(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 March 2025 (27.03.2025)

WIPO | PCT

(10) International Publication Number
WO 2025/062360 A1

(51) International Patent Classification:
G01N 23/04 (2018.01) *G01N 13/00* (2006.01)
G01N 23/06 (2018.01) *G01N 1/30* (2006.01)
G01N 23/083 (2018.01)

(21) International Application Number:
PCT/IB2024/059166

(22) International Filing Date:
20 September 2024 (20.09.2024)

(25) Filing Language: Italian

(26) Publication Language: English

(30) Priority Data:
102023000019599 22 September 2023 (22.09.2023) IT

(71) Applicants: **IMBERG SPA** [IT/IT]; Via Pietro Savoldini, 73, 24057 Martinengo (BG) (IT). **UNIVERSITA' DEGLI STUDI DI BERGAMO** [IT/IT]; Via Salvecchio, 19, 24129 Bergamo (BG) (IT).

(72) Inventors: **LUPO, Carmelo**; c/o Diapath S.p.A., Via Pietro Savoldini, 71, 24057 Martinengo (BG) (IT). **CASATTA, Nadia**; c/o Diapath S.p.A., Via Pietro Savoldini, 71, 24057 Martinengo (BG) (IT). **BATTISTEL, Alberto**; c/o Diapath S.p.A., Via Pietro Savoldini, 71, 24057 Martinengo (BG) (IT). **BERGAMINI, Vladimiro**; c/o Diapath S.p.A., Via Pietro Savoldini, 71, 24057 Martinengo (BG) (IT). **SANTINI, Maurizio**; c/o UNIVERSITA' DEGLI STUDI DI BERGAMO, Via Salvecchio, 19, 24129 Bergamo (BG) (IT). **FEST-SANTINI, Stephanie**; c/o UNIVERSITA' DEGLI STUDI DI BERGAMO, Via Salvecchio, 19, 24129 Bergamo (BG) (IT). **COSSALI, Gianpietro**; c/o UNIVERSITA' DEGLI STUDI DI BERGAMO, Via Salvecchio, 19, 24129 Bergamo (BG) (IT).

(74) Agent: **TRUPIANO, Federica** et al.; MARIETTI, GISLON E TRUPIANO S.R.L., Via Larga, 16, 20122 Milano (IT).

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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

WO 2025/062360 A1

(54) Title: SYSTEM AND METHOD FOR PROCESSING BIOLOGICAL SAMPLES

(57) Abstract: The present invention relates to a system and method for the processing of biological samples, for example, cytological, histological and autopsical samples, in particular to a process for optimizing the fixation, dehydration and clarification treatments necessary for the subsequent paraffin infiltration of said biological samples.

"System and method for processing biological samples"

****

Field of the invention

The present invention relates to a system and method for the processing of biological samples, for example, cytological, histological and autopsical samples, in particular to a system and process for optimizing the fixation, dehydration and clarification treatments necessary for the subsequent paraffin infiltration of said biological samples.

Technical background

The anatomo-pathological diagnosis is the result of the interpretation by the anatomo-pathologist of the morphological, macroscopical and microscopical characteristics of the biological sample under examination.

To provide an accurate and complete diagnosis, the sample excised from the patient must undergo a series of treatments with the purpose of ensuring its preservation over time. Such treatments essentially provide for:

i. fixation;
ii. dehydration;
iii. clarification; and
iv. paraffin infiltration.

The first stage of such processes consists in the fixation of the sample in a 10% buffered neutral formalin solution, which is able to minimize the degradation phenomena caused by the removal of the tissue from its vital environment and by the possible presence of microorganisms.

After the fixation, the sample is usually dehydrated by several subsequent steps with ethanol solutions of increasing concentration, up to 99% ethanol and subsequently clarified with xylene. The purpose of these steps is to remove water from the sample to allow its subsequent

infiltration in paraffin, which is a hydrophobic substance.

The final paraffin infiltration is necessary to make the sample solid enough to be cut into micrometer sections, to be subsequently stained and analyzed.

The purpose of the various steps of the dehydration treatment (with ethanol solutions) is to replace the water inside the tissue with solutions of increasing hydrophobicity to make possible the penetration of paraffin, which is a completely hydrophobic substance. Generally, after the formalin fixation, a dehydration and clarification treatment protocol provides for subjecting the sample to the following steps:

- treatment with 70% alcohol;
- treatment with 90% alcohol;
- treatment with 99% alcohol (three times); and
- clarifying treatment with xylene (three times).

After the clarification step with xylene, which is able to remove the alcohol and to clearly increase the compatibility with paraffin, in terms of hydrophobicity, the tissue is infiltrated, usually by three consecutive baths of melted paraffin at a temperature of about 60°C. The triple step allows to obtain an infiltrated tissue free of xylene, which is released from the sample especially in the first paraffin bath.

Then the sample can be included in a paraffin block and cut by a microtome, after which it can be further processed for analysis, for example, it can be subjected to staining to increase the contrast between different structures in the sample, to highlight different tissue and cellular components.

Proper processing allows the structural framework of the biological sample to be kept as unaltered as possible, thus ensuring accurate analysis of the sample.

In addition to protecting the physical structure of the sample, it is also important that all the

components on the sample that are useful for the diagnosis, such as antigens, markers and the like, are able to pass the processing treatments unaltered. Indeed, some of these components are complex molecules, such as for example proteins, and inappropriate treatments can lead to denaturation of their quaternary structure. Other components of the samples are thermolabile or easily degradable over time, and inappropriate processing jeopardizes their detection at the time of analysis.

Therefore, it is understood that only rapid and proper processing of the samples allows reliable diagnosis to be made.

To date, the processing treatments described above (basically fixation, dehydration and clarification) are carried out according to standard protocols under predetermined conditions, that is, with predetermined amounts of solvent and in predetermined infiltration time frames. However, such predetermined parameters do not in any way take into account the actual infiltration of the sample with the solvent. As a result, depending on their cellular or tissue structure and/or size, some biological samples may be poorly infused, for example, due to the use of an insufficient amount of solvent or insufficient processing time. Conversely, other samples may be subjected to infusion with excessive amounts of solvents and/or for longer times than necessary, resulting in damage to the structure and components of the sample.

In all of the above cases, the structure and composition of the biological sample may be altered and the resulting analysis and diagnosis may be incorrect.

In light of the above, it is understood that monitoring the diffusion of the solvents through a biological sample is essential to determine if said solvents have actually infused the entire sample and allows the risk of carrying out the analysis on altered samples to be minimized or at least limited.

Methods of monitoring the solvent diffusion into a biological sample by using optical or X-ray

tomography (OCT or CAT), or X-ray micro-tomography (micro-CT) are known in the art.

For example, a system for monitoring the quality of heavy metal staining and diffusion during preparation protocols of biological samples for electron microscopy is known from the document US2020/0150063. The disclosed approach uses X-rays via a commercially available micro-CT device, to observe and measure the diffusion and distribution of heavy metals during conventional staining procedures of biological samples for electron microscopy.

Tomography is an advanced displaying method that uses X-rays to obtain a three-dimensional volume of a biological sample downstream of a total acquisition of the sample rotated about an axis, and only then detailed cross-sectional images can be obtained non-destructively. Optical tomography creates a series of cross-sectional images that can be combined to produce a three-dimensional view of the biological sample being analyzed in a destructive manner.

