

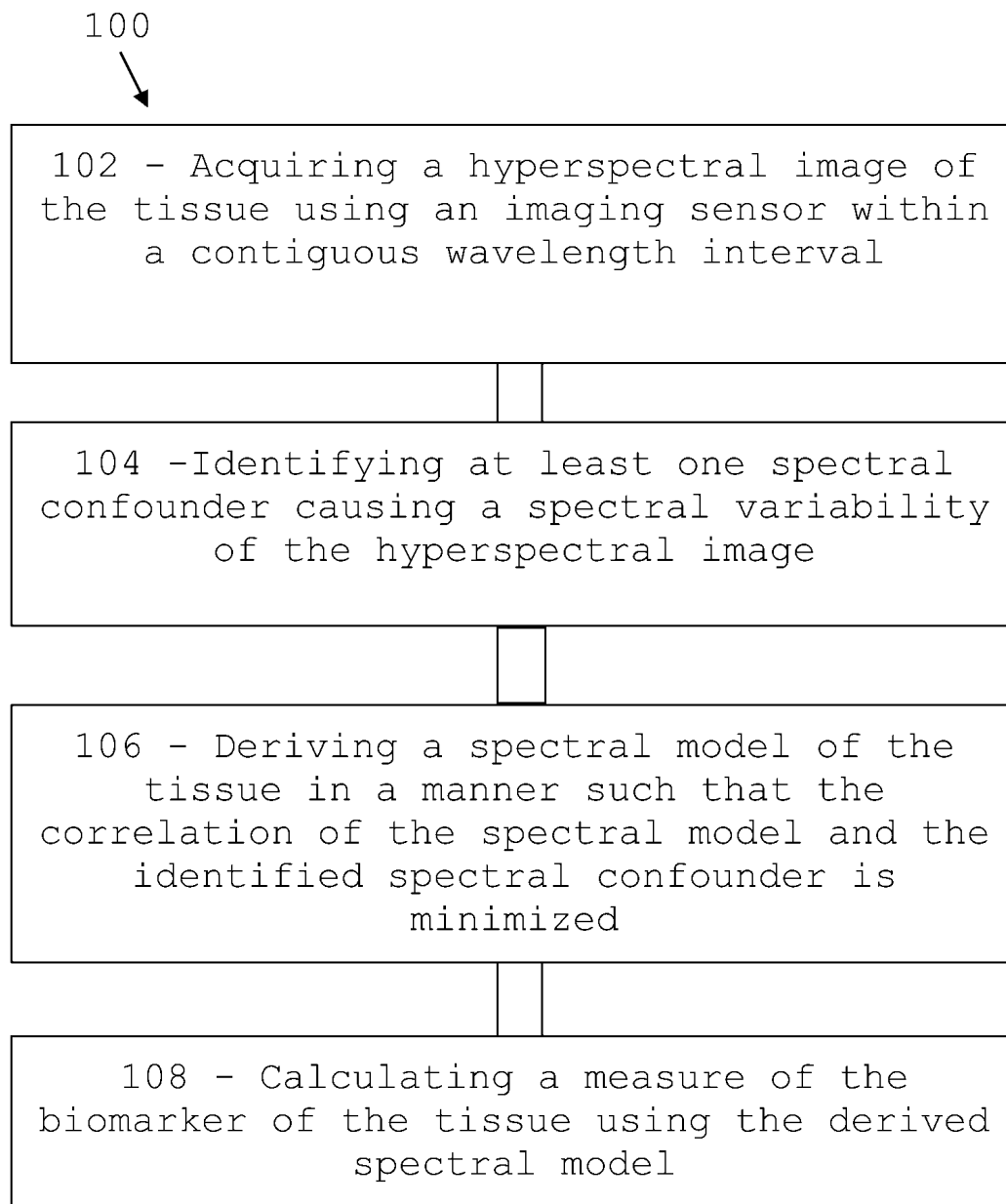


FIGURE 1

102 Acquérir une image hyperspectrale du tissu à l'aide d'un capteur d'imagerie dans un intervalle de longueur d'onde contigu

104 Identifier au moins un facteur parasite provoquant une variabilité spectrale de l'image hyperspectrale

106 Dériver un modèle spectral du tissu d'une manière telle que la corrélation du modèle spectral et du facteur parasite spectral identifié est réduite à un minimum

108 Calculer une mesure du biomarqueur du tissu à l'aide du modèle spectral dérivé

SUMMARY

[0006] Embodiments of the present invention provide techniques for quantifying a biomarker of a tissue using imaging and image data processing algorithms. In particular, aspects of the invention provide systems and methods to acquire and process hyperspectral image data from a biological tissue and quantify a biomarker in a tissue associated with a disease. Further aspects of the invention provide systems and methods to acquire multispectral image data from a tissue and process the image data to quantify the biomarker.

[0007] In accordance with a first aspect, the present invention provides a method for quantifying a biomarker of a tissue, the method comprising:

- acquiring a plurality of images of the tissue at discrete wavelengths within a main wavelength interval using an imaging sensor;

- determining, using a processor, at least two spectral sub-intervals in a manner such that combination of the image data in the at least two spectral sub-intervals is correlated with a clinical variable of interest; and

- calculating a measure of the biomarker of the tissue using the image data in the at least two spectral sub-intervals using the processor;

- wherein the main wavelength interval is broader than each of the at least two spectral sub-intervals and at least one spectral confounder causing a spectral variability of the acquired image is present in the tissue.

[0008] In accordance with a second aspect, the present invention provides A method for quantifying a biomarker of a tissue, the method comprising:

- determining, using a processor, at least two spectral sub-intervals from a plurality of images of the tissue acquired at discrete wavelengths within a main wavelength interval using an imaging sensor in a manner such that the combination of the image data in the at least two spectral sub-intervals is correlated with a clinical variable of interest;

- illuminating the tissue using one or more light sources with wavelengths within

the at least two spectral sub-intervals;

acquiring a measurement of the light reflected by the tissue using an imaging sensor; and

calculating a measure of the biomarker of the tissue using the acquired measurement;

wherein the main wavelength interval is broader than each of the at least two spectral sub-intervals and at least one spectral confounder causing a spectral variability of the acquired image is present in the tissue.

[0009] In an embodiment, the clinical variable of interest comprises any one or a combination of a disease, a severity or subtype of disease, a known biomarker or a physiological state.

[0010] In an embodiment, the main wavelength interval is such that the acquired images represent a hyperspectral or multispectral representation of the tissue.

[0011] In an embodiment, the method comprises acquiring a plurality of images comprising using one or more cameras operating at different wavelengths.

[0012] In an embodiment, the method comprises extracting a spectral parameter for each of the at least two spectral sub-intervals to form a multispectral representation of the tissue.

