



(43) International Publication Date  
27 September 2012 (27.09.2012)

WIPO | PCT

(10) International Publication Number  
**WO 2012/127378 A1**

(51) International Patent Classification:

G01N 21/47 (2006.01) A61B 5/00 (2006.01)  
G01N 21/64 (2006.01)

(21) International Application Number:

PCT/IB2012/051247

(22) International Filing Date:

15 March 2012 (15.03.2012)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

11159259.8 22 March 2011 (22.03.2011) EP

(71) Applicant (for all designated States except US):

KONINKLIJKE PHILIPS ELECTRONICS N.V.  
[NL/NL]; Groenewoudseweg 1, 5621 BA Eindhoven (NL).

(72) Inventors; and

(75) Inventors/Applicants (for US only): NACHABE, Rami

[LB/NL]; c/o High Tech Campus Building 44, 5656 AE Eindhoven (NL). HENDRIKS, Bernardus, Hendrikus, Wilhelmus [NL/NL]; c/o High Tech Campus Building 44, 5656 AE Eindhoven (NL). LUCASSEN, Gerhardus, Wilhelmus [NL/NL]; c/o High Tech Campus Building 44, 5656 AE Eindhoven (NL). VAN DER VOORT, Marjolein [NL/NL]; c/o High Tech Campus Building 44, 5656 AE Eindhoven (NL). HORIKX, Jeroen, Jan, Lambertus [NL/NL]; c/o High Tech Campus Building 44, 5656 AE

Eindhoven (NL). MÜLLER, Manfred [DE/NL]; c/o High Tech Campus Building 44, 5656 AE Eindhoven (NL). BIERHOFF, Waltherus, Cornelis, Jozef [NL/NL]; c/o High Tech Campus Building 44, 5656 AE Eindhoven (NL). DESJARDINS, Adrien, Emmanuel [CA/CA]; c/o High Tech Campus Building 44, 5656 AE Eindhoven (NL). RUERS, Theodoor, Jacques, Marie [NL/NL]; c/o High Tech Campus Building 44, 5656 AE Eindhoven (NL).

(74) Agents: VAN VELZEN, Maaïke, M. et al.; High Tech Campus, Building 44, 5656 AE Eindhoven (NL).

(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE,

[Continued on next page]

(54) Title: AN APPARATUS FOR OPTICAL ANALYSIS OF AN ASSOCIATED TISSUE SAMPLE

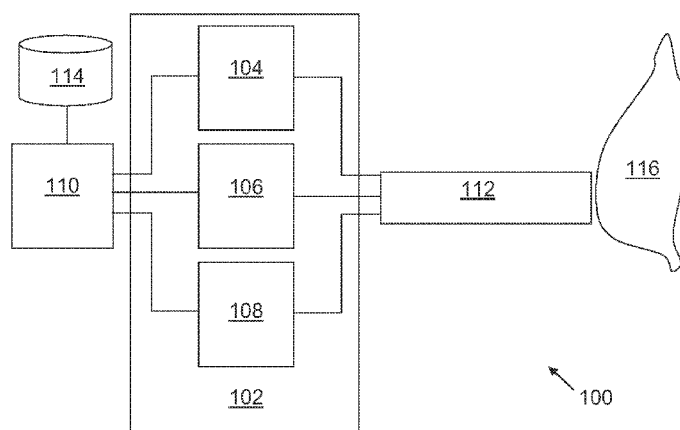


FIG. 1

(57) Abstract: The present invention relates to an apparatus (100) and, a method and a computer program for optical analysis of an associated tissue sample (116), the apparatus being adapted to determine a concentration of collagen and/or elastin with two independent methods. With two independent methods, the concentrations determined with each method may be held up against the other, and if the difference is too large a user may be informed that at least one of the measured values is not reliable. In a particular embodiment, the two independent methods are Diffuse Reflectance Spectroscopy (DRS) and fluorescence spectroscopy, and the absorption and scattering determined with DRS may be used when interpreting the fluorescence spectroscopy spectra so as to be able to obtain quantitative information regarding concentration of collagen and elastin from both methods. According to a specific embodiment, the apparatus further comprises an interventional device (112).



WO 2012/127378 A1



DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, **Published:**

LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE,

SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA,

GN, GQ, GW, ML, MR, NE, SN, TD, TG).

— *with international search report (Art. 21(3))*

An apparatus for optical analysis of an associated tissue sample

## FIELD OF THE INVENTION

The present invention relates to an apparatus for optical analysis of an associated tissue sample, and more specifically to an apparatus, a method and a computer program for determination of a first and second parameter indicative of a biomolecule in the associated tissue sample.

## BACKGROUND OF THE INVENTION

During interventions in the field of oncology it is important to be able to discriminate pathology tissue from normal tissue in order to know that the treatment is performed on the correct location. For instance ablation of a small tumor lesion requires accurate placement of the ablation needle tip. Image guidance by for instance X-ray or ultrasound can provide valuable feedback but these navigation means do not provide real time tissue feedback from the tip of the needle. This makes targeting small lesions difficult with these techniques.

Determination of physiological parameters, such as composition of a given tissue sample may be of benefit when determining the type of a tissue or when discriminating between tissues. However, in everyday situations, such as in laboratories and clinics, the accuracy of measurements may be limited for various reasons and the discrimination between tissues is consequently impeded or may even be erroneous.

An apparatus which could aid in discriminating tissue types and/or avoid erroneous assignment of a tissue type to a given tissue sample would thus be advantageous.

US 2004/0044287 A1 discloses a method for optically identifying human tissue type and comprises the steps of: illuminating a surface area of tissue to be identified with a source of white light and gathering diffuse reflectance light returned from the illuminated tissue area; illuminating the surface area of the tissue to be identified with a source of monochromatic light and gathering autofluorescent light emitted by the tissue area in response to the monochromatic light illumination, the illumination and gathering of the diffuse reflectance light and the autofluorescent light occurring in either order; generating a first ratio combination including a value of intensity of the diffuse reflectance light gathered

from the illuminated tissue area and a value of intensity of the autofluorescent light gathered from the illuminated tissue area; and using the first ratio combination to identify the type of tissue of the area illuminated. The reference appears not to provide measures for conditions where a risk of inaccuracies in the measured data is present.

Hence, an improved apparatus which could aid in discriminating tissue types and/or avoid erroneous assignment of a tissue type to a given tissue sample would thus be advantageous.

## SUMMARY OF THE INVENTION

It is a further object of the present invention to provide an alternative to the prior art.

In particular, it may be seen as an object of the present invention to provide an apparatus for optical analysis of an associated tissue sample that solves the above mentioned problems of the prior art with lack of accuracy and/or reliability in the determination of the physiological parameter, in particular in determination of a concentration of collagen and/or elastin.

Thus, the above described object and several other objects are intended to be obtained in a first aspect of the invention by providing an apparatus for optical analysis of an associated tissue sample, the apparatus comprising:

- a spectrometer for obtaining a first set of measured data representative of an optical spectrum of the associated tissue sample and a second set of measured data representative of an optical spectrum of the associated tissue sample, the spectrometer comprising:
  - a light source, and
  - an optical detector, and
  - a processor arranged for:
    - receiving the first set of measured data and the second set of measured data,
    - determining a first parameter being indicative of a concentration of a biomolecule in said associated tissue sample from the first set of measured data, and
    - determining a distortion parameter being representative of scattering and absorption in the associated tissue sample from the first set of measured data,
    - determining a second parameter being indicative of a

concentration of said biomolecule in said associated tissue sample from the second set of measured data and the distortion parameter,

wherein the biomolecule is chosen from the group comprising:  
collagen and elastin.

The invention is particularly, but not exclusively, advantageous for determining a concentration of collagen and/or elastin based on at least two independent methods so as to enable a user to assess based on the two measurements and subsequent determinations how reliable the measurements are. For example, in a situation where both determinations result in similar values, then both determinations may be assumed to be reliable. In the opposite situation, where one value differs significantly from the other, then the conclusion may be drawn that at least one of the two values is not reliable. For conditions where a risk of inaccuracies in the measured data is present, the present invention may thus be advantageous in that two independent measurements may be used to validate if similar or – in the opposite case – be used to obtain information that the information derived from the measurements, such as assignment of a tissue type, is of less reliable character. It may be seen as a basic idea of the invention that information (such as information related to absorption and scattering) derived from the first set of measured data may be utilized for deriving the second parameter from the second set of measured data (using the distortion parameter), where the first and second parameter are both related to the same biomolecule which may appear in relatively low concentrations. In this way information in the first set of measured data, which may have appeared not to be beneficial for improving reliability, is utilized for assessing and/or improving reliability by exploiting its usefulness in connection with the second set of measured data.

Light is to be broadly construed as electromagnetic radiation comprising wavelength intervals including visible, ultraviolet (UV), near infrared (NIR), infra red (IR), X-ray. The term optical is to be understood as relating to light.

An optical spectrum is understood to be information related to a plurality of wavelengths of light, such as an intensity parameter, an absorption parameter, a scattering parameter or a transmission parameter given for a plurality of wavelengths of light. A continuous spectrum represents spectral information, but it is further understood, that information related to light at discrete wavelengths may represent an optical spectrum.

A spectrometer is understood as is common in the art. It is understood, that the spectrometer comprises means for selecting wavelengths, such as transmission filters or gratings. Alternatively, wavelength specific light sources, such as light emitting diodes or

LASERS, may be used or wavelength specific optical detectors may be used. A spectral filtration may occur at different places in the system, for instance it may occur between the second light source and the interventional device, it may occur in the interventional device, or it may occur between the interventional device and the optical detector.

In the present context ‘distortion parameter’ is understood to depend on the contribution from scattering and absorption, and to be representative of scattering and absorption. It will be readily understood that the ‘distortion parameter’ is not limited to being a single number, but may be described as a number, a vector, a matrix, a table or a mathematical function, so as to enable the ‘distortion parameter’ to describe the distortion contributions from scattering and absorption for a number of constituents, such as biomolecules, across a number of wavelengths. It is noted that a possible advantage of knowing the distortion parameter may be that it renders it possible to take the distortion parameter into account, such as the distortion parameter determined from the first set of measured data enables removal of the effects of scattering and absorption from the second set of measured data. For example, an algorithm for disentangling contributions from scattering, absorption and fluorescence in a fluorescence spectrum of one or more different optically active constituents, such as chromophores, in a sample may not be able to correctly disentangle the contributions and correctly quantify the constituents if distortion (such as scattering and absorption) is present in the sample, unless the algorithm determines the distortion parameter and takes it into account. The distortion parameter may be a parameter enabling determination of intrinsic fluorescence in a fluorescence spectroscopy spectrum where the intrinsic fluorescence is entangled with the effects of scattering and/or absorption. In the present context ‘intrinsic fluorescence’ is defined as the fluorescence that is due only to fluorophores, without interference from the absorbers and scatterers that may be present in the associated tissue sample. In a particular embodiment the distortion parameter is based on any one of: scattering, absorption, a probe specific constant and the anisotropy parameter of the associated tissue sample.

In an embodiment, the first set of measured data is representative of an optical reflectance spectrum, such as a diffuse reflectance spectroscopy spectrum, and the distortion parameter is based on the reflectance spectrum. In a particular embodiment, the distortion parameter relates intrinsic fluorescence to measured fluorescence. An example of using such a distortion parameter to recover the intrinsic fluorescence is described in “*Turbidity-free fluorescence spectroscopy of biological tissue*”, by Qingguo Zhang, Markus G. Müller, Jun Wu, and Michael S. Feld in Optics Letters, Vol. 25, No. 19, October 1, 2000, page 1451,

which is hereby incorporated in entirety by reference and hereafter referred to as [Zhang2000]. Notice particularly that Eq. (4) in [Zhang2000] can be used to extract an intrinsic fluorescence excitation–emission matrix (EEM) from an experimentally measured fluorescence EEM. When the medium contains multiple fluorophores, one need only replace every occurrence of  $\mu_{fx} \phi_{xm}$  in Eqs. (2) and (3) with a summation of this quantity over all fluorophores. Doing so will result in the same expression as in Eq. (4).

