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(54) **ATHEROSCLEROTIC PLAQUE DETECTION**

(71) Applicant: **BAKER HEART AND DIABETES INSTITUTE**, Melbourne (AU)

(72) Inventors: **Karlheinz Peter**, Hawthorne (AU); **Nay Min Htun**, South Yara (AU); **Yung-Chih Chen**, Box Hill South (AU)

(73) Assignee: **BAKER HEART AND DIABETES INSTITUTE**, Melbourne (AU)

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Primary Examiner — Keith M Raymond
Assistant Examiner — Milton Truong
(74) *Attorney, Agent, or Firm* — KNOBBE, MARTENS, OLSON & BEAR, LLP

(57) **ABSTRACT**

An apparatus detects atherosclerotic plaques. The apparatus includes an electronic processing device that determines a level of fluorescence sensed by a sensor at a second infrared wavelength in response to exposure of at least part of an artery to radiation at a first infrared wavelength and determines a fluorescence indicator using the level of fluorescence. The fluorescence indicator is indicative of the presence, absence, or degree of an atherosclerotic plaque.

20 Claims, 11 Drawing Sheets
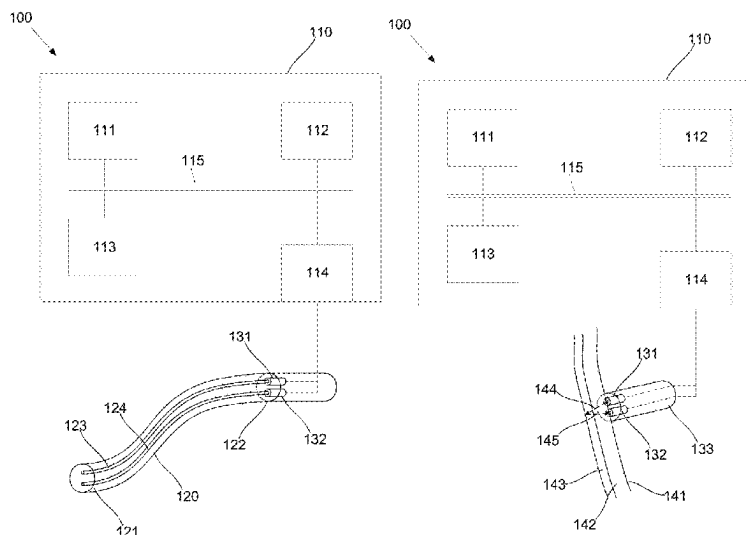
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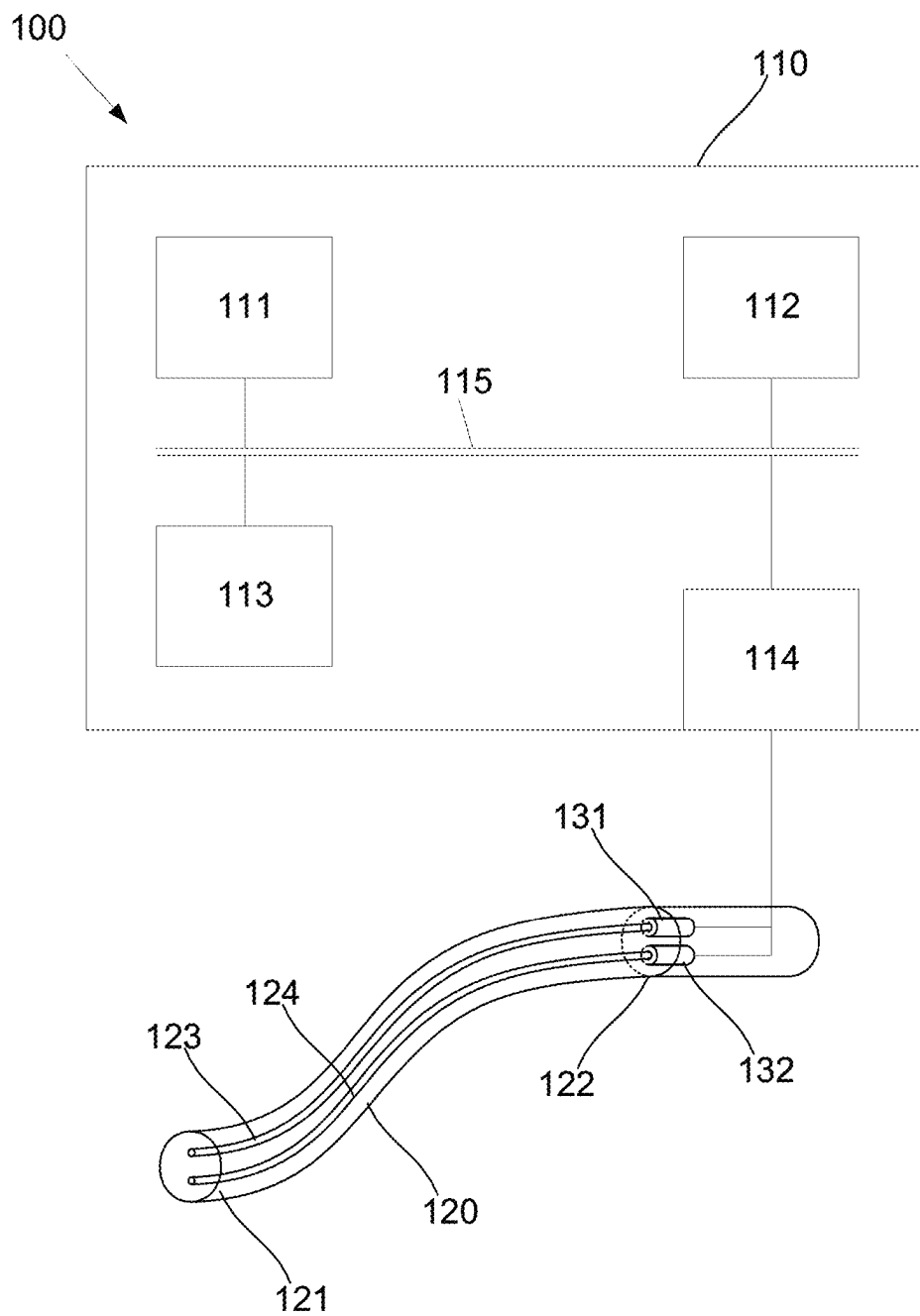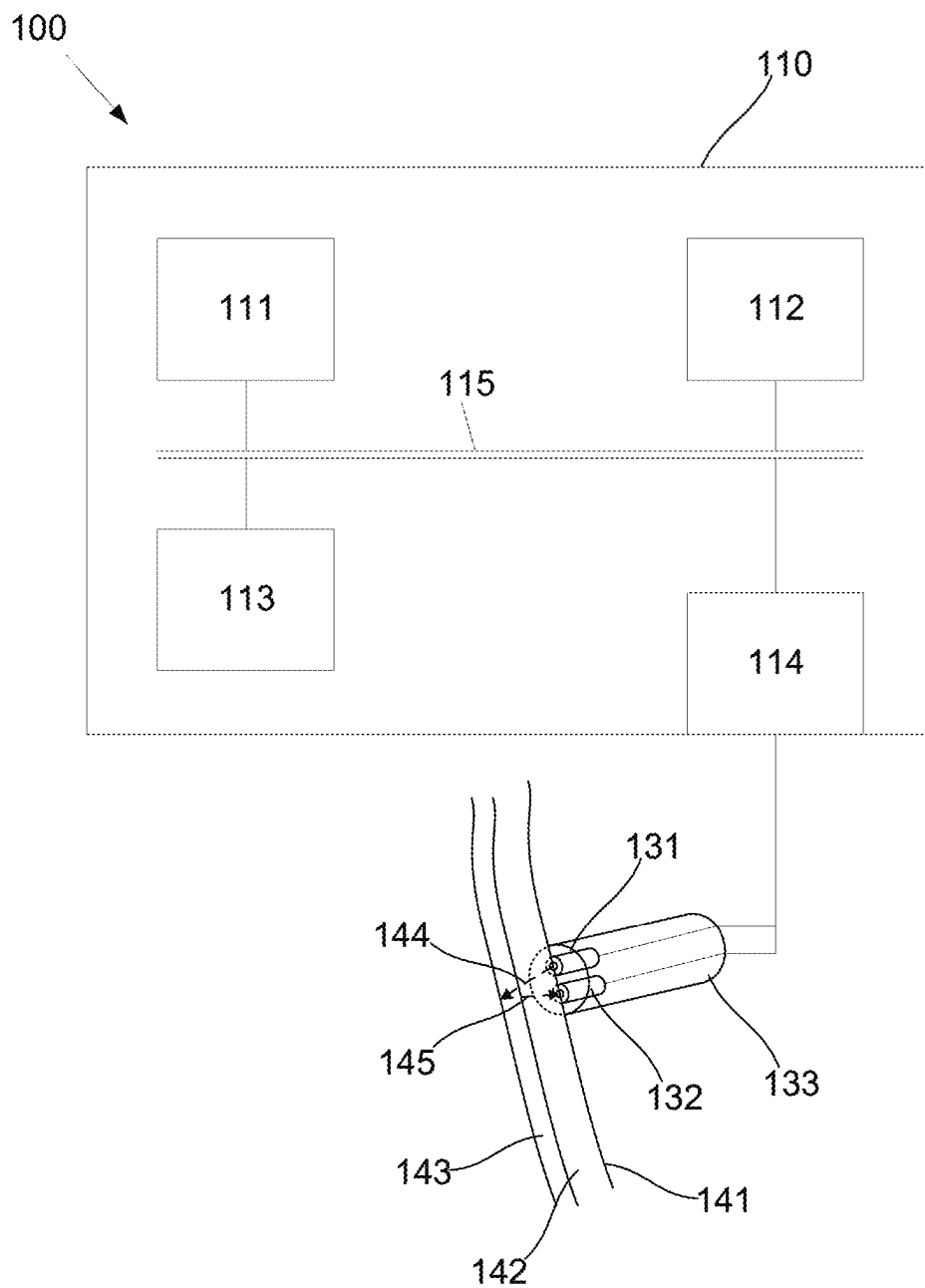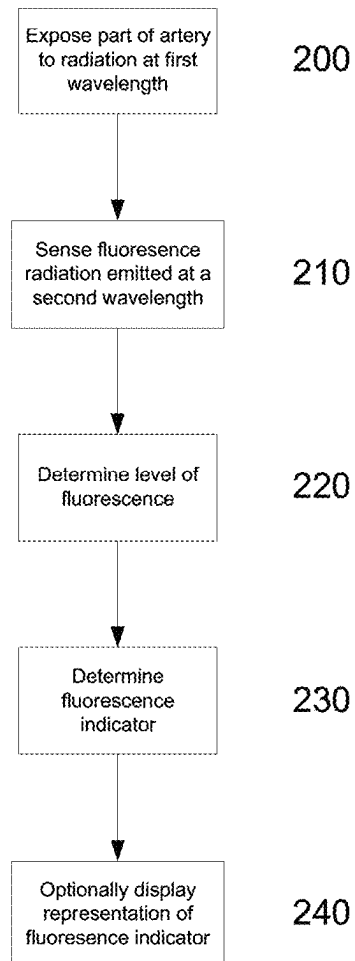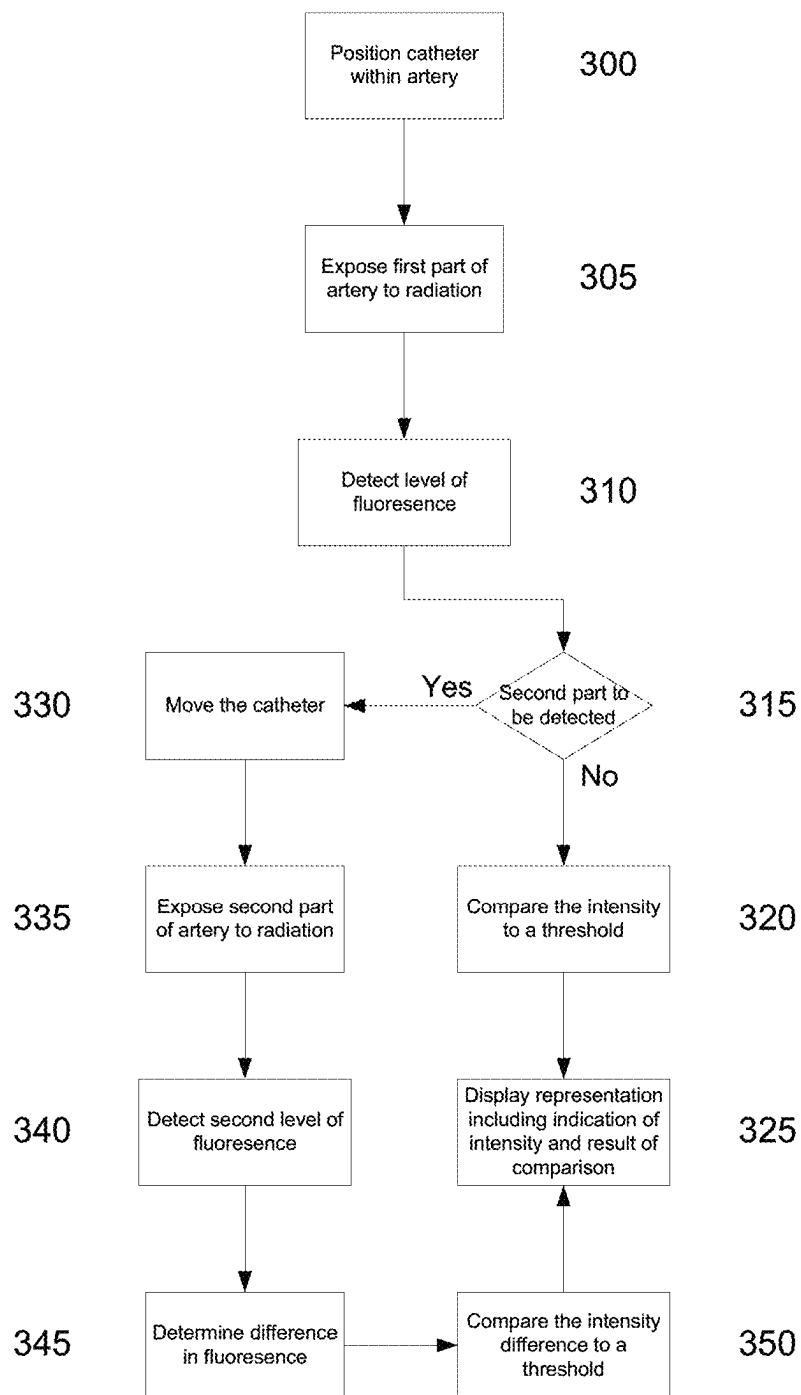
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Fig. 1A