One of the main advantages of tomography is its ability to provide very precise details thanks to the acquisition and subsequent combination of a large number of X-ray images.

However, such diagnostic technology also has several limitations: it is a time-consuming technique, due to the large number of X-ray images (at least about 1,200, for a significant resolution on biological samples) that must be acquired to produce a three-dimensional view of the biological sample under analysis: for an average-sized sample (for example, 1 cm x 1 cm x 1 cm sample), the time required is between 40 and 180 minutes and is certainly longer than the diffusion time of a reagent into the biological sample under analysis, which is on the order of a few minutes, or less.

Therefore, this does not allow a real time estimation of the progress of solvent diffusion into the biological sample, especially if the reagent in question diffuses rapidly into the biological sample or if the biological sample is not constituted by a homogeneous tissue, for example, one that comprises vessels for example capillaries and/or lymphatic vessels able to accelerate the

speed of solvent diffusion into the sample, or denser parts of tissue that, on the contrary, slow it down.

For the final result of monitoring by tomography the diffusion of the solvents into a biological sample to be reliable, it would be necessary to be certain that the sample is stable and homogeneous in terms of diffusion speed, in the long time-interval (40 to 180 minutes) required to perform the tomography.

A further disadvantage of tomography and micro-tomography is the high cost of known apparatuses: the high cost does not allow their use in ordinary analytical laboratories.

Thus, there is a need for methods and devices suitable for the effective and rapid processing of biological samples, for example cytological, histological and autopsical samples, that are cheaper than the apparatuses of the known art, that can be easily integrated into equipment in common use in analytical laboratories, and that, in general, overcome the limitations and disadvantages of the prior art.

Objects of the invention

An object of the invention is to provide a method suitable for the efficient and rapid processing of biological samples, such as cytological, histological and autopsical samples for their analysis.

Another object of the invention is to provide a system for implementing the method of the invention in laboratories for the processing of biological samples, such as cytological, histological and autopsical samples for their analysis.

Description of the invention

These and other objects are achieved by means of the invention developed by the Applicant, which comprises a system and method that provides information on the saturation of a biological sample with a reagent, and/or on the diffusion coefficient of different processing solvents for different types of organs, tissues and cells, in order to enable improved processing

and more accurate analysis of biological samples.

Furthermore, the invention provides a system that is easy to use in laboratories for processing and analyzing biological samples, which enables the rapid and efficient processing of said samples.

In particular, these and other objects are achieved by a system according to claim 1, by the related method according to claim 11, and by the related use according to claim 19.

Note that, according to the present invention, by "processing" is meant to denote the treatments of fixation and/or dehydration and/or clarification, whether done singly, sequentially or simultaneously. As an example, the dehydration and clarification steps can be carried out at the same time, by using appropriate reagent mixtures.

According to an embodiment, the processing according to the invention comprises the fixation and/or dehydration and/or clarification steps.

According to an embodiment, the processing according to the invention consists of the fixation and/or dehydration and/or clarification steps.

According to the present invention, by "biological sample" is meant to denote any biological, cytological, histological and autopsical sample to be subjected to an anatomo-pathological analysis.

According to an embodiment, the biological sample according to the invention is intended for the analysis under an optical microscope. According to an embodiment, the biological sample according to the invention is not a biological sample intended for the electron microscopic analysis.

According to the present invention, by "processing reagent" is preferably meant to denote a compound or mixture of compounds in the liquid state, which are suitable for processing biological samples, as defined herein. Such reagents include fixative agents, such as for

example fixatives, including, for example, formalin, preferably neutral buffered formalin, and dehydrating and clarifying agents, such as alcohols, for example, isopropyl alcohol or ethanol; hydrocarbons, for example, xylene, naphtha, octane, limonene, isoparaffin and the like. Reagent mixtures can also be used.

However, if desired or necessary, the system and method of the invention can be applied to other steps of the processing of biological samples, such as for example sample staining, where said staining does not include staining which comprises reagents containing heavy metals.

Indeed, according to the invention, the system and method described and claimed herein do not include the measurement of the diffusion and distribution of heavy metals used, for example, during staining procedures of biological samples; furthermore, the system and method described and claimed herein do not include the measurement of the diffusion and distribution of heavy metals used, for example, during staining procedures of biological samples by electron microscopy.

The term "heavy metals" is meant herein to refer to heavy metals, salts and heavy metal compounds used in the processing of biological samples. The staining and reagents containing heavy metals are known in art.

According to the invention, the system and method described and claimed herein do not include a tomograph or micro-tomograph.

The system according to the invention, for monitoring the development of the processing of a biological sample by perfusion with a processing reagent ,comprises a housing for a sample, an X-ray radiation source configured to emit an X-ray beam and adapted to perform a plurality of radiographies during the diffusion over time of said reagent into said biological sample, a signal detector to detect the radiation exiting the sample and acquire corresponding X-ray images, a processor implemented to process in real time a plurality of said X-ray images of the biological

sample under investigation, to estimate the average value of the attenuation over time of the X-ray radiation inside the sample under investigation, in order to monitor the completion of the saturation of said sample with said reagent.

Note that, by "X-ray images" is meant a series of two-dimensional images obtained by radiography.

As is known, radiography works by sending a short dose of ionizing radiation (X-rays) through the biological sample under investigation and detecting how much is absorbed by tissues and organs.

One of the main advantages of radiography is its speed and ease of use.

Images are captured quickly and can be immediately available for review.

Unlike tomography, which requires the acquisition of a series of cross-sectional images that can be combined to produce a three-dimensional view, radiography provides a single two-dimensional image.

Furthermore, it should be noted that by means of the system according to the invention, it is possible to acquire the necessary X-ray images in a predetermined time interval, regardless of the sample size, whereas for instruments that exploit tomography, the image acquisition time and the number of images needed varies according to the sample size.

In this regard, an additional advantage of the system according to the invention over the devices of the known art that exploit tomography is that the sample does not have to be rotated on itself for the image acquisition, but can be placed on a fixed sample holder.

Furthermore, the use of a radiographic system requires emissions of X-rays (a single radiography instead of a series of radiographies required to make a single tomographic reconstruction) with shorter time, and consequently requires reduced complication for the protections according to the law of these scientific apparatuses.

According to the invention, perfusion of the biological sample with a processing reagent is carried out in order to achieve the diffusion of said reagent into said sample and the subsequent replacement of the liquids contained in said sample with said reagent.

According to the invention, by "saturation" of the sample is meant the complete diffusion of the processing reagent into said sample, resulting in the complete replacement of the liquids previously contained in said sample with said reagent.

A fully perfused sample is therefore saturated (or perfused to saturation), that is, a sample in which the processing reagent has diffused into said sample by replacing all previously present liquids of said sample.

Advantageously, the system according to the invention allows to assess the state of the sample processing, that is, to evaluate the completion of the sample saturation by the reagent, only by analyzing X-ray images, which provides a simple and inexpensive tool for monitoring sample processing.

Furthermore, the system according to the invention makes it possible to evaluate the state of the sample processing, that is, to assess the completion of the saturation of the sample by the reagent in a much shorter time than systems of the known art that exploit tomography or micro-CT, thanks to the fact that it is necessary to acquire a few X-ray images (a minimum of two) to estimate the average value of the attenuation over time of the X-ray radiation inside the sample under investigation, to monitor the completion of the saturation of said sample with said reagent.