The first and second parameter may in some embodiments be understood each to be indicative of a concentration of either collagen or elastin, and in some embodiments the first and second parameter may be understood each to comprise a set of concentrations so as to be indicative of a concentration of both collagen and elastin.

In the present context, collagen is understood to be pure collagen, such as pure mammalian collagen or more specifically human collagen. When referring to concentration of collagen, it is understood that the concentration of collagen is to be measured relative to pure collagen, such as pure collagen type 1, such as pure human collagen type 1 or pure bovine collagen type 1. An example of reference collagen could be type I collagen from bovine achilles tendon (Sigma-Aldrich C9879). In other words, pure human collagen is our reference. Hence 100 vol% (volume percentage) collagen means pure collagen. It is understood that there are further types of collagen, however, their spectral properties are similar. It is understood that collagen may be a sum of different types of collagen, such as partially collagen type I and partially collagen type II. Collagen type I is a protein which may be identified by UniProt ID: P02452 (alpha I) or P08123 (alpha II).

Elastin is understood to be pure elastin, such as pure mammalian elastin or more specifically human elastin. When referring to concentration of elastin, it is understood that the concentration of elastin is to be measured relative to pure elastin, such as pure human elastin or pure bovine elastin. An example of reference elastin could be elastin from bovine neck ligament (Sigma-Aldrich E1625). In other words, pure elastin is our reference. Hence 100 vol% (volume percentage) elastin means pure elastin. Elastin is a protein which may be identified by UniProt ID: P15502 (human elastin).

The invention can be used in the field of oncology, or other healthcare applications where the determination of tissue type is relevant. The apparatus may be applicable for real-time intra-operative needle localization and ablation monitoring to improve ablation efficacy and disease free survival. It is noted, that collagen and elastin concentrations can be indicative for discriminating pathology tissue from normal tissue. As other examples of applications where embodiments of the present invention may be used

include applications any field where accurate determination of collagen and/or elastin is important. It may thus be used in fields where the determination of a quality parameter of food or skin is relevant.

According to another embodiment of the invention, there is provided an apparatus wherein the apparatus is arranged so as to obtain a Diffuse Reflectance Spectroscopy spectrum as the first set of measured data.

According to another embodiment of the invention, there is provided an apparatus wherein the apparatus is arranged so as to obtain a fluorescence spectroscopy spectrum as the second set of measured data.

According to another embodiment of the invention, there is provided an apparatus wherein the processor is arranged for disentangling the contributions from scattering and absorption from the intrinsic fluorescence by means of the distortion parameter and the fluorescence spectroscopy spectrum so as to obtain a spectrum representative of intrinsic fluorescence of the associated tissue sample.

According to another embodiment of the invention, there is provided an apparatus wherein the intrinsic fluorescence is disentangled from the scattering and absorption by dividing the fluorescence spectroscopy spectrum with the distortion parameter. In a particular embodiment, the distortion parameter is given as:

$$f_{xm} = \frac{F_{xm}}{\frac{1}{\mu_{sx}l} \left( \frac{R_{0x}R_{0m}}{\epsilon_x\epsilon_m} \right)^{1/2} \frac{R_x}{R_{0x}} \left( \frac{R_m}{R_{0m}} + \epsilon_m \right)}$$

This equation corresponds to equation (4) in [Zhang2000] where indices  $x$  and  $m$  denoted evaluation of the corresponding quantities at excitation frequency  $\nu_x$  and emission frequency  $\nu_m$ .  $f_{xm}$  corresponds to the intrinsic fluorescence,  $F_{xm}$  corresponds to the second set of measured data corresponding to the (measured) fluorescence spectroscopy spectrum,  $\mu_{sx}$  corresponds to a scattering parameter,  $R_m$  corresponds to a reflectance spectrum,  $R_0$  corresponds to reflectance spectrum in the absence of absorption,  $l$  corresponds to a measure of thickness of a measured part of the associated tissue sample,  $\epsilon$  corresponds to a parameter being dependent on a probe specific constant  $S$  and the anisotropy parameter  $g$  of the associated tissue sample.

According to further, specific embodiment of the invention there is provided an apparatus wherein the spectrometer is arranged to measure the second set of measured data by:

- illuminating the associated tissue sample with light having relatively high intensity in a first wavelength region corresponding to wavelengths below  $\lambda_1$ , and having relatively low intensity in a second wavelength region above the wavelength  $\lambda_1$ ,
- detecting light emitted from the associated tissue sample in the second wavelength region.

The illumination of the associated tissue sample with light having relatively high intensity in a first wavelength region corresponding to wavelengths below  $\lambda_1$ , and having relatively low intensity in a second wavelength region above the wavelength  $\lambda_1$  may be carried out with, for example a LASER emitting in frequencies below  $\lambda_1$ , or with a broad band source equipped with a filter cutting of frequencies above  $\lambda_1$ .

Methods based on determining these concentrations on autofluorescence are possible but not always reliable. To accurately determine the collagen and/or elastin it is here proposed to combine autofluorescence measurements with diffuse reflectance measurements in the near-infrared. The inventors of the present invention have made the insight that absorption in the near-infrared (wavelength  $>1000\text{nm}$ ) also allow discriminating these tissue constituents.

There are various chromophores that can have a significant influence on the light reaching the collection fibers and that could be used to discriminate pathology tissue from normal tissue. For instance blood will have strong absorption features at wavelength around 550 nm while lipids have strong absorption features around 1200 nm. Furthermore, some substances generate typical fluorescence signals when excited at wavelengths lower than 450 nm. Examples are collagen and elastin that in turn are parameters for oncology. A problem with the fluorescence signals is that they are obscured by the scattering and absorption of the tissue sample. As a result the fluorescence measured is not the intrinsic fluorescence as described in [Zhang2000] or the reference “Model-based spectroscopic analysis of the oral cavity: impact of anatomy”, by S. McGee, J. Mirkovic, V. Mardirossian, A. Elackattu, C-C. Yu, S. Kabani, G. Gallagher, R. Pistey, L. Galindo, K. Badizadegan, Z. Wang, R. Dasari and M.S. Feld, in J. Biomed. Opt. 13 (2008) p064034-1-15, which reference is hereby incorporated by reference, and hereafter referred to as [McGee2008]. Consequently, the measured fluorescence cannot be directly compared with the native fluorescence of the fluorophores on themselves. Algorithms are required to remove first the scattering and absorption effects from the measured fluorescence. This makes the extraction of the

concentrations of the fluorophores difficult and less accurate. However these chromophores like collagen and elastin are known to be important in the discrimination of tumors. Therefore, a more reliable determination of the collagen and elastin concentration is required.

From measurements in the near infrared, i.e., between 900 nm and 1800 nm the inventors of the present invention have found that elastin and collagen show distinct absorption features in the diffuse reflectance spectra in the near infrared (see FIGS 5-6). These features in the spectrum allow determination of the concentration of these chromophores. Since the main absorption peaks of elastin and collagen overlap with the absorption peaks of water and lipid in specific wavelengths ranges (i.e. from 900 to 1000 nm and 1150 to 1250 nm), it is therefore difficult to accurately estimate the concentration of elastin and collagen based on the reflectance spectra only. Our invention is a device capable of detecting the autofluorescence of tissue sample in the visible spectrum and capable of detecting the near infrared diffuse reflectance spectra. The algorithm which the processor may be adapted to run is capable of determining the collagen and elastin concentration based on the autofluorescence data as well as from the absorption features in the near infrared spectrum.

According to another embodiment of the invention, there is provided an apparatus wherein the processor is further arranged for calculating a reliability parameter based on the first parameter and the second parameter. By correlating the two measurements an accuracy parameter can be derived that allow the physician to make a better decision on whether tissue sample is abnormal or not. In a specific embodiment, the processor is arranged with an algorithm that compares the concentrations of collagen and elastin based on the two independent measurements. When the concentrations are in agreement with each other or when a change in concentration due to advancing the probe is in agreement with each other, the concentrations are displayed with a high reliability label indicating that the measurement can be trusted. When both are not in agreement, both concentrations are displayed with a low reliability label indicating that care should be taken by the doctor when interpreting the data.

According to a particular embodiment of the invention, the first and second parameter may after being determined be transmitted to a display unit where they are displayed, such as displayed together with an accuracy indicator, such as an accuracy value. The accuracy value may for instance be derived from a statistical test such as the Students t-test resulting in a p-value indicative for the statistical correlation of the two measured independent values. A further improvement can be achieved by also giving a weighting factor to each of the results from each of the at least two independent methods, such as according to

the diffuse reflectance prediction and the fluorescence prediction. In this case even when both methods show a deviation with respect to each other the prediction with the highest reliability so as to enable that the first or second parameter with the best accuracy can then be used.

According to another embodiment of the invention, there is provided an apparatus further comprising an interventional device, the interventional device comprising:

- a first guide for guiding photons from the light source to an exit position on a distal end of the interventional device, the photons being emittable from the exit position, and
- a second guide for guiding photons from an entry position (220) on the distal end of the interventional device and to the optical detector (106).

It is understood that in one particular embodiment, the first guide and the second guide may be one guide, such as the first guide is identical to the second guide. In another particular embodiment, the first guide and the second guide are two separate guides. An interventional device is generally known in the art, and may include any one of an endoscope, a catheter, a biopsy needle. Integrating optical fibers in the interventional device allows inspection of the optical characteristics of the tissue sample and may allow discrimination of pathology tissue from normal tissue. In a particular embodiment, there is provided an interventional device being suited both for Diffuse Reflectance Spectroscopy (DRS) and/or fluorescence spectroscopy. It is noted that the constraint that the interventional device should be applicable for fluorescence spectroscopy puts some additional constraints on the interventional device. For instance the fibers used for fluorescence spectroscopy must not produce too much autofluorescence themselves and the separation between fiber ends for the fibers respectively connected to source and detector may be shorter compared to the same distance for DRS.

In another embodiment according to the invention, the exit position and the entry position are spatially separated and spatially oriented so that the entry position is not intersected by ballistic photons emitted from the exit position, when the distal end of the interventional device is placed adjacent the associated sample. It is understood that the entry position is not intersected by ballistic photons emitted from the exit position, at least from a practical point of view. For all practical purposes, the number of ballistic photons hitting the entry position is non-zero but negligible.

Ballistic photons are construed as photons which move in straight lines without being scattered more than once, such as a photon used for imaging which is scattered once on the imaged object.

Diffusive photons are photons which experience multiple, scattering events, such as multiple random scattering events. The scattering events may be elastic, such as Rayleigh scattering, or inelastic, such as Raman scattering. Absorption of photons emitted at the exit position may take place at certain wavelengths giving rise to particular absorption bands being visible in the spectrum of the diffusive photons being collected at the entry position.

By arranging the entry and exit positions as described, a large majority of photons collected at the entry position will be diffusive photons which have traversed a relatively long and non-straight path between the exit and entry position. In total, when using a large number of photons, as will generally be the case, the information collected together with the photons collected at the entry position will be dependent on a second region, the second region being traversed by the diffusive photons emitted at the exit position, and the second region being larger than the imaged first region.

In yet another embodiment of the system, the photons emittable at the exit position and subsequently collectable at the entry position are diffusive photons. An advantage of collecting diffusive photons may be that in general they have traversed a larger region, compared to ballistic photons.

According to another embodiment of the invention, there is provided an apparatus wherein the processor is arranged for

- receiving the first set of measured data, wherein the first set of data comprises long wavelength measured data corresponding to data measured at wavelengths above 1100 nm,
- determining the first parameter based on the long wavelength measured data.

The inventors of the present invention have found that elastin and collagen show distinct absorption features in the diffuse reflectance spectra in the near infrared (see FIGS 5-6). Thus, it may be advantageous that the first set of data comprises long wavelength measured data corresponding to data measured at wavelengths above 1100 nm, such as above 1150 nm, such as within 1100-1300 nm, such as within 1150-1250 nm.

According to another embodiment of the invention, there is provided an apparatus wherein the apparatus further comprises any one of: a light source for providing therapeutic light and/or an ultrasound unit. A possible advantage of providing a therapeutic light source is that it enables therapy using light. An advantage of providing an ultrasound unit may be that it enables ablation, such as radio frequency ablation or imaging.