[0013] In an embodiment, the method comprises identifying at least one spectral confounder causing a spectral variability in the acquired images.

[0014] In an embodiment, determining at least two spectral sub-intervals is performed in a manner such that the spectral information of the combination of the at least two spectral sub-intervals and the spectral confounder are orthogonal, uncorrelated or independent.

[0015] In an embodiment, determining at least two spectral sub-intervals is performed by applying a band selection method, a machine learning algorithm or an artificial intelligence engine.

[0016] In an embodiment, the variable of interest comprises the amount or distribution of amyloid beta measured in a brain using positron emission tomography (PET), or measured

in cerebrospinal fluid (CSF).

[0017] In an embodiment, determining at least two spectral sub-intervals comprises using a multivariate statistics, machine learning or artificial intelligence techniques.

[0018] In an embodiment, calculating a measure of the biomarker uses multivariate statistics, machine learning or artificial intelligence techniques.

[0019] In an embodiment, the multivariate statistics include regression, logistic regression or discrimination.

[0020] In an embodiment, the machine learning or artificial intelligence techniques account for demographic information, clinical information, other variables of interest or multiple regions of interest in the image data.

[0021] In an embodiment, determining at least two spectral sub-intervals is performed by a machine learning algorithm that transforms multispectral data into a score that represents the biomarker.

[0022] In an embodiment, the method comprises applying an optical filter to the one or more light sources.

[0023] In an embodiment, the method comprises applying an optical filter along a light path of the light generated by the one or more light sources.

[0024] In an embodiment, the tissue is the retina of a person and the optical filter is embedded within a contact lens applied on an eye of the person.

[0025] In accordance with the second aspect, the present invention provides a system for quantifying a biomarker of a tissue, the system comprising:

an optical assembly comprising one or more light sources, the optical assembly being arranged to illuminate a portion of the tissue at wavelengths within at least two predetermined spectral intervals; and

an imaging assembly comprising an imaging sensor arranged to receive light reflected from the portion of the tissue and translate the light to digital data;

wherein the at least two predetermined spectral sub-intervals are specific to a known variable of interest.

[0026] In an embodiment, the system comprises a biomarker selection assembly arranged to select the at least two light spectral intervals based on a user selected biomarker of a tissue.

[0027] In an embodiment, the system comprises a processing module arranged to receive data from the imaging sensor and being configured to:

combine and process the data received from the imaging sensor to extract spectral information and form a multispectral representation of the tissue; and

process the multispectral representation of the tissue to quantify the biomarker.

[0028] In an embodiment, the tissue is a portion of the retina and the biomarker is a measure of amyloid beta (AB) for predicting/stratifying risk, screening or diagnosing Alzheimer's disease and the at least two spectral intervals consist of three wavebands located within about 100 nm of 475 nm, within about 100 nm of 545 nm, and within about 100 nm of 725 nm, and each with a bandwidth under about 200 nm.

[0029] In an embodiment, the optical assembly is arranged to generate light with a spectrum within the at least two spectral intervals.

[0030] In an embodiment, the illumination assembly comprises optical filters arranged to filter the generated light path or the reflected light path within the at least two sub-spectral intervals.

[0031] In an embodiment, the tissue is a portion of the retina and the variable of interest is brain or cerebrospinal fluid amyloid beta level related to Alzheimer's disease and the at least two spectral intervals consist of three wavebands located within about 100 nm of 475 nm, within about 100 nm of 545 nm, and within about 100 nm of 725 nm, and each with a bandwidth under about 200 nm.

[0032] In accordance with the third aspect, the present invention provides a method for quantifying a biomarker of a tissue, the method comprising:

acquiring a hyperspectral image of the tissue using an imaging sensor within a contiguous wavelength interval;

identifying at least one spectral confounder causing a spectral variability of the hyperspectral image;

deriving a spectral model of the tissue in a manner such that the correlation of the spectral model and the identified spectral confounder is minimized; and

calculating a measure of the biomarker of the tissue using the derived spectral model.

[0033] In an embodiment, the spectral model of the tissue is derived to be orthogonal, uncorrelated or independent to the identified spectral confounder.

[0034] In an embodiment, the spectral model is derived from the main spectral difference between two groups, each group having a distinct amount of the variable of interest.

[0035] In an embodiment, deriving a spectral model of the tissue comprises deriving a clean hyperspectral representation of the tissue by processing the hyperspectral representation of the tissue and a spectral signature of the at least one spectral confounder in a manner such that the clean hyperspectral representation of the tissue has reduced variability caused by the spectral signature of the at least one spectral confounder.

[0036] In an embodiment, acquiring an hyperspectral image of the tissue comprises sequentially illuminating the tissue, using one or more light sources, with monochromatic or narrow bandwidth light within a contiguous predetermined range of wavelengths and, for each wavelength, using the imaging sensor to acquire a measurement of the light reflected by the tissue.

[0037] In an embodiment, identifying at least one spectral confounder comprises reading from a spectral database one or more spectral profiles of spectral confounders and processing the spectral information from the hyperspectral image and the one or more spectral profile.

[0038] In an embodiment, deriving a clean hyperspectral representation comprises removing, from the spectral information, a spectral variability caused by the spectral profile

of the at least one spectral confounder.

[0039] In an embodiment, identifying at least one spectral confounder comprises using a specific spectral confounder as a reference cofounder and calculating a delta hyperspectral image which represents a pixel-by-pixel and wavelength-by-wavelength difference between two hyperspectral images.

[0040] In an embodiment, the spectral information is processed in accordance with the following steps:

- computing the inter-class spectral variability between groups that differ with respect to the variable of interest;

- extracting a first set of principal eigenvectors of the intra-class spectral database; and

- removing one or more eigenvectors using an orthogonal projection to produce an intermediate dataset in which the intra-class variability is substantially removed;

- wherein the biomarker is quantified using a spectral dimension reduction based on the clean spectral representation of the tissue.

[0041] In an embodiment, the spectral information is processed in accordance with the following steps:

- computing the inter-class spectral variability between distinct groups of the spectral database;

- extracting a first set of principal eigenvectors of the inter-class variability between the distinct groups;

- removing the inter-class variability using an orthogonal projection based on the first set of principal eigenvectors from a combination of the distinct groups to produce an intermediate dataset in which the inter-class variability is substantially removed;

- computing intra-class variability within the intermediate dataset;

extracting a second set of principal eigenvectors of the intra-class variability of the intermediate dataset; and

extracting a third set of principal eigenvectors of a plurality of clean spectral presentations in the statistical database;

wherein removing, from the interim spectral information, the spectral variability caused by the at least one non-specific confounder comprises removing, from the interim spectral information, the intra-class variability using an orthogonal projection based on the second set of principal eigenvectors to create the clean spectral representation of the tissue; and

wherein the biomarker is quantified using a projection based on the third set of principal eigenvectors on the clean spectral representation of the tissue.