In contrast, as mentioned, the optical tomography requires the acquisition of a large number of images (at least about 1,200) and their processing to produce a three-dimensional view of the biological sample under analysis. It follows that such analysis technique is not appropriate for real-time perfusion testing of biological samples. Advantageously, the presence of an X-ray

radiation source allows to verify the diffusion coefficient in real time by analyzing and processing simple two-dimensional radiographies acquired by the signal detector, following the radiation of the sample under investigation by the X-ray source.

Indeed, advantageously, the system according to the invention allows a processing time of X-ray images comparable to the diffusion time of a reagent in the biological sample under investigation, which is in the order of few minutes, therefore, this allows a real time estimation of the progress of solvent diffusion into the biological sample, even if the reagent in question, as stated, diffuses rapidly into the biological sample, or if the biological sample is not constituted by a homogeneous tissue, for example one that comprises vessels, for example capillaries and/or lymphatic vessels, that are able to accelerate the speed of solvent diffusion into the sample, or denser parts of tissue that, on the contrary, slow it down.

According to an aspect, the processor is implemented to calculate the estimated processing time of said sample with said reagent, as a function of said estimated average attenuation value of the X-ray radiation.

Advantageously, the system allows to estimate the time required for each sample to be properly and completely perfused, up to saturation, based only on the analysis and processing of X-ray images.

According to an aspect, the processor is implemented to estimate in real time the value of the diffusion coefficient of said reagent into said sample, as a function of said average value of the attenuation over time of the X-ray radiation inside the sample under investigation, for monitoring the development of the processing of said sample.

According to an aspect, the processor is implemented to calculate the estimated processing time of said sample with said reagent, as a function of said value of diffusion coefficient.

Advantageously, the real-time estimation of the diffusion coefficient allows estimation, in real

time precisely, of the actual processing time required for the completion of diffusion of the reagent into the sample. Following this estimation, an operator can adjust the processing time and/or speed.

According to an aspect, the system comprises an output device that displays the estimated processing speed and/or estimated processing time of the sample with the reagent.

Advantageously, the presence of an output device allows an operator to view and monitor the estimated processing time.

Furthermore, according to an aspect, the X-ray radiation source operates in a range between 110 kV and 130 kV, preferably 120 kV, and in a range between 40 μA and 60 μA, preferably 50 μA, the endpoints of the ranges being included.

According to an aspect, the sample scans are acquired at a scan rate of about 1 Hertz for a total time of about 30 minutes.

Advantageously, such system allows high-resolution X-ray images of the sample under investigation to be obtained without being destructive to the sample.

According to an aspect, the system comprises a memory coupled to the processor, wherein a plurality of standard values of diffusion coefficients of a plurality of porous tissue samples, each associated with a respective standard value of speed and/or diffusion time of said reagent into said porous tissue sample are stored in the memory, wherein the processor is implemented to perform the operations comprising:

(a) receiving as input one of the values of the standard diffusion coefficient stored in the memory, associated with the tissue sample under investigation,

(b) comparing said value of the standard diffusion coefficient selected from said memory, associated with the sample under investigation, with the diffusion coefficient value estimated in real time by scanning the sample with the X-ray source.

According to such aspect, the processor is implemented to calculate the estimated processing time of said sample with said reagent, as a function of said value of the standard diffusion coefficient received as input, and preferably as a function of the size of said sample.

Advantageously, the system allows to estimate the time required for each sample to be correctly and completely perfused, based on previously estimated values of standard diffusion coefficients for each specific type of organ, and stored on the system's memory, and based on the size of the extracted histological sample.

Compared with the known art, thanks to the system and method described, the time required for each sample to be correctly and completely perfused is no longer estimated solely on the basis of experimental evidence and is dependent on the type of organ and the size of the histological sample extracted.

The use of standard diffusion coefficients, stored in the system's memory, allows to optimize the processing of the sample, thus reducing processing time compared to systems available in the known art, decreasing the amount of chemicals required and reducing the possibility of failure at each step.

Real-time verification of the diffusion coefficient allows to compare, in real time exactly, the actual processing time required to complete the diffusion of the reagent into the sample, with the estimated processing time obtained on the basis of the standard diffusion coefficient selected by the operator and received as input from the processor and of the sample size. As a result of this comparison, an operator can make a time and/or processing speed correction, or a time and/or processing speed correction can be operated automatically by the processor.

According to such aspect, the processor is implemented to compare said value of standard diffusion coefficient selected from the memory and received as input from the processor, associated with the sample under investigation, with the value of the diffusion coefficient

estimated in real time by scanning the sample with the X-ray source, and to adjust the processing time to achieve the completion of the diffusion of said reagent into said sample, that is, the saturation of the sample with the reagent.

Advantageously, the system allows a real-time verification of the previously estimated processing time based on the value of standard diffusion coefficient inputted to the processor and enables correction of the actual processing time in case the actual processing time is different from the estimated time.

According to an aspect, the output device displays the value of the diffusion time remaining to achieve the completion of the diffusion of the said reagent into the said sample (until "saturation" of the sample).

As mentioned, the present invention is furthermore aimed to a method for monitoring the development of the processing of a biological sample by perfusion with a processing reagent in order to diffuse said reagent into said sample, by means of a system according to the invention, said method comprising the following steps:

- acquiring, by means of said detector, a plurality of X-ray images of said sample, by performing a plurality of scans of the sample by means of said X-ray radiation source during the diffusion over time of said reagent into said sample;
- processing said X-ray images in real time by said processor, to estimate in real time the average value of the attenuation of X-ray radiation at different radial distances inside the sample under investigation,
- monitoring the completion of the saturation of the sample with the reagent, based on the estimated real-time average value of the attenuation over time of X-ray radiation.

According to an aspect, the method comprises a step of adjusting the processing time to achieve the completion of the saturation of said sample with said reagent.

According to an aspect, the method comprises the step of displaying, by means of the output device, the value of estimated processing speed and/or estimated processing time of said sample with said reagent.

According to an aspect, the method comprises the step of estimating in real time the value of the diffusion coefficient of said reagent into said sample to monitor the development of the processing of said sample.

According to an aspect, the method comprises the step of estimating the processing time of said sample with said reagent, as a function of said value of diffusion coefficient.

According to an aspect, the value of the diffusion coefficient is estimated in real time by a measurement of the change in the reagent concentration in the sample under investigation, correlated with said average value of the attenuation over time of the X-ray intensity inside the sample under investigation.

According to an aspect, the value of the diffusion coefficient is estimated in real time by the following steps:

- acquiring said X-ray images and calculating the attenuation value over time of the intensity of the X-ray radiation in the sample under investigation;
- calculating the value of the change in reagent concentration in the sample under investigation as a function of the obtained value of effective intensity attenuation of X-ray radiation in the sample under investigation as a function of time;
- calculating in real time the value of the diffusion coefficient of said reagent into said sample, as a function of that value of the change in reagent concentration in the sample under investigation.