According to another embodiment of the invention, there is provided an apparatus wherein the processor is further arranged for determining from the first parameter and/or the second parameter a third parameter being indicative of tissue type.

According to another embodiment of the invention, there is provided an apparatus wherein the exit position of the first guide and the entry position of the second guide are spatially separated and spatially oriented so that, upon positioning the distal end of the interventional device adjacent to the associated tissue sample, an average spectral information of a region of the associated tissue sample is obtainable from photons collectable at the entry position. An advantage of this may be that photons emitted at the exit position of the first guide and collected at the entry position of the second guide may have travelled a distance outside of the interventional device, such as in the associated tissue sample.

According to another embodiment of the invention, there is provided an apparatus wherein the photons exiting the first guide at the exit position are non-focused. A possible advantage of this is that the energy of the photons is divided over a broader area of the associated tissue sample due to the defocusing, and as a result there is less risk of damaging the adjacent tissue.

According to another embodiment of the invention, there is provided an apparatus wherein the apparatus further comprises a database, the database being operably connected to the processor. In a particular embodiment, the processor is further arranged to access a database comprising information regarding various tissue types, and identify which tissue type or tissue types the sample is most likely to comprise, and wherein the identification is based on concentration of collagen and/or elastin. An advantage of this may be that valuable information regarding the tissue type might be obtained this way.

According to another embodiment of the invention, there is provided an apparatus wherein the database comprises predetermined data representative of an optical spectrum. Having predetermined data representative of an optical spectrum stored in the database may be beneficial for determining from the measured data, such as from the first set of measured data and/or from the second set of measured data, a first parameter respectively a second parameter being indicative of a concentration of a biomolecule in the associated tissue sample. The predetermined data may be representative of spectra of a tissue type, or the predetermined data may be representative of an optical spectrum of a chromophore expected to be in the associated tissue sample, which may be useful, e.g., as an input parameter in a mathematical model. The predetermined optical spectra may include spectra which have been calculated theoretically, such as by mathematical models, or spectra which

have been measured on phantoms, such as samples prepared by mixing constituents expected to be in the associated tissue sample. Multivariate analysis is commonly known in the art and understood to include Principal Components Analysis (PCA) and least squares discriminant analysis.

According to another embodiment of the invention, there is provided an apparatus wherein the predetermined data is representative of an optical spectrum of any one optical spectrum chosen from the group of: an optical spectrum of collagen and an optical spectrum of elastin. This may be beneficial, e.g., for disentangling the contributions to the measured data from different chromophores. This may also be beneficial for enabling determination of a quantitative estimate of a concentration of collagen and/or elastin in the associated tissue sample.

According to a second aspect of the invention, the invention further relates to a method for optically analyzing an associated tissue sample 116, the method comprising the steps of:

- obtaining S1 a first set of measured data representative of an optical spectrum of the associated tissue sample,
- obtaining S2 a second set of measured data representative of an optical spectrum of the associated tissue sample,
- determining S3a from the first set of measured data a first parameter being indicative of a concentration of a biomolecule in said associated tissue sample,
- determining S3b from the first set of measured data a distortion parameter,
- determining S4 from the second set of measured data and the distortion parameter a second parameter being indicative of a concentration of the biomolecule in said associated tissue sample,

wherein the biomolecule is chosen from the group comprising: collagen and elastin.

This aspect of the invention is particularly, but not exclusively, advantageous in that the method according to the present invention may be implemented using an apparatus according to the first aspect of the invention.

The may be carried out in the order listed, however, the order in which the steps are listed or carried out is not important. The method does not require interactions with a patient's body nor does it require involvement of a medical practitioner.

The method does not require interaction with a patient's body or involvement of a medical practitioner. In general, the invention is not about providing a diagnosis or

treating a patient, but the invention provides a technical solution for assisting a physician in reaching a diagnosis or treating a patient.

In a further embodiment the invention relates to a method for optical analysis of an associated tissue sample, wherein the determination of the first parameter includes any one of fitting S5a the measured data to a mathematical model, performing S5b multivariate statistical analysis, such as PCA or partial least squares discriminant analysis, and assessing S5c a look-up-table comprising predetermined optical spectra, such as predetermined optical spectra generated from phantoms.

In one embodiment a method for optical analysis of an associated tissue sample is provided, wherein the determination of the first parameter includes fitting the measured data to a mathematical model. A mathematical model is in the present context understood to be a theoretical expression which for a given set of input parameters having influence on the optical spectrum, for example quantities of chromophores present and amount of scattering may as output yields data representative of an optical spectrum. Fitting is understood to be the process of adjusting the input parameters so as minimize a difference between a measured optical spectrum and a theoretically given optical spectrum. An advantage of fitting is that fitting may be used to quantitatively estimate the input parameters.

According to a third aspect of the invention, the invention further relates to a computer program product being adapted to enable a computer system comprising at least one computer having data storage means associated therewith to operate a processor arranged for carrying out the method according to the second aspect of the invention.

The first, second and third aspect of the present invention may each be combined with any of the other aspects. These and other aspects of the invention will be apparent from and elucidated with reference to the embodiments described hereinafter.

## BRIEF DESCRIPTION OF THE FIGURES

The apparatus for optical analysis of an associated tissue sample and corresponding method and computer program product according to the invention will now be described in more detail with regard to the accompanying figures. The figures show one way of implementing the present invention and is not to be construed as being limiting to other possible embodiments falling within the scope of the attached claim set.

FIG 1 shows a schematic of an embodiment of the invention,

FIG 2 shows a schematic of an interventional device,

FIG 3 shows absorption spectra for blood, water and lipid,

FIG 4 shows absorption spectra for collagen and elastin,

FIG 5 shows absorption spectrum of water, lipid and collagen,

FIG 6 shows absorption spectrum of water, lipid, collagen and elastin,

FIG 7 shows measured optical spectra on various tissue types and fitting results with and without collagen absorption taken into account,

FIG 8 shows measured optical spectra on various tissue types and fitting results with and without collagen absorption taken into account,

FIG 9 is a flow-chart of a method according to the invention.

FIG 10 is an example of using disentangling distortion intrinsic fluorescence from a fluorescence spectroscopy spectrum using a distortion parameter obtained from a DRS spectrum.

## DETAILED DESCRIPTION OF AN EMBODIMENT

FIG 1 shows a schematic of an embodiment of the invention by showing an apparatus according to an embodiment of the invention comprising a spectrometer 102 comprising a light source 104, an optical detector 106 and an interventional device 112, where the interventional device 112 has one or more guides, such as optical elements, such as optical waveguides, capable of guiding light from the light source 104 to a distal end of the interventional device so as to emit the light at the distal end of the interventional device, and furthermore capable of guiding light back from the distal end of the interventional device to the optical detector 106. The light guides enable light to enter an associated tissue sample 116 and the light guides further enable light exiting the associated tissue sample to be collected and led to the optical detector. The apparatus thus enables procurement of measured data representative of an optical spectrum of the associated tissue sample 116. The optical detector 106 may be controlled by processor 110 so as to acquire the measured data. The processor may have access to a database 114. In a specific embodiment, the apparatus is further arranged to access the database 114, where the database comprises predetermined data representative of an optical spectrum, such as an optical spectrum of a biomolecule, such as collagen and/or elastin, such as a plurality of optical spectra of different chromophores. This may enable the processor to better determine any one of the first parameter, the distortion parameter and the second parameter.

In the specific embodiment shown there is also a second light source 108. In this embodiment the first light source 104 is a lamp suited for Diffuse Reflectance Spectroscopy (DRS) and the second light source 108 is a LASER suited for fluorescence

spectroscopy. In an alternative embodiment, there may be only a single light source, such as a single lamp which may then be used in combination with a switchable filter serving to limit the range of frequencies emitted and thereby narrowing the bandwidth and thereby obtaining an appropriate bandwidth for doing fluorescence spectroscopy.

FIG 2 shows a perspective illustration of an embodiment of an interventional device 112, which interventional device comprises a first guide 219, a second guide 221, a third guide 223 and a fourth guide 225. The figure shows an exit position 219 on distal end of the first guide and an entry position 221 on a distal end of the second guide. Similarly, there is shown an exit position 223 on distal end of the third guide and an entry position 225 on a distal end of the fourth guide. The drawing is not to scale. The first, second, third and fourth guide are understood to be light guides, such as optical fibers, such as optical waveguides. Furthermore is indicated the distance  $d1$  between an exit position 219 on the first guide 218 and an entry position 221 on the second guide 220. Still further is shown a distance  $d2$  between an exit position 223 on the third guide 222 and an entry position 225 on the fourth guide 224. Note that in a particular embodiment the interventional device may be constructed so as to optimize  $d1$  for Diffuse Reflectance Spectroscopy. In another particular embodiment the interventional device may be constructed so as to optimize  $d2$  for fluorescence spectroscopy.

In a specific embodiment there is provided an optical probe, such as the interventional device 112, is a needle with optical fibers 218, 220, 222, 224 that can be connected to an optical console, such as the spectrometer 102. The optical console contains a light source 104 enabling light to be provided via one of the fibers to the distal end of the optical probe. The scattered light is collected by another fiber and is guided towards the detector 106. The optical console may also contain a LASER source 108 with a wavelength lower than 450 nm in order to induce autofluorescence in the tissue sample. The obtained data, such as the first and/or second set of measured data are processed by processor 110 using a dedicated algorithm. For instance light is coupled out of the distal tip through at least one fiber, which serves as a source, and the wavelength is swept from e.g. 500-1600 nm or a broadband light source is used. The corresponding wavelength-dependent reflection is measured by at least one other fiber, which is spatially separated from the source, such as a distance  $d1$  of at least 0.5, such as at least 1, such as at least 2 mm apart, such as at least 5 mm apart. The amount of reflected light measured at the “detection” fiber, is determined by the absorption and scattering properties of the probed structure (e.g. tissue sample). From this signal we can deduce the concentration of the chromophores such as collagen and elastin.

The autofluorescence is measured through a fiber that is in close vicinity with the excitation fiber, such as within a distance  $d_2$  being less than 5 mm, such as less than 2 mm, such as less than 1 mm, such as less than 0.5 mm, such as less than 0.25 mm.. The measured autofluorescence is corrected for scattering and absorption such that the estimated intrinsic fluorescence is obtained. From this the concentration of collagen and elastin can be measured.

In a specific embodiment, the apparatus comprises a light source 104 in the form of a halogen broadband light source with an embedded shutter, an interventional device 112 with four guides and an optical detector 106 that can resolve light across a span of wavelengths, such as substantially in the visible and infrared regions of the wavelength spectrum, such as from 400 nm to 1700 nm. The apparatus may furthermore comprise a filter that rejects light for wavelengths below 465 nm which filter may be mounted in front of the optical detector 106 to reject second order light at the optical detectors during diffuse reflectance spectroscopy. The interventional device 112 has a first guide connected to the light source, the second guide connected to the optical detector 106. The centre-to-centre distance separation  $d_1$  between the exit position 219 on the first (emitting) guide 218 and the exit position 221 on the second (collecting) guide 220 may be in the millimeter range, such as at least 1 mm, such as at least 2 mm, such as 2.48 mm. All guides may be low-OH fibers of core diameters in the micron range, such as core diameter of 200 microns. Fibers containing low-OH, sometimes also called VIS-NIR fibers, are typically suitable for the visible (VIS) and near infrared (NIR) part of the optical spectrum.

In an alternative embodiment a plurality of optical detectors are applied, such as two optical detectors that can resolve light in different length regions, such as substantially in the visible and infrared regions of the wavelength spectrum respectively, such as from 400 nm to 1100 nm and from 800 nm to 1700 nm respectively.

In a particular embodiment diffuse reflectance spectroscopy is used for obtaining the first set of measured data representative of an optical spectrum and fluorescence spectroscopy is used for obtaining the second set of measured data representative of an optical spectrum. Other optical methods can be envisioned, such as fluorescence spectroscopy measurements, diffuse optical tomography by employing a plurality of optical fibers, differential path length spectroscopy, or Raman spectroscopy.