[0042] In an embodiment, the at least one spectral confounder comprises at least one ocular confounder and the quantified biomarker indicates the presence or absence of a variable of interest in the retina of the person.

[0043] In an embodiment, the at least one ocular spectral confounder is comprised of variations in melanin content, cataract, discolouration of the lens, hemoglobin content, retinopathy, retinal pigment epithelial thickness or density, RNFL thickness, choroidal thickness, macular pigment content and/or a combination thereof.

[0044] In accordance with a fifth aspect, the present invention provides a system for quantifying a biomarker of a tissue, the system comprising:

an illumination assembly arranged to successively illuminate a portion of a tissue with monochromatic or narrow bandwidth light within a contiguous range of wavelengths;

an imaging assembly comprising an imaging sensor arranged to receive light reflected by the portion of the tissue and translate the light to digital data; and

a processing module and a memory module arranged to receive data from the imaging sensor and being configured to execute instructions to perform a method in accordance with the fourth aspect.

[0045] The present invention is suitable for any measuring any biomarker that is indicative of a disease state or predisposition for a disease. Examples include, but not limited to, amyloid beta for Alzheimer's , varying levels of cataract and melanin content in the retina within the subject (within the eye and between the two eyes), and between subjects, amyloid beta in the brain measured by PET scan, or amyloid beta in the cerebrospinal fluid, RNFL thickness, retinal thickness, blood within or beneath the retina.

[0046] The foregoing and other features will become more apparent upon reading of the following non-restrictive description of illustrative embodiments thereof, given by way of example only with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0047] Embodiments of the disclosure will be described by way of example only with reference to the accompanying drawings, in which:

[0048] Figure 1 shows a flow diagram with method steps used to quantify a biomarker of a tissue based on hyperspectral imaging;

[0049] Figure 2 shows spectral data before and after removing spectral effects from cataract and pigmentation;

[0050] Figures 3 and 10 show schematic diagrams of systems for quantifying biomarker of a tissue;

[0051] Figure 4 shows representative HS montages of four eyes showing the inherent variability for individuals with positive brain amyloid beta PET scans (Alzheimer's disease) and those without (PET negative controls) data within- and between-eyes due the retinal and choroidal vasculature, ocular pigment and clarity of ocular media (A-D); the sampled foveal locations, superior & inferior to the temporal vascular arcades with segmentation of the major inner retinal blood vessels (E) and the normalized reflectance spectra at the different sampling locations for controls and cases (F to K);

[0052] Figure 5 shows a graph of the estimation of two main spectral confounders for retinal imaging data from individuals with positive brain amyloid beta PET scans (Alzheimer's disease) and those without (PET negative controls). (A), a graph showing the

corrected reflectance spectra at a specified sampling location (B), a graph showing the P-values for two-sided unpaired t-tests between groups using false discovery rate control for significance across all the wavelengths (C), and a graph of the spectral model at the specified sampling location corresponding to the main spectral difference between the two groups (D);

[0053] Figure 6 shows a graph depicting the discrimination between individuals with positive brain amyloid beta PET scans (Alzheimer's disease) and those without (PET negative controls) using HS scores at the different sampling locations;

[0054] Figure 7 shows graphs depicting how the HS scores are able to discriminate between individuals with positive brain amyloid beta PET scans (Alzheimer's disease) and those without (PET negative controls) in validation datasets.

[0055] Figure 8 shows in (A) correlation between HS scores of the study and fellow eye, and in (B) correlation between HS scores prior to and following cataract surgery;

[0056] Figure 9 shows a flow diagram with method steps used to quantify a biomarker of a biological tissue based on multispectral imaging;

[0057] Figure 10 shows a schematic diagram of a system for quantifying a biomarker of a biological tissue, based on multispectral imaging;

[0058] Figure 11 shows a schematic diagram of retinal multispectral imaging with bandpass filter(s) in different positions in the optical light path of a fundus camera which has a broadband light source and a conventional color array at an imaging sensor;

[0059] Figure 12 shows a schematic diagram of retinal multispectral imaging using a light source and customized and modified filter array at an imaging sensor;

[0060] Figure 13 shows a schematic diagram of retinal multispectral imaging using illumination with relatively narrow band(s) of light and a conventional color array at an imaging sensor;

[0061] Figure 14 shows a schematic diagram of retinal multispectral imaging using sequential waveband illumination with relatively narrow band(s) and an imaging sensor;

[0062] Figure 15 shows selection of three wavebands for retinal multispectral imaging for

Alzheimer's disease. ADSF showing which wavebands (Filter 1, 2, 3) are utilized in this scenario of detecting amyloid in the retina for the dataset examined;

[0063] Figure 16 shows association between scores obtained with hyperspectral (HS) and multispectral (MS) retinal imaging of individuals with positive brain amyloid beta PET scans (Alzheimer's disease) and those without (PET negative controls);

[0064] Figures 17 shows the representation of contrast enhancement of the MS score image (right) compared to the conventional colour (RGB) image (left) respectively from two patients with glaucoma (A and B), and the comparisons of contrast enhancement achieved between the scores from each separate RGB colour channel from the conventional photograph, the score from the RGB fundus image, and the score from the MS image (C);

[0065] Figure 18 shows an image of the retinal nerve fibre layer (RNFL) thickness measured by OCT (A), an image of the RNFL thickness estimated using MS imaging with three spectral intervals (B), and a graph showing the correlation between RNFL thickness measured by OCT versus thickness estimated using MS imaging (C);

[0066] Figure 19 shows that subretinal and intraretinal fluid is difficult to detect on conventional RGB fundus images as depicted in (A). Fluid is clearly apparent using MS imaging (B);

[0067] Figure 20 shows that choroidal neovascularization (CNV) is often difficult to detect with conventional RGB fundus imaging as depicted in (A). CNV is more apparent using MS imaging (B); and

[0068] Figure 21 is a graph depicting age prediction on the basis of retinal MS images.

DETAILED DESCRIPTION

[0069] Various aspects of the present disclosure generally address one or more of the problems related to the presence of confounders that may impair the performance of medical imaging techniques.

[0070] Broadly stated, a confounder, also called a confounding factor, can be any factor that impacts the content and/or quality of a biometric measurement when this factor is not

directly related to the quantity or variable that is intended to be measured. In the particular case of hyperspectral (HS) or multispectral (MS) imaging of the retina of a subject, where it is desired to acquire spectral information about the retina or about a part of the retina, various factors present in the eye of the subject may impact this spectral information. For example, a subject having blue eyes has very little melanin in the stroma of his/her iris and fundus. In contrast, the stroma of a subject having brown eyes has a high melanin content. The melanin content tends to alter the spectral content of the HS image, this alteration being unrelated to the actual condition of the retina. Without limitation, other confounders may include cataract, discolouration of the lens, blood hemoglobin levels and thickness, retinopathy, spectral density, RNFL thickness, and macular pigment content. A given subject may have any number of these confounders.

