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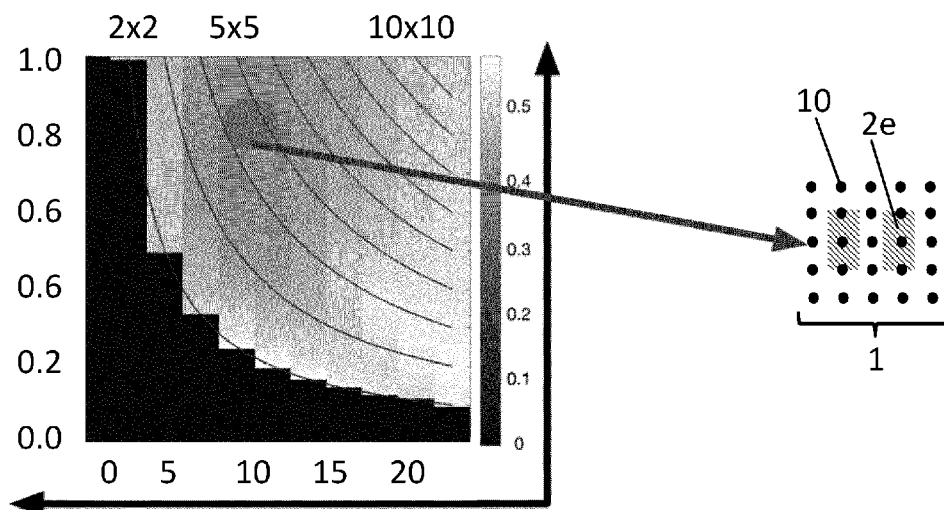
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(54) Title: METHOD FOR SELECTING AN ILLUMINATION PATTERN FOR A FLUORESCENCE MOLECULAR TOMOGRAPHY MEASUREMENT

Fig. 2



(57) **Abstract:** The invention relates for selecting an illumination pattern (1) for a fluorescence molecular tomography (FMT) measurement, comprising the steps of: a) Providing (100) a sample model (3) for a sample (4) to be evaluated using fluorescence molecular tomography, wherein the sample model (3) comprises information about an anatomy of the sample (3), wherein the sample model further comprises an expected fluorophore distribution (2e) spatially associated with the information about the anatomy, b) Determining (100) a plurality of different illumination patterns (1) consisting of illuminated regions and non-illuminated regions of excitation light (60), wherein each illumination pattern (1) of the plurality of illumination patterns (1) is different from the remaining illumination patterns (1) of the plurality of illumination patterns (1), wherein each illumination pattern (1) consists of illumination portions (10) that are surrounded by a non-illumination region, c) For each of the illumination pattern (1) simulating (200) a fluorescence molecular tomography measurement of the sample model (3) with the illumination pattern (1), wherein the simulation is configured to reflect that the sample model (3) is illuminated sequentially with the illumination portions (10) of the illumination pattern (1), d) Reconstructing (202) a fluorophore distribution (2r) in the sample model (3) from each of the simulated measurements, e) Comparing (205) the

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reconstructed fluorophore distributions (2r) with the expected fluorophore distribution (2e) by means of a score function, wherein the score function determines a score indicative for a correspondence of the reconstructed and the expected fluorophore distribution (2r, 2e), particularly wherein the score decreases with increasing correspondence, f) Selecting (206) the illumination pattern (1) associated to the simulated measurement (200) yielding the score indicating the highest correspondence, g) Performing (300) a measurement of the sample (4) with the selected illumination pattern (1), by sequentially illuminating the sample (4) with the illumination portions (10) of the selected illumination pattern, h) Determining (400) a fluorophore distribution (2s) in the sample (4) from the measurement. The invention furthermore relates to a computer program for executing the method.

Method for selecting an illumination pattern for a fluorescence molecular tomography measurement

Specification

The invention relates to a method for selecting an illumination pattern for a fluorescence molecular tomography (FMT) measurement.

For an FMT measurement typically the following components are needed:

A light detector for imaging a sample, the detector particularly comprising a two-dimensional detection area comprising a plurality of detector pixels arranged in a detector array, an illumination source, such as a laser for illuminating the sample, a sample and a computer for recording measurement data, comprising detector data, and for evaluating the measurement data and particularly for reconstructing the measurement data into a three-dimensional image.

WO 2007/109678 A2 discloses a method for non-contact fluorescent optical tomography using patterned illumination. According to WO 2007/109678 A2 a fluorescent sample is illuminated projecting at least two illumination motifs of excitation light on the surface of sample. For each motif the method records the reflected excitation light for generating an excitation data set. Furthermore, the fluorescence emission is recorded for each motif, such that for each motif also a fluorescence emission data set is generated. From the data acquired from all motifs, a single three-dimensional image of the fluorescent sample is generated (reconstructed) by applying an iterative algorithm for minimizing a difference between a predicted data set based on a mathematical model and the excitation and emission data sets.

The goal of using a plurality of illumination motifs and to record a plurality of excitation and emission data sets is to reconstruct the fluorescent portions in the sample as good as possible, particularly if the fluorescent portions are located at different depths within the sample.

While the teaching of WO 2007/109678 A2 improves fluorescence optical tomography in certain scenarios, for a day-to-day user a challenging task is to decide, which of the countless possible illumination motifs and other illumination parameters might provide the reconstruction having the highest resolution and confidence for a specific sample.

Simply trying out a plurality of illumination motifs and exposing the sample to many measurement cycles is not feasible in terms of computational power and time as well as from an experimental perspective, where available measurement cycles and time is often limited.

An object of the present invention is to provide a method that allows for determining and selecting an illumination pattern for recovering a fluorophore distribution inside a sample. The object is achieved by the device having the features of claim 1.

Advantageous embodiments are described in the subclaims.

According to claim 1 a method for adjusting and selecting an illumination pattern for a particularly non-contact fluorescence molecular tomography (FMT) measurement, comprises at least the steps of:

- a) Providing particularly an information on a sample model for a sample to be evaluated by, particularly measured with fluorescence molecular tomography, wherein the sample model comprises information about an anatomy, particularly geometrical and structural information of the sample, wherein the sample model further comprises an expected, particularly three-dimensional fluorophore distribution spatially associated with the sample model,
 - a1) Particularly generating a volumetric mesh to model the geometry and structure of the sample, particularly wherein information on the volumetric mesh is comprised in the sample model, wherein the volumetric mesh comprises faces, vertices (also referred to as nodes) and edges for defining the volumetric mesh,
 - a2) Particularly assigning values of optical properties of the sample model, such as scattering and absorption coefficients, and/or refractive indices to each vertex and/or face of the volumetric mesh comprised by the sample model,
 - a3) Particularly selecting a measurement portion that is evaluated using FMT of the sample model and the sample,
 - a4) Particularly setting a position, a pixel size, and/or a numerical aperture of the detector,
 - a5) Particularly selecting a reconstruction method for reconstructing the fluorophore distribution,
 - a6) Particularly adjusting parameters for the reconstruction method,

- b) Determining, for example generating or selecting from a database, a plurality of different illumination patterns consisting of illuminated regions and non-illuminated regions of illumination light, wherein each illumination pattern of the plurality of illumination patterns is different from the remaining illumination patterns of the plurality of illumination patterns, wherein each illumination pattern comprises particularly consists of illumination portions that are particularly completely surrounded by a non-illumination region,
- c) For each of the illumination pattern simulating a fluorescence molecular tomography measurement of the sample model, particularly the measurement portion of the sample model with the illumination pattern, wherein the simulation is configured to reflect that the sample model is illuminated sequentially, particularly one by one, with the illumination portions of the illumination pattern, wherein for each illumination portion an excitation image and an emission image is virtually recorded particularly by a virtual detector,
- d) Reconstructing a fluorophore distribution in the sample model from each of the simulated measurements, particularly wherein for each illumination pattern a fluorophore distribution is determined from the plurality of excitation and emission images that have been virtually recorded sequentially for each illumination portion of the illumination pattern,
- e) Comparing the reconstructed fluorophore distributions with the expected fluorophore distribution by means of a score function, wherein the score function determines a score indicative for a correspondence of the reconstructed and the expected fluorophore distribution, particularly wherein the score decreases with increasing correspondence,
- f) Selecting particularly a single illumination pattern associated to the simulated measurement yielding the score indicating the highest correspondence,
- g) Performing, particularly executing a fluorescence molecular tomography measurement of the sample particularly of the measurement portion of the sample with the particularly single selected illumination pattern, by sequentially illuminating the sample with the illumination portions of the selected illumination pattern, and particularly record for each illumination portion an excitation image and an emission image,
- h) Determining, particularly reconstructing a fluorophore distribution in the sample from the fluorescence molecular tomography measurement,

particularly by evaluating the excitation and emission images recorded during the measurement of the sample,

- i) Particularly, outputting or displaying the determined fluorophore distribution.

The sample model is particularly a virtual sample model that is described by means of digital information relating the structural, anatomical, optical and/or geometrical properties of the sample model.

The provision of the sample model particularly comprises the generation of the sample model, particularly by means of generating a volumetric mesh representing the sample geometry and/or anatomy.

The sample model can comprise information about the shape and location of an anatomical portion such as a tumor site, the liver or the kidney or another organ or organelle of the sample model. Moreover, the sample model can comprise information regarding a spatial distribution of an absorption, a fluorescence, a luminescence, a refractive index, and/or a scattering strength for describing optical properties of the sample model that are important parameters for FMT imaging.

In the context of the specification a distinction between a task being performed or executed in a simulated manner, i.e. during simulation on the sample model, i.e. in silico, or being performed by executing said task on the sample is not always made, as for example it is clear to the person skilled in the art that for example the sample model cannot be illuminated ex silico, but only in a simulated fashion..