Preferably, the optical console allows for the fluorescence excitation wavelength to be changed. This could be accomplished with multiple sources that are switched or multiplexed (e.g. frequency modulated) or with a tunable source. Measuring

different fluorescence emission spectra at different excitation wavelengths would provide information that is potentially relevant for differentiating collagen and elastin (and additionally different types of collagen).

Two-photon fluorescence excitation could also be utilized. This may have the benefits of deeper penetration depth relative to one-photon excitation. The volumes probed with two-photon fluorescence measurements may be more similar to the volumes probed for diffuse reflectance measurements in the infrared.

### Algorithm

In the following an algorithm for extracting information from Diffuse Reflectance Spectroscopy spectra is described. The inventors of the present application have participated in developing an algorithm that can be used to derive optical tissue properties such as the scattering coefficient and absorption coefficient of different tissue chromophores: e.g. hemoglobin, oxygenated haemoglobin, water, lipid, collagen and elastin from the diffuse reflectance spectra. These properties may be different between normal and pathologic tissues.

In more detail the algorithm can be described as follows. The spectral fitting will be performed by making use of an analytically derived formula for reflectance spectroscopy which has recently been described in a scientific article featuring the inventors of the present application as authors “*Estimation of lipid and water concentrations in scattering media with diffuse optical spectroscopy from 900 to 1600 nm*”, Nachabé *et al.*, Journal of Biomedical Optics 15(3), 1 (May/June 2010), the article is hereby incorporated by reference in entirety and hereafter referred to as [Nachabé2010]. The diffuse reflectance model is described in section 2 of [Nachabé2010], more particularly in section 2.3. The reflectance distribution  $R$  is given by

$$\begin{aligned}
 R(\rho) &= \int_0^{\infty} R(\rho, z_0) \delta(z_0 - 1/\mu_t') dz_0 \\
 &= \frac{d}{4\pi} \left[ \frac{1}{\mu_t'} \left( \mu_{eff} + \frac{1}{\tilde{r}_1} \right) \frac{e^{-\mu_{eff}\tilde{r}_1}}{\tilde{r}_1^2} + \left( \frac{1}{\mu_t'} + 2z_b \right) \left( \mu_{eff} + \frac{1}{\tilde{r}_2} \right) \frac{e^{-\mu_{eff}\tilde{r}_2}}{\tilde{r}_2^2} \right]
 \end{aligned} \tag{1}$$

where

$$\begin{aligned}
 \tilde{r}_1 &= [\rho^2 + (1/\mu_t')^2]^{1/2} \\
 \tilde{r}_2 &= [\rho^2 + (1/\mu_t') + 2z_b]^2]^{1/2} \\
 \mu_{eff} &= \sqrt{3\mu_a[\mu_a + \mu_s(1-g)]}
 \end{aligned}$$

In this formula the three macroscopic parameters describing the probability of interaction with tissue are: the *absorption coefficient*  $\mu_a$  and the *scattering coefficient*  $\mu_s$  both in  $\text{cm}^{-1}$  as well as by  $g$  which is the mean cosine of the scattering angle. Furthermore, we have the total reduced attenuation coefficient  $\mu_t'$  that gives the total chance for interaction with tissue

$$\mu_t' = \mu_a + \mu_s(1 - g). \quad (2)$$

The *albedo*  $a'$  is the probability of scattering relative to the total probability of interaction

$$a' = \mu_s / \mu_t'. \quad (3)$$

We assume a point source at a depth  $z_0 = 1/\mu_t'$  and no boundary mismatch hence  $z_b = 2/(3\mu_t')$ . Furthermore, we assume that the scattering coefficient can be written as

$$\mu_s'(\lambda) = a\lambda^{-b}. \quad (4)$$

where  $\lambda$  is the wavelength and  $a$  and  $b$  fixed parameters. The main absorbing constituents in normal tissue dominating the absorption in the visible and near-infrared range are blood (i.e. hemoglobin), water and lipid.

The total absorption coefficient is a linear combination of the absorption coefficients of chromophores in a probed sample, for instance blood, water and lipid as depicted in FIG. 3. Hence for each component the value of that shown in FIG 3 must be multiplied by its volume fraction. By fitting the above formula while using the power law for scattering it is possible to determine the volume fractions of chromophores present, for example blood, water, lipid, collagen and elastin as well as the scattering coefficient. With this method it is thus possible to translate the measured spectra in physiological parameters that can be used to discriminate different tissues.

It is noted that the measurement of the first and second set of measured data representative of optical spectra can be carried out in various ways, such as by means of various filter systems in different positions of the optical path, one or more light sources emitting in one or more delimited wavelength bands, or detectors for different delimited wavelength bands. This is understood to be commonly known by the skilled person. It is also possible to modulate the various wavelength bands with different modulation frequencies at the source and demodulate these at the detector, (this technique is described in the published patent application WO2009/153719 which is hereby incorporated by reference in its entirety).

Various other modifications can be envisioned without departing from the scope of the invention for instance using more than one detector or using more than one light source with different wavelength band, such as Light Emitting Diodes (LEDs) or LASER sources.

FIG 3 shows absorption spectra for blood, water and lipid. The graph shows absorption coefficients of the chromophores from deoxygenated haemoglobin (Hb) 324, oxygenated haemoglobin (HbO<sub>2</sub>) 326, water 328 and lipid 330 as a function of the wavelength. Note that blood dominates the absorption in the visible range, while water and lipids dominate in the near infrared range. The graph has on its first, horizontal axis, the wavelength ( $\lambda$ , lambda) given in nanometer (nm), and on its second, vertical axis, the absorption coefficient  $\mu_a$  ( $\mu_a$ ) given in reciprocal centimeters (1/cm).

FIG 4 shows absorption spectra for collagen and elastin. The graph shows absorption coefficients of collagen 432 and elastin 434 as a function of the wavelength. The measured collagen corresponds to type I collagen from bovine achilles tendon (Sigma-Aldrich C9879). In human breast tissue, collagen type I represents 70% of the total collagen content and the rest corresponds to collagen type III. The measured elastin corresponds to elastin from bovine neck ligament (sigma-aldrich E1625). For a measurement the collagen, or respectively elastin, were packed as much as possible so as to constitute pure collagen or elastin. Both biological substances were measured using an optical spectrophotograph with a large diameter integrating sphere (a highly diffuse reflecting sphere also known as an Ulbricht sphere). The absorption measurement can be separated from the scattering by placing thin cuvettes (sub-millimeter thickness) inside the integrating sphere far away from the detector. The detector measures a certain fraction of the light flux filling the sphere. When a sample is mounted inside the sphere, the loss of light compared to the sample inside the cuvette is mainly due to absorption by the sample related to the absorption coefficient. When the forward transmitted light can escape out of an exit port in the back end of the sphere, the scattered light from the sample mounted inside the sphere is measured, and the scattering coefficient can be determined.

FIG 5 shows absorption spectra of water, lipid and collagen. The graph shows absorption coefficients of water (H<sub>2</sub>O) 528, lipid 530 and collagen 532 as a function of the wavelength.

FIG 6 shows absorption spectra of water, lipid, collagen and elastin. The graph shows absorption coefficients of water (H<sub>2</sub>O) 628, lipid 630, collagen 632 and elastin 634 as a function of the wavelength.

FIGS 5-6 show that the absorption coefficients in the near infrared (NIR) of the water, lipid, collagen and elastin are all different. The differences enable determination, such as quantification within a certain accuracy, of these chromophores with optical analysis, such as Diffuse Reflectance Spectroscopy (DRS) measurements.

The total absorption coefficient is a linear combination of the absorption coefficients of the present chromophores, such as blood, water, lipid, collagen and elastin (hence for each component the value of that shown in FIGS 3-6 must be multiplied by its volume fraction). By fitting the above formula (1) while using the power law for scattering it is possible to determine the volume fractions of the blood, water, lipid, collagen and elastin as well as the scattering coefficient. With this method it is thus possible to translate the measured set data, such as optical spectra, into physiological parameters such as a concentration of collagen and/or elastin. A possible advantage of determining physiological parameters is that they can be used to discriminate between different tissues.

FIGS 7A-B show measured optical spectra on various tissue types and fitting results with and without collagen absorption taken into account. In each of FIGS 7A-B there is shown a set of measured data 736, a fit 738 to the set of measured data without taking collagen into account, the residual 740 between this fit and the set of measured data, a fit 742 to the set of measured data which does take collagen into account and the residual 744 between this fit and the set of measured data. The legend in FIG 7B applies to FIG 7A as well. As can be observed in FIGS 7A-B the effect of collagen on the diffuse reflectance spectroscopy spectrum is small but present. Accurate determination of collagen from the diffuse reflectance spectroscopy spectrum is possible. However, it is also noted that it requires accurate measurements and that uncertainties in the measurements may propagate through the calculations and result in an uncertainty in the determination of the physiological parameters. As a result an independent method in conjunction to that of this method could be advantageous for obtaining robustness in the determination of the physiological parameter, such as the determination of a concentration of collagen and/or elastin.

Although fitting has been described above as a way to discriminate differences in spectra, other ways to do so are understood to be encompassed by the present invention. An example of another way to discriminate differences in spectra is making use of multivariate statistical analysis methods, such as for example principal component analysis or partial least squares discriminant analysis which renders classification of differences in spectra and thus allows discrimination between tissues. Yet another alternative to fitting with an analytical model and using statistical classification is to create a table of spectra generated

from measurements of phantoms with different concentrations of the chromophores of interest that can be used as vector space for regression of an acquired spectrum in tissue. The reference Rajaram N, Nguyen TH, Tunnell JW., “*Lookup table-based inverse model for determining the optical properties of turbid media*”, Journal of Biomedical Optics 13(5):050501, 2008, describes this approach and is hereby incorporated by reference.

FIGS 8A-C show measured optical spectra on various tissue types and fitting results with and without collagen absorption taken into account. FIGS 8A-C show optical spectroscopy measurements on normal (FIG 8A), glandular (FIG 8B) and tumor (FIG 8C) tissues. Measurement values are represented by dotted curves. A fit was applied to the three measurements in order to extract the concentration of chromophores. The gray line 842 shows the fit taking collagen into account and the black line 838 is the fit without taking into account the absorption profile of collagen.

Table I compares the estimated parameters of water, lipid and collagen for the three different types of tissue in FIGS 8A-C (where the number between brackets correspond to the estimation of parameters when collagen is not taken into account).

|          | Normal (FIG 9) | Gland (FIG 10) | Tumor (FIG 11) |
|----------|----------------|----------------|----------------|
| Water    | 7% (7%)        | 56% (53%)      | 86% (93%)      |
| Lipid    | 83% (93%)      | 20% (37%)      | 0% (7%)        |
| Collagen | 10% (0%)       | 25% (0%)       | 14% (0%)       |

Table I

The results of this table show that the concentration of collagen is highest in glandular tissue and lowest in normal tissue. The differences between spectra of different tissues, which differences can be attributed to different concentrations of the respective chromophores, entails that knowing the physiological parameters may aid in discriminating between the different tissue types.

The second set of measured data may be obtained with fluorescence spectroscopy. Deducing the intrinsic fluorescence (autofluorescence) from the measured fluorescence is not straightforward. It is hampered by the effects of scattering and absorption in turbid media such as tissue. Various ways may be employed in order to determine the autofluorescence but all rely on certain assumptions that influence the accuracy. Examples of extraction of the intrinsic fluorescence is described in the references [McGee2008] and [Zhang2000]. The reference shows that the difference between the various fluorophores is

small and the determination can be done with a certain accuracy. The more absorbing chromophores are present in the tissue the less accurate the method becomes.