[0071] One aspect of the technology described herein involves obtaining a hyperspectral image of a biological tissue, for example the retina of a subject, and alleviating the effects of one or more potential ocular confounders on this image by using statistical information obtained from a plurality of subjects, some of whom having similar spectral confounders. A spectral representation of the biological tissue is obtained in view of quantifying a biomarker, for example in view of determining the presence of amyloid beta in the retina of a subject. Alternatively, a multispectral image of a biological tissue, for example the retina of a subject can be taken by selecting appropriate wavelength intervals that allow alleviating the effects of one or more potential ocular confounders. Numerical parameters are associated with each spectral interval to quantify a physiological parameter, for example in view of determining the presence of amyloid beta in the retina of a subject.

[0072] In Alzheimer's disease (AD), for example, a protein called amyloid beta accumulates in the brain and in the eye over many years. While there are tests that can be used to confirm a diagnosis of AD once memory impairment occurs, these tests are costly, not widely available and invasive. None of these tests are used to screen people without memory impairment to identify those at risk of the disease. This is important as if treatments are to be effective for AD, it is likely that they need to be started before the disease is too advanced.

[0073] Referring now to Figure 1 there is shown a flow diagram 100 with method steps

used to quantify a biomarker of a tissue starting from a HS images. At step 102, at least one hyperspectral image of the tissue is acquired using an imaging sensor within a contiguous wavelength interval. At step 104, at least one spectral confounder causing a spectral variability of the hyperspectral image is identified. At step 106, a spectral model of the tissue is derived in a manner such that the correlation of the spectral model and the identified spectral confounder is minimized. At step 108, a measure of the biomarker of the tissue is calculated using the derived spectral model.

[0074] A preferred way of deriving the spectral model required the calculation of a clean HS representation of the biological tissue. This representation can be calculated by processing the HS data with a spectral signature of the at least one spectral confounder with the aim of minimising the variability caused by the spectral signature of the at least one spectral confounder.

[0075] The HS image comprises a plurality of images of the retina of the subject, each of the images being obtained at a distinct wavelength. The image may be pre-processed and the pre-processing can include, for example, a normalization of light intensities at the various wavelengths of the HS image. Image registration may take place to ensure that pixels of images taken at any given wavelength are aligned with corresponding pixels of images taken at any other wavelength so that corresponding pixels represent a same element of the retina of the subject. The registration can overcome misalignments caused by a movement of the eye of the subject as the images are taken at the various wavelengths. Co-registration between images of the retina of the subject taken at different times may take, for example, when images are taken before and after an intervention.

[0076] A preferred way of deriving the spectral model required the calculation of a clean HS representation of the biological tissue. This representation can be calculated by processing the HS data with a spectral signature of the at least one spectral confounder with the aim of minimising the variability caused by the spectral signature of the at least one spectral confounder.

[0077] The HS image comprises a plurality of images of the retina of the subject, each of the images being obtained at a distinct wavelength. The image may be pre-processed and the pre-processing can include, for example, a normalization of light intensities at the

various wavelengths of the HS image. Image registration may take place to ensure that pixels of images taken at any given wavelength are aligned with corresponding pixels of images taken at any other wavelength so that corresponding pixels represent a same element of the retina of the subject. The registration can overcome misalignments caused by a movement of the eye of the subject as the images are taken at the various wavelengths.

[0078] The HS image of the retina may be segmented in order to identify a specific region of interest (ROI). For example, the segmentation may lead to selecting a specific region of a biological tissue, such as the retina, of the subject as the ROI when it is desired to determine whether or not amyloid beta is present in the tissue of the subject.

[0079] To study the variability of a specific spectral signal of a given group at different times, for example when images are taken before and after cataract surgery, to evaluate a specific confounder such as, in this example, discoloration of the natural lens, the specific spectral signal of this confounder may be removed in the evaluation of AD or of another disease. The variability of the specific spectral signal within a group of subjects having undergone cataract surgery can be determined, for example, using non-negative matrix factorization (NMF), principal component analysis (PCA), linear discrimination analysis (LDA), singular value discrimination (SVD) or unmixing. These techniques enable extracting meaningful spectral features from the HS images, these spectral features being related to the actual confounder (e.g. cataract) and explaining a large percentage of variability of HS image spectra. Effects of such spectral features can be eliminated from various HS images so that the resulting data can be evaluated for AD and/or other illnesses, the effects of the specific confounder being effectively minimized or removed.

[0080] To remove the effects of a specific confounder, a so-called “cube” is calculated. The cube is a representation of a HS image in which a plurality of single wavelength 2D images are stacked on top of one another, a third dimension being defined by the cumulative wavelengths of the images, thereby forming a 3D representation, or “cube”.

[0081] HS imaging of the eye involves receiving spectral information from all the ocular structures the light interacts with before returning to the sensor. To reduce inter-subject, within subject, inter-session variability to determine the different biomarkers in the retinal

tissue across the population, this allows for development as a proper screening tool and also use for longitudinal monitoring. Biomarkers include but are not limited to, varying levels of cataract and melanin content in the retina within the subject (within the eye and between the two eyes), and between subjects.

[0082] Referring now to figures 2A to 2C, the specific confounder corresponding to a cataract was obtained by imaging using the hyperspectral camera n=9 patients before and after undergoing cataract surgery. The hyperspectral images were co-registered so that the same pixels in both images corresponded to the same retinal locations. The HS image corresponding to the ratio before and after cataract surgery was computed and the resulting images were log transformed. Regions of the HS image with artifacts due or outliers were excluded and the remaining data fed into a principal component analysis. The principal axis responsible for most of the data variance was saved into memory.

[0083] The specific confounder corresponding to pigmentation was obtained by imaging n=7 healthy participants with various degrees of retinal pigmentation and no visible signs of a cataract on clinical examination. Spectral data from the hyperspectral images were combined, log transformed and fed into a principal component analysis. The principal axis responsible for the largest source of data variance was saved into memory.

[0084] Hyperspectral data from n=9 patients with various degrees of retinal pigmentation and visible cataract on clinical examination were acquired and log transformed (figure 2a to c, black curves). For each participant, the spectral data from the foveal region of the retina was sampled for illustration of the cleaning procedure. This spectral data was first projected orthogonal to the main spectral effect of cataract removal figure 2A), to the main spectral effect of retinal pigmentation removal (figure 2B), and to both spectral confounders (figure 2C).

