 -page 4, line 32; table 1	1,12,17, 18,20, 23-26,36

INTERNATIONAL SEARCH REPORT

formation on patent family members

Inte nal Application No
PCT/GB 01/04438

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		WO 9844841 A1	15-10-1998

专利名称(译)	用于测量生物结构的装置和方法		
公开(公告)号	EP1322218A1	公开(公告)日	2003-07-02
申请号	EP2001974456	申请日	2001-10-05
[标]申请(专利权)人(译)	格拉斯哥卡利多尼亚UNIV法院		
申请(专利权)人(译)	格拉斯哥卡利多尼亚大学法院		
当前申请(专利权)人(译)	格拉斯哥卡利多尼亚大学法院		
[标]发明人	HILLIER CHRISTOPHER BUNTON DAVID COATS PAUL SMITH PETER		
发明人	HILLIER, CHRISTOPHER BUNTON, DAVID COATS, PAUL SMITH, PETER		
IPC分类号	A61B5/00 A61B5/107		
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摘要(译)

本发明涉及一种用于测量生物结构尺寸的装置和方法。特别地，但非排他地，本发明涉及响应于诸如激素，激动剂，潜在药物的刺激而测量管状生物结构（例如血管）的物理尺寸随时间的变化。该方法可用于确定基因的作用。