According to an aspect, the method comprises the following steps:

- selecting a standard diffusion coefficient value associated with the sample under

investigation, and inputting it to said processor;

- calculating, by means of said processor, the processing time of said sample with said reagent, as a function of said selected value of standard diffusion coefficient, and preferably as a function of the size of said sample.

Finally, the present invention is aimed to the use of an X-ray radiation source in a system and method for processing a biological sample by perfusion with a processing reagent as described above.

Brief description of the Figures

The characteristics and advantages of the system and method according to the invention will be evident from the description hereunder related to its preferred embodiments and given by way of example and not limitation, with reference to the attached figures, in which:

- Figure 1 shows a system according to an embodiment of the invention;
- Figure 2 schematically shows a sample under investigation scanned by an X-ray radiation source according to the invention;
- Figure 3A shows a plot of the X-ray attenuation over time, measured at different radial distances from the center of the sample and obtained from ten ROIs (Regions Of Interest) aligned in the same X-ray image of a sample;
- Figure 3B shows ten ROIs (Regions Of Interest) aligned in an X-ray image of the sample;
- Figure 4 shows a plot of the value of the estimated diffusion coefficient for different ROIs at different radial distances from the center of the sample.

Detailed description of the invention

With reference to the attached figures, some possible embodiments of the system 1 according to the present invention will be described.

Note that the samples that can be processed by the system 1 according to the invention can be samples of tissues, organs, cells, as well as whole organs or portions of tissues, of different shapes and sizes, as well as of different morphology, density and texture.

The system 1 according to the invention, for monitoring the development of the processing of a biological sample by perfusion with a processing reagent, in order to achieve the diffusion of said reagent into said sample and the subsequent replacement of the liquids contained in said sample with said reagent, comprises a housing 4 for a sample and a processor 5.

The system 1 according to the invention comprises an X-ray radiation source 2 configured to emit an X-ray beam and suitable for performing a plurality of scans during the diffusion over time of the reagent into the sample, and a signal detector 3 to detect the radiation exiting the sample and acquire corresponding X-ray images.

In a possible embodiment, there are two X-ray images acquired and they are acquired with a time interval of about one second.

In a further embodiment, 2 to 1200 X-ray images are acquired and they are acquired with a time interval calculated based on the estimation of the effective diffusion rate into the sample and which can be perfectly and easily adapted to the needs. Such estimation is within the reach of the skilled in the art.

Preferably, the X-ray source is a micro-focus source.

In a possible embodiment, the X-ray source is an open-tube source with micro-focus operating at voltages between 110 kV and 130 kV, preferably 120 kV, and currents between 40 μA and 60 μA, preferably 50 μA, the endpoints of the ranges being included.

In some embodiments, the focusing spot is selected to be about 5 μm. However, it is possible to use a smaller focal spot size, for example when higher resolution is needed.

The scans of the sample can be acquired at a scan rate of about 1 Hertz for a total time of about

30 minutes or more, until the sample is saturated.

The X-ray beam is acquired by the X-ray detector 3, which is suitable for acquiring two-dimensional X-ray images of the sample under investigation.

Preferably, the detector device 3 comprises a photo-detector in the visible field and optically coupled to a scintillator to convert the X-ray beam that has passed through the sample into an optical signal that impinges on the photo-detector.

For example, the detector 3 may be a detector of the type comprising a panel consisting of single-substrate amorphous silicon TFT active diode arrays, and a direct deposition thallium-doped Cesium iodide (CsI(TI)) scintillator, having a pixel size of 100 μm and a total size of 4096x4096 pixels, at 16 bits (equal to 65536 gray levels).

The X-ray images acquired by the X-ray detector 3 are then processed to optimize the contrast and define the *region of interest* (ROI) of radiation diffusion into the sample, subsequently processed by using a code that can be developed through any program, including opensource, by way of non-limiting example a Matlab® code.

The detector 3 and source 2 can be mounted on respective support bases to allow optical alignment between the elements of the apparatus.

In a preferred embodiment shown in Figure 2, the emitted X-ray beam is conical in shape and a main axis of the beam emitted by the source can be defined, which in the system 1 shown in Figure 1 coincides with a main direction of incidence of the beam on the sample, denoted by the X-axis in Figure 2.

A sample is placed between the source 2 and the detector 3 so that it is crossed by the X-ray beam emitted by the source 2.

In some embodiments, the sample is placed on a sample holder P, which in turn is mounted on the housing 4.

The processor 5 of the system 1 according to the invention is implemented to process in real time a plurality of X-ray images of the biological sample under investigation, which are acquired by the signal detector 3 by making a plurality of scans of the sample by the X-ray radiation source 2 during the diffusion over time of the reagent into the sample.

As mentioned, in an embodiment, two consecutive X-ray images are sufficient.

The X-ray images are processed by the processor 5 to estimate in real time the average value of the attenuation $I_{eff}(t)$ of X-ray radiation at different radial distances inside the sample under investigation, to monitor the completion of the saturation of the sample with the reagent.

Note that the X-ray images detected by the detector 3 are preferably acquired as digital images by the processor 5, which is logically connected to the detector 3 in order to receive the output signals.

The X-ray images that are digital, or digitized in case they are acquired by the processor 5 in analog form, are recorded as two-dimensional arrays of pixels, whose number of pixels and size of a single pixel depend mainly on the spatial resolution of the detector device 3.

In an embodiment, the processor 5 is also implemented to calculate the estimated processing time t_0 of the sample with the reagent, as a function of the estimated average attenuation value $I_{eff}(t)$ of the X-ray radiation.

With reference to Figure 1, the system 1 can comprise an output device 7 that displays the estimated processing speed v_0 and/or estimated processing time t_0 of the sample with the reagent. In such a way an operator can see the speed and/or estimated processing time.

In an embodiment, the processor 5 is also implemented to estimate in real time the value of the diffusion coefficient D_{eff} of the reagent into the sample, as a function of the average value of the attenuation over time $I_{eff}(t)$ of the X-ray radiation inside the sample under investigation, for monitoring the development of the processing of the sample.

With reference to the method according to the invention, the way in which the diffusion coefficient D_{eff} is estimated in real time will be better explained below.

In a possible embodiment, the processor 5 is also implemented to calculate the estimated processing time t_0 and/or estimated processing speed v_0 of the sample with the reagent, as a function of the value of diffusion coefficient D_{eff}. In an embodiment, the system 1 comprises a memory 6 coupled to the processor 5.

A plurality of standard values of diffusion coefficients D_{std} of a plurality of porous tissue samples, each associated with a respective standard value of speed v_{std} and/or diffusion time t_{std} of the reagent into the porous tissue sample under investigation, are stored in the memory 6.

Note that standard values of diffusion coefficients D_{std} of the different porous tissue samples recorded in the memory 6 can be calculated by the system 1 according to the invention, which exploits the processing and analysis of X-ray images obtained from samples of different organs, tissues or cells.

In such embodiment, the processor 5 is further implemented to perform the operations comprising:

(a) receiving as input one of the values of the standard diffusion coefficient D_{std} stored in the memory 6, associated with the tissue sample under investigation,

(b) comparing the value of standard diffusion coefficient D_{std} selected from the memory 6, associated with the sample under investigation, with the diffusion coefficient value D_{eff} estimated in real time by scanning the sample with the X-ray source 2.