Fig. 1B

Expose part of artery to radiation at first wavelength   200

Sense fluoresence radiation emitted at a second wavelength   210

Determine level of fluorescence   220

Determine fluorescence indicator   230

Optionally display representation of fluoresence indicator   240

Fig. 2

Position catheter within artery   300

Expose first part of artery to radiation   305

Detect level of fluoresence   310

330   Move the catheter   Yes   Second part to be detected   315

No

335   Expose second part of artery to radiation

320   Compare the intensity to a threshold

340   Detect second level of fluoresence

325   Display representation including indication of intensity and result of comparison

345   Determine difference in fluoresence

350   Compare the intensity difference to a threshold

Fig. 3

Fig. 4A

Fig. 4B

Fig. 5A

Fig. 5B

Fig. 5C

Fig. 5D

Ramen 785nm

H&E
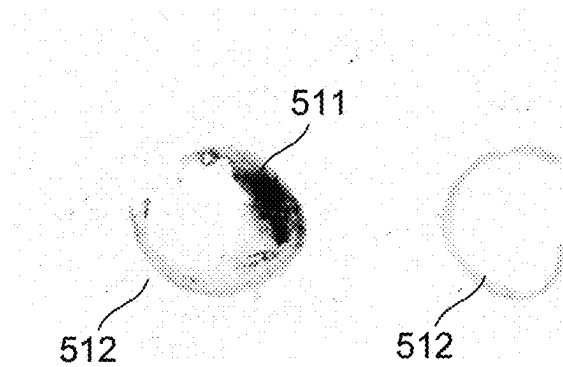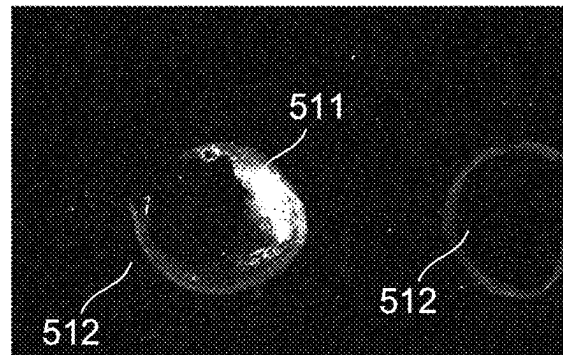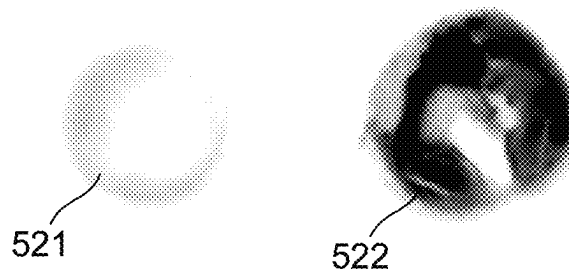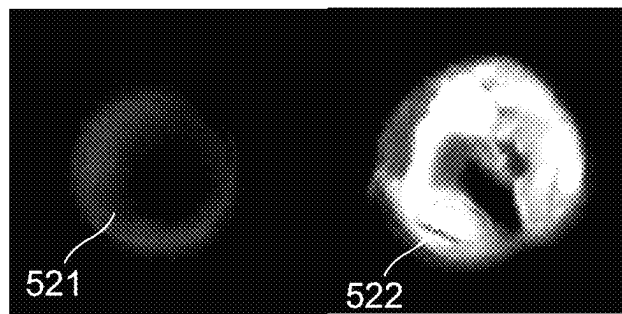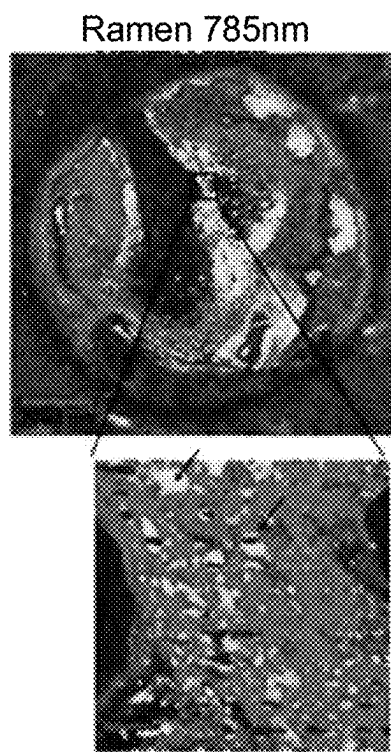
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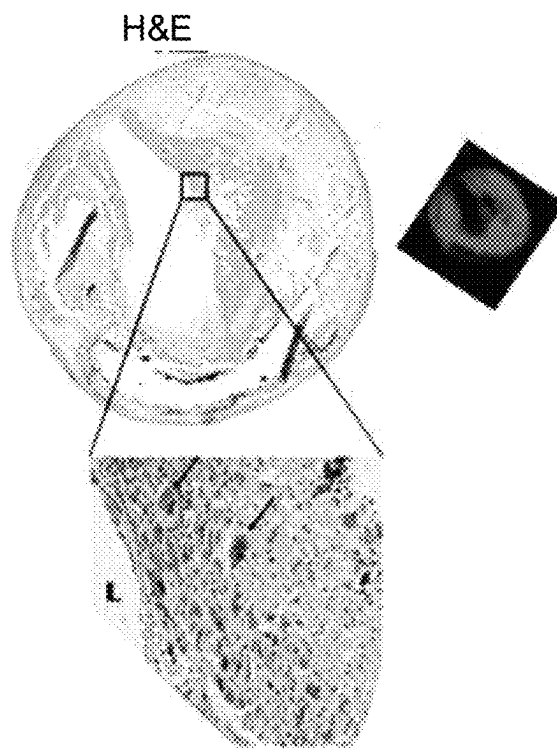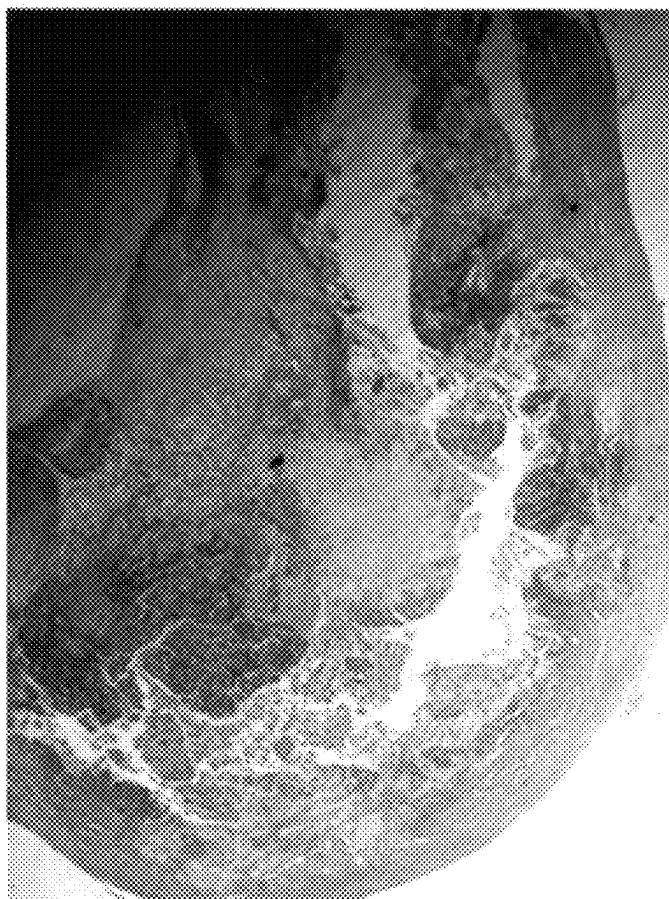
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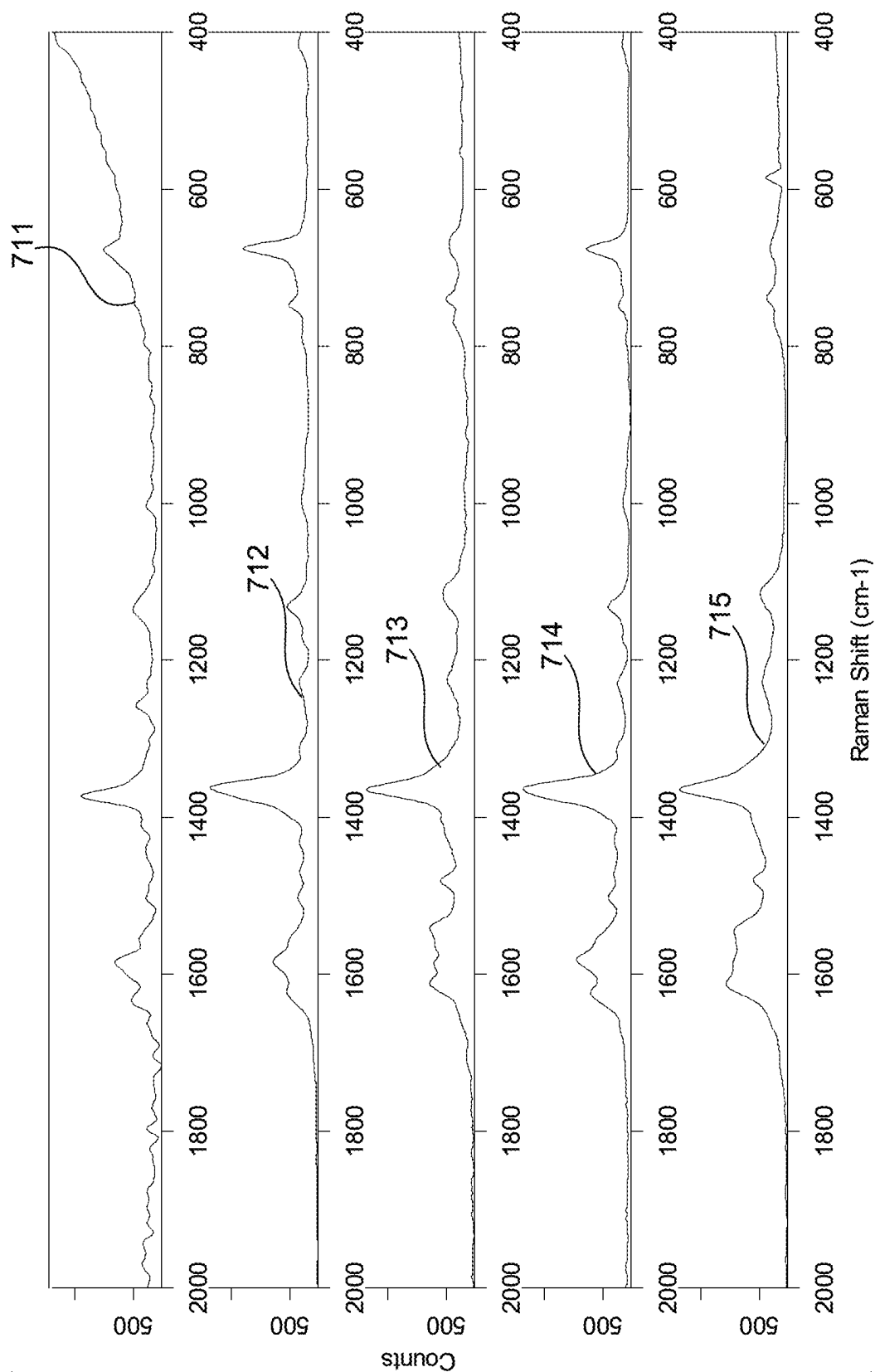
Fig. 7A
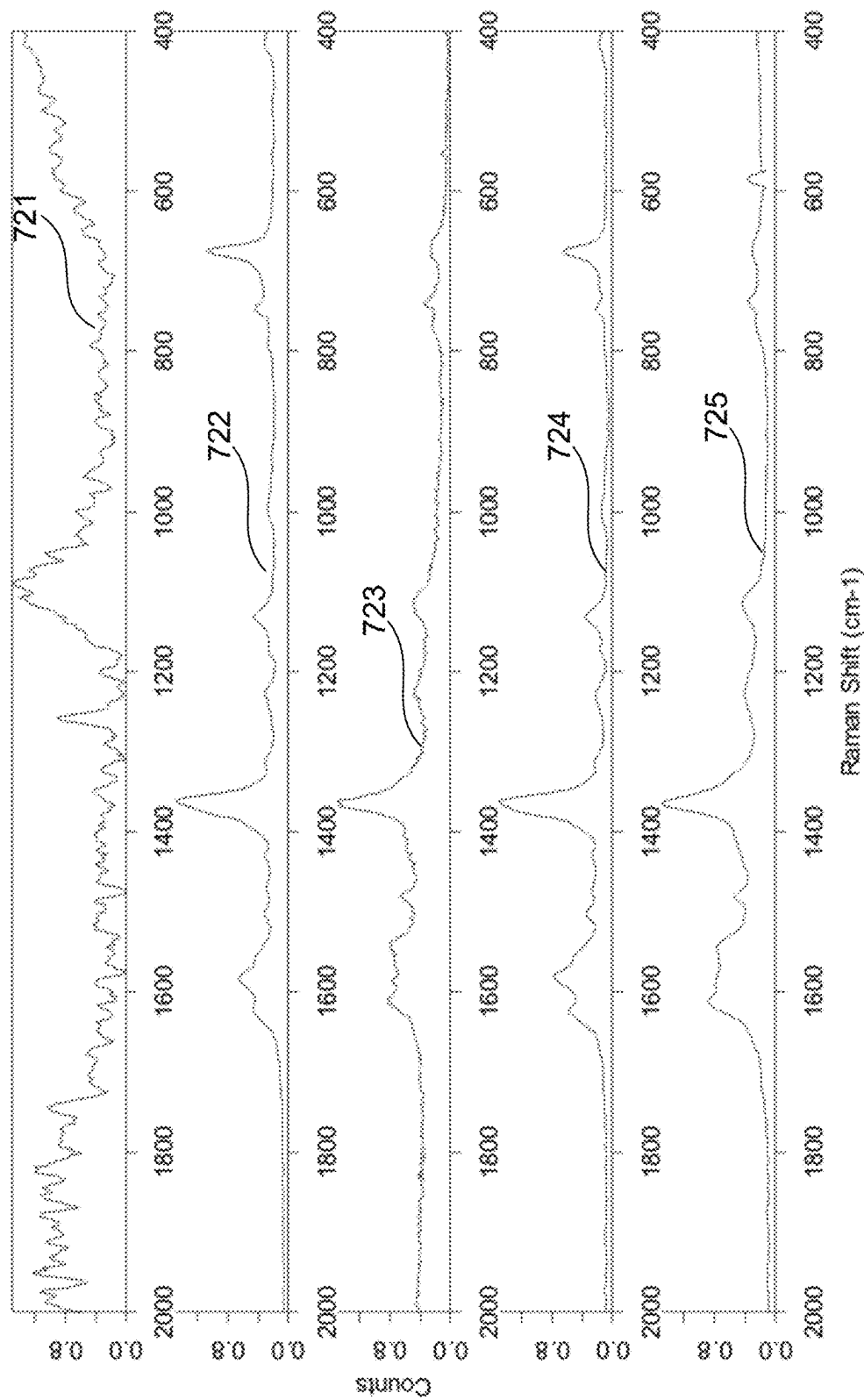
Fig. 7B