[0085] Referring now to figure to Figure 3, there is shown a schematic diagram of a system 300 for quantifying a biomarker of a biological tissue, based on HS imaging. The system has an optical illumination assembly 310 which provides illumination of a portion of a biological tissue with monochromatic light within a contiguous range of wavelengths. The optical assembly 310 comprises one or more light sources. For each wavelength, the generated light 312 is directed from the optical assembly 310 towards the biological tissue

which, in the example of Figure 2, is retinal tissue 314. For each wavelength, an imaging assembly 316 receives light reflected by the portion of the retinal tissue and translates the light to digital data.

[0086] A processing module 318 is provided to receive data from the imaging sensor 316 and combine and process the data received from the imaging sensor to extract spectral information and form a hyperspectral representation of the biological tissue. In addition, the processing module 318 retrieves, from a memory, a spectral signature of at least one image confounder and derives a spectral model of the biological tissue by processing the hyperspectral representation of the biological tissue and a spectral signature of the at least one spectral confounder. The spectral model is calculated by the processing module so that the correlation with the cofounder is minimized or, preferably, to be orthogonal to the identified spectral confounder. Furthermore, the processing module processes the hyperspectral representation of the biological tissue based on the spectral model to quantify the physiological parameter.

The processing module 318 can be configured to communicate with an external statistical database, through a communication interface and a communication network to retrieve spectral signatures of the at least one image confounder.

[0087] Whilst the imaging sensor module 316 shown in figure 3 includes synchronized filters, it should be appreciated that the filters could also be placed just after the contiguous light source, or elsewhere in the optical pathway.

[0088] The optical illumination assembly 310 can be configured to provide light between 400nm to a 1000nm. The light sources can be provided as wide band LEDs, a xenon flash light sources, tungsten sources, supercontinuum lasers and can be used in cooperation with wavelength selection mechanism, such as a synchronized filter.

[0089] Referring now to figure 4, there is shown the natural spectral variation between eyes that precludes discrimination between cases and controls with respect to amyloid beta status on PET. (A-D) Representative HS montages of 4 eyes (n = 2 controls, n = 2 cases from 450 – 900 nm in 5 nm steps) showing the inherent variability within- and between-eyes due to the retinal and choroidal vasculature, ocular pigment and clarity of ocular

media. (E) Systematic sampling method used to analyse HS images including two foveal locations (F1, F2), superior (S1, S2) & inferior (I1, I2) to the temporal vascular arcades with segmentation of the major inner retinal blood vessels. (F-K) Normalised reflectance spectra at the different sampling locations for controls ($n = 20$, darker) and cases ($n = 15$, lighter) highlighting the large degree of inter-subject variability using uncorrected spectral data, which precludes discrimination between cases and controls. The data is shown as mean $\pm$ SEM.

[0090] To account for within-subject variability and avoid selection bias, six regions of the retina based on well-defined anatomical landmarks were systematically sampled. The foveola was sampled using a circular area of interest (60-pixel diameter) centered on the fovea (F1). The parafoveola was sampled using an annulus (100 – 200 pixels in diameter) centered on the fovea (F2). Areas in the temporal vascular arcades superior (S1) and inferior to the fovea (I1) as well as superior (S2) and inferior to the optic nerve head (I2) were sampled using squares (200 x 200 pixels), on a fixed template horizontally orientated with the temporal raphe.

[0091] In each sampling location, blood vessels visible in the inner retina are automatically segmented using the green channel of the false-colored image. A difference of Gaussian (DoG) image was calculated using variance parameters 1 and 20 to increase contrast between the blood vessels and background whilst removing noise and artefacts. To be conservative, 40% of pixels in the DoG image with the highest intensity are considered to be blood vessels and were excluded from analysis. Reflectance spectra normalised to the average spectrum of a first cohort are displayed in figures F to K, highlighting the difference in spectra observed at the 6 sampling locations due to variations in retinal structures at each location. A large degree of inter-subject spectral variability is found. Although a trend can be observed in every location, no statistically significant difference is found between cases and controls on the basis of uncorrected reflectance data. These findings indicate that the effects of variability in key determinants of ocular reflectance must be accounted for before meaningful comparisons can be made between individuals.

[0092] Referring now to figure 5, there is shown how the spectral confounder and the spectral model are derived. (A) shows the estimation of two main spectral components

of within-subject variability. These components could be largely explained by a combination of spectra from known ocular constituents and can be used to correct each reflectance spectrum. (B) depicts corrected reflectance spectra at sampling location S1 ($n = 20$ controls, $n = 15$ cases, data shown as mean $\pm$ SEM) unmasked the spectral difference between the two groups. (C) shows P-values for two-sided unpaired t-tests between groups using false discovery rate (FDR) control for significance across all the wavelengths (n.s. stands for non-significant). And in (D) is depicted the spectral model at sampling location S1 corresponding to the main spectral difference between the two groups.

[0093] To transform the multiplicative interaction between retinal structure and reflectance, measured spectra can first be log-transformed. The average spectrum at each sampling location can then be computed and smoothed using spectral smoothing procedure such as a Savitzky-Golay filter with a 5th order kernel of width of 13. At each location, a single HS score is obtained by combining the spectral variables using Dimension Reduction by Orthogonal Projection for Discrimination (DROP-D). This linear discrimination method was used to derive the HS scores as it is robust to over-fitting and well-suited to problems in which the number of observations (participants) is small compared to the number of variables (wavelengths). In brief, DROP-D works in two steps. First, it estimates (A) and removes (B) the main spectral axes of within-class variability from the data, i.e., spectral sources of variability that are detrimental to the discrimination problem. Second, it derives a model (D) by computing the principal spectral axis of between-class variability on the corrected data. A HS score (single number) for a given spectrum is obtained by taking its inner product with the spectral model. Note that by construction, the HS scores are not sensitive to any linear combination of the removed within-class axis. To further avoid over-fitting, leave-one-out cross-validation was used to identify the number of spectral axes to optimise discrimination between groups.

[0094] Referring now to Figure 6 there is shown the discrimination between individuals with positive brain amyloid beta PET scans (Alzheimer's disease, cases) and those without (PET negative controls) using retinal HS scores. HS scores obtained at the different sampling locations were on average higher for the cases compared to controls ($F_{1,33} = 7.1$, $p = 0.01$, two-way repeated measures ANOVA). Error bars represent 95% CI of the

mean for each group. Pairwise comparisons show significant differences between sampling locations F1 and S1.

[0095] The spectral model derived for each sampling location was used to derive a HS score for each participant. Overall, these HS scores were higher in cases compared to controls across all retinal locations ($F_{1,33} = 7.1$, $p = 0.01$, two-way repeated measures ANOVA), indicating a general spectral difference between cases and controls, which was only visible after correcting for the inherent spectral variation between eyes.