The sample as well as the sample model comprises at least one fluorescent portion that is to be imaged by FMT. The fluorescent portion comprises fluorophores and/or other luminescent probes that are excitable by an illumination light source.

The expected fluorophore distribution can be for example a spatial portion in the sample model that comprises the fluorophores. However, the fluorophore distribution does not necessary relate to positions of single fluorophores but comprises information relating to the spatial distribution of fluorescence in the sample model.

It is noted that the expected fluorophore distribution is spatially associated to the sample model for example, by means of an anatomical portion, such as the liver or kidney of the sample model that is expected to be fluorescent.

The sample model can be derived or generated from measurement data acquired from the sample.

Said measurement data are particularly acquired by means of an imaging method, such as magnet resonance imaging, positron emission tomography, computer

tomography or another imaging method that is deemed suitable to generate the sample model.

According to one embodiment of the invention the sample model has a surface, wherein said surface is represented by a mesh model or a surface of a volumetric mesh.

The volumetric mesh comprises a plurality of vertices, edges and faces, wherein the mesh is configured to model the optical properties of the sample locally. This can for example be done by assigning each edge, vertex and/or face of the volumetric mesh a values relating to the local optical property, such that for example the absorption and/or scattering coefficient as well as the refractive index of the sample can be described and modelled locally.

The volumetric mesh particularly extends through the entire sample model volume and also models the sample surface.

According to the invention, an illumination pattern consists of illuminated regions and non-illuminated regions of excitation light. Illumination light can for example be provided by a laser, an LED or a lamp. Illumination light is particularly configured to excite the fluorophores comprised in the fluorescent portion such that fluorescence emission is triggered upon illumination.

The term “excitation light” particularly refers to illumination light.

The illumination pattern consists of a plurality of illumination portions. During measurement or simulation these portions are projected sequentially on the sample or the sample model respectively. This can be done for example by turning on and off the illumination light and by scanning an appropriate light beam over the sample, for example by using a scan system. Thus, the illumination pattern is particularly not present at the same instance, as the illumination portions are not illuminated at the same instance but sequentially. Thus, particularly a time averaged representation of the illumination pattern would represent all illumination portions simultaneously. In order to allow a sensible description, the sequential nature of illuminating the sample model or sample model with illumination portions is not always explicitly mentioned.

It is also noted that a simulation configured to simulate the sequential illumination of the sample model with the illumination portion, by no means have to sequentially process the sequential illumination, but it can also be done by parallel computing or programming this task accordingly parallel.

The illumination pattern is particularly characterized by a spatially varying light distribution particularly in case all illumination portions would light up at the same

time. Moreover, the illumination pattern is particularly characterized by a lateral, i.e. two-dimensional spatially and temporally varying light distribution, when the excitation light hits or is scanned over the surface of the sample or a planar surface.

Thus, for example the time-averaged representation of the illumination pattern would cover an extended surface area, comprising a plurality of illumination portions that are arranged spatially isolated, i.e. surrounded by a non-illuminated region.

The light from the illumination pattern is particularly focussed on the surface of the sample.

The plurality of illumination patterns differ for example in their spatial distribution. Therefore, some patterns might cover a larger area than other patterns. Some patterns might have illuminated regions having different shapes than others.

An illumination pattern is particularly characterized by the spatial distribution of illumination light, particularly by the spatial distribution of illuminated regions and non-illuminated regions of the surface of the sample (or sample model), when all illumination portions are considered to be highlighted or represented in a time-averaged manner.

Therefore, a spatial pattern particularly differs in at least one property, such as density of illuminated regions, size of the illuminated surface area of the sample or sample model respectively, or other properties particularly relating to the spatial distribution of the excitation light.

A simulation of a fluorescence molecular tomography measurement particularly comprises the steps of defining and/or providing simulation parameters such as:

- parameters or information relating to a light detector used for the particularly simulated FMT experiment,
- parameters or information relating to the sample model such as an anatomy, a geometry and/or a structure of the sample, optical property maps, such as an absorption coefficient map, a scattering coefficient map, and a refractive index map comprising information about the spatial distribution of these optical parameters. The sample model particularly comprises a virtual mesh, with information for nodes and elements for defining a surface of the sample model.
- parameters relating to illumination, such as an intensity, a wavelength or a wavelength range, an illumination pattern, such as point-shaped, Gaussian shaped.

An initial pattern of the raster scanning illumination and/or a minimum gap between two adjacent light point sources can be provided as well.

- parameters relating to the reconstruction of the three-dimensional image, such as an output data dimension (or a reconstruction grid), a reconstruction method, regularization terms, maximum number of iteration loops.
- parameters defining a region of reconstruction interest, i.e. a portion where the fluorophores are expected to be located in the sample model or sample, for example, at a tumour site, a kidney, etc.

The parameters relating to the detector particularly comprise information about a position, a dimension, and a sensitivity of each pixel on the detector. The detector can be a non-contact CCD or CMOS type camera.

With these parameters that can be estimated or determined by the person skilled in the art, a simulation of the FMT experiment can be performed particularly yielding a three-dimensional fluorophore distribution.

An FMT measurement, simulated or performed on the sample, particularly acquires a plurality of excitation images, particularly two-dimensional excitation images, detected at the wavelength of the illumination light and a plurality of emission images, particularly two-dimensional emission images, detected at a fluorescent wavelength covering the fluorescence of the fluorophores of the fluorescent portion in the sample. The fluorophore distribution is reconstructed using the plurality of excitation and emission images.

According to another embodiment of the invention, for each illumination portion of the illumination pattern an excitation and an emission image is generated during simulation or recorded during the FMT measurement. As the illumination portions are projected, particularly virtually projected on the sample and/or the sample model in a sequence, each excitation and emission image is acquired sequentially as well. The simulation of the FMT measurement for each pattern is particularly performed on a computer or on a plurality of computers, i.e. in silico.

The simulation of the FMT measurement is performed particularly using the photon diffusion equation (DE) and its Robin boundary condition. The DE is particularly solved by a finite element method (FEM). For each point source, particularly leading to a scattering, a refractive interaction, a fluorescence emission and/or an absorption process in the diffusion equation, particularly two images are obtained: an excitation image and an emission image (the latter also referred to as the fluorescence image).

Particularly for each illumination pattern, a plurality of excitation images and a plurality of emission images are generated for reconstruction.

Performing a plurality of simulations with the illumination patterns rather than performing the experiment on the sample itself allows conserving the sample, but also an exact determination of the illumination pattern that is likely to yield the fluorophore distribution that has the highest correspondence with a true fluorophore distribution in the sample. This is particularly because the expected fluorophore distribution in the sample model is known a priori and thus a comparison of the resulting, reconstructed (estimated from the simulations) fluorophore distribution in the sample model can be quantitatively compared for each illumination pattern with the expected fluorophore distribution.

As the sample model is a representation of the sample particularly with respect to the optical and anatomical properties, it can be assumed that the illumination pattern providing the reconstructed fluorophore distribution exhibiting the largest correspondence with or the least deviation from the expected fluorophore distribution, will yield a fluorophore distribution as determined from the measurement of the sample that has the largest correspondence with a true fluorophore distribution in the sample.

It is again noted that fluorophore distributions do not necessarily comprise positions of individual fluorophores but can for example represent spatial distributions of a fluorescence intensity.

It is further noted that in certain embodiments the fluorophore distribution comprises the spatial distribution of individual fluorophores, fluorescent compounds, or fluorescent portions particularly together with an estimated spatial fluorescence intensity distribution.

The step of reconstructing a fluorophore distribution (also referred to as fluorescent dye distribution in the specification) in the sample model from each of the simulated measurements is for example implemented by particularly minimizing an error or weighting function upon addition of a regularization term, with possibility for acceleration by using conjugation gradient methods for example.

The reconstruction step is particularly performed using a reconstruction method.

The score function provides a score that is indicative particularly of the degree of correspondence (or deviation) of the reconstructed fluorophore distribution and the expected fluorophore distribution.

The correspondence between two fluorophore distributions is for example measured by a spatial overlap of the fluorophore distribution and/or a similar or identical fluorescence intensity distribution.

The score for quantifying such correspondence comprises or is for example a root-mean-square value or a variance. The root-mean-square value is well suited to provide information about a degree of correspondence or deviation of two entities.

Particularly after simulating and reconstructing the fluorophore distributions for all illumination patterns of the plurality of illumination patterns, an optimal illumination pattern is selected by means of the score, wherein the illumination pattern from the plurality of illumination patterns is chosen that results in a score that indicates the largest degree of correspondence between the expected fluorophore distribution and the reconstructed fluorophore distribution.

The illumination patterns particularly differ from each other either by an illumination density, i.e. the density of the illuminated regions or by a lateral length or extent of the illumination pattern.

As the illumination pattern is particularly generated by a raster scanning device and method, the length or extend of the illumination pattern can be adjusted by the length or extent of the scan field of excitation light. The illumination density and lateral extent of the illumination pattern are particularly the only parameters that differ between the plurality of illumination patterns.

Once the optimal illumination pattern has been determined, the sample is illuminated with said illumination pattern and the signals in response to the illumination of the sample are recorded by the detector.

The signals particularly comprise scattered, reflected and fluorescent light. The signals can be split such that the scattered and reflected light are recorded separately from the fluorescent light, particularly using two different detectors or a single detector using appropriate filters.

From the recorded signals the fluorophore distribution in the sample is determined.

The determination of the fluorophore distribution is particularly done analogously to the reconstruction of the simulated data, particularly using the same reconstruction methods. The fluorophore distribution in the sample is therefore particularly reconstructed from the measurement data, particularly using the same reconstruction method.