Fig. 7D

Fig. 7C

ATHEROSCLEROTIC PLAQUE DETECTION

Priority and Cross-Reference to Related Applications

This application is a Continuation Application of U.S. application Ser. No. 14/394,057, filed Oct. 10, 2014, which is a U.S. National Phase Application under 35 U.S.C. § 371 of International Application No. PCT/AU2013/000373, filed Apr. 12, 2013, designating the U.S., and published in English as WO 2013/152395 on Oct. 17, 2013, which claims the benefit of Australian Patent Application No. 2012901476, filed Apr. 13, 2012. The complete disclosures of the foregoing applications are hereby incorporated by reference.

BACKGROUND

The present invention relates to a method and apparatus for use in detecting atherosclerotic plaques using fluorescence spectroscopy, and in one example to detecting and/or quantifying the vulnerability of atherosclerotic plaques.

DESCRIPTION OF RELATED ART

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that the prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

Atherosclerosis is currently a leading cause of mortality and morbidity worldwide. Atherosclerotic plaques can affect different arteries at different sites of the body, potentially leading to a multitude of clinical presentations. These lesions can be totally asymptomatic for years, can present with stable symptoms like exertional angina, or can suddenly lead to life-threatening complications such as myocardial infarction or stroke.

Fatty streaks composed of foam cells (lipid-filled macrophages) are the earliest lesions in atherosclerosis. These fatty streaks are harmless and fully reversible. Only some of these progress to more advanced stages of atherosclerosis. More advanced plaques become irreversible and contain varying proportions of cells (including smooth muscle cells, macrophages and T-cells), extracellular matrix (including collagen, elastin and proteoglycans) and lipids (intracellular and extracellular). Different morphological types of plaques emerge depending on their composition. Some are fibrous plaques consisting of fibrous tissues and calcifications without lipid-rich core and some become fibroatheroma with lipid-rich core covered by a thin fibrous cap.

Atherosclerosis is widely regarded as an inflammatory disease. Inflammatory cells play a key role not only in the pathogenesis of plaque progression but also in destabilising the plaque (inflammatory cytokines and proteolytic enzymes cause the disruption of the fibrous cap leading to plaque rupture and complications).

The development of atherosclerotic plaques is quite diverse. Some plaques remain quiescent for years and some become complicated leading to plaque rupture or erosions. Complicated plaques are the cause of thrombosis and occlusion of the arteries leading to potentially catastrophic outcomes. Plaque rupture is the commonest cause of coronary thrombosis.

The plaques which are prone to rapid progression and thrombosis or rupture, commonly known as rupture prone, are called "high risk/vulnerable plaques". Thin-cap fibroatheroma (TCFA), which is the hallmark of vulnerable plaque is described to have a few classical morphological features, namely a thin fibrous cap of <65 µm, a large necrotic core, increased plaque inflammation, positive vascular remodelling, increased vasa-vasorum neovascularisation and intraplaque haemorrhage.

However, despite extensive advances in the field of cardiovascular medicine, the ability for early detection of the complication-prone plaques (high risk/vulnerable plaques) before they rupture, remains elusive.

Several invasive plaque imaging techniques are being developed to detect TCFA. These methods are far from ideal at the current state and could detect only some, not all, features of vulnerable plaques. For example, IVUS (intravascular ultrasound) is able to detect positive remodelling but with its resolution of 100 to 250 µm, it is impossible to detect fibrous cap thickness or plaque inflammation. IVUS-VH (Virtual Histology) uses IVUS backscatter radiofrequency signal to detect the composition of the plaque, especially necrotic core. Meanwhile, optical Coherence Tomography (OCT) with its high resolution (10-15 µm) has been proposed for detection of thin fibrous cap, macrophages and necrotic core but is not suitable for positive remodelling.

Other emerging methods include intravascular MRI (to detect the necrotic core), angioscopy (to visualise surface appearance of the plaque), thermography (to detect metabolic activity of the plaque) and spectroscopy.

Different types of spectroscopic imaging have been used for the characterization and detection of atherosclerotic plaques. Chemical composition of atherosclerosis has been successfully analysed by Raman spectroscopy and Fourier transform Infrared (FT-IR) spectroscopy in various ex-vivo human atherosclerotic plaques.

Infrared reflection spectroscopy has been shown to be able to differentiate the different plaque components based on their characteristic absorbance/reflectance properties both in-vivo and in-vitro. InfraReDx, Inc. (Burlington, Massachusetts, USA) developed an intracoronary probe using this technique which was later combined with IVUS (LipiScan IVUS system). The SPECTACL (SPECTroscopic Assessment of Coronary Lipid) trial was the first human multicenter study which demonstrated safety and feasibility of detection of lipid core-containing plaques by an intravascular NIRS (Near Infra-Red Spectroscopy) system in living patients.

UV (Ultra-Violet) fluorescence spectroscopy is used as another modality of spectroscopic assessment of atherosclerotic plaques. Some of the components of plaques such as collagen, elastin, some extracellular lipids and ceroids/lipofuscin have been shown to have intrinsic fluorescence when excited by ultraviolet light and visible light in the range of violet and blue (325 to 475 nm). However, UV fluorescence suffers from a number of drawbacks, including photon absorption and substantial tissue autofluorescence, making detection of plaques in this manner problematic.

WO2005/052558 describes methods and apparatus for classifying cancerous tissue using features of Raman spectra and background fluorescent spectra. The spectra may be acquired in the near-infrared wavelengths. Principal component analysis and linear discriminant analysis of reference spectra may be used to obtain a classification function that accepts features of the Raman and background fluorescence spectra for test tissue and yields an indication as to the

likelihood that the test tissue is abnormal. The methods and apparatus are applied to screening for skin cancers or other diseases.

SUMMARY

In a first broad form the present invention seeks to provide apparatus for use in detecting atherosclerotic plaques, the apparatus including an electronic processing device that:

a) determines a level of fluorescence sensed by a sensing device at a second infrared wavelength in response to exposure of at least part of an artery to radiation at a first infrared wavelength; and,

b) determines a fluorescence indicator using the level of fluorescence, the fluorescence indicator being indicative of the presence, absence or degree of an atherosclerotic plaque.

Typically the apparatus includes:

a) a radiation source for generating radiation to thereby expose at least part of the artery to radiation at a first infrared wavelength; and,

b) a sensing device for sensing radiation emitted from at least part of the artery at a second infrared wavelength.

Typically the radiation source includes a laser.

Typically the sensing device includes an infrared photodetector.

Typically the apparatus includes optics for focusing radiation.

Typically the apparatus includes a catheter including one or more optical fibres extending between proximal and distal ends, the distal end being for insertion into an artery, and the radiation source and sensing device being coupled to the proximal end.

Typically the apparatus includes a bandpass filter.

Typically the first wavelength is at least one of:

a) 650 nm±50 nm;

b) 700 nm±50 nm;

c) 750 nm±50 nm;

d) 800 nm±50 nm; and,

e) 850 nm±50 nm.

Typically the first wavelength is at least one of:

a) between 650 nm and 900 nm;

b) 685 nm; and

c) 785 nm.

Typically the second wavelength is different to the first wavelength.

Typically the second wavelength is at least one of:

a) 700 nm±50 nm;

b) 750 nm±50 nm;

c) 800 nm±50 nm;

d) 850 nm±50 nm;

e) 900 nm±50 nm;

f) 950 nm±50 nm;

g) 1000 nm±50 nm;

h) 1050 nm±50 nm; and,

i) 1100 nm±50 nm.

Typically the second wavelength is at least one of:

a) between 650 nm and 1144 nm;

b) between 800 nm and 820 nm;

c) 700 nm; and

d) 800 nm.

Typically the electronic processing device:

a) compares the level of fluorescence to a threshold; and,

b) determines the fluorescence indicator using the results of the comparison.

Typically the electronic processing device:

a) determines a first level of fluorescence sensed by the sensing device at the second infrared wavelength in response to exposure of a first part of the artery to radiation at the first infrared wavelength;

b) determines a second level of fluorescence sensed by the sensing device at the second infrared wavelength in response to exposure of a second part of the artery to radiation at the first infrared wavelength; and,

c) determines the fluorescence indicator using the first level and the second level.

Typically the electronic processing device:

a) compares the first level to the second level; and,

b) determines the fluorescence indicator using the results of the comparison.

Typically the first part is an at risk part, the at risk part being an area at risk of having an unstable plaque and the second part is a healthy part.

Typically the method includes determining if the first level of radiation is greater than the second level of radiation by a threshold factor of at least 2.

Typically the threshold factor is at least one of:

a) 3 or more;

b) 5 or more;

c) 10 or more; and,

d) 15 or more.

Typically the level of fluorescence is based on an intensity of radiation emitted by the at least one part.

Typically the apparatus is for detecting the vulnerability of atherosclerotic plaque, wherein the fluorescence indicator is indicative of the vulnerability of an atherosclerotic plaque.