[0096] Using pairwise comparisons at each location, it was shown that the greatest difference between groups was found at the S1 sampling location ($p = 0.002$, 95% CI: 0.06 – 0.22). This is consistent with the findings of post-mortem retinal immunohistochemical studies which indicate that amyloid beta accumulation appears to be greatest in the superior retina. Significant differences between cases and controls were also found at the F1 location.

[0097] Referring now to Figure 7, there is shown how HS scores effectively discriminate between individuals with positive brain amyloid beta PET scans (Alzheimer's disease) and those without (PET negative controls) in validation datasets.

[0098] Validation of the spectral model. (A) Hyperspectral (HS) score obtained for each dataset (mean about 95% CI for each group). HS images of the principal cohort (study eye and fellow eye) and the validation cohort were acquired on two different cameras of the same model, using the same imaging methods. Significant differences (two-sided unpaired t-tests controlled for false discovery rate for each dataset) were obtained between cases and controls in both cohorts. (B) Receiver operating characteristic curve (ROC) and area under the curve (AUC) for the principal cohort (black) and for the validation cohort (orange) show good discrimination between cases and controls. (C) Scatterplot of quantitative PET amyloid beta load and HS score showing significant positive correlation between the two metrics.

[0099] Referring now to Figure 8, there is shown robust agreement between eyes of the same individual despite lens status (presence of natural or artificial lens) following the application of the processing method described.

[00100] Figure 8A depicts correlation showing a good agreement between the HS scores of the study and fellow eye. Figure 8B depicts correlation showing good agreement between the HS scores pre- and post-cataract surgery for individuals, following the application of the processing method described.

[00101] To assess the robustness of the model to the presence of lens opacification (cataract) and artificial intraocular lenses, a cohort of 10 participants scheduled to undergo elective outpatient cataract surgery were studied. Retinal HS images were taken before and 8 weeks after cataract surgery. The spectral model obtained from the principal cohort was used to derive the HS scores pre- and post-cataract surgery. Differences in HS scores pre- and post-operatively were tested using a paired two-sided t-test. The agreement of the HS scores pre- and post-operatively was estimated using Pearson's correlation coefficient.

[00102] To test the robustness of the model, the HS scores from the study and fellow eyes of each individual in the principal cohort were correlated. In some individuals, the fellow eye had more ocular pathology (e.g., presence of drusen) than the study eye. Despite differences between some fellow and study eyes, there was a significant correlation ($r = 0.6$, $p = 0.0002$, 95% CI = $0.32 - 0.78$) between the HS scores for study and fellow eyes (Figure 8A), indicating that the HS score is robust to a degree of variation in ocular pathology.

[00103] In addition to this, the effect of lens status on the HS score was investigated. Individuals undergoing routine surgery were recruited for visually significant cataracts and imaged them prior to and following cataract extraction and artificial intraocular lens implantation. No differences were found in HS score pre- and post-operatively ($p = 0.88$, 95% CI = $-0.15 - 0.18$, two-sided paired t-test). Importantly, a strong correlation was found between HS scores measured pre- and post-operatively ($r = 0.8$, $p = 0.01$, 95% CI: $0.23 - 0.94$, Figure panel B of figure 8), indicating that lens status (cataract or artificial intraocular lens) had minimal effect on HS scores.

[00104] Referring now to Figure 9, there is shown a flow diagram 800 with method steps used to quantify a biomarker of a tissue based on multispectral imaging.

[00105] Multispectral imaging is used for detection of biomarker(s), e.g. amyloid in the retina. In some embodiments, multispectral imaging is advantageous in scaling down from HS imaging, for example, in the cost of device, in the complexity of data acquisition and data analysis.

[00106] At step 902 at least two spectral sub-intervals are determined, using a processor, from a plurality of images of the tissue acquired at discrete wavelengths within a main wavelength interval using an imaging sensor. The sub-intervals are determined so that the combination of the image data in the at least two spectral sub-intervals is correlated with a clinical variable of interest.

[00107] At step 904, the tissue is illuminated, using a one or more light sources with wavelengths within the at least two spectral sub-intervals. At step 906, an image of the reflected light is acquired using an imaging sensor. At step 908, a spectral parameter is extracted for each of the at least two spectral intervals to form a multispectral representation of the biological tissue. At step 910, a measure of the biomarker of the tissue is calculated using the acquired measurement.

[00108] The plurality of images of the tissue acquired at discrete wavelengths within a main wavelength interval could consist of one or more hyperspectral images of different participants and/or the same participant at different times. Extraction of spectral data from the image(s) with potential average over a predefined region of interest. Establishing one or more dependent variables using for example clinical data (e.g., burden of disease, presence or absence of disease) and/or demographic (e.g., age, sex) data. Feeding the spectral data and the dependent variables into an ADSF algorithm (and/or other variable/wavelength/spectral interval selection algorithms). The output of such algorithms corresponds to an optimized spectral interval(s) for a given prediction problem. Establishing the optimal combination of spectral interval which may involve using the dependent variables, statistical modeling techniques (multivariate regression, logistic regression, discriminant analysis or the like) and/or machine learning techniques (Support vector machine, Partial least squares, Artificial neural networks, or the like). The optimal combination may also involve the use of a previously recorded image, spatial information present in the image (segmentation of blood vessel, location of optic nerve head, fovea,

texture analysis) or other images taken with other multispectral, hyperspectral or other medical imaging (e.g., OCT, PET scan) and non-imaging modalities, such as clinical data (age, blood pressure, questionnaires).

[00109] Referring now to Figure 10, there is shown a schematic diagram of a system 950 for quantifying a physiological parameter of a biological tissue, based on multispectral imaging.

[00110] The system has an optical illumination assembly 952 which provides illumination to a portion of a biological tissue 954, in this case a portion of the retina of an eye. Assembly 952 comprises one or more light sources being arranged to illuminate a portion of the tissue at wavelengths within at least two predetermined spectral intervals.

[00111] The spectral intervals are selected by a biomarker selection assembly based on the biomarker being investigated. In some embodiments, the system may be setup with a specific optical illumination assembly suitable for investigating one or more specific biomarkers. In these instances the biomarker selection assembly is not present.

[00112] The generated light is directed from the illumination assembly 952 towards the biological tissue 954. For each wavelength, an imaging assembly 956 receives light reflected by the portion of the biological tissue and translates the light to digital data. The data can be locally processed by the system's processor 958 or made available for processing, in accordance with the methodologies described herein, by the system.

[00113] The system 950 may also be able to save image data locally and to a central database. The system may update the prediction capability (machine learning) with the accumulation of new data. The system may also be able to tell which multispectral filter is used/introduced at time of acquisition and send this information to the local/central database. The system may be able to measure/collect/store/transfer spectral and/or spatial calibration data. The system could have a subsystem to help alignment of the imaging system with the patient eye (with or without feedback to user and/or indication to move the camera in/out, left/right, up/down). The system may have a subsystem that manually or automatically focus the image of the retina received by the imaging sensor.