FIG 9 is a flow-chart of a method for optically analyzing an associated tissue sample comprising the steps of obtaining (S1) a first set of measured data representative of an optical spectrum of the associated tissue sample, obtaining (S2) a second set of measured data representative of an optical spectrum of the associated tissue sample, determining (S3a) from the first set of measured data a first parameter being indicative of a concentration of a biomolecule in said associated tissue sample, determining (S3b) from the first set of measured data a distortion parameter, determining (S4) a second parameter from the second set of measured data and the distortion parameter, the second parameter being indicative of a concentration of the biomolecule in said associated tissue sample, wherein the biomolecule is chosen from the group comprising: collagen and elastin. The steps of determining (S3a) the first parameter from the first set of measured data, determining (S3b) the distortion parameter from the first set of measured data and determining (S4) the second parameter from the second set of measured data may each comprise any one of fitting (S5a) the measured data to a mathematical model, performing (S5b) multivariate statistical analysis, such as PCA or partial least squares discriminant analysis, and assessing (S5c) a look-up-table comprising predetermined optical spectra, such as predetermined optical spectra generated from phantoms. In a specific embodiment, the method may further comprise a step of calculating (S6) a reliability parameter based on the first and the second parameter. In another specific embodiment, the method comprises transmitting (S7) a third parameter representative being indicative of a concentration of a biomolecule in said associated tissue sample. The third parameter may be based on the first and second parameter, such as being the first or the second parameter or being a weighted average, such as a weighted average dependent on the reliability parameter. In another embodiment, the reliability parameter is also transmitted. The reliability parameter may in a particular embodiment be the discrepancy between the first and second parameter such as the absolute discrepancy or the relative discrepancy.

FIGS 10A-D show an example where the first set of data depicted in FIG 10A is representative of an optical diffuse reflectance spectroscopy spectrum of an associated tissue sample. The distortion parameter is based on the diffuse reflectance spectroscopy spectrum of FIG 10A. FIG 10B shows a second set of data which in the present example are representative of a measured fluorescence spectroscopy spectrum of the associated tissue sample. FIG 10C shows intrinsic, known fluorescence spectra of the fluorophores collagen 1032, elastin 1034, NADH 1046 and FAD 1048. In the present example the distortion

parameter relates intrinsic fluorescence to measured fluorescence, thus the intrinsic fluorescence of the associated tissue sample can be determined and is depicted in FIG 10D in which figure are shown the thus derived intrinsic fluorescence 1050 and a fit 1052, where the fit enables quantification of the fluorophores present in the associated tissue sample. In all FIGS 10A-D the horizontal axis represents wavelength and spans from 400 nm at the left end to 800 nm at the right end. The vertical axis represents intensity (Int.) measured in arbitrary units (arb.).

To sum up the present invention relates to an apparatus 100 and, a method and a computer program for optical analysis of an associated tissue sample 116, the apparatus being adapted to determine a concentration of collagen and/or elastin with two independent methods. With two independent methods, the concentrations determined with each method may be held up against the other, and if the difference is too large a user may be informed that at least one of the measured values is not reliable. In a particular embodiment, the two independent methods are Diffuse Reflectance Spectroscopy (DRS) and fluorescence spectroscopy, and the absorption and scattering determined with DRS may be used when interpreting the fluorescence spectroscopy spectra so as to be able to obtain quantitative information regarding concentration of collagen and elastin from both methods. According to a specific embodiment, the apparatus further comprises an interventional device 112.

Although the present invention has been described in connection with the specified embodiments, it should not be construed as being in any way limited to the presented examples. The scope of the present invention is set out by the accompanying claim set. In the context of the claims, the terms “comprising” or “comprises” do not exclude other possible elements or steps. Also, the mentioning of references such as “a” or “an” etc. should not be construed as excluding a plurality. The use of reference signs in the claims with respect to elements indicated in the figures shall also not be construed as limiting the scope of the invention. Furthermore, individual features mentioned in different claims, may possibly be advantageously combined, and the mentioning of these features in different claims does not exclude that a combination of features is not possible and advantageous.

## CLAIMS:

1. An apparatus (100) for optical analysis of an associated tissue sample (116), the apparatus comprising:
  - a spectrometer (102) for obtaining a first set of measured data representative of an optical spectrum of the associated tissue sample and a second set of measured data representative of an optical spectrum of the associated tissue sample, the spectrometer comprising:
    - a light source (104), and
    - an optical detector (106), and
    - a processor (110) arranged for:
      - receiving the first set of measured data and the second set of measured data,
      - determining a first parameter being indicative of a concentration of a biomolecule in said associated tissue sample from the first set of measured data, and
      - determining a distortion parameter being representative of scattering and absorption in the associated tissue sample from the first set of measured data,
      - determining a second parameter being indicative of a concentration of said biomolecule in said associated tissue sample from the second set of measured data and the distortion parameter,wherein the biomolecule is chosen from the group comprising: collagen and elastin.
2. An apparatus according to claim 1, wherein the apparatus is arranged so as to obtain a Diffuse Reflectance Spectroscopy spectrum as the first set of measured data.
3. An apparatus according to claim 1, wherein the apparatus is arranged so as to obtain a fluorescence spectroscopy spectrum as the second set of measured data.

4. An apparatus according to claim 3, wherein the processor is arranged for disentangling the contributions from scattering and absorption from the intrinsic fluorescence by means of the distortion parameter and the fluorescence spectroscopy spectrum so as to obtain a spectrum representative of intrinsic fluorescence of the associated tissue sample.
5. An apparatus according to claim 4, wherein the intrinsic fluorescence is disentangled from the scattering and absorption by dividing the fluorescence spectroscopy spectrum with the distortion parameter.
6. An apparatus according to claim 1, wherein the processor (110) is further arranged for:
  - calculating a reliability parameter based on the first parameter and the second parameter.
7. An apparatus according to claim 1, further comprising an interventional device (112), the interventional device comprising:
  - a first guide (218) for guiding photons from the light source to an exit position (219) on a distal end of the interventional device, the photons being emittable from the exit position, and
  - a second guide (220) for guiding photons from an entry position (221) on the distal end of the interventional device and to the optical detector (106).
8. An apparatus according to claim 1, wherein the processor (110) is arranged for
  - receiving the first set of measured data, wherein the first set of data comprises long wavelength measured data corresponding to data measured at wavelengths above 1100 nm,
  - determining the first parameter based on the long wavelength measured data.
9. An apparatus according to claim 7, wherein the exit position (219) of the first guide (218) and the entry position (221) of the second guide (220) are spatially separated and spatially oriented so that, upon positioning the distal end of the interventional device (112) adjacent to the associated tissue sample (116), an average spectral information of a region of the associated tissue sample is obtainable from photons collectable at the entry position.

10. An apparatus according to claim 1, wherein the apparatus (100) further comprises a database (114), the database being operably connected to the processor (110).

11. An apparatus according to claim 10, wherein the database (114) comprises predetermined data representative of an optical spectrum.

12. An apparatus according to claim 11, wherein the predetermined data is representative of an optical spectrum of any one optical spectrum chosen from the group of: an optical spectrum of collagen and an optical spectrum of elastin.

13. A method for optically analyzing an associated tissue sample (116), the method comprising the steps of:

- obtaining (S1) a first set of measured data representative of an optical spectrum of the associated tissue sample,
  - obtaining (S2) a second set of measured data representative of an optical spectrum of the associated tissue sample,
  - determining (S3a) from the first set of measured data a first parameter being indicative of a concentration of a biomolecule in said associated tissue sample,
  - determining (S3b) from the first set of measured data a distortion parameter,
  - determining (S4) from the second set of measured data and the distortion parameter a second parameter being indicative of a concentration of the biomolecule in said associated tissue sample,
- wherein the biomolecule is chosen from the group comprising: collagen and elastin.

14. A method according to claim 13 for optical analysis of an associated tissue sample, wherein the determination of the first parameter includes any one of:

- fitting (S5a) the measured data to a mathematical model,
- performing (S5b) multivariate statistical analysis, such as PCA or partial least squares discriminant analysis, and
- assessing (S5c) a look-up-table comprising predetermined optical spectra, such as predetermined optical spectra generated from phantoms.

15. A computer program product being adapted to enable a computer system comprising at least one computer having data storage means associated therewith to operate a processor (110) arranged for carrying out the method of claim 13.