Typically the representation includes at least one of:

a) a numerical value indicative of the fluorescence indicator;

b) a symbolic value indicative of the fluorescence indicator;

c) a graphical indicator indicative of the fluorescence indicator; and,

d) an indicator of at least one threshold.

Typically the electronic processing device:

a) determines a detected marker associated with a pathological condition by introducing an agent that detects the marker into the at least part of the artery; and,

b) determines the fluorescence indicator at least partially using the detected marker.

In a second broad form the present invention seeks to provide a method for use in detecting atherosclerotic plaques, the method including, in an electronic processing device:

a) determining a level of fluorescence sensed by a sensing device at a second infrared wavelength in response to exposure of at least part of an artery to radiation at a first infrared wavelength; and,

b) determining a fluorescence indicator using the level of fluorescence, the fluorescence indicator being indicative of the presence, absence or degree of atherosclerotic plaques.

In a third broad form the present invention seeks to provide a method for use in detecting vulnerable atherosclerotic plaques, the method including:

a) exposing at least part of an artery to radiation at a first infrared wavelength;

b) determining a level of fluorescence sensed by a sensing device from the at least part of the artery at a second infrared wavelength; and,

c) determining a fluorescence indicator using the level of fluorescence, the fluorescence indicator being indicative of the presence, absence or degree of atherosclerotic plaques.

Typically the method includes:

a) introducing an agent that detects a marker into the at least part of the artery, the marker being associated with a pathological condition;

b) determining the detected marker in the at least part of the artery; and,

c) determining the fluorescence indicator at least partially using the detected marker.

In a fourth broad form the present invention seeks to provide apparatus for use in analysing fluorescence measurements of atherosclerotic plaques, the apparatus including an electronic processing device that:

a) determines a level of fluorescence sensed by a sensing device at a second infrared wavelength, the radiation being sensed in response to exposure of an atherosclerotic plaque to radiation at a first infrared wavelength; and,

b) determines a fluorescence indicator using the level of fluorescence, the fluorescence indicator being indicative of a degree of fluorescence of the atherosclerotic plaque.

In a fifth broad form the present invention seeks to provide a method for use in analysing fluorescence measurements of atherosclerotic plaques, the method including, in an electronic processing device:

a) determining a level of fluorescence sensed by a sensing device at a second infrared wavelength, the radiation being sensed in response to exposure of an atherosclerotic plaque to radiation at a first infrared wavelength; and,

b) determining a fluorescence indicator using the level of fluorescence, the fluorescence indicator being indicative of a degree of fluorescence of the atherosclerotic plaque.

In a sixth broad form the present invention seeks to provide apparatus for use in detecting the vulnerability of atherosclerotic plaques, the apparatus including:

a) a radiation source for generating radiation to thereby expose at least part of the artery to radiation at a first infrared wavelength;

b) a sensing device for sensing radiation emitted from at least part of the artery at a second infrared wavelength;

c) an electronic processing device coupled to the sensing device that:

i) determines a level of fluorescence sensed by the sensing device; and,

ii) determines a fluorescence indicator using the level of fluorescence, the fluorescence indicator being indicative of the vulnerability of an atherosclerotic plaque.

Typically the apparatus includes a catheter including one or more optical fibres extending between proximal and distal ends, the distal end being for insertion into an artery, and the radiation source and sensing device being coupled to the proximal end.

BRIEF DESCRIPTION OF THE DRAWINGS

An example of the present invention will now be described with reference to the accompanying drawings, in which:

FIG. 1A is a schematic diagram of a first example of an apparatus for use in detecting atherosclerotic plaques;

FIG. 1B is a schematic diagram of a second example of an apparatus for use in detecting atherosclerotic plaques;

FIG. 2 is a flowchart of an example of a process for use in detecting atherosclerotic plaques;

FIG. 3 is a flowchart of a second example of a process for use in detecting atherosclerotic plaques;

FIG. 4A is an image of samples from mice models defined by histological examination;

FIG. 4B shows positive and negative images of NIR (Near Infra-Red) Fluorescence of the samples of FIG. 4A;

FIG. 5A is an image of an example of a freshly excised carotid endarterectomy specimen;

FIG. 5B shows positive and negative images of NIR Fluorescence of the specimen of FIG. 5A;

FIG. 5C shows positive and negative NIR Fluorescence images of a cryosectioned excised carotid endarterectomy specimen;

FIG. 5D shows positive and negative NIR Fluorescence images of a stable and vulnerable paraffin-embedded excised carotid endarterectomy specimen;

FIG. 6A shows example images of autofluorescence of areas of vulnerable plaques on excitation with 785 nm radiation (Raman spectrometer);

FIG. 6B shows example images of vulnerable plaques with H&E staining showing infiltration of inflammatory cells and some intraplaque haemorrhage (right);

FIG. 7A is a trace of an example of Raman signals from an area of fluorescence in a vulnerable plaque section in comparison with Raman signals from purified haeme, methaeme and protoporphrin IX compounds;

FIG. 7B is a trace of an example of Raman signals from stable plaque section without autofluorescence in comparison with Raman signals from purified haeme, methaeme and protoporphrin IX compounds;

FIG. 7C is an image of an example of trichrome staining of intraplaque haemorrhages in the plaque; and

FIG. 7D is an image of an example of autofluorescence of the intraplaque haemorrhages of FIG. 7C.

DETAILED DESCRIPTION

An example of an apparatus for use in detecting atherosclerotic plaques will now be described with reference to FIGS. 1A and 1B.

In this example, the apparatus 100 includes a processing system 110 including an electronic processing device, such as a processor 111, that determines a level of fluorescence sensed by a sensing device at a second infrared wavelength in response to exposure of at least part of an artery to radiation at a first infrared wavelength and determines a fluorescence indicator using the level of fluorescence, the fluorescence indicator being indicative of the presence, absence or degree of an atherosclerotic plaque.

Thus, the above described apparatus is capable of analysing a level of infrared fluorescence determined by a sensing device, and using this to detect a presence, absence or degree of an atherosclerotic plaque. In one particular example, this can be used to provide an indicator that can be used by a practitioner to ascertain the degree of vulnerability of a detected atherosclerotic plaque, hence allowing a medical practitioner to identify a suitable treatment regime.

Sensing of fluorescence is typically carried out in response to exposure of a suspected atherosclerotic plaque to infrared radiation of a first wavelength, with detection of fluorescence being performed at a second different infrared wavelength. This is performed to allow radiation used to expose the tissue to be distinguished from fluorescence, and

In use, the processor **111** operates in accordance with input commands and/or instructions in the form of applications software stored in the memory **112**, to allow signals from the sensing device **132** to be interpreted and optionally used, for example to detect or quantify the vulnerability of atherosclerotic plaques, as will be described in more detail below. Accordingly, for the purposes of the following description, it will be appreciated that actions performed by the processing system **110** are typically performed by the processor **111** under control of instructions stored in the memory **112**, and this will not therefore be described in further detail below.

It will be further appreciated that the processing system **110** may be formed from any suitably programmed processing system, such as a suitably programmed PC, Internet terminal, lap-top, hand-held PC, tablet PC, slate PC, iPad™, mobile phone, smart phone, PDA (Personal Data Assistant), or other processing device. Accordingly, the processor **111** can be any form of electronic processing device such as a microprocessor, microchip processor, logic gate configuration, firmware optionally associated with implementing logic such as an FPGA (Field Programmable Gate Array), or any other electronic device, system or arrangement capable of interacting with the sensing device **132** and optionally the radiation source **131**.

It will also be appreciated that whilst a single physical processing system is shown, multiple processing systems could be used, with for example some preliminary processing being performed in custom configured hardware, such as an FPGA, and with the generation and display of a fluorescence indicator being performed using a general purpose computer system.

Operation of the apparatus **100** for use in detecting atherosclerotic plaques will now be described with reference to FIG. **2**.

In this example, at step **200**, part of an artery is exposed to radiation at a first infrared wavelength, the radiation being generated by the radiation source **131**, and transmitted into the artery via the catheter **120**. At step **210**, the sensing device **132** operates to sense fluorescence radiation emitted from the part of the artery at a second infrared wavelength which is different to the first infrared wavelength, for example, the second infrared wavelength may be longer than the first infrared wavelength.

At step **220**, the processing system **110** uses signals acquired from the sensing device **132** to determine a level of fluorescence, which is then used to determine a fluorescence indicator at step **230**. The fluorescence indicator can be determined using any suitable technique, and may therefore be based on an absolute, relative or normalised intensity of fluorescence. Alternatively, the fluorescence indicator can be determined at least in part based on a comparison of the detected fluorescence to a threshold, such as one or more reference values, as will be described in more detail below.

At step **240**, a representation of the fluorescence indicator may optionally be displayed allowing this to be used by a medical practitioner, for example in diagnosing the presence, absence, degree or vulnerability of atherosclerotic plaque.

The nature of the fluorescence indicator and, in particular the manner in which it is displayed to an operator, will vary depending upon the preferred implementation. In one example, the fluorescence indicator is in the form of a numerical value, indicative of the absolute level of fluorescence. Alternatively however, a symbolic value, such as including letters or other symbols, or a graphical indicator can be displayed which is representative of the numerical

value. Additionally, an indication of a threshold and optionally the result of a comparison thereto can be displayed allowing this to be used by a medical practitioner in order to assist in diagnosing the presence, absence or degree of vulnerability of an atherosclerotic plaque.

It will be appreciated that whilst the above described method and apparatus arrangements are described for use in-vivo, alternatively the apparatus and method could be performed in-vitro, on samples excised from a subject. In this example, the catheter is not required and the processing system **110**, radiation source **131**, and sensing device **132** can form part of an infrared imaging system, which can be used to acquire fluorescence information from section samples, as will be described in more detail below.