[00114] In some embodiments, retinal imaging using only selected wavebands

may have potential to achieve similar results to HS imaging (i.e. confirmation of PET status/disease state). Thus, this may produce an opportunity to create a downscaled device but with similar capabilities of the HS camera at detecting biomarkers or diagnostic features of various diseases such as Alzheimer's disease.

[00115] In some embodiments, the apparatus, device/camera, system, and method herein can acquire images at select wavebands. In some embodiments, the customized apparatus, device/camera, system, and method herein can acquire images at select wavebands, optionally with various options of improvements using existing devices/cameras. Nonlimiting examples of such improvements include: (1) adding onto an existing camera a custom filter set (e.g. a single multi bandpass filter or multiple filters allowing multiple bandpass at specified wavebands), which can be placed at but not limited to the positions along the optical path of the camera as shown in Figure 11 (the filter(s) may be alternatively placed at other positions along the optical path including in a contact lens position of the cornea of an individual not shown in Figure 11); (2) building a custom device/camera which for example may include any combinations of: (a) modified imaging sensor and/or modified color filter array, (b) modified light source, and/or (c) sequential waveband illumination as shown in Figures 12-14; and (3) a camera that is a combination of options from (1) and (2).

[00116] In some embodiments, one or more multispectral images using the different light wavebands can be combined and/or analysed together to improve diagnostic ability and/or robustness (e.g. an Alzheimer's disease filter for amyloid beta and an Alzheimer's disease filter for tau; an Alzheimer's disease filter for amyloid beta and a filter for different types of intraocular lenses).

[00117] In some embodiments, a measure of the biomarker (for example, a multispectral score) is obtained at the point of care, optionally within a short time period, using the devices, systems, and methods herein. In some embodiments, obtaining a measure of the biomarker includes acquiring one or more images of a subject's retina using the device/system herein and/or analyzing the image data acquired during a single or multiple imaging sessions if required. In some embodiments, the measure of the biomarker can be tracked over time by acquiring one or more images of a subject's retina using the

device/system herein and/or analyzing the image data acquired over multiple imaging sessions if required. In some embodiments, such longitudinal studies over time may facilitate monitoring of progression of disease and/or evaluation of treatment over time.

[00118] In some embodiments, ADSF is used to determine the wavebands required from the raw reflectance hyperspectral data as shown in Figure 15A. In this scenario for detection of amyloid in the retina, at least three wavebands are selected. The spectral information derived from these three bandpass filters of varying shape and width are combined in a weighted fashion (black trace) to derive a multispectral score. ADSF may not be the only method that can be used to determine the wavebands to use for multispectral imaging. Additionally, the shape/profile of the wavebands (e.g., an approximate Gaussian distribution), may change (as shown in Figure 15B with the example of a rectangular-shaped band) as well as the number of wavebands required and its bandwidth (Figure 15C) and/or center frequency can vary. In some cases, the wavebands, aside from differing in number may also have differing widths. In some embodiments, the wavebands may not overlap with each other as in Figures 15B-15C. In some embodiments, two or more of the light wavebands partially overlap e.g. Filters 1 and 2. In some embodiments, none of the specified light wavebands overlaps with other light wavebands. In some embodiments, none of the specified light wavebands comprises a center frequency that is approximately the same as another one of the specified light waveband. In some embodiments, the center frequency, and/or the full width half maximum bandwidth of each of the specified light wavebands varies based on population, age, presence of confounding factors or other factors. In some embodiments, the wavebands of filters are applied simultaneously during light generation, image acquisition. In some embodiments, the wavebands of filters are applied in sequence during light generation, image acquisition, or image analysis.

[00119] In some embodiments, the spectral information determined from these wavebands is mathematically combined in a weighted fashion. In some embodiments, a score (measure of the level of biomarker) is computed from the weighed combination to describe the spectral information from the selected wavebands, optionally in a single number. In this case of imaging Alzheimer's disease participants, the multispectral imaging

score is linked to retinal amyloid beta load.

[00120] The computed biomarker levels can be compared to the HS scores as in Figure 15. In this case, despite only using 3 wavebands to derive the the measure of biomarker levels from the raw reflectance data (using only a few select wavelengths with a wider band, the the measure of biomarker level closely resembles that derived using HS), strong agreement is found between the HS and multispectral imaging scores ($r = 0.89$, $p = 8.1 \times 10^{-13}$) suggesting that the the measure of biomarker level, herein as a downscaled version using spectral information from less wavelengths, is a good approximation of that derived from the full hyperspectral data.

[00121] Spectral changes occurring in a biological tissue such as the retina can be used to detect changes in a range of disease not limited to Alzheimer's disease. In some embodiments, the number/bandwidth of wavebands and the wavelengths may be the same or different to what can be used in multispectral imaging of AD. Other than AD, applications of multispectral imaging may include but are not limited to: detecting structural changes to retinal tissue in eye conditions such as (but not exclusive to) glaucoma, age-related macular degeneration and diabetic retinopathy. Multispectral imaging may also be used in other conditions such as Lewy body dementia, traumatic brain injury, Parkinson's disease, and detecting heavy metals in tissue and drug and/or toxin deposition.

[00122] In addition to the aforementioned embodiments, the inventors determined that increasing the contrast of the optic nerve neuroretinal rim (the neural tissue lining the edge of the optic disc with the cup in the centre) from the cup via multispectral imaging, allows for easier detection of tissue loss than conventional fundus photography for screening/detection and longitudinal monitoring of diseases causing retinal nerve fibre layer loss such as, but not exclusive to, glaucoma.

[00123] In this embodiment, hyperspectral images of $n=7$ patients with established glaucoma were taken. In each image the spectra for ten points on the neuroretinal rim and ten from the central cup were extracted. Using these spectra, it was estimated (using the ADSF method) which spectral intervals would be optimal for discriminating the neuroretinal rim from the cup. Spectral intervals were then selected from the hyperspectral image to obtain a multispectral image. Thereafter, the multispectral channels were combined to

provide a score image exhibiting increased contrast between the neuroretinal rim and cup. Figures 17a and 17b each show a representation of the increased contrast of the neuroretinal rim which was derived from the MS score image compared to conventional fundus photography for two respective patients with glaucoma. Statistical comparisons of the change in contrast between neuroretinal rim and cup was performed between: each channel of the RGB image, the score image derived from combining the RGB channels and the score image derived from the multispectral image (Figure 17c).

[00124] As is evident, in the conventional RGB image, the contrast is greater in the blue channel compared to the green and red. Combining the RGB channels together is only as good as the blue channel on its own. The MS image contrast is significantly larger than that obtained from the conventional RGB image, allowing for easier discrimination of the neuroretinal rim.