The determined fluorophore distribution is particularly displayed or otherwise visualized. This can be done on a suitable display, such as a computer display connected to the computer processing the measurement.

According to another embodiment of the invention, each illumination pattern is selected from a spatially regular illumination pattern. This particularly allows for a simplified generation of the illumination patterns and a simplified handling of the reconstruction and simulation.

The term regular particularly refers to a repeating and/or symmetrical illumination pattern. The symmetry is particularly one of a central symmetry or an axial symmetry along a symmetry axis.

According to another embodiment of the invention, each illumination portion is a point-like illumination region, namely an illumination point, wherein the illumination portions are spatially non-overlapping or touching.

The size of one point-like illumination region, particularly of one illumination point is particularly in the range of 0.2 mm to 2 mm, more particularly in the range of 0.5 mm to 1 mm.

The illumination pattern is particularly generated by means of focusing a laser illumination source to the specified point-like illumination region.

The intensity profile of the illumination portion is particularly a Gaussian intensity profile.

The FMT device particularly comprises such a laser illumination source and particularly a scanner for moving the sample relative to the illumination pattern and/or for scanning the laser so that the illumination portions of the illumination pattern can be positioned on the sample surface.

The illumination portions are particularly not connected with each other, i.e. they are disjoint, isolated illumination portions that are completely surrounded by a non-illuminated region.

According to another embodiment of the invention, the illumination portions particularly of at least one, particularly each illumination pattern are arranged in a regular grid particularly forming the illumination pattern, wherein the regular grid is composed of at least one identical grid unit forming the regular grid.

As mentioned above, the illumination portions are projected in a sequential manner on the sample or sample model, such that the regular grid would become visible when using for example a time averaged representation of the illumination pattern.

The identical grid unit can for example be a triangle, a square, a rectangle or other geometrical, particularly two-dimensional shape that particularly upon repetition forms the illumination pattern.

The term “regular” particularly refers to a property of a pattern that comprises at least two portions that are identical, such that the regular pattern is formed by repetition of the portion. Said portion is referred to as the identical grid unit.

This embodiment allows for a defined illumination of the sample model or the sample, wherein particularly a plurality of illumination patterns can be generated by altering one or two properties, such as the side length of the identical grid unit and/or the number of the identical grid units, of the regular patterns without changing the symmetry or general layout of the pattern. This in turn allows for synergetic computational effects in reconstructing the fluorophore distribution for the different illumination patterns. Moreover, the parameter space for varying the illumination pattern is well-defined and the effects of altering one parameter on the reconstruction quality, i.e. the score can be assessed straightforwardly.

According to another embodiment of the invention, wherein the illumination portions of at least one, particularly each illumination pattern are arranged in a rectangular or square grid, with the identical grid unit being a rectangle or a square, wherein the corners of each grid unit are formed by the illumination portions.

For each illumination pattern for example the rectangle size (e.g. the side length) and/or the number of rectangle can be varied.

For example, if the grid is a square grid, the illumination pattern comprises at least four point-like illumination portions, such as illumination points, wherein each illumination forms a corner of the square grid.

The square grid can comprise a plurality of squares, wherein the corner of each square is formed by a point-like illumination portion.

For example a square grid can comprise 2 by 2, 3 by 3, or M by N illumination portions, particularly wherein the identical grid unit is a square, wherein N and M are natural numbers and particularly wherein N equals M.

In case of a rectangular grid, the grid can comprise M by N illumination portions, wherein particularly the identical grid unit is a rectangle, wherein N and M are natural numbers and particularly wherein N equals M.

Such square or rectangular grid can for example be generated by projecting the illumination portions of the illumination pattern sequentially on the sample surface or

the sample model surface respectively. Particularly for each projected portion an emission and excitation image is recorded.

According to another embodiment of the invention, the sample and/or the sample model is illuminated with the illumination portions repeatedly, i.e. particularly the same illumination portion is particularly projected twice or more times on the sample or the sample model.

According to another embodiment of the invention, the plurality of illumination patterns is selected from illumination patterns with illumination portions arranged in a regular grid, particularly in a rectangular or in a square grid, wherein a number of grid units forming the regular grid and/or a side length of each grid unit is varied for different illumination patterns.

This embodiment allows for an efficient handling and computation of the reconstruction of the fluorophore distribution.

According to another embodiment of the invention, at least some illumination patterns are selected from illumination patterns having a different number of illumination portions.

This embodiment allows for example using non-regular illumination patterns or no or more than one identical grid unit, allowing for a greater flexibility for illumination patterns, such that complex sample geometries or fluorophore distributions, such as fluorophore distributions covering a large three-dimensional portion in the sample or the sample model, can be addressed.

According to another embodiment of the invention, at least some, particularly all illumination patterns are selected from illumination patterns having a different illumination pattern size.

According to this embodiment, the method according to the invention can be executed simply by varying the size, i.e. the particularly two-dimensional extent of the illumination pattern. This size of the illumination pattern is for example defined by the area the illumination pattern covers on the surface of the sample model or the sample. The area can for example be defined either by the area covered by the illuminated regions or by an area defined by an envelope that encompasses the illuminated and non-illuminated regions of the illumination pattern. The illumination pattern size is particularly also the scan size of the illumination pattern.

According to another embodiment of the invention, wherein the score function comprises an error function calculating a deviation between the expected fluorophore distribution and the reconstructed fluorophore distribution.

An error function is a function that provides a quantitative, particularly continuous measure for the deviation between the expected fluorophore distribution and the reconstructed fluorophore distribution. The smaller the deviation between the expected fluorophore distribution and the reconstructed fluorophore distribution is the larger the correspondence.

The error function particularly provides a single integer for estimating the score. The error function is for example a root-mean-square function.

According to another embodiment of the invention, the score function comprises a variance, particularly wherein the score function comprises or is a root-mean-square error between the expected fluorophore distribution and the reconstructed fluorophore distribution.

These score functions are particularly suitable for evaluating the correspondence / deviation of the expected fluorophore distribution and the reconstructed fluorophore distribution.

According to another embodiment of the invention, the sample model further comprises an information about optical properties of the sample, wherein said information comprises information on a spatial distribution of an absorption and a scattering coefficient as well as information on a spatial distribution of an index of refraction, particularly wherein said information about optical properties is an estimated information.

The sample model particularly comprises a mesh, particularly a volumetric mesh for defining the geometric and particularly anatomic properties of the sample. The properties of the mesh have been elaborated at a different section of the specification.

The sample model properties are for example used for reconstructing the fluorophore distribution in the sample model.

This is for example done by applying the photon diffusion equation modelling the propagation of light under the given, particularly local optical conditions in the sample model.

For different tissues, organs and organelles the absorption, particularly the absorption coefficient, the refractive index, and/or the scattering coefficient can vary. Taking into account said variations with the sample model, allows for a precise modeling and a reliable outcome of the reconstructed fluorophore distribution.

The optical properties of the sample model can be for example looked-up in a data storage that relates the optical properties to the organelles and attributed to the sample model accordingly.

Alternatively or additionally, the optical properties are measured or determined by an imaging method employing for example MRI (magnet resonance imaging), CT (computer tomography), PET (positron emission tomography), or X-ray.

According to another embodiment of the invention, from each simulated measurement of the sample model a plurality excitation images and a plurality of corresponding emission images are generated, particularly one for each illumination portion, wherein the excitation images and the emission images from the simulated measurements are used for reconstructing the fluorophore distribution for the respective simulated measurement in the sample model, and wherein from the measurement of the sample a plurality of excitation images and a plurality of corresponding emission images are generated, particularly one for each illumination portion, wherein the excitation images and the emission images from the FMT measurement are used for determining the fluorophore distribution in the sample.

An excitation image is particularly a data set that comprises the detected light that is of non-fluorescent origin, such as for example scattered or reflected light.

In contrast, the emission image is particularly a data set that comprises detected light that is of fluorescent or otherwise luminescent origin, such as from example light emitted by the fluorophores excited by the excitation light of the FMT device or system.

The emission and excitation images are particularly used to reconstruct the fluorophore distribution in the sample model and/or the sample.

According to another embodiment of the invention, each simulated measurement is based on the evaluation of the photon diffusion equation with regard to the information about the sample model and particularly a Robin boundary condition, wherein the photon diffusion equation is solved by means of a finite element method.

According to this embodiment the emission and excitation images are evaluated by means of the photo diffusion equation and particularly by using the so-called Robin boundary conditions.

This embodiment makes use of the knowledge about light propagation in turbid media. In the frequency domain, the photon diffusion equation can expressed as:

$$\nabla \cdot \kappa(r) \nabla \phi(r, \omega) + \left[\mu_a(r) + \frac{i\omega}{c} \right] \phi(r, \omega) = 0 \quad r \in \Omega \quad \text{Eq. (1)}$$

wherein particularly the absorption coefficient $\mu_a(r)$ and the diffusion coefficient $\kappa(r)$ depend on the location r within the sample model Ω . The field $\phi(r, \omega)$ denotes the photon density distribution, c the speed of light in the medium and ω the modulation frequency. At tissue interfaces, particularly when considering non-contact FMT measurements, a Robin type boundary condition can be applied:

$$\phi(m, \omega) + 2\zeta(c)\kappa(m)\frac{\partial\phi(m, \omega)}{\partial v} = q(m, \omega) \quad m \in \partial\Omega \quad \text{Eq. (2)}$$

with a term $\zeta(c)$ addressing a refractive index mismatch and $q(m, \omega)$ accounting for the source term at the boundary domain $\partial\Omega$. ∂v denotes an outward normal at the surface of the sample model.