Note that, in a possible embodiment, the processor 5 is implemented to calculate the estimated processing time t_0 of the sample with the reagent, as a function of the value of standard diffusion coefficient D_{std} received as input, and preferably as a function of the size of the sample.

As a result of the comparison of the diffusion coefficient D_{eff} estimated in real time with the standard diffusion coefficient D_{std} selected from the memory 6, a user can adjust the processing time accordingly to achieve the completion of the perfusion of the said reagent in the said sample.

In a possible embodiment, the system 1 may comprise a user interface 100, by which an operator can select the sample under investigation type associated with a respective value of standard diffusion coefficient D_{std} stored in the memory 6, such that the processor 5 receives as input such selected value of standard diffusion coefficient D_{std}.

Furthermore, the system 1 can comprise an output device 7 that displays the estimated processing speed v_0 and/or estimated processing time t_0 of the sample with the reagent. In such a way an operator can see the speed and/or estimated processing time.

In a possible embodiment, the processor 5 is also implemented to adjust the processing time and/or processing speed after this comparison, to achieve the completion of the reagent diffusion into the sample.

In this regard, in a possible embodiment, the output device 7 displays the value of remaining diffusion time $t_{r,\,eff}$ to achieve the completion of the diffusion of the reagent into the sample.

As mentioned, the present invention is aimed to a method for the processing of biological samples, in particular for monitoring the development of the processing of a biological sample by perfusion with a processing reagent, by using a system according to the invention.

The method according to the invention comprises a first step (a) of acquiring a plurality of high resolution X-ray images of the sample by means of the detector 3, by performing a plurality of scans of the sample by means of the X-ray radiation source 2 during the diffusion over time of the reagent into the sample.

In a preferred embodiment, the scans are performed during the diffusion over time of the reagent

into the sample at a scan frequency of about 1 Hertz, for a total time of about 30 minutes.

In a possible embodiment, by the processor 5, it is possible to program the acquisition times of the X-ray images and/or the number of images acquired and/or the optical parameters of the X-ray beam emitted by the source 2.

The method according to the invention further comprises a second step (b) of processing the X-ray images acquired in real time by the processor 5, to estimate in real time the average value of the attenuation $I_{eff}(t)$ of the X-ray radiation over time at different radial distances inside the sample under investigation.

As shown for illustrative purpose with reference to Figures 3A and 3B, ten aligned ROIs (Regions Of Interest) can be identified in the same X-ray image at different radial distances from the center of the sample (Figure 3B), and for each of such regions the corresponding value of the X-ray attenuation over time, measured at the corresponding different radial distances from the center of the sample, can be calculated.

Furthermore, the method according to the invention comprises a step of monitoring the completion of the saturation of the sample with the reagent, based on the average value estimated in real time of the attenuation over time $I_{eff}(t)$ of X-ray radiation.

In an embodiment, the method comprises a step of adjusting the processing time (t_0) to achieve the completion of the saturation of the sample with the reagent.

In an embodiment, the method comprises the step of estimating in real time the value of the diffusion coefficient D_{eff} of the reagent into the sample, for monitoring the development of the processing of said sample: thus the processing time t_0 of the sample with the reagent can be estimated as a function of the value of diffusion coefficient D_{eff}.

As shown as an example in Figure 4, the value of the diffusion coefficient D_{eff} can be estimated for different ROIs, at different radial distances from the center of the sample.

Note that in a possible embodiment, the value of the diffusion coefficient D_{eff} is estimated in real time by processing the X-ray images acquired by the X-ray detector 3 to optimize the contrast and define the ROI (*region of interest*) of radiation diffusion into the sample, and then using a code that can be developed through any program, including opensource, by way of non-limiting example a Matlab® code.

In particular, the detector 3 records a series of two-dimensional X-ray images generated by the attenuation over time of X-ray radiation through the sample such that each X-ray image acquired corresponds to a step of the saturation of the sample with the reagent.

The processor 5 receives a plurality of X-ray images acquired from the detector 3, preferably while the sample is positioned along an axis transverse to the main direction of beam incidence on the sample.

Note that the 2D X-ray images detected by the detector 3 are preferably acquired as digital images by the processor 5, which is logically connected to the detector 3 in order to receive the output signals.

The X-ray images that are digital, or digitized in case they are acquired by the processor in analog form, are recorded as two-dimensional arrays of pixels, whose number of pixels and size of a single pixel depend mainly on the spatial resolution of the detector device.

In order to determine in real time the value of the diffusion coefficient D_{eff} of the reconstructed volume of the sample, in an embodiment the method proceeds with an automatic image processing procedure so as to define and highlight the sample portions perfused by the reagent, to obtain a measurement of the concentration change χ in the reagent in the sample under investigation, which measurement is correlated with the value of the attenuation over time of the intensity $I_{eff}(t)$ of X-rays within the sample under investigation.

In particular, preferably, the value of the diffusion coefficient D_{eff} is estimated in real time by

a measurement of the concentration change χ in the reagent in the sample under investigation, which measurement is correlated with the value of the attenuation over time of the X-ray intensity $I_{eff}(t)$ inside the sample under investigation, detected by analyzing the plurality of high-resolution X-ray images acquired.

In more detail, according to a possible embodiment, during the estimation step in real time of the value of the diffusion coefficient D_{eff}, the processor 5 also performs the following steps:

- acquiring the X-ray images digitized and calculating the attenuation value over time of the intensity $I_{eff}(t)$ of the X-ray radiation, as a function of the time in the sample under investigation, as shown as an example in Figures 3A and 3B;
- calculating the value of the concentration change χ in the reagent in the sample under investigation, as a function of the obtained value of effective intensity attenuation $I_{eff}(t)$ of X-ray radiation in the sample under investigation as a function of time;
- calculating the actual value of the diffusion coefficient D_{eff} of said reagent into said sample, as shown as an example in Figure 4, as a function of such value of the concentration change χ in the reagent in the sample under investigation.

Note that the diffusion into the biological sample is modeled by assuming isotropic behavior and cylindrical symmetry of the sample, with a constant and uniform effective diffusion coefficient. Therefore, the diffusion equation in cylindrical coordinates is:

$$\frac{\partial \rho}{\partial t} = D_{eff}\left[\frac{\partial^2 \rho}{\partial r^2} + \frac{1}{r}\frac{\partial \rho}{\partial r} + \frac{\partial^2 \rho}{\partial z^2}\right]$$

In such equation, with reference to Figure 2, we have $r \leq R_0$ and $z \leq z_0$, where R_0 is the sample radius, $2z_0$ is its length along the vertical axis, and ρ is the concentration of the diffusing reagent in the sample.