[00125] The inventors have found that the methodology in accordance with the invention can be used to estimate the RNFL tissue thickness from a multispectral image. To test the proposition, the retina of a patient with glaucoma was imaged with Optical Coherence Tomography (OCT) and the hyperspectral camera. The thickness of RNFL layer was segmented by the OCT camera software and as illustrated in figure 18a, a pseudo-coloured tissue thickness map was created of the OCT image (top left panel). This OCT-derived thickness map was co-registered with the hyperspectral image. Every pixel of the hyperspectral data for which an RNFL thickness was available from the OCT thickness map was extracted and fed into the ADSF method to estimate the 3 optimal spectral intervals for RNFL thickness estimation.

[00126] These spectral intervals were then selected from the hyperspectral image to obtain a multispectral image. Multivariate linear regression using the OCT thickness as the dependent variable and the channels of the MS image as explanatory variables was subsequently computed. Figure 18b (bottom left panel) shows a pseudo-coloured map of the estimated RNFL thickness from the MS image is shown in the figure. Figure 18c shows the pixel-wise correlation between the thickness measured by OCT and that estimated using MS imaging.

[00127] In a further embodiment of the invention, the inventors have determined that the

methodology using MS imaging can be applied to detect retinal/subretinal fluid. To test the application of the methodology, a patient with diabetic retinopathy and retinal fluid, in the form of macular oedema, visible on OCT was imaged with the hyperspectral camera. Spectra were selected in the area where fluid was present (based on the OCT image) and in adjacent retinal tissue where there was not. The 3 spectral intervals that were optimal for increasing the visibility between retinal locations with and without fluid were selected using ADSF. A MS image was obtained from the hyperspectral image using these spectral intervals. A MS score image was computed by projecting the MS channels on the subspace that optimally discriminate between the affected and non-affected locations of the retina. Figure 19a illustrates a conventional fundus image, as is evident, it is almost impossible to determine the location of fluid in the retina however as is clearly visible in figure 19b, it becomes obvious when observing the multispectral score image.

[00128] To test the proposition that the methodology was able to be used to validate new blood vessel growth originating from the choroid (choroidal neovascularization [CNV]), a patient with age-related macular degeneration (AMD) and CNV development, visible on a conventional OCT was imaged with the hyperspectral camera.

[00129] Spectra were selected in the area where the CNV was present (based on OCT images) and in adjacent retinal tissue where CNV was absent. The 3 spectral intervals that were optimal for increasing the visibility between retinal locations with and without CNV were selected using ADSF. A MS image was obtained from the hyperspectral image using these spectral intervals. A MS score image was computed by projecting the MS channels on the subspace that optimally discriminated between the affected and non-affected locations of the retina. As depicted in Figure 20a it is difficult to determine the location of CNV with conventional fundus photography but it becomes obvious when observing the multispectral score image (see figure 20b).

[00130] In a still further embodiment, the methodology is able to be used to provide a retinal biomarker of ageing. To validate the application of the methodology, thirty nine patients were imaged using the hyperspectral camera. The average macular spectral data was extracted for each patient. The 4 spectral intervals that were optimal for predicting patients age were selected using ADSF. A MS image was then obtained from the

hyperspectral image using these spectral intervals, and a MS score image was computed by projecting the MS channels on the subspace that was optimal for predicting the patient's age. The predicted age using the MS score at the sample location was used to create the illustrated prediction model shown in figure 21 which is plotted against the real patient's age.

[00131] In accordance with the present disclosure, the components, process operations, and/or data structures described herein may be implemented using various types of operating systems, computing platforms, network devices, computer programs, and/or general purpose machines. In addition, those of ordinary skill in the art will recognize that devices of a less general purpose nature, such as hardwired devices, field programmable gate arrays (FPGAs), application specific integrated circuits (ASICs), or the like, may also be used. Where a method comprising a series of operations is implemented by a computer, a processor operatively connected to a memory, or a machine, those operations may be stored as a series of instructions readable by the machine, processor or computer, and may be stored on a non-transitory, tangible medium.

[00132] Systems and modules described herein may comprise software, firmware, hardware, or any combination(s) of software, firmware, or hardware suitable for the purposes described herein. Software and other modules may be executed by a processor and reside on a memory of servers, workstations, personal computers, computerized tablets, personal digital assistants (PDA), smartphone, and other devices suitable for the purposes described herein. Software and other modules may be accessible via local memory, via a network, via a browser or other application or via other means suitable for the purposes described herein. Data structures described herein may comprise computer files, variables, programming arrays, programming structures, or any electronic information storage schemes or methods, or any combinations thereof, suitable for the purposes described herein.

[00133] The present disclosure has been described in the foregoing specification by means of non-restrictive illustrative embodiments provided as examples. These illustrative embodiments may be modified at will. The scope of the claims should not be limited by the embodiments set forth in the examples, but should be given the broadest interpretation

consistent with the description as a whole.

TERMS AND DEFINITIONS

[00134] Clinical variable: any measurable characteristic or property of an individual that relates to a physiological, pathological or disease state including disease subtype or severity and that can have different quantitative or qualitative values between and within individuals. Clinical variables may be used to make clinical decisions for people with a given disease.

[00135] Biomarker: an indicator of some biological state or condition by which a particular pathological or physiological process or disease that can be identified or measured.

[00136] Hyperspectral imaging (HS): where each pixel in an image acquires the light intensity for a large number (can be dozens to thousands) of contiguous, narrow (<20 nm) equally-spaced wavelength bands such that each pixel contains a continuous spectrum.

[00137] Multispectral imaging (MS): refers to imaging using more than 1 wavelength band of a certain bandwidth. These bands need not be equally wide or equally spaced bands unlike hyperspectral imaging.

[00138] Wavebands: bands of light at a certain range of wavelengths that has a certain bandwidth and a center frequency.

[00139] Multi bandpass filter: a bandpass filter, bandstop filter(s), or a combination of multiple filters allowing desired multiple wavebands through.

[00140] Multispectral imaging score (a nonlimiting example of the measure of biomarker level): Multispectral score is a linear or non-linear combination of weighted spectral information from select wavebands of light.

[00141] ADSF: Application Dedicated for Selection of Filters, algorithm used to select optimal wavelengths for a particular application (in this case, wavelength selection for multispectral imaging for detection of amyloid in the retina). This may not be the only method that can be used to determine the optimal wavelengths to use. Additionally, the

shape of the wavebands may vary.

[00142] AD: Alzheimer's disease.

[00143] PET: positron emission tomography.

[00144] PET Centiloid: Centiloid is the generic score to combine the SUVR (standardized uptake value ratio) results derived from different radioactive tracers used in PET imaging so that meaningful comparisons could be made.

[00145] A β : amyloid beta, a protein known to be present in Alzheimer's disease.

[00146] AUC: area under the curve of receiver operating characteristic curves.