When continuous wave mode FMT is used, ω can be set to zero.

According to another embodiment of the invention, at least some of the illumination patterns differ in a different exposure time of the sample model and/or for a different excitation intensity of the sample model.

This allows for more parameters potentially affecting the reconstruction quality of the fluorophore distribution to be varied.

The problem is furthermore solved by a computer program and particularly also a computer program product.

According to this aspect, the computer program and/or the computer program product comprises instructions for example stored on a non-transitory medium, which, when the program is executed by a computer, causes the computer to carry out the steps of the method according to any of the preceding claims, wherein the computer is connected to or integrated in a fluorescence molecular tomography device or system such as to control the fluorescence molecular tomography device to carry out the steps necessary for a fluorescence molecular tomography measurement.

The computer therefore particularly comprises a computation unit configured to compute the simulated measurements as well as the reconstruction of the fluorophore distribution and a control unit configured to control the FMT device such as to perform the measurement on the sample, including, illumination of the sample with the selected illumination pattern, recording the light from the sample and storing the detector signals or detector data on a non-transitory medium in order to process said data for reconstructing the fluorophore distribution.

A computer program comprises particularly computer program modules for

- i) optimizing the FMT experiment parameters, i.e. a measurement simulation tool (a simulator),
- ii) acquiring data based on the selected experiment design from the simulation (a controller),
- iii) reconstructing the three-dimensional fluorophore distribution (a reconstructor),
- iv) evaluating the experimental results (a viewer), i.e. displaying the reconstructed fluorophore distribution and particularly the score.
- v) Setting the problem and feedback for iterative adjustments (a configurator).

A virtual FMT protocol can test the feasibility of a specific FMT measurement and generate the optimal experimental design, i.e. particularly define an optimized illumination pattern. This optimized pattern will then be used for the experimental FMT protocol.

Particularly, exemplary embodiments are described below in conjunction with the Figures. The Figures are appended to the claims and are accompanied by text explaining individual features of the shown embodiments and aspects of the present invention. Each individual feature shown in the Figures and/or mentioned in said text of the Figures may be incorporated also in an isolated fashion into a claim relating to the method or computer program according to the present invention.

In the following, further features as well as embodiments of the present invention are described with reference to the Figures that are appended to the claims, wherein:

- Fig. 1 shows a flow chart depicting the method according to the invention;
- Fig. 2 shows the score for different illumination patterns depending on the pattern size and density;
- Fig. 3 shows a portion of the sample model in form of a volumetric mesh and an illumination pattern;
- Fig. 4 shows two different illumination patterns and the associated reconstructed fluorophore distribution; and
- Fig. 5 shows a typical FMT measurement system for executing the method or the computer program according to the invention;

In Fig. 1 a flowchart of one embodiment of the method according to the invention is shown.

On the left side extending from top to bottom, the main steps of executing the method or the computer program are shown.

In a first step 100, the parameters for the experiment and the simulation are set. This step is also referred to as problem setting. The parameters that have to be adjusted are for example, defining or selecting the plurality of illumination patterns, illumination intensity, creating the sample model from image data acquired with other imaging methods. In the case of a regular and square illumination pattern consisting of a plurality of point-like illumination portions arranged in squares, the patterns are selected regarding a density δ of illumination points, a length l_{SF} of the illumination pattern, i.e. an area that is covered or scanned by the illumination pattern, as well as the number of illumination points N_{illu} , wherein these three parameters are intertwined by the following relation:

$$\delta = \frac{N_{illu}-1}{l_{SF}}$$

Thus a plurality of illumination patterns is generated that differs in at least one of these three parameters from each other. This way an illumination pattern can be described by the number of illumination points and the density of illumination points (cf. e.g. Fig. 3).

The step of problem setting particularly comprises the meshing of the sample model, i.e. to generate a virtual description of the surface and the interior of the sample model, particularly by means of a volumetric mesh. The volumetric mesh comprises edges, faces and vertices, wherein the physical and particularly optical properties of the sample model are assigned to the faces, edges or vertices of the mesh, such that local variations of the optical properties can be taken account for.

The step of meshing the sample model is independent of the selection of the illumination patterns and is performed isolated from other problem setting steps.

It is noted that the sample model is modelled such that it provides a virtual replica of the sample with regard to the optical properties, the geometry and the anatomy, particularly wherein the sample model comprises particularly the volumetric mesh for coarsening the virtual representation in comparison to the sample, due to computational constraints.

The volumetric mesh can be derived from image data acquired with other imaging method, as listed above.

Furthermore, in the problem setting step, detector properties and illumination properties are configured for simulation.

Moreover, the sample model comprises a known, i.e. expected fluorophore distribution that is to be recovered during simulation. The expected fluorophore distribution should ideally resemble the fluorophore distribution that is to be expected in the sample, at least in terms of its anatomical location.

In the next step 200, simulated FMT measurements are performed on the sample model. The simulation 201 comprises for each of the plurality of illumination patterns a virtual FMT measurement on the sample model. For each simulated measurement, the resulting fluorophore distribution is reconstructed 202. Whenever 203 an illumination patterns has been used (measurement and reconstruction) in the simulation, the next illumination pattern is chosen 204 and the virtual measurement and the reconstruction is repeated.

Once, the fluorophore distributions for all illumination patterns have been reconstructed, the fluorophore distributions are compared 205 to the expected fluorophore distribution comprised by the sample model.

The comparison is done by means of a score function that is configured to be indicative to the correspondence of two fluorophore distributions.

The illumination pattern yielding the score indicating the highest correspondence (or least deviation) with the expected fluorophore distribution is selected for a subsequent real measurement 300 on the real sample. The acquired data from the real measurement is reconstructed 400 using the same reconstruction method as for the simulated measurements.

In Fig. 2 a diagram is shown for selecting the supposedly best suited illumination pattern for measuring the sample. On the x-axis (extending horizontally) the lateral length l_{SF} of the square illumination pattern is shown in units of mm. On the y-axis extending along the left side of the diagram the density of the illumination pattern, here with illumination points arranged in squares, is depicted in units of illumination points per 2.3 mm. The intensity scale on the right of the diagram provides the value of the score provided by the score function. The lower the score, i.e. the darker the color, the higher is the correspondence between the reconstructed fluorophore distribution of the particular illumination pattern and the expected fluorophore distribution 2e. It is noted that values of zero in the diagram indicate that no simulation was performed for the specific combination of density and lateral length of the illumination pattern. The x-axis on top of the diagram refers to the number of

illumination points for the specific illumination pattern, ranging from 4 (2×2) to 121 (11×11). As can be seen, for the specific sample model and expected fluorophore distribution, the illumination pattern having 5×5 illumination points arranged over an area of $9.2 \text{ mm} \times 9.2 \text{ mm}$ ($l_{SF} = 9.2 \text{ mm}$) yields the highest correspondence with the expected fluorophore distribution 2e. This has been also verified by a controlled measurement on a phantom with a known fluorophore distribution. The gray arrow points to the selected illumination pattern. The expected fluorophore distribution 2e is indicated with the two parallel extending rectangles.

Fig. 3 shows a portion of the sample model 3, wherein the sample model comprises a volumetric mesh 5 that model the local variations of optical properties (and thus particularly the relevant anatomical) as well as the geometry of the sample model 3.

The illumination pattern 1 is virtually projected on the surface of the sample model 3. In this example the illumination pattern consists of a plurality of illumination points 10 that are spaced regularly in squares forming a square illumination pattern with a side length or scan length of l_{SF} . The distance between two adjacent illumination points 10 is given by $1/\delta$. In total there are 49 illumination points 10 comprised by the illumination pattern 1. Each illumination point is completely surrounded by a non-illuminated region, even in the time averaged representation of the illumination pattern of Fig. 3.

In Fig. 4 two results from a simulated measurement and reconstruction are shown. In the upper panel, two illumination patterns 1 are schematically depicted, wherein the illumination pattern 1 on the left consists of 2×2 illumination points 10, wherein the illumination pattern 1 on the right consists of 5×5 illumination points 10. Both illumination patterns has the same density δ and thus a different lateral length l_{SF} . The expected fluorophore distribution 2e of the sample model is also shown as two rectangles (the expected fluorophore distribution has a three-dimensional extent and is cubic in this example). The expected fluorophore distribution 2e is incorporated in the sample model at a specific depth of the sample model.

In the middle and lower panel of Fig. 4, the results for the reconstructed fluorophore distribution 2r both illumination patterns 1 based on the simulated measurements are shown. The middle panel shows an intensity image of a lateral view of the reconstructed fluorophore distribution and the lower panel shows an axial view of the reconstructed fluorophore distribution. The gray values in the intensity images refer to the intensity of the fluorescence signal. As can be seen by comparison of the reconstructed fluorophore distribution with the expected fluorophore distribution, the

reconstructed fluorophore distribution for the illumination pattern on the right column of Fig. 4 has a higher correspondence with the expected fluorophore distribution and thus yields a score indicative for the better correspondence.

In Fig. 5 a typical setup for a FMT measurement is shown, comprising the sample 4 with a fluorescent portion 2s that is to be recovered by the FMT measurement.

The sample 4 is illuminated with laser light 60 provided by a laser 6. The laser light 60 is scanned over the sample 4 by means of two scanners 8. The light that is scattered, reflected or emitted by the sample 4 is detected and recorded by a detector unit 7. The measurement is controlled 9c by a computer 9. The computer particularly controls the laser, the scanners and the detectors, such that the method according to the invention can be executed by the computer 9. The computer is further configured to perform the simulated measurement and the reconstruction of the fluorophore distribution 2r.