ρ_0 is the uniform initial value of ρ, and ρ_1 the value on the sample surface (assumed uniform

and constant). Therefore, the initial and boundary conditions are then

$$\rho(r,0) = \rho_0$$

$$\rho(R_0,t) = \rho_1 \; ; \rho(z,t) = \rho_1$$
$$\left(\frac{\partial \rho}{\partial r}\right)_{r=0} = 0 \; ; \left(\frac{\partial \rho}{\partial z}\right)_{z=0} = 0$$

By substituting:

$$\chi = \frac{\rho - \rho_1}{\rho_0 - \rho_1}$$

the substitution produces the following form of the problem:

$$\chi = \sum_{n,i} \frac{\partial \chi}{\partial t} = D_{eff}\left[\frac{\partial^2 \chi}{\partial r^2} + \frac{1}{r}\frac{\partial \chi}{\partial r} + \alpha \frac{\partial^2 \chi}{\partial z^2}\right]$$
$$\chi(R,z,0) = 1 \; ; \text{IC}$$
$$\chi(R,z,t) = 0 \; ; \left(\frac{\partial \chi}{\partial r}\right)_{r=0} = 0 \; ; \text{BC1}$$
$$\chi(r,z_0,0) = 0 \; ; \left(\frac{\partial \chi}{\partial z}\right)_{z=0} = 0 \; ; \text{BC2}$$

The general solution to this problem can be found as:

where j_n are the zeros of $J_0(S)$, the Bessel function of first type of zero order. The initial conditions are satisfied by

$$a_{n,k} = (-1)^k \frac{4}{j_n\left(\frac{\pi}{2} + k\pi\right) J_1(j_n)}$$

X-ray absorption in the plane of symmetry z = 0 can be calculated from the integral

$$a(\xi_1, \xi_2) = \int_{\xi_1}^{\xi_2} \rho(\xi) d\xi$$

where ξ is the coordinate along the direction of the ray (Figure 2).

By considering Beer's law, the intensity $I(t)$ at the signal detector 3 can be related to the instantaneous value of the quantity $a(\xi_1, \xi_2)$, and the quantity $Y(t) = log \frac{I(t)}{I(0)}$ can be evaluated

$$Y(t) = c_0^* + \sum_{n,k} c_{n,k}^* e^{-\frac{D_{eff}}{R_0^2}\left[\frac{1}{\varepsilon^2}\left(\frac{\pi}{2}+k\pi\right)^2 + j_n^2\right]t'}$$

by using the solution obtained for the value χ of the concentration change of the reagent diffusing into the sample, thus obtaining the following relationship:

where t' = (t + t_0) takes into account the fact that the measurement start time is different from the start of diffusion (the sample must be placed in the measurement region in a finite time), while $\varepsilon = \frac{z_0}{R_0}$ is the asymptotic value of b (i.e., when $t \to \infty$), e $c_{n,k}^*$ are coefficients that also absorb the constant t_0.

The value of D_{eff} can therefore be obtained by exponential fitting of Y(t) (Figure 4).

Furthermore, the method according to the invention further comprises a step of comparing in real time the value of standard diffusion coefficient D_{std} and the value of diffusion coefficient D_{eff} estimated in real time, and a step of adjusting the processing time to achieve the completion of the diffusion of the reagent into the sample.

Note that in a possible embodiment, the processing time can be changed manually by an operator, who can increase or decrease the processing time or can stop processing.

Alternatively, the processing time can be adjusted automatically by the system.

In an embodiment, the method first comprises selecting a value of standard diffusion coefficient D_{std} associated with the sample under investigation, which is sent to the processor 5.

Preferably, in such embodiment the system 1 comprises a memory 6 as described above, wherein a plurality of standard diffusion coefficients representative of different samples are recorded.

In a preferred embodiment, an operator can select, for example from a user interface 100, the type of sample under investigation. Such selection is associated with a respective standard diffusion coefficient D_{std} stored in the memory 6, which is inputted to the processor 5.

Furthermore, the operator can also select the size and/or weight of the sample under investigation, and this information can be inputted to the processor 5.

The method then has in that case an additional step of calculating, by means of the processor 5, the processing time of the sample with the reagent, as a function of the value of standard diffusion coefficient D_{std} selected and as a function of the sample size.

Note that, in a possible embodiment, the method for monitoring the development of the processing comprises the step of displaying, by means of the output device 7, the value of estimated processing speed v_0 and/or estimated processing time (t_0) of said sample with said reagent.

Claims

1. A system (1) for monitoring the development of the processing of a biological sample by perfusion with a processing reagent in order to diffuse said reagent into said sample, said system comprising a housing (4) for a sample, an X-ray radiation source (2) configured to emit an X-ray beam and adapted to perform a plurality of scans during the diffusion over time of said reagent into said biological sample, a signal detector (3) to detect the radiation exiting the sample and acquire corresponding X-ray images, a processor (5) implemented to process in real time a plurality of said X-ray images of the biological sample under investigation, to estimate in real time the average value of the attenuation over time ($I_{eff}(t)$) of the X-ray radiation at different radial distances inside the sample under investigation, in order to monitor the development of the processing until the saturation of said sample with said reagent is completed,

   said system being characterized in that said processing reagent is not a staining reagent which comprises heavy metals.

2. The system according to claim 1, in which said processor (5) is implemented to calculate the estimated processing time (t_0) of said sample with said reagent, as a function of said estimated average attenuation value $I_{eff}(t)$ of the X-ray radiation.

3. The system according to claim 1 or 2, wherein said processor (5) is implemented to estimate in real time the value of the diffusion coefficient (D_{eff}) of said reagent into said sample, as a function of said average value of the attenuation over time ($I_{eff}(t)$) of the X-ray radiation inside the sample under investigation, to monitor the development of the processing of said sample.

4. The system according to claim 3, wherein said processor (5) is implemented to calculate the estimated processing time (t_0) of said sample with said reagent, as a function of said

value of diffusion coefficient (D_{eff}).

5. The system according to one of the preceding claims, comprising an output device (7) that displays the estimated processing speed (v_0) and/or estimated processing time (t_0) of said sample with said reagent.

6. The system according to one of the preceding claims, wherein said X-ray radiation source (2) operates in a range between 110 kV and 130 kV, preferably 120 kV and in a range between 40 μA and 60 μA, preferably 50 μA, the endpoints of the ranges being included.

7. The system according to one of the preceding claims, wherein the sample scans are acquired at a scan rate of about 1 Hertz for a total time of about 30 minutes.

8. The system (1) according to one of the preceding claims, comprising a memory (6) coupled to the processor (5), wherein a plurality of standard values of diffusion coefficients (D_{std}) of a plurality of porous tissue samples, each associated with a respective standard value of speed (v_{std}) and/or diffusion time (t_{std}) of said reagent into said porous tissue sample are stored in the memory (6), wherein the processor (5) is implemented to perform the operations comprising:

   (a) receiving as input one of the values of standard diffusion coefficient (D_{std}) stored in the memory (6), associated with the tissue sample under investigation,

   (b) comparing said value of standard diffusion coefficient (D_{std}) selected from said memory (6), associated with the sample under investigation, with the diffusion coefficient value (D_{eff}) estimated in real time by scanning the sample with the X-ray source (2).

9. The system (1) according to claim 8, wherein the processor (5) is implemented to calculate the estimated processing time (t_0) of said sample with said reagent, as a function of said value of standard diffusion coefficient (D_{std}) received as input, and preferably as a function

of the size of said sample.

10. The system (1) according to one of the preceding claims, wherein said output device (7) displays the value of remaining diffusion time ($t_{r,eff}$) to achieve the completion of the diffusion of said reagent into said sample.

