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(54) Title: METHOD FOR DETERMINING/CORRECTING DEFECTS IN SECTIONS OF A SAMPLE AND ASSOCIATED DE-
VICES TO RECONSTRUCT THREE-DIMENSIONAL VOLUME IMAGES

(57) Abstract: The present invention concerns a method for determining/correcting defects in sec-
tion of a sample, the defects being generated when sectioning the sample into sections of the sample,
the method being based on two imaging modalities, one being spectroscopy. The present invention
enables to reconstruct a three-dimensional quantitative chemical image of samples.

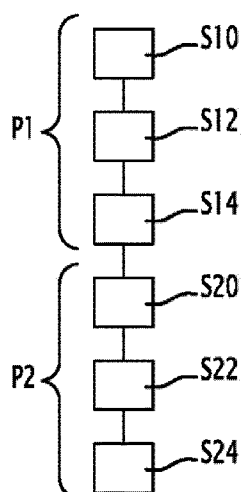


FIG.3

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METHOD FOR DETERMINING/CORRECTING DEFECTS IN SECTIONS OF A SAMPLE AND ASSOCIATED DEVICES TO RECONSTRUCT THREE-DIMENSIONAL VOLUME IMAGES

5 TECHNICAL FIELD OF THE INVENTION

The present invention concerns a method for determining defects in sections of a sample. The present invention also relates to a method for correcting defects in sections of a sample. The present invention also describes an imaging method. The present invention also concerns an associated computer program product, an associated
10 computer readable medium, an associated device for determining defects, an associated apparatus for correcting defects and an associated imaging system.

BACKGROUND OF THE INVENTION

Many research fields are using sections of a sample to investigate.

15 As a specific example, histological sections are a prerequisite for the analysis of a tissue sample with its cyto-architectonic areas, layers, and cell networks represented at a microscopic scale. Histological sections which are notably used in histopathology refer to the microscopic examination of tissue in order to study the manifestations of disease. Specifically, in clinical medicine, histopathology refers to the examination of a biopsy or
20 surgical specimen by a pathologist, after the specimen has been processed and histological sections have been placed onto glass slides. In contrast, cytopathology examines free cells or tissue fragments.

As another example, in the domain of plant, the analysis of the internal architecture to confirm sample functions and contents implies using section of the plants.

25 Such requirement also applies to complex materials, polymers or fabrics.

In each of the previous examples, the sectioning of the sample is required to obtain sections to analyze. The analysis of the sections enables obtaining information about the sample.

30 However, when carrying out the sectioning, the sample may be compressed or stretched, this resulting in global defects of the sample shape with respect to its initial dimensions in the body.

It is therefore desirable that a sample section image be acquired and manipulated for correcting these defects.

35 The corrections of tissue section defects induced by sample manipulation and sectioning are currently limiting the development of three-dimensional (3D) digital histology methods, for example for automated pathology or diagnostics, also called

sometimes e-pathology. The corrections must also be automated for allowing the development high-throughput digital histology, that is where a systematic control of corrections is no longer required for both two-dimensional (2D) and 3D analyses.

5 SUMMARY OF THE INVENTION

There is therefore a need for a method enabling to determine and/or correcting the defects generated when sectioning a sample into sections of the sample.

One key-feature of 3D reconstruction methods is to correct defects induced by sample sectioning, which is required to allow an accurate alignment of sections for a
10 reliable 3D volume rendering.

To this end, it is proposed a method for determining defects in sections of a sample of a subject, the defects being generated when sectioning the sample into sections of the sample, the method for determining comprising at least a phase of constructing a first image of a section, the phase of constructing the first image comprising at least a step of
15 providing a measurement of the signal of radiations at several wavelengths by at least one part of a section of the sample, for obtaining a global signal, a step of dividing the at least one part in pixels to be analyzed, and a step of assigning an expected signal for each pixel, the expected signal for each pixel being obtained by distributing the global signal over each pixel the association of the expected signal to each pixel defining a first image
20 of the section. The method for determining comprises the constructing a second image of the same section. The phase of constructing the second image comprises at least a step of providing an image of the sample, the image of the sample being a three-dimensional image and being obtained by using a first imaging modality, and a step of extracting from the image of the sample, a two-dimensional image of the section by using data relative to
25 the position of the section with relation to the sample, the two-dimensional image being the second image. The method for determining comprises a phase determining the presence of a distortion defect, the phase of determining comprising at least a step of obtaining the first boundaries of the section in the first image, a step of obtaining the second boundaries of the section in the second image, a step of comparing the distances
30 of the boundaries with relation to a reference point, a distortion defect being determined as present if the difference in absolute value between 1 and the ratio of the compared distances is superior to a predefined threshold.