In the following the theoretical basis as well as an exemplary embodiment of the method according to the invention is given. References given herein relate to one of the corresponding figures.

In a typical FMT experiment, a point light source 6 is scanned 60 across the sample 4 and the light distribution on the sample surface both at the excitation and fluorescence wavelength is recorded with a detector 7. Collecting data from a sufficient number of source positions allows three-dimensional reconstruction of the fluorophore distribution 2s within the sample 4.

Data acquisition in FMT is an interactive procedure: the user has to define an illumination pattern 1 on the sample surface. Data reconstruction is based on sophisticated forward modelling and inversion methods. Proper selection of reconstruction strategy requires expert knowledge. Verification of reconstruction methods remains an issue as there are no standardized tools available for calibration such as tissue mimicking phantoms. Moreover, data acquisition, image reconstruction and validation are interlinked; for example the measurement configuration may impact the choice of reconstruction methods. A trade-off between measurement/ computational time and reconstruction accuracy has to be found.

With regard to the light propagation model applied, software solutions for diffuse optical tomography (DOT) and FMT can be classified into two major categories: finite-element method (FEM)-based and Monte-Carlo (MC)-based methods. In FEM-based methods, the object is discretized into a 2D or 3D finite mesh and the diffusion equation (DE), a second order partial differential equation, is solved for each nodal

point. Considering the computational expense, FEM is still the most commonly used algorithm for treating DOT and FMT problems with NIRFast [3] and Toast++ [4], [5] being the most established tools. Both software packages can handle heterogeneous optical properties inside the object, irregular air/tissue boundaries, multispectral data, and different modes of operation (frequency/time domain). Regarding inversion, both NIRFast and Toast++ provide a range of regularized approaches.

Apart from software tools provided with commercial systems, there is currently no single software package that could handle the whole FMT procedure from acquisition control to data reconstruction. Typically, the acquisition module is a part of hardware development and based on fast prototyping tools like LabVIEW [1], [2]. There is no immediate link between measurement and reconstruction algorithm in most cases. A seamless integration of reconstruction and measurement would allow generating virtual fluorophore distributions prior to a real data acquisition, thereby enabling the optimization of experimental protocols.

According to the invention, a Smart Toolkit for Fluorescence Tomography (STIFT) is disclosed, a computer program and a method comprising the whole FMT procedure. STIFT particularly includes five modules: i) a tool for optimizing the design of the FMT experiment (a simulator), ii) a data acquisition tool based on the optimal design (a controller), iii) a module for robust 3D reconstruction (a reconstructor), iv) a quality control feature (a viewer), and v) a setting and feedback module for iterative adjustments (a configurator). Virtual FMT protocol can test the feasibility of a specific FMT measurement and generate the optimal experimental design, i.e., define an optimized illumination pattern. This optimized pattern will then be used for the experimental FMT protocol. Performance evaluation of STIFT included studies with phantoms of different levels of complexity and yielded robust reconstruction results.

A. Theoretical background

Light propagation in turbid media can be approximated by the diffusion equation (DE) as set out in Eq. 1. Moreover, a Robin type boundary condition can be chosen as elaborated in connection with Eq. 2.

In the FEM formalism, the Galerkins method [3] simplifies the DE to a linear equation, $S\Phi = Q$, with S denoting the system matrix, Φ a vector comprising the nodal value of field ϕ , and Q a vector comprising the nodal source term. For a standard FMT measurement composed of excitation and emission procedures, the resulted coupled DEs may hence be written as:

$$S_x \Phi_{x,i} = Q_{x,i}, \quad i = 1, 2, \dots, n_1 \quad \text{Eq. 3}$$

$$S_m \Phi_{m,i} = Q_{m,i}, \quad i = 1, 2, \dots, n_l \quad \text{Eq. 4}$$

The subscripts x and m stand for the excitation and emission procedures respectively, while i indicates the source number. During the excitation, source term $Q_{x,i}$ is determined by the laser profile L_i , while for the emission, $Q_{m,i} = \eta \cdot \text{diag}(C_d) \Phi_{x,i}$, where C_d is the vector containing the nodal values of fluorescence concentration and η the fluorescence quantum yield. The operation $\text{diag}(C_d)$ converts the column vector C_d into a diagonal matrix.

To more accurately predict the signal M received by the detectors at the excitation and emission wavelength, a transportation matrix Γ is introduced to describe free-space light propagation from the object surface to the virtual detector plane

$$M_x = \Gamma^T \Phi_x \quad \text{Eq. 5}$$

$$M_m = \Gamma^T \Phi_m \quad \text{Eq. 6}$$

Γ incorporates a visibility term describing whether a surface element is visible from a detector element, the numerical aperture of lens, the distance from the surface to the detector, the area of a surface element and the geometry of object surface [7]. The normalized value $Y = M_m / M_x$ given as the ratio between M_m , the emission measurement and M_x , the corresponding excitation measurement, is used for the final reconstruction [8]. To control the reconstruction accuracy and ease the data fusion with data derived from another modality, a mapping matrix is introduced T_c to link C_d in the context of a tetrahedral mesh to C_{recon} referring to a Cartesian grid.

$$C_d = T_c^T C_{\text{recon}} \quad \text{Eq. 7}$$

Finally, Y can be expressed by the product of the weighting matrix, W times the unknown variable vector, C_{recon} .

$$Y = W C_{\text{recon}} \quad \text{Eq. 8}$$

where $W = W_d T_c^T$ with the elements $W_d(h, n)$

$$W_d(h, n) = \frac{\eta \Gamma_k^T g_{m,n} \Phi_{x,i}(n)}{\Gamma_k^T \Phi_{x,i}} \quad h = n_m(i-1) + k \text{ and } n = 1, 2, \dots, n_d \quad \text{Eq. 9}$$

n_m is the number of pixels on the detector plane, and n_d the number of the unknowns. $g_{m,i}$ are the column vectors of the S_m^{-1} , introduced in Eq. 4.

$$S_m^{-1} = [g_{m,1}, g_{m,2}, \dots, g_{m,n_d}] \quad \text{Eq. 10}$$

The recovery of the fluorescence concentration C_{recon} is implemented by minimizing a cost function $\Psi(C_{\text{recon}})$ upon addition of a regularization term $\lambda \|C_{\text{recon}}\|^2$ using the conjugation gradient (CG) method [6].

$$\Psi(C_{\text{recon}}) = \frac{1}{2} \|WC_{\text{recon}} - \tilde{Y}\|^2 + \lambda \|C_{\text{recon}}\|^2 \quad \text{Eq. 11}$$

B. The framework of STIFT

Input parameters of STIFT contain physical parameters defining the problem (optical properties of the sample, illumination, e.g. laser, detector, noise) and modelling parameters for the reconstruction (meshing, regularization). Output values include the weighting matrix and local fluorescence dye concentration. STIFT uses the concept of state machines: instead of giving a rigid workflow for all different studies, STIFT allows users to select different protocols and define an optimized setting for the measurement and reconstruction. STIFT comprises five modules: configurator, simulator, controller, reconstructor, and viewer. These five modules contain several functional methods that are combined into three protocols (Fig. 1):

- a) Virtual FMT: configurator => simulator => reconstructor => viewer
- b) Experimental setup and data acquisition: configurator => controller => (experimental data)
- c) Reconstruction of experimental data: configurator (experimental data) => reconstructor => viewer.

C. Features of STIFT

Four essential features of STIFT are described including 1) self-adaptive meshing, 2) image registration, 3) free-space detection, and 4) mapping technique.

1) Self-adaptive meshing 5. While the accuracy of FEM can be improved by using small elements, there is always a trade-off between accuracy and computational cost. Adaptive meshing is used to remedy this problem. First, a binary image dataset from the structural reference data (typically derived from CT or MRI) is generated. Second, the binary image is used for generating a coarse mesh. Finally, the coarse mesh is further refined by using:

- a) a geometry-adaptive refinement: a Laplacian operator is applied to the voxelized image stack to identify the edges of inner structures. The edge map in the grid is then mapped to the coarse mesh. Each element mapped with a binary edge signal is then split into finer elements.
- b) an illumination-adaptive refinement: the laser sources are first assigned to the surface of the coarse mesh. Forward modelling of the excitation procedure is then carried out on the coarse mesh. The residual for each element is then calculated based on the DE. The elements with higher residual values are broken up into more

sub-elements than those with lower residual values. The number of sub-elements that one element generates is inversely proportional to the residuals.

2) Image Registration: STIFT provides a simple landmark based registration function to fuse the structural reference with functional image. For slab phantoms, the landmarks are normally selected at corners, whereas for mice the landmarks are selected as clearly identifiable anatomical structures such as eyes, ears or nose tip, landmarks that can be easily identified on both the white-light image and the topological MRI map.

3) Free-space detection: A non-contact configuration of FMT reduces complexity of instrumentation and eases the experimental manipulation. In this case, in addition to describing photon propagation within the biological tissue, the model has to account for free-space photon propagation between the sample surface and the detector. This is achieved by projecting the individual elements of the CCD sensor to a virtual imaging plane (focal plane). All the normals of virtual pixels were calculated. The outward optical flux obeys Lambert's cosine law [7], [9].

4) Mapping technique: As the prior information is derived from a structural imaging modality (e.g. CT or MRI) with data typically given in Cartesian ordinates, it is necessary to relate it to the optimized mesh for FMT modelling and reconstruction. For the convenience of data fusion and result analysis, the FMT reconstruction results are transformed from the mesh based nodal format into a Cartesian grid. The final weighting matrix W in Eq. 8 contains the mapping matrix T_c defined in Eq. 7 such that the number of unknowns can be adjusted. STIFT uses linear interpolation to transform the detector plane, the assignment of optical parameters, and the distribution of fluorescence dye from the optimized mesh into the Cartesian grid coordinates.