In any event, it has been discovered that if atherosclerotic plaques are exposed to infrared radiation, they can undergo auto-fluorescence, depending on the status, and in particular the stability or vulnerability of the plaque. In this regard, if plaques are stable, the level of auto-fluorescence is minimal. However, if plaques are unstable, commonly referred to as "vulnerable", then the degree of auto-fluorescence is significantly increased. As a result, by determining a fluorescence indicator, this can be indicative of the presence, absence or degree of an atherosclerotic plaque, for example, indicative of the vulnerability of the plaque, such as being at high risk of rupture or rupture prone.

Accordingly, the above described method and apparatus can be used to allow a fluorescence indicator to be derived based on the fluorescence of at least part of an artery, in response to exposure by infrared radiation. The part of the artery is typically a part including a lesion, and can be analysed in situ, or can be excised from the subject and analysed remotely. The level of fluorescence can be used to derive a fluorescence indicator, which can in turn be indicative of the presence, absence or degree of stability of an atherosclerotic plaque.

Accordingly, this provides a straightforward method to allow medical practitioners to identify, and subsequently classify atherosclerotic plaques, in turn allowing the medical practitioner to assess what if any treatment may be required. Furthermore, this process relies on autofluorescence of the plaque, avoiding the need for the introduction of external markers, such as fluorochromes targeting some specific molecules (such as proteases) in the plaques.

A number of further features will now be described.

In one example, the first wavelength is typically between 650 nm and 900 nm. In one particular example, the first wavelength is either 685 nm or 785 nm, although it will be appreciated that other wavelengths may be used. Similarly, the second wavelength is typically in the region of 650 nm to 1144 nm and particularly is typically either 700 nm or 800 nm, or in the range 800 nm to 820 nm. The wavelengths are selected to maximise the fluorescent response of the plaques, as will be described in more detail below.

In some embodiments, the first wavelength is in a range selected from: 650 nm-850 nm, 700 nm-850 nm, 750 nm-850 nm, 800 nm-850 nm, 650 nm-800 nm, 700 nm-800 nm, 750 nm-800 nm, 650 nm-700 nm, 650 nm-750 nm, 650 nm-800 nm, or 700 nm-750 nm.

In some embodiments, the second wavelength is in a range selected from: 700 nm-1150 nm, 750 nm-1150 nm, 800 nm-1150 nm, 850 nm-1150 nm, 900 nm-1150 nm, 950 nm-1150 nm, 1000 nm-1150 nm, 1050 nm-1150 nm, 1100 nm-1150 nm, 700 nm-1100 nm, 750 nm-1100 nm, 800 nm-1100 nm, 850 nm-1100 nm, 900 nm-1100 nm, 950 nm-1100 nm, 1000 nm-1100 nm, 1050 nm-1100 nm, 700 nm-1050 nm, 750 nm-1050 nm, 800 nm-1050 nm, 850

nm-1050 nm, 900 nm-1050 nm, 950 nm-1050 nm, 1000 nm-1050 nm, 700 nm-1000 nm, 750 nm-1000 nm, 800 nm-1000 nm, 850 nm-1000 nm, 900 nm-1000 nm, 950 nm-1000 nm, 700 nm-950 nm, 750 nm-950 nm, 800 nm-950 nm, 850 nm-950 nm, 900 nm-950 nm, 700 nm-900 nm, 750 nm-900 nm, 800 nm-900 nm, 850 nm-900 nm, 700 nm-850 nm, 750 nm-850 nm, 800 nm-850 nm, 700 nm-800 nm, 750 nm-800 nm or 700 nm-750 nm.

It will be understood that the term "between" when used in reference to a range of numerical values encompasses the numerical values at each endpoint of the range. For example, a wavelength of between 650 nm and 900 nm is inclusive of a wavelength of 650 nm and a wavelength of 900 nm.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit, is also encompassed, unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range and is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention.

Each embodiment described herein is to be applied mutatis mutandis to each and every embodiment unless specifically stated otherwise.

As mentioned above, the catheter may include optical elements in order to allow suitable focusing of radiation within the artery and detection therefrom. Additionally, the apparatus may include bandpass filters for filtering radiation emitted from the radiation source or to be received by the sensing device to thereby limit unwanted background signals.

In order to determine the fluorescence indicator, the processing system 110 may perform a partial assessment of the level of fluorescence, for example by comparing this to a threshold and determining whether the level of fluorescence exceeds or falls below the threshold. This can be used to indicate a state or, in particular, a degree of vulnerability of an atherosclerotic plaque.

Additionally, and as will be described in more detail below, the processing system 110 may operate to compare the level of fluorescence detected at a healthy site to the level of fluorescence detected at a site where there is a suspected atherosclerotic plaque, which can in turn be used to confirm whether an atherosclerotic plaque is present, and optionally the degree of vulnerability.

A second example of the process for use in detecting atherosclerotic plaques will now be described with reference to FIG. 3. During this process, it is assumed that the processing system 110 is executing applications software for controlling the radiation source 131 and for analysing signals from the sensing device 132.

In this example, at step 300, the catheter is introduced into the artery of a subject, with the distil end 121 of the catheter 120 being positioned adjacent a part of the artery to be measured. Typically, this is a part of the artery which is thought to be at risk of having a vulnerable plaque.

At step 305, a first part of the artery is exposed to radiation at the first wavelength. Typically this is achieved by having an operator provide an input command to the processing system 110, confirming that the catheter is positioned as required, allowing the processing system 110 to activate the radiation source 131. At step 310, the processing system 110 acquires signals from the sensing device 132 that are indica-

tive of the level, and in particular the intensity, of fluorescence in the first part of the artery.

At step 315, the processing system 110 determines if fluorescence in a second part of the artery is to be determined. This can be achieved depending on a selected measurement protocol, or in accordance with an input command from an operator. This is typically performed to allow a comparison to be performed between readings at different locations within the artery, for example to assist in establishing if a fluorescence level is indicative of a vulnerable plaque or to take into account background levels of fluorescence in healthy tissue.

In this regard, the fluorescence in the second part of the artery may be determined at any suitable part of the artery, or alternatively other blood vessels, or a phantom vessel that includes fluorescence properties analogous to healthy tissue, and stable and/or unstable plaques. In one example, a location of the second part of the artery, or other vessel, which includes substantially healthy tissue or a stable plaque, may be determined based upon a location indicator, such as an image. In this respect, the method includes determining a location indicator, and determining fluorescence in the second part of the artery using at least partially the location indicator. In this respect, the image may be acquired according to any suitable internal or external imaging modality, such as angiography, x-ray, video imaging, OCT, computed tomography (CT), magnetic resonance imaging (MRI), near infrared spectroscopy (NIR), Raman spectroscopy, UV fluorescence, or the like.

In a further example, the second part of the artery may correspond to a location in the artery, or other vessel, which typically includes a low or lower probability of exhibiting vulnerable plaques, such as, based on a sample population, a scientific inference, or the like. For example, in a population with heart disease, typically a few unstable plaques develop in at least some of the arteries in the arm.

Additionally, the second level of fluorescence may be determined based on a plurality of measurements that are indicative of the level of fluorescence, where the measurements are acquired as discussed in any one of the above examples. In this respect, the plurality of measurements may correspond to a plurality of parts of the artery or another vessel, and hence the second level of fluorescence may be based on an average, median, mode, or other suitable indicator indicative of the plurality of measurements. In one example, the plurality of measurements are determined at a plurality of parts of the femoral artery, however this is not essential, and the plurality of measurements may be determined in different parts of any suitable vessel/s, or alternatively at different times in the same part of the artery or other vessel.

Alternatively, the intensity of the fluorescence may be compared to a threshold, for example the threshold may be indicative of the intensity of the fluorescence of healthy tissue or a stable plaque, and this is discussed in more detail below.

It will be appreciated that these steps, for example determining a location indicator, determining an average of a plurality of measurements, and the like, may not be limited to determining the second level of fluorescence and in this respect may also be applied in any of the above examples, such as determining the first level of fluorescence. For example, a location of the first part of the artery to be tested may be determined using a location indicator.

At step 320, if no other part is to be examined, the intensity of fluorescence can be compared to a threshold. The threshold may be established based on measurements

from reference samples of vulnerable and stable atherosclerotic plaques, identified by histological examination, previous in vivo measurements from a sample population, previous measurements on the first part of the artery, previous measurements from other blood vessels of a same or similar subject, or any other suitable technique.

This allows a threshold to be established which is indicative of whether an atherosclerotic plaque is vulnerable. It will be appreciated that multiple thresholds could also be established to allow a degree of vulnerability to be established. Alternatively, the intensity of fluorescence can be analysed using any other suitable approach.

At step 325, a representation of a fluorescence indicator is displayed, for example using the I/O device 113. The fluorescence indicator can be a numerical or graphical representation of the intensity of fluorescence and/or the result of the comparison to a threshold. An indication of the threshold may also be displayed, allowing the medical practitioner viewing the results to make an easy assessment of whether a vulnerable atherosclerotic plaque exists. A visual indication of an image of the fluorescence may also be displayed, assuming the sensing device is capable of capturing an image.

In the event that a second part of the artery is to be examined, the processing system 110 can generate an instruction to the medical practitioner operating the catheter causing them to reposition the catheter at step 330. The practitioner can confirm once this has been completed, by providing an appropriate input command, allowing the processing system 110 to activate the radiation source 131 and expose the second part of the artery to radiation at step 335. At step 340, the processing system 110 acquires signals from the sensing device 132 that are indicative of a second level, and in particular intensity, of fluorescence in the second part of the artery.

At step 345, the processing system determines a difference in the first and second levels of fluorescence, for example by subtracting one value from the other, by determining a ratio between the first and second levels of fluorescence, or the like. This allows the impact of fluorescence in a healthy part of the artery, representing a background fluorescence, to be taken into account. In this regard, the term healthy part is taken to include a part of the artery without any plaque and/or parts of the artery including stable and/or non-vulnerable plaques. At step 350 the difference in intensity can be compared to a threshold, which again may be established based on measurements from reference samples, as will be described in more detail below.