11. A method for monitoring the development of the processing of a biological sample by perfusion with a processing reagent in order to diffuse said reagent into said sample, by means of a system according to claims 1 – 10, said method comprising the following steps:

- acquiring, by means of said detector (3), a plurality of X-ray images of said sample, by performing a plurality of scans of the sample by means of said X-ray radiation source (2) during the diffusion over time of said reagent into said sample;
- processing said X-ray images in real time by said processor (5), to estimate in real time the average value of the attenuation $I_{eff}(t)$ of X-ray radiation at different radial distances inside the sample under investigation,
- monitoring the completion of the saturation of the sample with the reagent, based on the estimated real-time average value of the attenuation over time $I_{eff}(t)$ of X-ray radiation,

said method being characterized in that said processing reagent is not a staining reagent which comprises heavy metals.

12. The method according to claim 11, comprising a step of adjusting the processing time (t_0) to achieve the completion of the saturation of said sample with said reagent.

13. The method according to claim 11 or 12, comprising the step of displaying, by means of the output device (7), the value of estimated processing speed (v_0) and/or estimated processing time (t_0) of said sample with said reagent.

14. The method according to one of claims 11 - 13, comprising the step of estimating in real

time the value of the diffusion coefficient (D_{eff}) of said reagent into said sample to monitor the development of the processing of said sample.

15. The method according to claim 14, comprising the step of estimating the processing time (t_0) of said sample with said reagent, as a function of said value of diffusion coefficient (D_{eff}).

16. The method according to claim 14 or 15, wherein said value of the diffusion coefficient (D_{eff}) is estimated in real time by a measurement of the change in reagent concentration (χ) in the sample under investigation, correlated with said average value of the attenuation over time $I_{eff}(t)$ of the X-ray intensity inside the sample under investigation.

17. The method according to one of claims 13 to 16, wherein the value of the diffusion coefficient (D_{eff}) is estimated in real time by the following steps:

- acquiring said X-ray images and calculating the attenuation value over time of the intensity ($I_{eff}(t)$) of the X-ray radiation in the sample under investigation;
- calculating the value of the change in reagent concentration (χ) in the sample under investigation as a function of the obtained value of effective intensity attenuation ($I_{eff}(t)$) of X-ray radiation in the sample under investigation as a function of time;
- calculating in real time the value of the diffusion coefficient (D_{eff}) of said reagent into said sample, as a function of that value of the change in reagent concentration (χ) in the sample under investigation.

18. The method according to one of claims 11 to 17, comprising the following steps:

- selecting a value of standard diffusion coefficient (D_{std}) associated with the sample under investigation, and inputting it to said processor (5);
- calculating, by means of said processor (5), the processing time of said sample with said reagent, as a function of said selected value of standard diffusion coefficient

(D_{std}), and preferably as a function of the size of said sample.

19. The system according to one of claims 1 to 9 and the method according to one of claims 10 to 18, characterized in that said processing comprises the steps of fixating and/or dehydrating and/or clarifying.

20. The system according to one of claims 1 to 9 and the method according to one of claims 10 to 18, characterized in that said biological sample is intended for analysis by optical microscope.