1/7

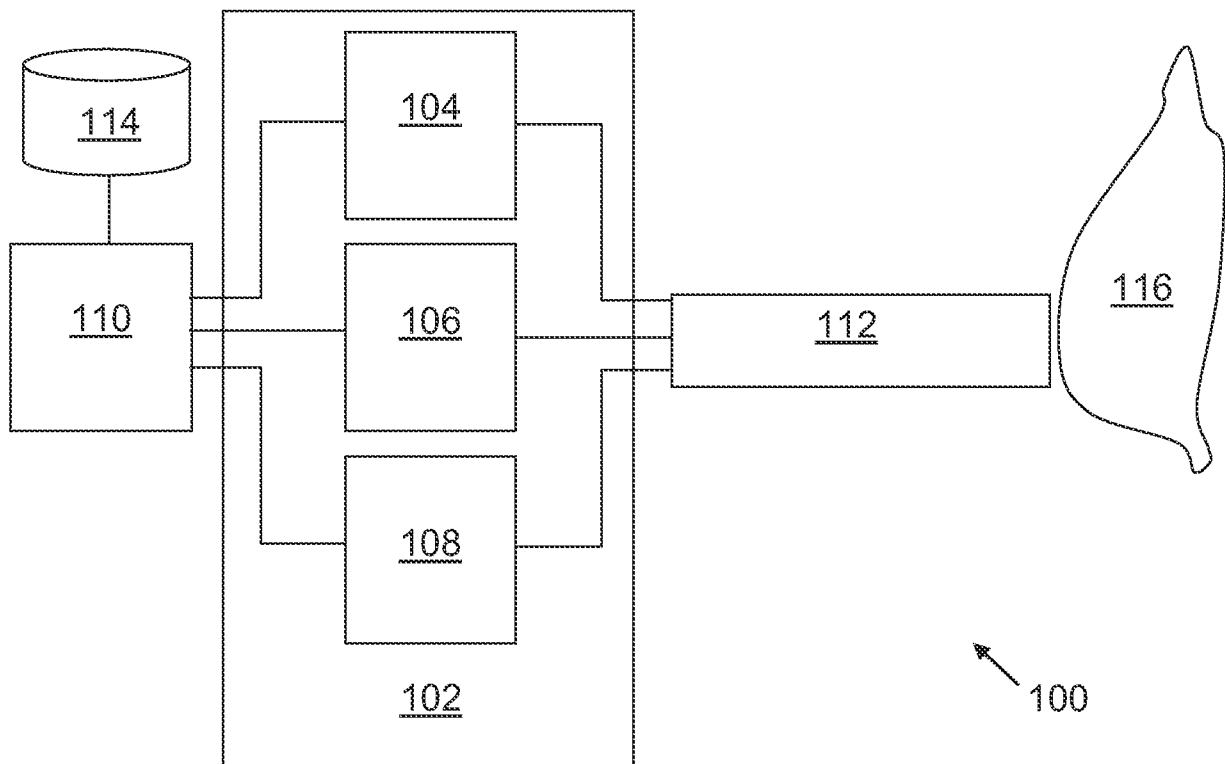


FIG. 1

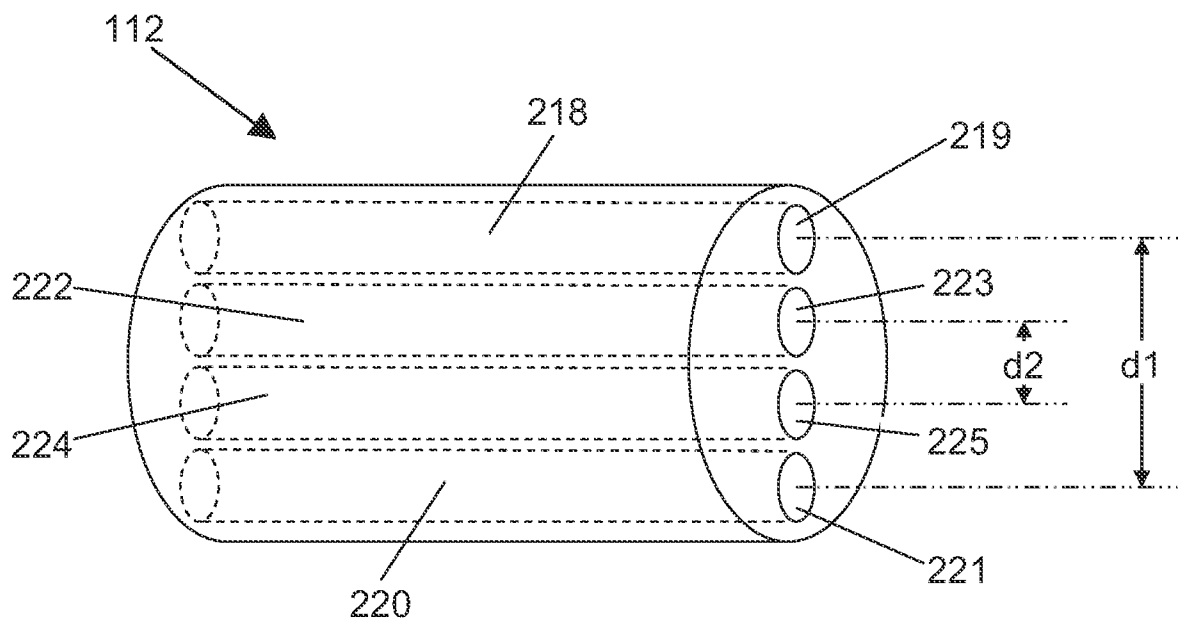


FIG. 2

2/7

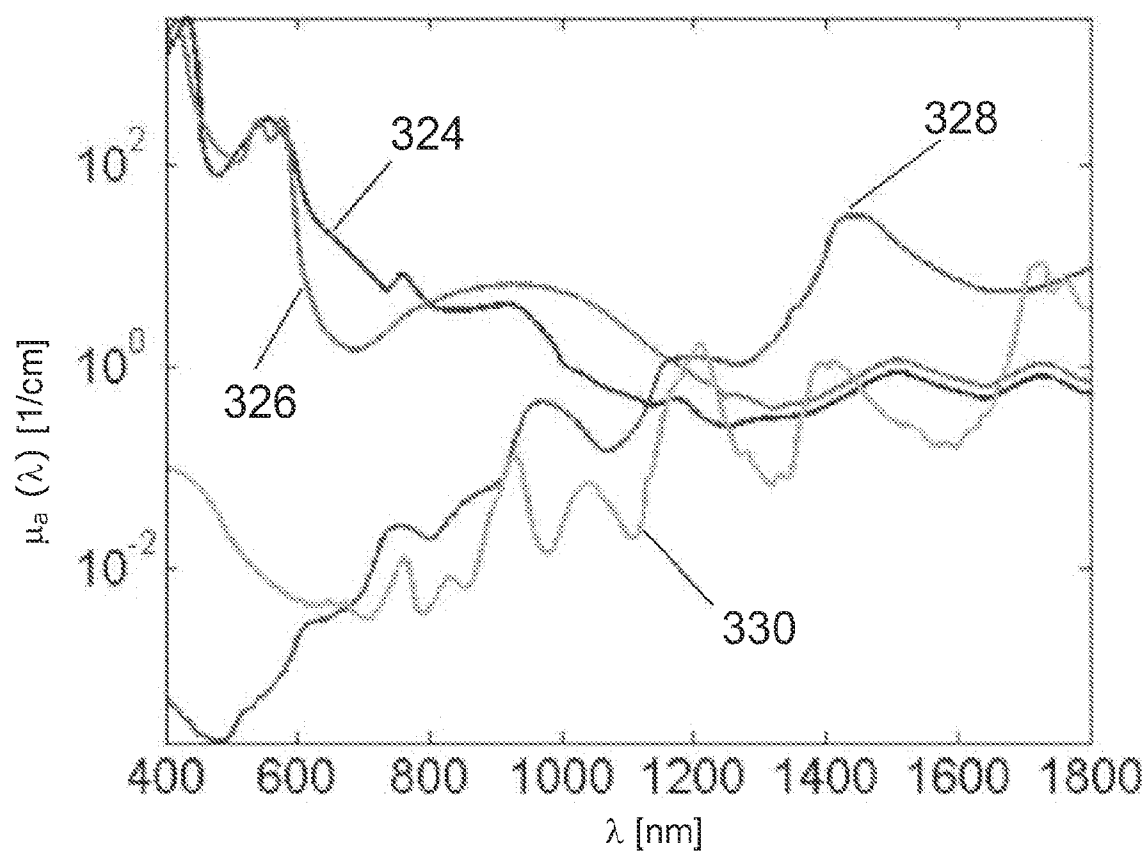


FIG. 3

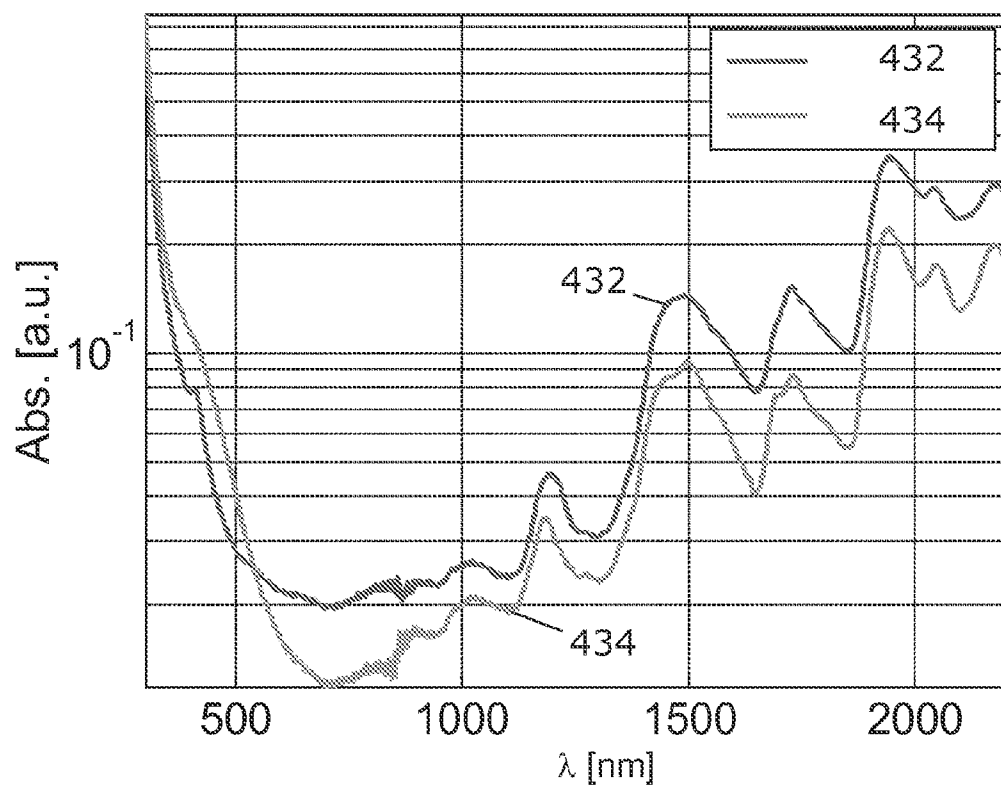


FIG. 4

3/7

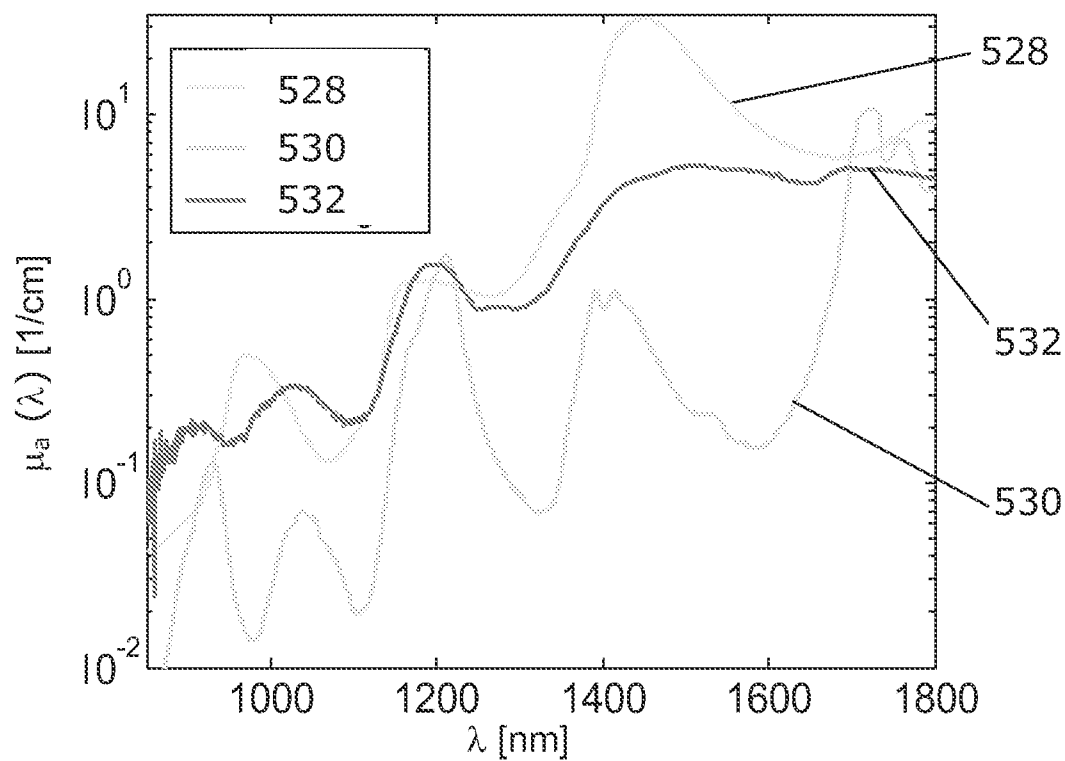


FIG. 5