At step 325, the fluorescence indicator can be displayed substantially as described above, allowing the fluorescence indicator to be interpreted by the medical practitioner, and in particular to allow the medical practitioner to assess the degree of vulnerability of any atherosclerotic plaque, and hence assess whether any intervention is required.

It will be appreciated that a number of variations of the above described process may be implemented. For example, the or each part of the artery can be exposed to radiation at a number of different first wavelengths, with the fluorescence being measured at a corresponding number of different second wavelengths. This can be used to help further classify the nature of any atherosclerotic plaque. Furthermore, multiple different thresholds can be used to allow the vulnerability of the plaques to be accurately identified.

The method may further include, for example, detecting a marker associated with a pathological condition and using the detected marker, for instance as a guide, to determine the fluorescence indicator in the at least part of the artery.

Suitably, the marker is detected by introducing an agent that detects the marker, into the at least part of the artery.

In this respect, the detected marker may provide a location indicator for, or guide to the position of, healthy tissue, one or more stable plaques, and/or one or more unstable plaques. Additionally or alternatively, the detected marker may be used with the level of fluorescence to determine the fluorescence indicator, such as by weighting the level of fluorescence in accordance with a numerical value indicative of the detected marker.

In one example, the pathological condition is associated with a presence or risk of an unstable plaque, and in this regard the marker may include any one or more of a cell, cell debris, tissue damage, cellular components, chemicals, or the like. In this respect, the pathological condition may include activated platelets, the marker may include a glycoprotein IIb/IIIa receptor and the agent may include an anti-LIBS (ligand induced binding site) antibody or microparticles of iron oxide (MIPO), for example for targeting LIBSs of the glycoprotein IIb/IIIa receptor. In a further example, the pathological condition may include inflamed endothelial tissue, and in this respect the agent may include an anti-interleukin-1 antibody and the marker may include interleukin-1. However, this is not essential, and optionally the marker may be associated with stable plaques, or healthy tissue, or alternatively no agent may be used.

In addition, determining the detected marker may be achieved in any suitable manner, for example an image of the detected marker may be acquired using an imaging modality corresponding to the properties of the detected marker. In this respect the imaging modality may include fluorescence, x-ray, computed tomography (CT), gamma-scintigraphy, positron emission tomography (PET), single photon emission computed tomography (SPECT), MRI, and combined imaging techniques.

Experimental evidence of the effectiveness of the above described auto-fluorescence of vulnerable plaques will now be described.

For the purpose of a first experimental example, animal data is derived using a "tandem stenosis model" of atherosclerosis in mice, which closely resembles the atherosclerosis process in humans.

A surgical ligation procedure (serial ligation of segments of the carotid arteries) was performed to create a tandem stenosis to the carotid artery of ApoE$^{-/-}$ mice fed with high fat diet. After 7 and 11 weeks postoperatively, disruption of fibrous caps, intraplaque haemorrhage, intraluminal thrombosis, neovascularisation and other characteristics of plaque instability/rupture in humans were seen in these mice.

The fresh carotid artery specimens from these mice, shown in FIG. 4A, were examined for autofluorescence under an Odyssey Infrared Imaging System which has 2 excitation channels 685 nm (detection on 700 nm) and 785 nm (detection 800 nm). Vulnerable plaques were identified by histological examination.

The results of fluorescence imaging are shown in FIG. 4B, which shows minimal autofluorescence in stable atherosclerotic plaque in aortic arch and carotid arteries 401, no autofluorescence in healthy carotid artery 402, significant autofluorescence in an unstable carotid plaque with intraplaque haemorrhage 403 and significant autofluorescence in a vulnerable carotid plaque with positive remodelling 404. Thus, the vulnerable atherosclerotic plaques 403, 404 show significant autofluorescence in both channels, in contrast to the healthy vessels and stable plaques 401, 402, which showed no intrinsic fluorescence.

For the purpose of a second experimental example, human data is derived from patients undergoing carotid endarterectomy at the Alfred Hospital (Melbourne, Australia). The project was approved by the Alfred Ethics Committee. Carotid endarterectomy specimens were collected immediately after being excised from the patients.

Freshly excised carotid endarterectomy specimens, an example of which is shown in FIG. **5A**, were placed on the glass slides and examined for autofluorescence under an Odyssey Infrared Imaging System as described above. As shown in FIG. **5B**, the areas of the vulnerable atherosclerotic plaques **501** showed significant autofluorescence in both channels in contrast to the areas of normal intima and stable plaques, which showed no intrinsic fluorescence.

The fresh carotid endarterectomy specimens were then snap-frozen and kept at −80° C. Parts of the snap-frozen specimens were then embedded in an optimal cutting temperature compound for cry sectioning. 5 μm tissue sections were performed and examined for autofluorescence under Odyssey Infrared Imaging System. As shown in FIG. **5C**, only areas of vulnerable plaques **511** but not stable and normal areas **512** were found to have significant autofluorescence in both channels of excitation and emission as above.

Parts of the snap-frozen specimens were treated with 10% formalin and then embedded in paraffin. 5 μm tissue sections were examined for autofluorescence as shown in FIG. **5D**, with autofluorescence of stable plaque **521** compared to vulnerable plaque **522**.

For the purpose of a third experimental example, shown in FIGS. **6A** and **6B**, analysis of atherosclerotic plaques was performed using an AFM Raman Spectrometer. This experiment used an NT-MDT Integra AFM Raman System with two Raman laser sources (532 nm and 785 nm excitation) to confirm the autofluorescence of these vulnerable plaques.

Paraffin embedded sections were excited with a 785 nm laser source and the emission was studied throughout the whole detection range (from 800 nm to 1144 nm). The strongest signal of emission was detected between 800-820 nm on AFM Raman system.

In a fourth experimental example, an Odyssey Infrared Imaging System was used to quantify the fluorescence detected from healthy tissue and unstable plaques. In this example, samples used were fresh macroscopic carotid endarterectomy tissues, which were scanned immediately after being taken out from the patient. The imager was used to scan the tissues with 21 μm resolution and at an intensity setting of "4" using both 700 nm and 800 nm excitation and detection channels. The images taken were then normalised with gamma=1.

An example of the measured intensities for healthy tissue and unstable plaques are shown in Table 1 below.

TABLE 1

Healthy sample #	Autofluorescence intensity units	Unstable sample #	Autofluorescence intensity units	Fold change
1	10	1	260	26
2	4	2	64	16
3	1	3	10	10
4	2	4	42	21
5	28	5	203	7.25
6	5	6	100	20
7	10	7	198	19.8
8	30	8	120	4
9	2	9	34	17
10	10	10	33	3.3
11	5	11	16	3.2

TABLE 1-continued

Healthy sample #	Autofluorescence intensity units	Unstable sample #	Autofluorescence intensity units	Fold change
12	10	12	64	6.4
13	4	13	15	3.75
14	6	14	32	5.3
15	11	15	59	5.36
16	5	16	15	3
17	5	17	17	3.4
18	7	18	103	14.71
19	8	19	29	3.62
20	9	20	41	4.56
Mean +/− SD	8.6 +/− 7.6	Mean +/− SD	72.75 +/− 71.6	9.88 +/− 7.5

Accordingly, the above study of **20** human plaques demonstrates a 9.88+/−7.5 (mean ±SD) fold difference in intensity between healthy tissue and unstable plaques.

It will be appreciated from this, that by comparing the intensity of fluorescence measured for a plaque of unknown status (unknown plaque) to a healthy reference, this allows the stability of the unknown plaque to be assessed. In particular, if the measured level of fluorescence for the unknown plaque is greater than the level of fluorescence from an artery with no plaque or a stable plaque, by a threshold factor generally of at least 2, and typically of at least 3, at least 5, at least 10 or at least 15, then this can be used to determine if the unknown plaque is unstable, and if so the degree of stability.

It will be appreciated that the value of the factor of increase in fluorescence intensity for a healthy sample, or an unstable plaque, can be further refined based on additional sample data, and that by collecting data from reference samples having different degrees of stability, this will allow thresholds for different degrees of stability to be identified.

Whilst not wishing to be bound by any particular theory or mode of action, it has been discovered following experimentation that autofluorescence in unstable plaques may be at least partially caused by a molecular species including haeme-degradation products, for example, haeme, meth-aeme, and protoporphyrin IX. In view of this, the experimental evidence may suggest an at least partial correlation between haeme-degradation products and autofluorescence of unstable plaques, and this will now be described.

Renishaw Invia confocal micro-Raman system using Raman laser source of Innova Ar/Kr ion laser 514/413 nm was used to assess Raman signals from the areas of auto-fluorescence in the vulnerable plaque sections. The Raman signals reveal that the source of autofluorescence is haeme-degradation products, which are abundant in the areas of intraplaque haemorrhage (microscopic or macroscopic). Intraplaque haemorrhages are well established as the triggers for plaque vulnerability (Jean-Baptise Michel et al. (2011) *Eur Heart J.* 32 (16): 1977-85). FIG. **7A** is an example of Raman signals from the area of fluorescence **711** in the vulnerable plaque section in comparison with Raman signals from purified haeme **714**, met-haeme **712** and two examples of Raman signals of the same sample of proto-porphrin IX compounds **713**, **715**. FIG. **7B** is an example of Raman signals from a stable plaque section without auto-fluorescence **721** in comparison with Raman signals from purified haeme **724**, met-haeme **722** and two examples of Raman signals of the same sample of protoporphrin IX compounds **723**, **725**. FIG. **7C** shows an example of a trichrome staining image of an "intraplaque haemorrhages in

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the plaque", and FIG. 7D shows an autofluorescence image of the "intraplaque haemorrhages in the plaque" of the example in FIG. 7C.