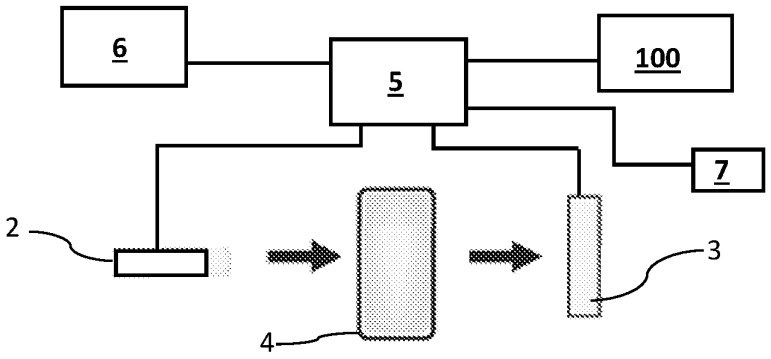

Figure 1

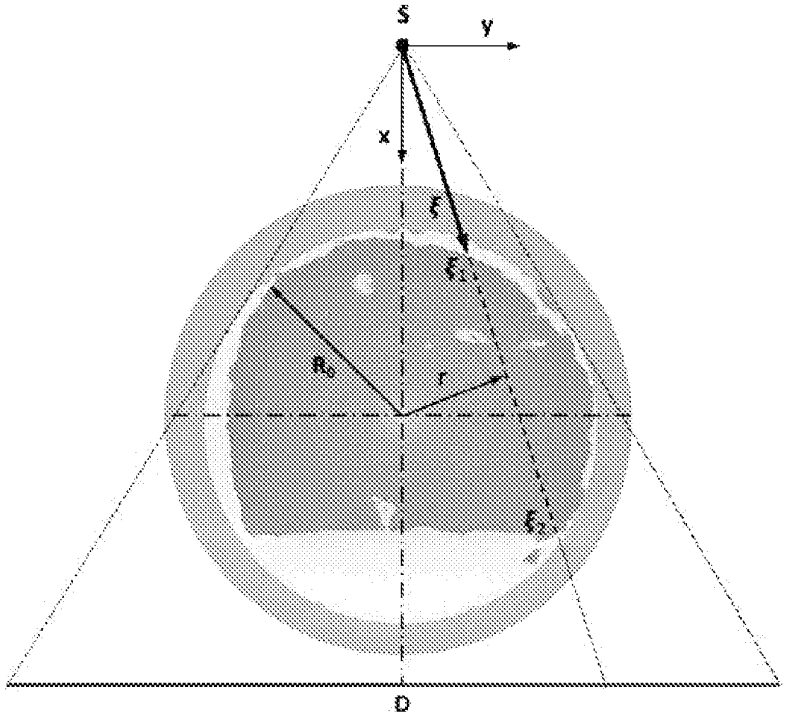

Figure 2

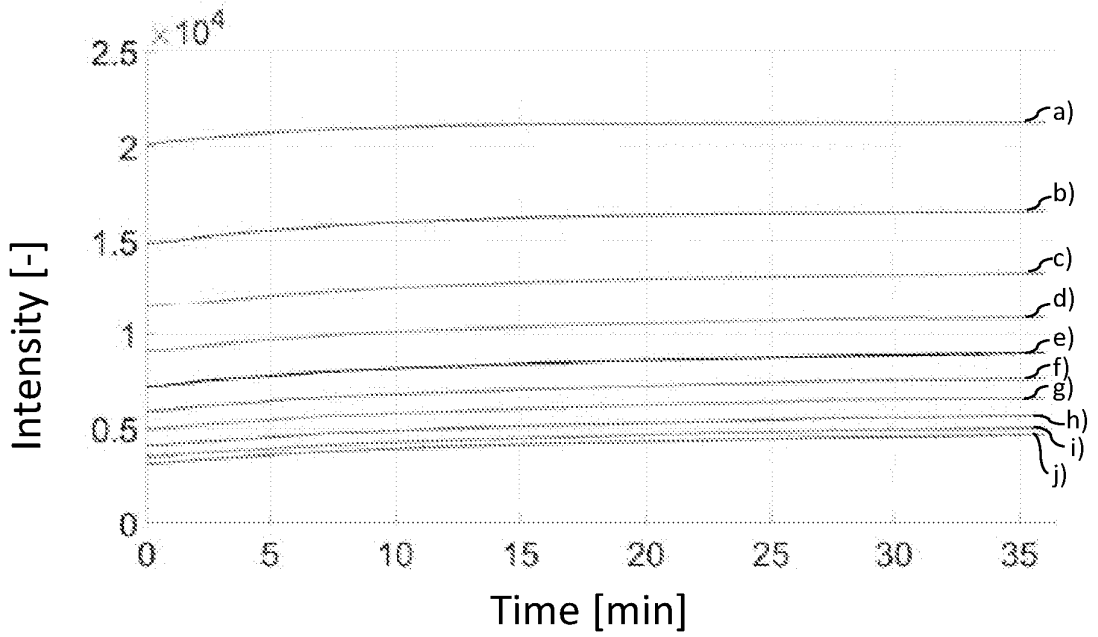

Figure 3A

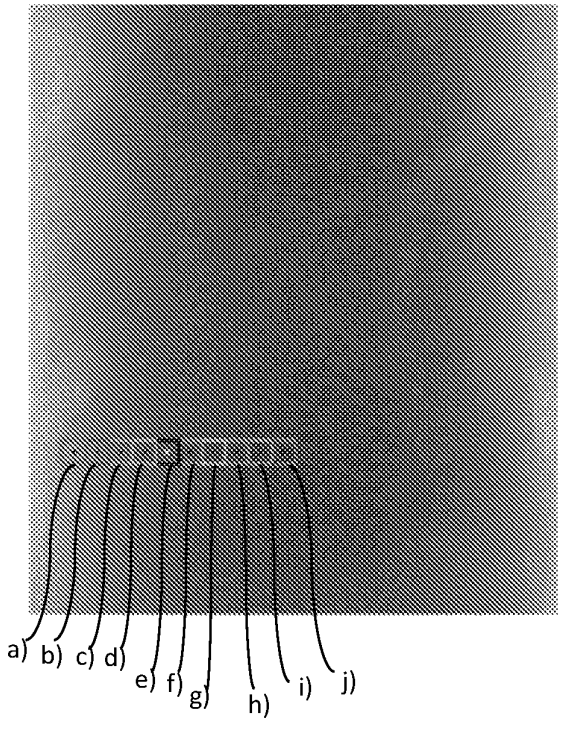

Figure 3B

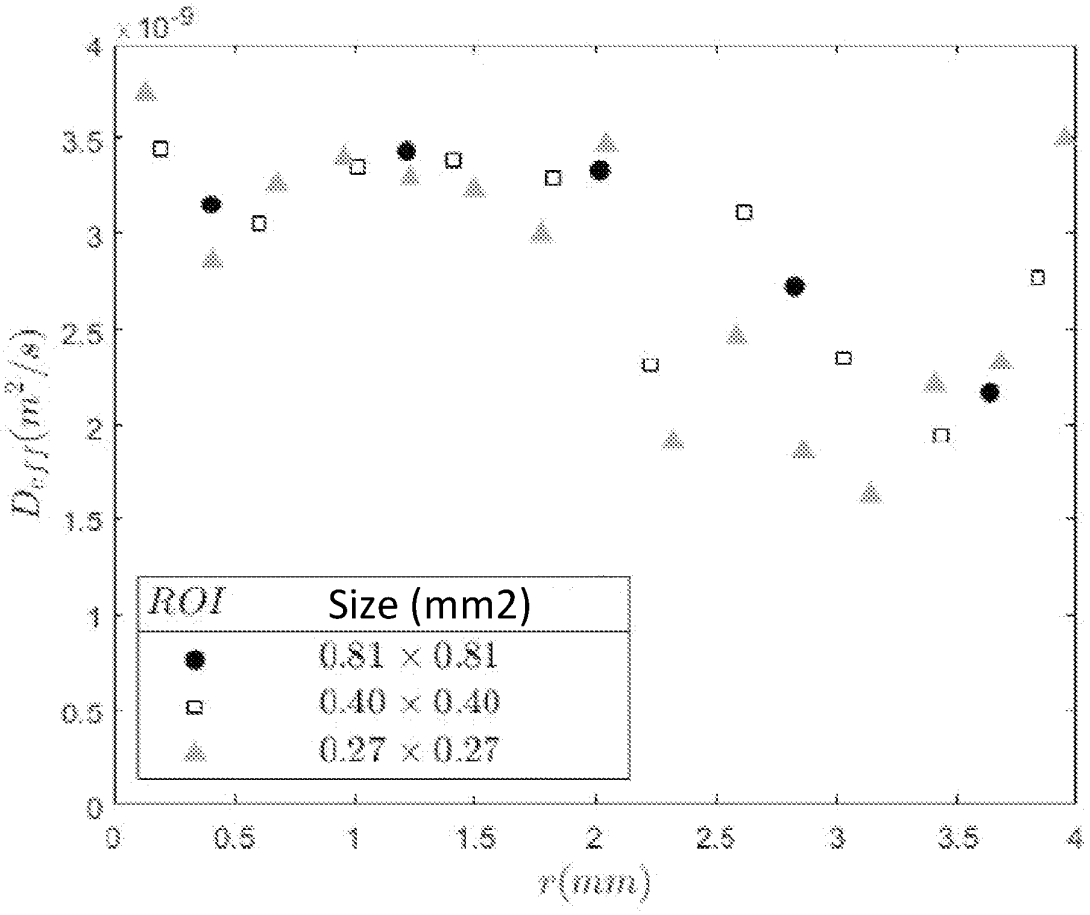
× 10^-9
4
3.5
3
2.5
2
1.5
1
0.5
0
0
0.5
1
1.5
2
2.5
3
3.5
4
D_sf(m^2/s)
r(mm)
ROI
Size (mm2)
0.81 × 0.81
0.40 × 0.40
0.27 × 0.27

Figure 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2024/059166

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N23/04 G01N23/06 G01N23/083 G01N13/00
ADD. G01N1/30

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

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.
X	US 2020/150063 A1 (WANNER ADRIAN A [US] ET AL) 14 May 2020 (2020-05-14)	1-10
Y	paragraph [0010] paragraph [0035] paragraph [0038] paragraph [0041] paragraph [0045] - paragraph [0048] paragraph [0054] - paragraph [0062] figure 3 figure 1 figure 2B figure 2A	11-20

-/--

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 December 2024	02/01/2025

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Traversa, Marzia

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2024/059166

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BAUER DANIEL R. ET AL: "Making a science out of preanalytics: An analytical method to determine optimal tissue fixation in real-time", PLOS ONE, vol. 16, no. 10, 14 October 2021 (2021-10-14), page e0258495, XP093122891, US ISSN: 1932-6203, DOI: 10.1371/journal.pone.0258495 abstract page 3, paragraph time of flight measurments page 4, paragraph Imaging and image processing page 4 - page 7, paragraph Results figure 2 figure 1	11-20
	- - - - -	
A	WO 2016/145366 A1 (RAGAN TIMOTHY [US]; DIMANT CHIAM MOSHE [US]) 15 September 2016 (2016-09-15) abstract claims 1,28	1-20
	- - - - -	

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2024/059166

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2020150063 A1	14-05-2020	NONE	
WO 2016145366 A1	15-09-2016	EP 3268715 A1	17-01-2018
		US 2018045623 A1	15-02-2018
		US 2021199545 A1	01-07-2021
		US 2023296484 A1	21-09-2023
		WO 2016145366 A1	15-09-2016