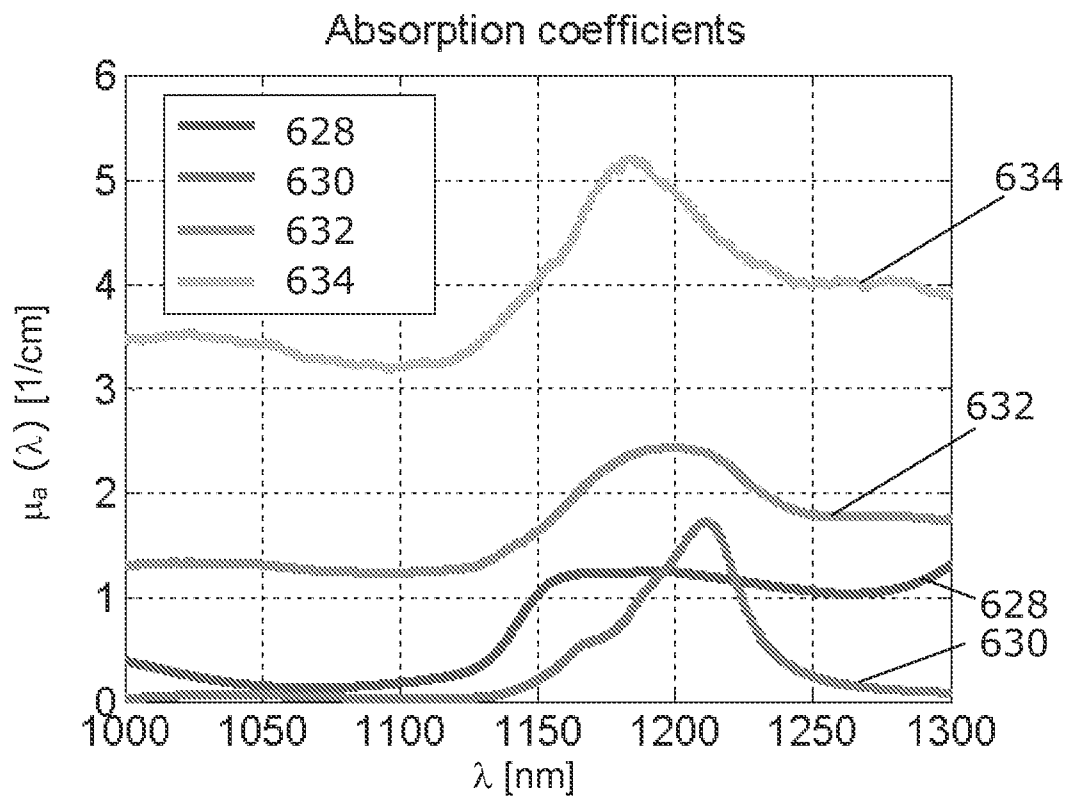


FIG. 6

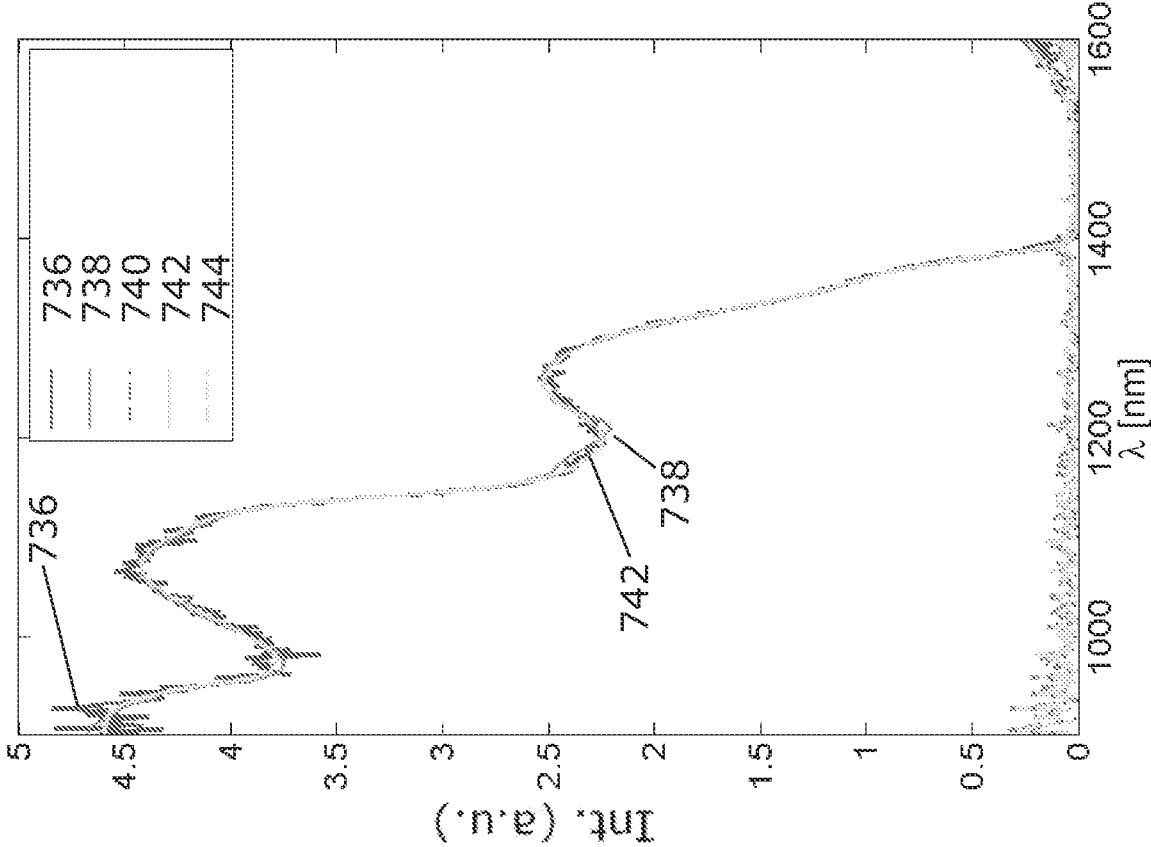


FIG. 7B

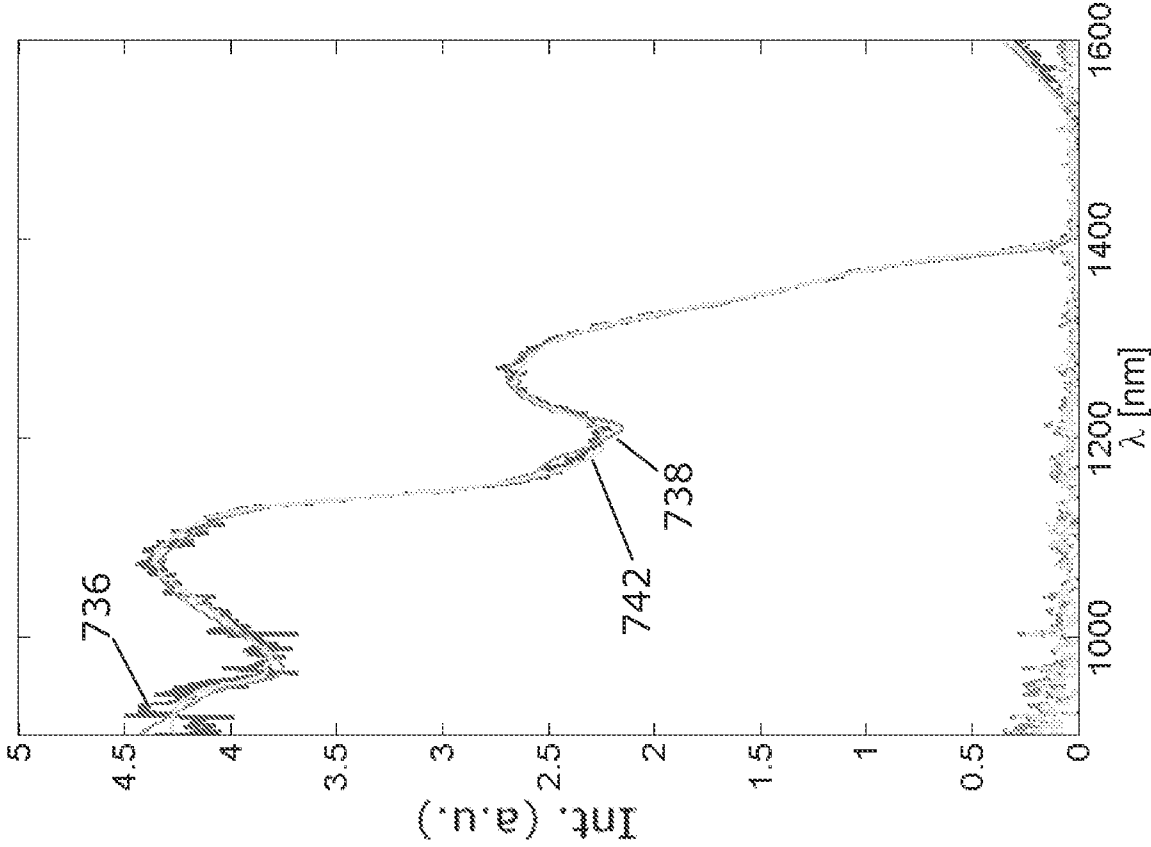
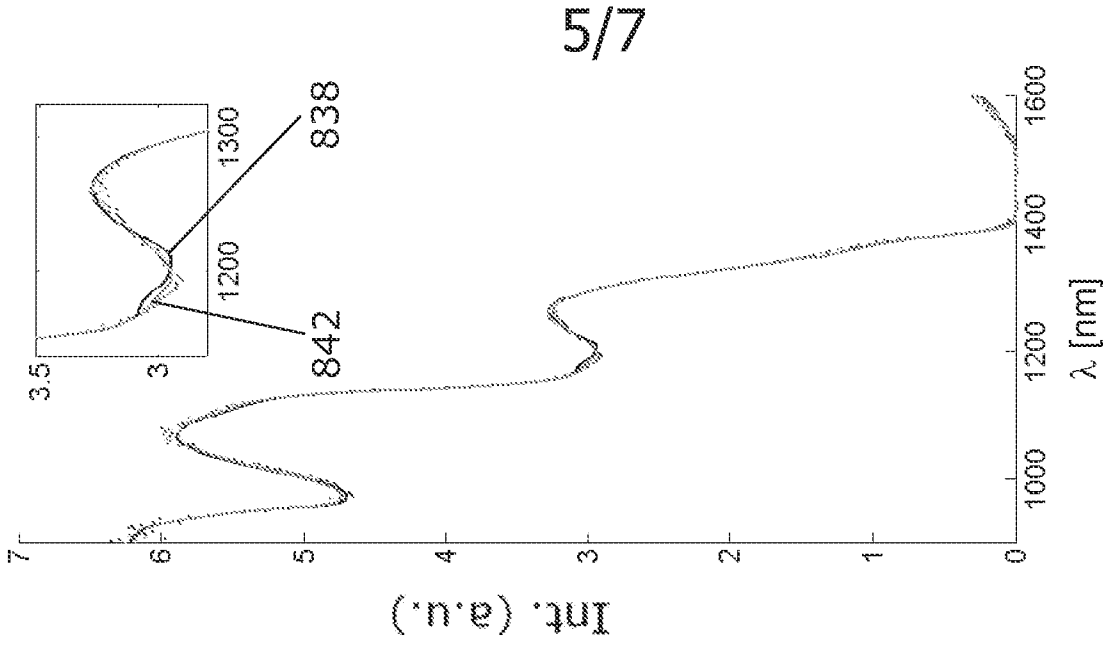
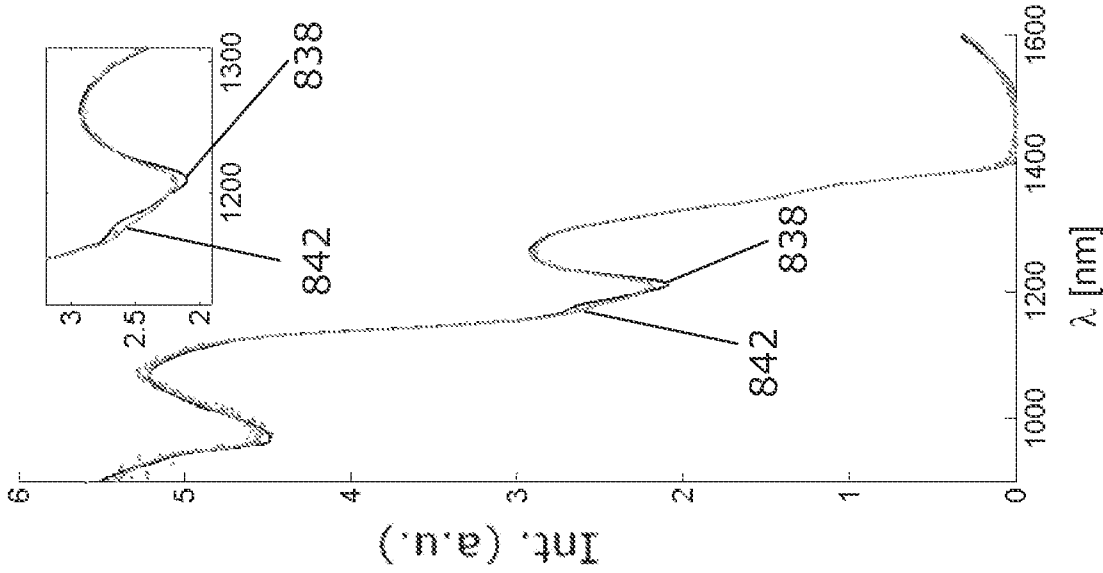
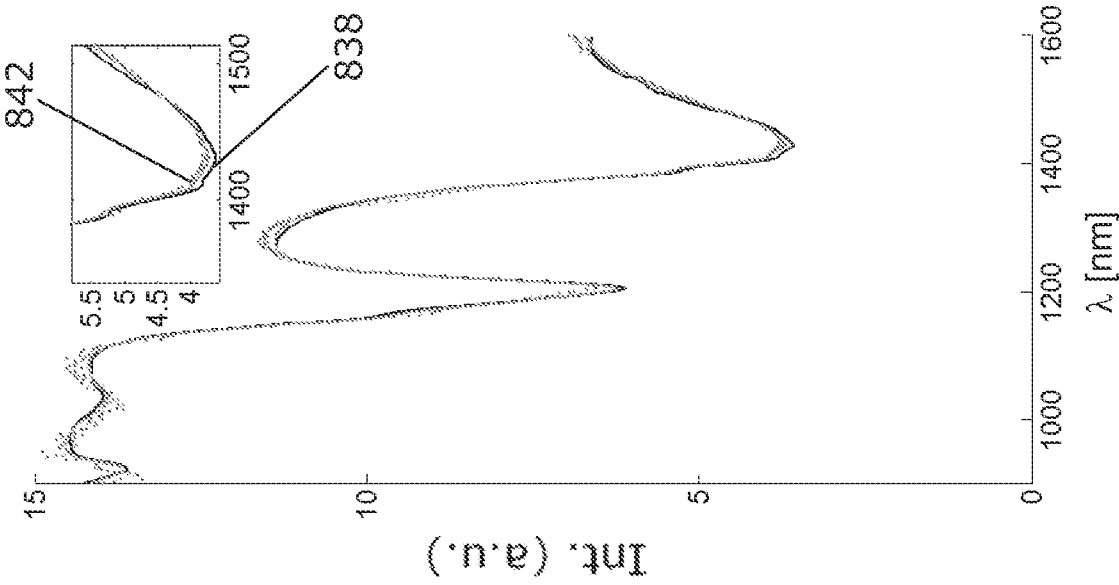