It will be appreciated that given the above, it would be considered routine for a person skilled in the art to further characterise the optimal exposure and sensing wavelengths, or first and second wavelengths, of these molecular species.

Accordingly, the above-described method uses an auto-fluorescence property of vulnerable atherosclerotic plaques in NIR range without the use of any external fluorochromes. NIR spectroscopy has a number of advantages over other detection techniques allowing for the detection of vulnerable atherosclerotic plaques in the arteries such as coronary arteries and carotid arteries.

Throughout this specification and claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers or steps but not the exclusion of any other integer or group of integers.

Persons skilled in the art will appreciate that numerous variations and modifications will become apparent. All such variations and modifications which become apparent to persons skilled in the art, should be considered to fall within the spirit and scope that the invention broadly appearing before described.

What is claimed is:

1. An apparatus for detecting atherosclerotic plaques, the apparatus comprising:
   a) a radiation source for generating radiation to thereby expose at least part of an artery to radiation at a first infrared wavelength, wherein the first infrared wave-length is in a range from 600 nm to 900 nm;
   b) a sensor configured to sense radiation emitted from at least part of the artery at a second infrared wavelength;
   c) an electronic processor configured to:
      i) determine a level of autofluorescence sensed by the sensor at the second infrared wavelength in response to exposure of at least part of the artery to radiation at the first infrared wavelength; and
      ii) determine a fluorescence indicator using the level of autofluorescence, the fluorescence indicator being indicative of a presence, absence, degree or vulner-ability of an atherosclerotic plaque.

2. The apparatus according to claim 1, wherein:
   a) the radiation source comprises a laser;
   b) the sensor comprises an infrared photodetector; and/or
   c) the apparatus comprises optics for focusing radiation.

3. The apparatus according to claim 1, wherein the apparatus comprises a catheter comprising one or more optical fibers extending between proximal and distal ends, the distal end being for insertion into the artery, and the radiation source and sensor being coupled to the proximal end.

4. The apparatus according to claim 1, wherein the apparatus comprises a bandpass filter.

5. The apparatus according to claim 1, wherein the first infrared wavelength is selected from the group consisting of:
   a) 650 nm±50 nm;
   b) 700 nm±50 nm;
   c) 750 nm±50 nm;
   d) 800 nm±50 nm;
   e) 850 nm±50 nm;
   f) 650 nm–850 nm;
   g) 700 nm–850 nm;
   h) 750 nm–850 nm;
   i) 800 nm–850 nm;

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   j) 650 nm–800 nm;
   k) 700 nm–800 nm;
   l) 750 nm–800 nm;
   m) 650 nm–700 nm;
   n) 650 nm–750 nm;
   o) 650 nm–800 nm;
   p) 700 nm–750 nm;
   q) 685 nm; and
   r) 785 nm.

6. The apparatus according to claim 1, wherein the second infrared wavelength is selected from the group consisting of:
   a) different to the first infrared wavelength; and
   b) longer than the first infrared wavelength.

7. The apparatus according to claim 1, wherein the second infrared wavelength is selected from the group consisting of:
   a) 700 nm±50 nm;
   b) 750 nm±50 nm;
   c) 800 nm±50 nm;
   d) 850 nm±50 nm;
   e) 900 nm±50 nm;
   f) 950 nm±50 nm;
   g) 1000 nm±50 nm;
   h) 1050 nm±50 nm;
   i) 1100 nm±50 nm;
   j) 700 nm–1150 nm;
   k) 750 nm–1150 nm;
   l) 800 nm–1150 nm;
   m) 850 nm–1150 nm;
   n) 900 nm–1150 nm;
   o) 950 nm–1150 nm;
   p) 1000 nm–1150 nm;
   q) 1050 nm–1150 nm;
   r) 1100 nm–1150 nm;
   s) 700 nm–1100 nm;
   t) 750 nm–1100 nm;
   u) 800 nm–1100 nm;
   v) 850 nm–1100 nm;
   w) 900 nm–1100 nm;
   x) 950 nm–1100 nm;
   y) 1000 nm–1100 nm;
   z) 1050 nm–1100 nm;
   aa) 700 nm–1050 nm;
   bb) 750 nm–150 nm;
   cc) 800 nm–1050 nm;
   dd) 850 nm–150 nm;
   ee) 900 nm–150 nm;
   ff) 950 nm–150 nm;
   gg) 1000 nm–150 nm;
   hh) 700 nm–1000 nm;
   ii) 750 nm–1000 nm;
   jj) 800 nm–1000 nm;
   kk) 850 nm–1000 nm;
   11) 900 nm–1000 nm;
   mm) 950 nm–1000 nm;
   nn) 700 nm–950 nm;
   oo) 750 nm–950 nm;
   pp) 800 nm–950 nm;
   qq) 850 nm–950 nm;
   rr) 900 nm–950 nm;
   ss) 700 nm–900 nm;
   tt) 750 nm–900 nm;
   uu) 800 nm–900 nm;
   vv) 850 nm–900 nm;
   ww) 700 nm–850 nm;
   xx) 750 nm–850 nm;
   yy) 800 nm–850 nm;
   zz) 700 nm–800 nm;

aaa) 750 nm–800 nm;

bbb) 700 nm–750 nm;

ccc) between 650 nm and 1144 nm;

ddd) between 800 nm and 820 nm;

eee) 700 nm; and

fff) 800 nm.

8. The apparatus according to claim 1, wherein the electronic processor is configured to:

a) compare the level of autofluorescence to a threshold; and

b) determine the fluorescence indicator using the results of the comparison.

9. The apparatus according to claim 1, wherein the electronic processor is configured to:

a) determine an at risk level of autofluorescence sensed by the sensor at the second infrared wavelength in response to exposure of an at risk part of the artery to radiation at the first infrared wavelength, the at risk part being an area at risk of having an unstable plaque;

b) determine a healthy level of autofluorescence sensed by the sensor at the second infrared wavelength in response to exposure of a healthy part of the artery to radiation at the first infrared wavelength; and

c) determine the fluorescence indicator using the at risk level and the healthy level.

10. The apparatus according to claim 9, wherein the electronic processor is configured to:

a) compare the at risk level to the healthy level; and

b) determine the fluorescence indicator using the results of the comparison.

11. The apparatus according to claim 9, comprising determining if the at risk level of autofluorescence is greater than the healthy level of autofluorescence by a threshold factor of:

a) at least 2;

b) 3 or more;

c) 5 or more;

d) 10 or more; and/or

e) 15 or more.

12. The apparatus according to claim 1, wherein the level of autofluorescence is based on an intensity of radiation emitted by the at least one part.

13. The apparatus according to claim 1, wherein the apparatus is for detecting the vulnerability of atherosclerotic plaque, and wherein the fluorescence indicator is indicative of the vulnerability of an atherosclerotic plaque.

14. The apparatus according to claim 1, wherein the apparatus is configured to provide representation comprising:

a) a numerical value indicative of the fluorescence indicator;

b) a symbolic value indicative of the fluorescence indicator;

c) a graphical indicator indicative of the fluorescence indicator; and/or

d) an indicator of at least one threshold.

15. The apparatus according to claim 1, wherein the electronic processor is configured to:

a) determine a detected marker associated with a pathological condition by introducing an agent that detects the marker into the at least part of the artery; and

b) determine the fluorescence indicator at least partially using the at least one detected marker.

16. An apparatus for detecting a vulnerability of atherosclerotic plaques, the apparatus comprising:

a) a radiation source for generating radiation to thereby expose at least part of an artery to radiation at a first infrared wavelength, wherein the first infrared wavelength is in a range from 600 nm to 900 nm;

b) a sensor for sensing radiation emitted from at least part of the artery at a second infrared wavelength;

c) an electronic processor coupled to the sensor configured to:

i) determine a level of autofluorescence sensed by the sensor; and

ii) determine a fluorescence indicator using the level of autofluorescence, the fluorescence indicator being indicative of the vulnerability of an atherosclerotic plaque.

17. The apparatus according to claim 16, wherein the apparatus comprises a catheter comprising one or more optical fibers extending between proximal and distal ends, the distal end being for insertion into the artery, and the radiation source and sensor being coupled to the proximal end.

18. The apparatus according to claim 1, wherein the fluorescence indicator is indicative of one or more of:

a) intraplaque hemorrhage;

b) intraluminal thrombosis;

c) neovascularization;

d) one or more characteristics of plaque instability/rupture;

e) a high risk of plaque rupture;

f) a rupture prone plaque;

g) a presence of heme-degradation products;

h) a presence of heme;

i) a presence of metheme; or

j) a presence of protoporphyrin IX.

19. The apparatus according to claim 1, wherein the apparatus is configured to:

a) determine levels of autofluorescence sensed by the sensor at one or more second infrared wavelengths in response to exposure of at least part of the artery to radiation at a plurality of first infrared wavelengths; and,

b) determine a fluorescence indicator using the levels of autofluorescence.

20. A method for detecting atherosclerotic plaques, the method comprising:

a) providing

i) a radiation source for generating radiation to thereby expose at least part of an artery to radiation at a first infrared wavelength, wherein the first infrared wavelength is in a range from 600 nm to 900 nm,

ii) a sensor for sensing radiation emitted from at least part of the artery at a second infrared wavelength, and

iii) an electronic processor

b) exposing at least part of the artery to radiation at the first infrared wavelength with the radiation source;

c) determining, with the electronic processor, a level of autofluorescence sensed by the sensor at the second infrared wavelength in response to exposure of at least part of the artery to radiation at the first infrared wavelength; and

d) determining, with the electronic processor, a fluorescence indicator using the level of autofluorescence, the fluorescence indicator being indicative of a presence, absence, degree or vulnerability of atherosclerotic plaques.

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