Carrying out a virtual FMT protocol allows examining the feasibility of actual FMT experiments and optimizing the experimental design. This is illustrated for the homogeneous phantom #1 containing fluorophore distributions 2s p1 and p2, for which virtual FMT was used to optimize the illumination pattern 1, a key setting for the FMT experiment. Configuration of the problem constitutes the first step in the modelling process. The sample 4 was defined as slab phantom with the size of 60 mm × 30 mm × 15 mm and homogeneous optical properties, i.e., an absorption coefficient μ_a of 0.007 mm⁻¹ and a scattering coefficient μ_s of 0.87 mm⁻¹.

The virtual detector array comprised 60 × 30 elements. The area of individual detector elements was 1 mm × 1 mm, i.e., the detection array covered the whole

surface (x, y-plane) of the phantom. The measurement was performed in the reflection mode, indicating that the images were recorded from the same side as the illumination. The Cartesian grid for assigning reconstruction values had the dimension of 36 x 18 x 9. White noise of an amplitude of 1% of the overall mean intensity was added.

To accurately describe the illumination pattern 1, three parameters were introduced: i) the lateral number of illumination points N_{illu} , ii) the lateral length l_{SF} of the scanning field (SF), and iii) the illumination density indicating the number of illumination points per millimeter and given by Eq. 12. All of the illumination patterns 1 are square-shaped, consisting of a $N_{\text{illu}} \times N_{\text{illu}}$ grid of point sources 10, covering an area of $l_{\text{SF}} \times l_{\text{SF}}$ (Fig. 3).

$$\delta = \frac{N_{\text{illu}} - 1}{l_{\text{SF}}} \quad \text{Eq. 12}$$

Multiple illumination patterns were simulated for optimization purpose. A minimum step for steering laser beams is d (here $d = 2.307 \pm 0.001$ mm). Each SF was centered at the midpoint of the phantom located above the inclusion in p1 and l_{SF} was given by $n \cdot d$ ($n = 1, 2, \dots, 10$). For a given l_{SF} , different illumination density parameters were tested with $N_{\text{illu}} = 2, 3, \dots, (n + 1)$. For each illumination pattern 1, the concentration of fluorescent dye was reconstructed using the reconstruction module of STIFT. Reconstruction results were visualized for several illumination patterns (for a fixed illumination density, $\delta = 1/d$, $N_{\text{illu}} = 2, 5, 8, 10$). Root mean-square error (RMSE) [10] were calculated to evaluate the reconstruction quality for each illumination pattern (cf e.g. Fig. 2). The setting, which generates the minimal value of RMSE, is considered as the optimal design for the experimental FMT.

2) Validation of experimental FMT function: Following virtual FMT simulations, experiments have been carried out using the three phantoms and FMT system [11], cf. e.g. Fig. 5. The system includes a 16-bit CCD camera (ANDOR Corporation, Belfast, Northern Ireland) with 1024 x 1024 pixels for detection 7, a galvanometric driven mirror system 8 for steering the laser beam, a solid-state laser generator 6 with 670 nm wavelength for illumination and a sample support [11]. The fluorescence source consisted of a 1 mm diameter capillary filled with a drop of cyanine5.5 (Cy5.5) with a concentration of 2 nmol/ml. The wavelength of filters for excitation and emission procedures were set to 680 nm and 700 nm respectively.

The purpose of the experimental part of FMT was i) to validate the result of virtual FMT optimization procedure and ii) to assess the accuracy of FMT reconstruction for

different complexity of object. For validating the optimization result, the fixed δ of $1/d$ and N_{illu} values ranging from 2 to 11, and/or l_{SF} from 2.3 mm to 23.1 mm were used. The settings for phantom geometry, optical properties, detector array, and reconstruction grid remain identical to the virtual FMT part. For each illumination pattern 1, the fluorescence signal was reconstructed and the RMSE calculated with regard to the dye distribution on the basis of the phantom geometry. Finally, RMSE values from virtual and experimental FMT protocols were compared to validate the results of the optimization procedure. Identical Cy5.5-filled capillaries were inserted into p1 position in all three phantoms. FMT experiments were carried out both in reflection and transmission mode for the three phantoms, resulting in six sets of reconstruction data. For the reflection mode, a 7 x 7 illumination pattern was used covering an area of 20 mm x 20 mm, whereas for the transmission mode experiments, a 5 x 5 grid covering an area 15 mm x 15 mm was selected. The scanning area for transmission mode is smaller than the reflection one because of the limited dimension of the window embedded in the animal support. For the same phantom, the measurements with reflection and transmission modes were performed sequentially, without moving the phantom.

The virtual FMT protocol was used to optimize the experimental design during real FMT measurements using #1 with fluorophore inclusion at positions p1 and p2. For all patterns, the inclusion p1 underneath the center of SF could be resolved. However, due to limited information available for the smallest 2 x 2 excitation grid covering SF = 2.3 mm x 2.3 mm the signal of the p2 inclusion could not be recovered. The two inclusions located 5 mm apart could be separated better in both lateral and axial planes for the 5 x 5 (SF = 11.5 mm x 11.5 mm) excitation grid than that from 8 x 8 (SF = 18.4 mm x 18.4 mm) and 10 x 10 (SF = 23 mm x 23 mm) grids. This became also apparent when analyzing lateral profiles at $y = 15$ mm and $z = 12$ mm, and depth profiles at $y = 15$ mm and $x = 30$ mm.

In order to find the optimal illumination matrix for the actual FMT experiment, all meaningful combinations of δ and l_{SF} have to be considered. For a certain area of SF with $l_{\text{SF}} = nd$, the number of illumination points increases with $N_{\text{illu}} 2, 3, \dots, n + 1$, and the corresponding illumination density increases with $\delta = \frac{1}{nd}, \frac{1}{(n-1)d}, \dots, 1/d$. For each illumination pattern, the concentration of fluorescent dye was reconstructed and RMSE was used to evaluate the reconstruction quality (Fig. 2). As indicated by the color bar, locations displaying darker gray values indicate lower RMSE, implying smaller deviations of reconstruction results from the ground truth. Based on this

analysis, it is concluded that for the specific phantom used $SF = 9.2 \text{ mm} \times 9.2 \text{ mm}$ and $\delta = 1/2d$ would yield the best reconstruction result. Assuming that at each excitation point 10 the exposure time T_{exp} is fixed, the temporal resolution for a $N_{\text{illu}} \times N_{\text{illu}}$ excitation grid is $T_{\text{exp}} \cdot N_{\text{illu}}^2$. By comparing values of RMSE along temporal-resolution curves having the same temporal resolution, an optimal combination of illumination density and SF covering $l_{SF} \times l_{SF}$ can be recognized, leading to the least error. For example, if we apply a 5×5 excitation grid, a SF covering an area of about $9.2 \text{ mm} \times 9.2 \text{ mm}$ is expected to generate the most robust result.

In a subsequent measurement with the selected illumination pattern good agreement with optimal N_{illu} values of 5 and 4 were found for virtual and experimental FMT protocols, respectively. Too few or too many illumination points resulted in reconstructed signal that were either blurred or missing features.

With the method according to the invention it is possible to automatically identify and select the best suited illumination pattern for a given sample.

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Claims

1. A method for selecting an illumination pattern (1) for a fluorescence molecular tomography (FMT) measurement, comprising the steps of:
 - a) Providing (100) a sample model (3) for a sample (4) to be evaluated using fluorescence molecular tomography, wherein the sample model (3) comprises information about an anatomy of the sample (3), wherein the sample model further comprises an expected fluorophore distribution (2e) spatially associated with the information about the anatomy,
 - b) Determining (100) a plurality of different illumination patterns (1) comprising or consisting of illuminated regions and non-illuminated regions of excitation light (60), wherein each illumination pattern (1) of the plurality of illumination patterns (1) is different from the remaining illumination patterns (1) of the plurality of illumination patterns (1), wherein each illumination pattern (1) consists of illumination portions (10) that are surrounded by a non-illumination region,
 - c) For each of the illumination pattern (1) simulating (200) a fluorescence molecular tomography measurement of the sample model (3) with the illumination pattern (1), wherein the simulation is configured to reflect that the sample model (3) is illuminated sequentially with the illumination portions (10) of the illumination pattern (1),
 - d) Reconstructing (202) a fluorophore distribution (2r) in the sample model (3) from each of the simulated measurements,
 - e) Comparing (205) the reconstructed fluorophore distributions (2r) with the expected fluorophore distribution (2e) by means of a score function, wherein the score function determines a score indicative for a correspondence of the reconstructed and the expected fluorophore distribution (2r, 2e), particularly wherein the score decreases with increasing correspondence,
 - f) Selecting (206) the illumination pattern (1) associated to the simulated measurement (200) yielding the score indicating the highest correspondence,
 - g) Performing (300) a measurement of the sample (4) with the selected illumination pattern (1), by sequentially illuminating the sample (4) with the illumination portions (10) of the selected illumination pattern,

- h) Determining (400) a fluorophore distribution (2s) in the sample (4) from the measurement.
2. Method according to claim 1, wherein each illumination pattern (1) is selected from a set of spatially regular illumination patterns.
 3. Method according to one of the preceding claims, wherein each illumination portion (10) of each illumination pattern is a point-like illuminated region, namely an illumination point.
 4. Method according to one of the preceding claims, wherein the illumination portions (10) are arranged in a regular grid, wherein the regular grid is composed of at least one identical grid unit forming the regular grid.
 5. Method according to one of the preceding claims, wherein the illumination portions (10) are arranged in a rectangular or square grid, with the identical grid unit being a rectangle or a square, wherein the corners of each grid unit are formed by the illumination portions (10).
 6. Method according to one of the preceding claims, wherein the plurality of illumination patterns (1) is selected from illumination patterns (1) with illumination portions (10) arranged in a regular grid, particularly in a rectangular or in a square grid, wherein a number of grid units forming the regular grid and/or a side length of each grid unit is varied for different illumination patterns (1).
 7. Method according to one of the preceding claims, wherein at least some illumination patterns (1) are selected from illumination patterns (1) having a different number (N_{illu}) of illumination portions (10).
 8. Method according to one of the preceding claims, wherein at least some illumination patterns (1) are selected from illumination patterns (1) having a different illumination pattern size (l_{SF}).
 9. Method according to one of the preceding claims, wherein the score function comprises an error function calculating a deviation between the expected fluorophore distribution (2e) and the reconstructed fluorophore distribution (2r).
 10. Method according to one of the preceding claims, wherein the score function comprises a variance, particularly wherein the score function comprises or is a

root-mean-square error between the expected fluorophore distribution (2e) and the reconstructed fluorophore distribution (2r).