FIG. 7A



6/7

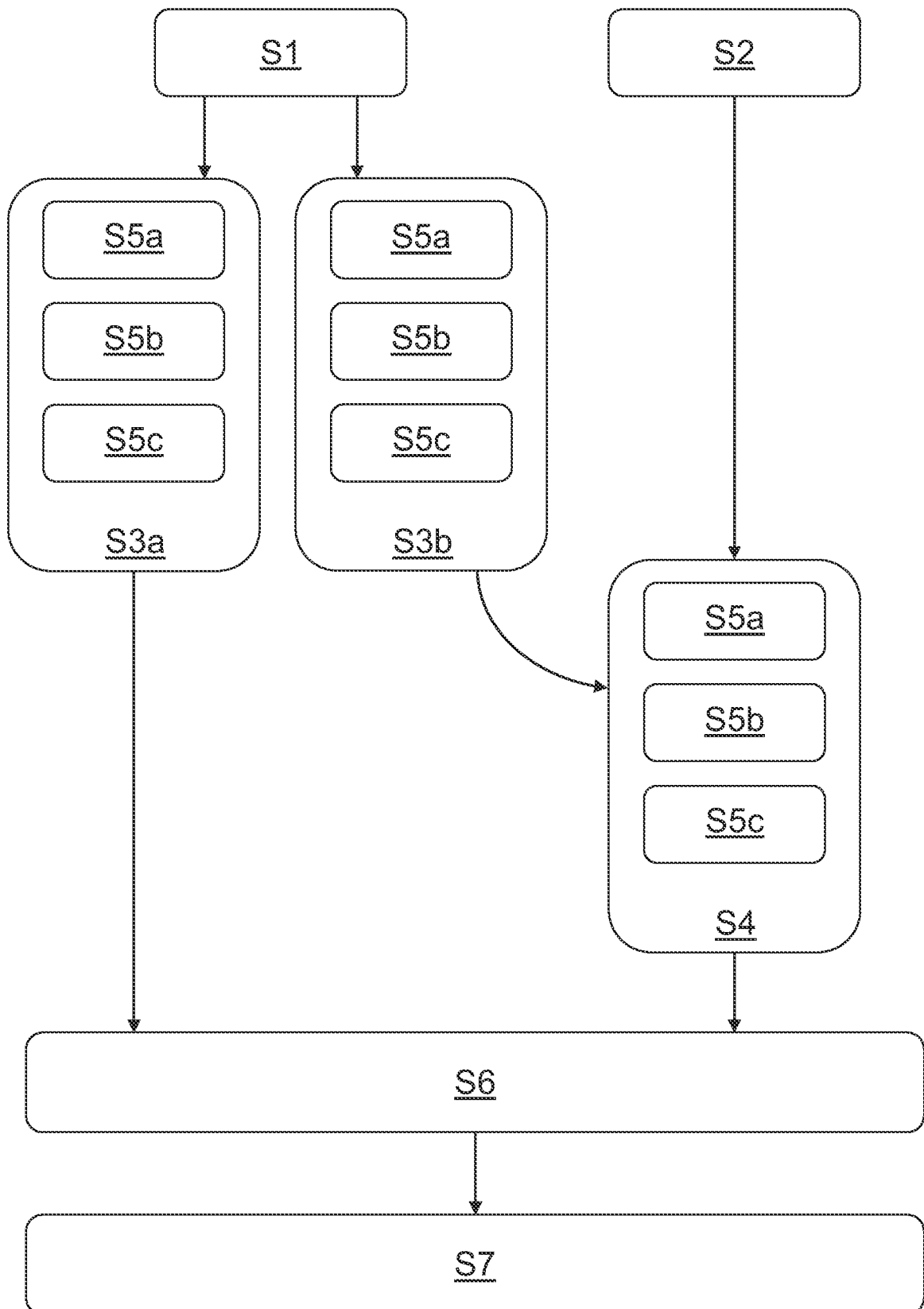


FIG. 9

7/7

FIG. 10A

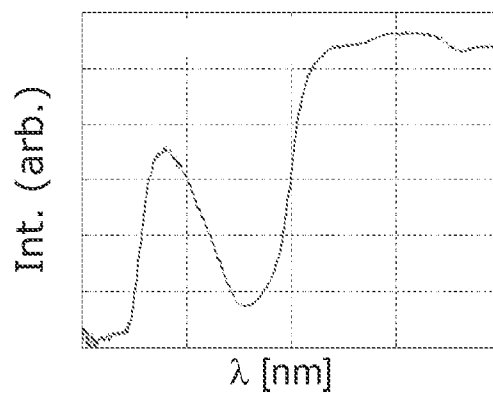


FIG. 10B

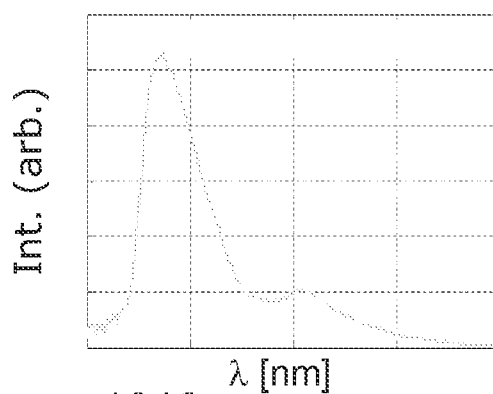


FIG. 10C

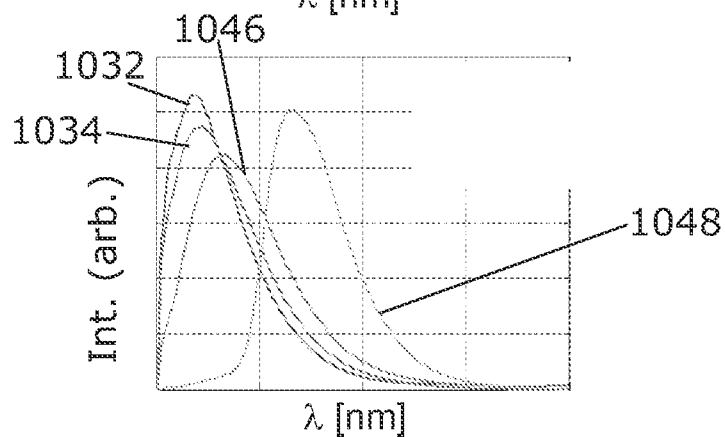
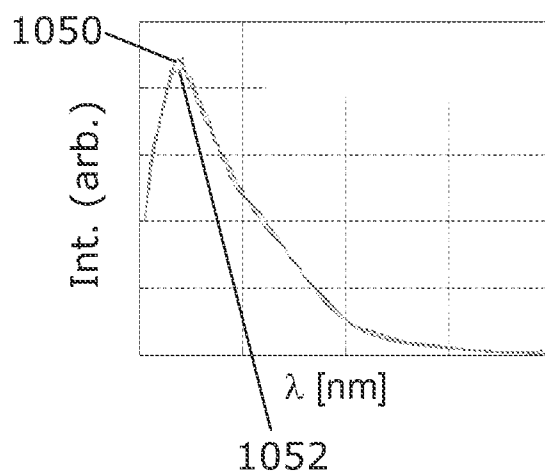


FIG. 10D



## INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2012/051247

A. CLASSIFICATION OF SUBJECT MATTER  
 INV. G01N21/47 G01N21/64 A61B5/00  
 ADD.

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

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N 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 practicable, search terms used)

EPO-Internal, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                               | Relevant to claim No. |
|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X         | WO 2009/043045 A1 (UNIV DUKE [US];<br>RAMANUJAM NIRMALA [US]; YU BING [US];<br>BROWN J QUINCY [])<br>2 April 2009 (2009-04-02)<br>page 5, lines 9-26; page 8, line 16 to<br>page 9, line 21; page 13, lines<br>4-30; figures 1, 2A, 2D,<br>----- | 1-7,<br>9-13, 15      |
| X         | US 2009/317856 A1 (MYCEK MARY-ANN [US] ET<br>AL) 24 December 2009 (2009-12-24)<br>paragraphs [0012], [0059] - [0071],<br>[0076], [0105], [0126]; figure 5<br>-----                                                                               | 1-7,<br>10-15         |
| X         | US 2009/137908 A1 (PATWARDHAN SACHIN V<br>[US]) 28 May 2009 (2009-05-28)<br>paragraphs [0003] - [0005], [0029],<br>[0037], [0075]; figures 1A, 3, 4,<br>-----<br>-/-                                                                             | 1-6, 8,<br>10-15      |



Further documents are listed in the continuation of Box C.



See patent family annex.

\* Special categories of cited documents :

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

"E" earlier application or patent but published on or after the international filing date

"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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

15 June 2012

Date of mailing of the international search report

22/06/2012

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2  
 NL - 2280 HV Rijswijk  
 Tel. (+31-70) 340-2040,  
 Fax: (+31-70) 340-3016

Authorized officer

Weinberger, Thorsten

## INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2012/051247

| C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                                                                                                                                                                   |                       |
|------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Category*                                            | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                | Relevant to claim No. |
| A                                                    | <p>R. S BRADLEY ET AL: "A review of attenuation correction techniques for tissue fluorescence",<br/>JOURNAL OF THE ROYAL SOCIETY INTERFACE,<br/>vol. 3, no. 6,<br/>22 February 2006 (2006-02-22), pages 1-13,<br/>XP55029341,<br/>ISSN: 1742-5689, DOI:<br/>10.1098/rsif.2005.0066<br/>section 2.4</p> <p>-----</p>                                               | 1,13                  |
| A                                                    | <p>ANTHONY KIM ET AL: "Quantification of in vivo fluorescence decoupled from the effects of tissue optical properties using fiber-optic spectroscopy measurements",<br/>JOURNAL OF BIOMEDICAL OPTICS,<br/>vol. 15, no. 6,<br/>1 January 2010 (2010-01-01), page 067006,<br/>XP55029337,<br/>ISSN: 1083-3668, DOI: 10.1117/1.3523616<br/>abstract</p> <p>-----</p> | 1,13                  |
| A                                                    | <p>MARKUS G. MÜLLER ET AL: "Intrinsic fluorescence spectroscopy in turbid media: disentangling effects of scattering and absorption",<br/>APPLIED OPTICS,<br/>vol. 40, no. 25,<br/>1 January 2001 (2001-01-01), page 4633,<br/>XP55029334,<br/>ISSN: 0003-6935, DOI: 10.1364/AO.40.004633<br/>the whole document</p> <p>-----</p>                                 | 1,13                  |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2012/051247

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s)                            | Publication<br>date                    |
|-------------------------------------------|---------------------|-------------------------------------------------------|----------------------------------------|
| WO 2009043045 A1                          | 02-04-2009          | EP 2194848 A1<br>US 2011112435 A1<br>WO 2009043045 A1 | 16-06-2010<br>12-05-2011<br>02-04-2009 |
| US 2009317856 A1                          | 24-12-2009          | NONE                                                  |                                        |
| US 2009137908 A1                          | 28-05-2009          | US 2009137908 A1<br>WO 2009070370 A2                  | 28-05-2009<br>04-06-2009               |