11. Method according to one of the preceding claims, wherein the sample model (3) further comprises information about optical properties of the sample (4), wherein said information comprises information on a spatial distribution of an absorption and a scattering coefficient as well as information on a spatial distribution of an index of refraction.
12. Method according to any of the preceding claims, wherein from each simulated measurement (202) of the sample model (3) a plurality excitation images and a plurality of corresponding emission images are generated, wherein the excitation images and the emission images from the simulated measurements are used for reconstructing the fluorophore distribution (2r) for the simulated measurement (202), and wherein from the measurement (300) of the sample (4) a plurality of excitation images and a plurality of corresponding emission images are generated, wherein the excitation images and the emission images from the measurement are used for determining the fluorophore distribution (2s) in the sample (4).
13. Method according to one of the preceding claims, wherein each simulated measurement (200) is based on the evaluation of the photon diffusion equation with regard to the information about the sample model (3), wherein the photon diffusion equation is solved by means of a finite element method.
14. Method according to one of the preceding claims, wherein at least some of the illumination patterns (1) differ in a different exposure time and/or for a different excitation intensity.
15. Computer program comprising instructions which, when the program is executed by a computer (9), cause the computer (9) to carry out the steps of the method according to any of the preceding claims, wherein the computer (9) is connected to or integrated in a fluorescence molecular tomography device such as to control the fluorescence molecular tomography device to carry out the steps necessary for an fluorescence molecular tomography measurement (300).

Fig. 1

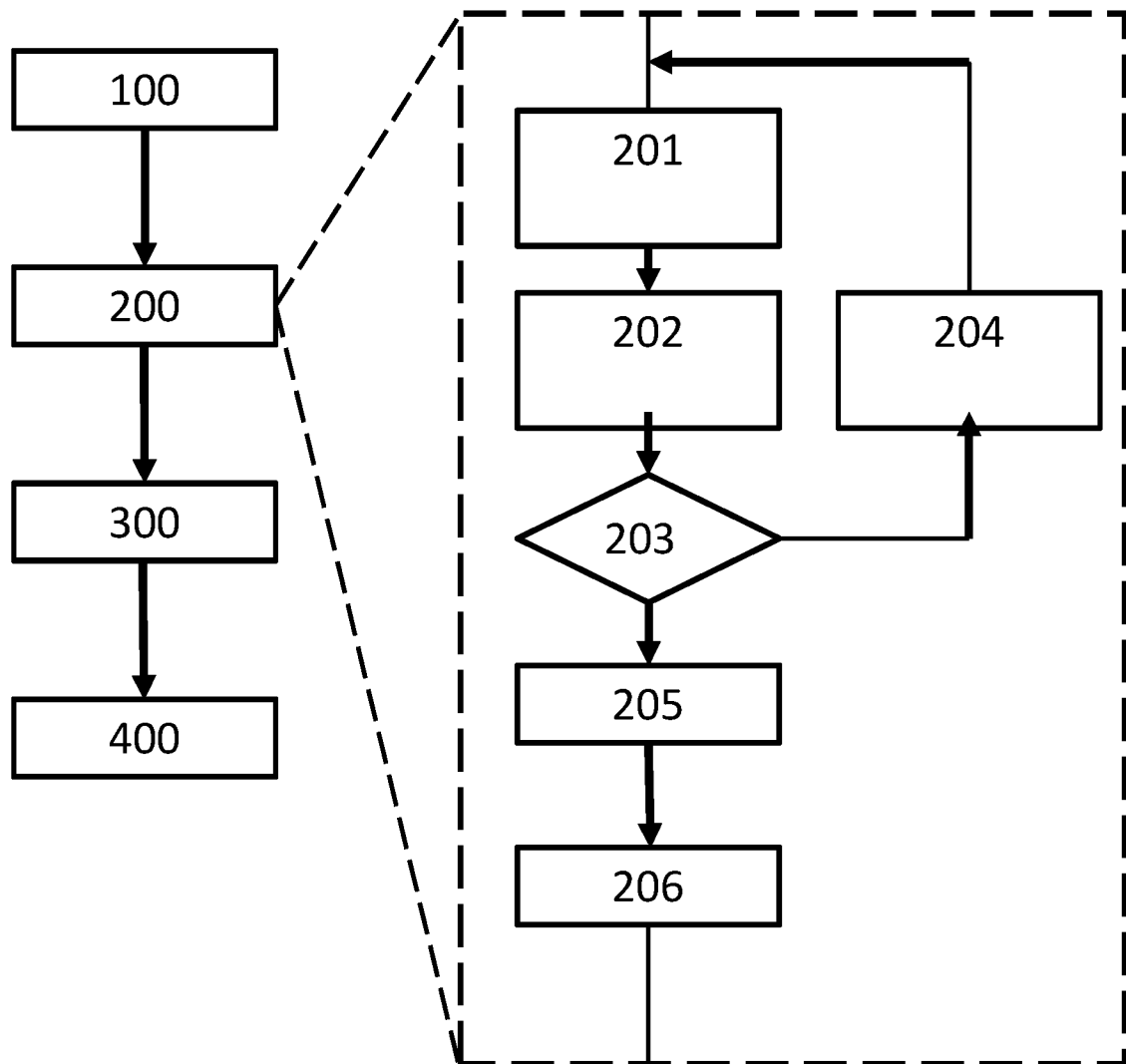


Fig. 2

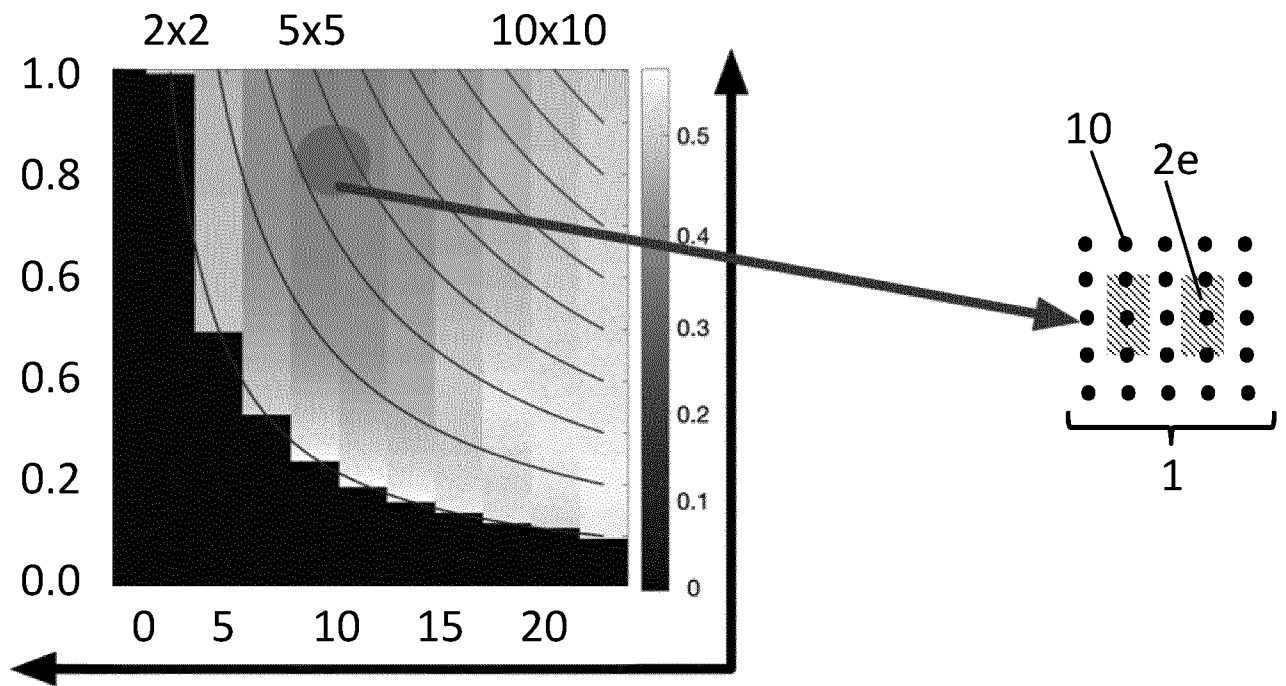


Fig. 3

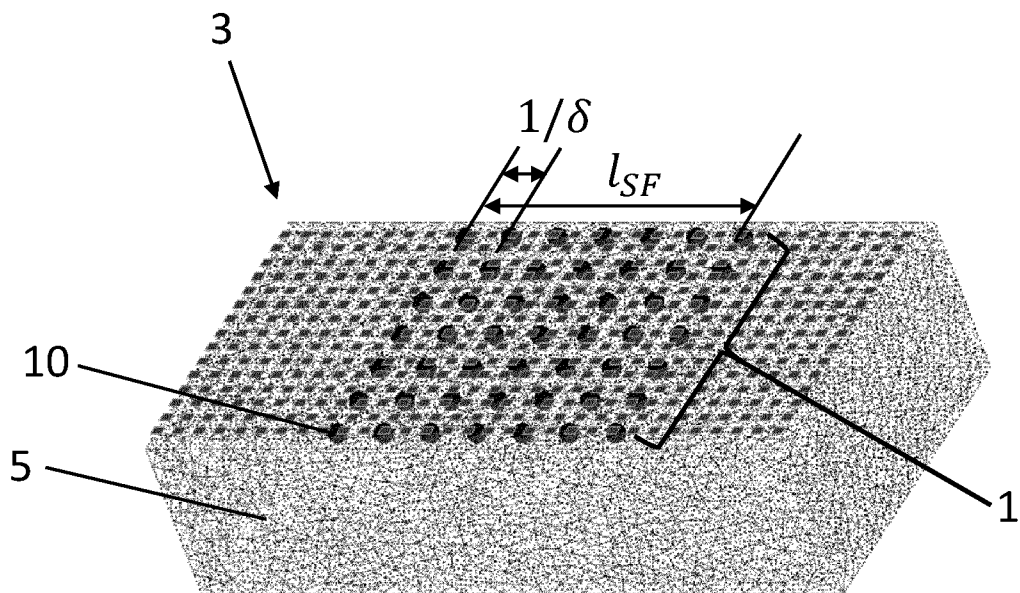


Fig. 4

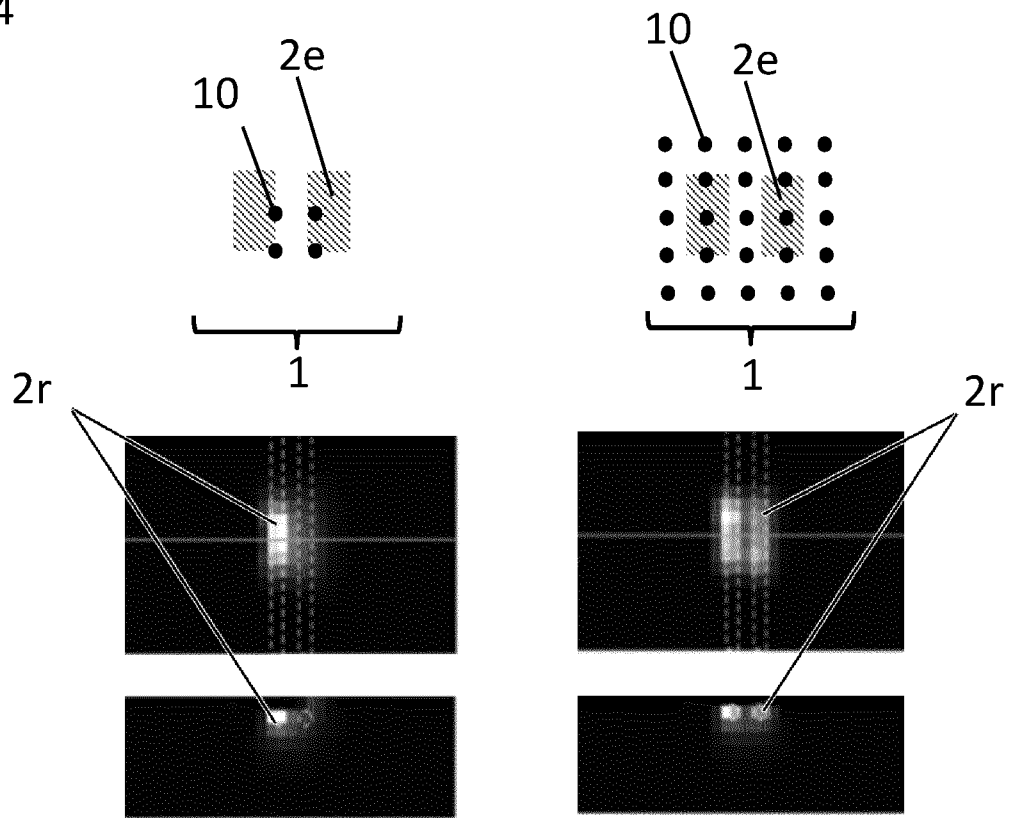
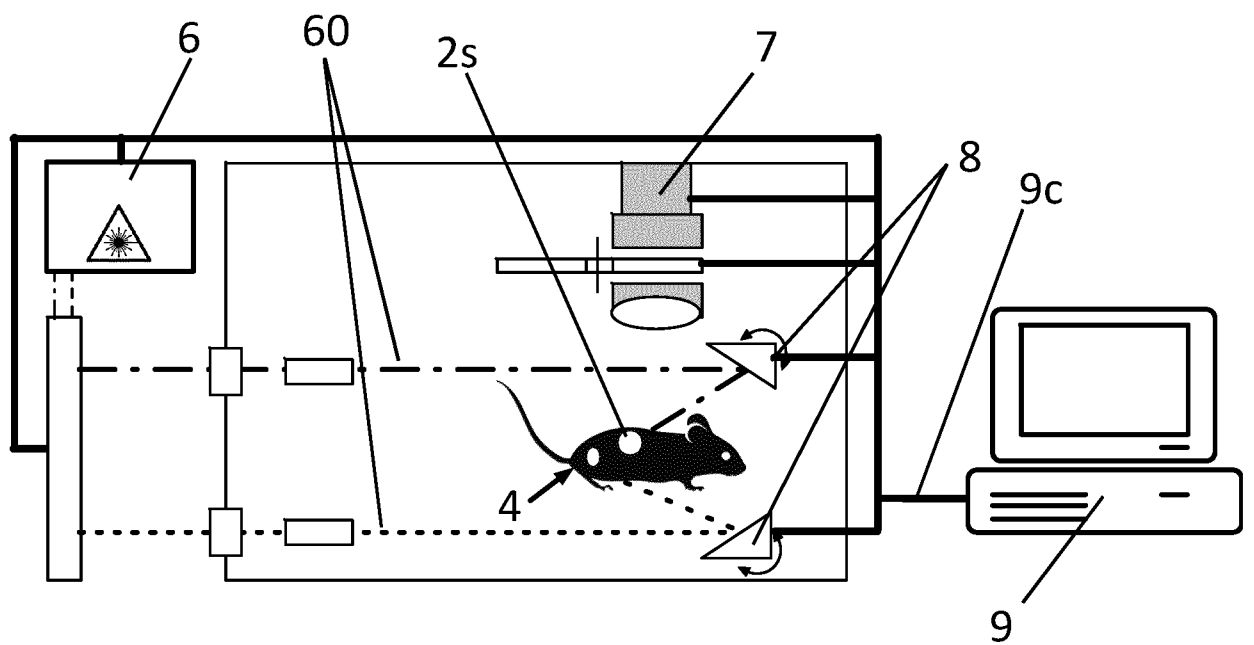


Fig. 5



INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2020/051779

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61B5/00 G01N21/64
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)

A61B G01N

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JIN AN ET AL: "Light Illumination and Detection Patterns for Fluorescence Diffuse Optical Tomography Based on Compressive Sensing", IEEE TRANSACTIONS ON IMAGE PROCESSING, IEEE SERVICE CENTER, PISCATAWAY, NJ, US, vol. 23, no. 6, June 2014 (2014-06), pages 2609-2624, XP011547604, ISSN: 1057-7149, DOI: 10.1109/TIP.2014.2300756 [retrieved on 2014-05-07] the whole document ----- -/-	1-15



Further documents are listed in the continuation of Box C.



See patent family annex.

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"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

19 March 2020

Date of mailing of the international search report

29/04/2020

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Riblet, Philippe

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2020/051779

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOYITA DUTTA ET AL: "Illumination pattern optimization for fluorescence tomography: theory and simulation studies; Illumination pattern optimization for fluorescence tomography", PHYSICS IN MEDICINE AND BIOLOGY, INSTITUTE OF PHYSICS PUBLISHING, BRISTOL GB, vol. 55, no. 10, 21 May 2010 (2010-05-21), pages 2961-2982, XP020171655, ISSN: 0031-9155 Sections 3-5.; figure 1	1-15
A	VENUGOPAL VIVEK ET AL: "Adaptive wide-field optical tomography", JOURNAL OF BIOMEDICAL OPTICS, SPIE, vol. 18, no. 3, 8 March 2013 (2013-03-08), page 36006, XP060024092, ISSN: 1083-3668, DOI: 10.1117/1.JBO.18.3.036006 [retrieved on 2013-03-08] Section 3.; figures 3-4	1-15
A	RUOYANG YAO ET AL: "Wide-field fluorescence molecular tomography with compressive sensing based preconditioning", BIOMEDICAL OPTICS EXPRESS, vol. 6, no. 12, 17 November 2015 (2015-11-17), page 4887, XP055592051, United States ISSN: 2156-7085, DOI: 10.1364/BOE.6.004887 Sections 2-4.	1-15
A	ROBERT W. HOLT ET AL: "Methodology to optimize detector geometry in fluorescence tomography of tissue using the minimized curvature of the summed diffuse sensitivity projections", JOURNAL OF THE OPTICAL SOCIETY OF AMERICA A, vol. 30, no. 8, 19 July 2013 (2013-07-19), page 1613, XP055592129, US ISSN: 1084-7529, DOI: 10.1364/JOSAA.30.001613 the whole document	1-15