Compared to a warping technique, such method for determining defects enables to obtain with a better accuracy the defects generated when sectioning a sample into
35 sections of the sample.

According to further aspects of the method for determining defects which are advantageous but not compulsory, the method for determining defects might incorporate one or several of the following features, taken in any technically admissible combination:

- the first imaging modality is chosen in the group consisting of magnetic resonance imaging, X-ray imaging and positron emission tomography.
- the wavelengths of the radiations are included in a wavelength range, the providing step comprising calculating the integral of the measured signal over the wavelength range, the global signal being the result of the integral.

The specification also relates to a method for correcting defects in a section of a sample of a subject, the defects being generated when sectioning the sample into sections of the sample, the method for correcting comprising at least a phase of correcting the distortion defect, the phase of correcting the distortion defect comprising at least a step of determining the presence of a distortion defect by carrying out a method for determining defects in the section of the sample as previously described, and a step of applying a transformation to the first image by using the ratio of the compared distances.

It is also proposed an imaging method comprising at least a step of, for each section of a sample of a subject, obtaining a corrected first image by using the method for correcting as previously described, and a step of reconstructing a three-dimensional image based on the corrected first images of each section, the three-dimensional image corresponding to a quantitative chemical image of the sample.

The specification also relates to a computer program product comprising instructions for carrying out at least one step of a method as previously described when said computer program product is executed on a suitable computer device.

The specification also concerns a computer readable medium having encoded thereon a computer program product as previously described.

It is also proposed a device for determining defects in a section of a sample of a subject, the defects being generated when sectioning the sample into sections of the sample, the device for determining comprising a spectrometer, a three-dimensional imager and a calculator, the device for determining being adapted to carry out a method for determining defects in sections of the sample of the subject, the method for determining comprising at least a phase of constructing a first image of a section, the phase of constructing the first image comprising at least a step of providing a measurement of the signal of radiations at several wavelengths by at least one part of a section of the sample, for obtaining a global signal, a step of dividing the at least one part in pixels to be analyzed, and a step of assigning an expected signal for each pixel, the expected signal for each pixel being obtained by distributing the global signal over each

pixel the association of the expected signal to each pixel defining a first image of the section. The method for determining comprises the constructing a second image of the same section. The phase of constructing the second image comprises at least a step of providing an image of the sample, the image of the sample being a three-dimensional image and being obtained by using a first imaging modality, and a step of extracting from the image of the sample, a two-dimensional image of the section by using data relative to the position of the section with relation to the sample, the two-dimensional image being the second image. The method for determining comprises a phase determining the presence of a distortion defect, the phase of determining comprising at least a step of obtaining the first boundaries of the section in the first image, a step of obtaining the second boundaries of the section in the second image, a step of comparing the distances of the boundaries with relation to a reference point, a distortion defect being determined as present if the difference in absolute value between 1 and the ratio of the compared distances is superior to a predefined threshold.

The specification also concerns an apparatus for correcting defects in a section of a sample of a subject, the defects being generated when sectioning the sample into sections of the sample, the apparatus for correcting comprising a device for determining as previously described, the device determining the presence of a distortion defect, the apparatus being further adapted to carry out a method for correcting defects in the section of the sample of the subject, the method for correcting comprising at least a phase of correcting the distortion defect, the phase of correcting the distortion defect comprising at least a step of applying a transformation to the first image by using the ratio of the compared distances.

The invention also concerns an imaging system comprising the apparatus as previously described, the imaging system being further adapted to carry out an imaging method comprising at least a step of for each section of a sample of a subject, obtaining a corrected first image by using the apparatus, and reconstructing a three-dimensional image based on the corrected first images of each section, the three-dimensional image corresponding to a quantitative chemical image of the sample.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be better understood on the basis of the following description which is given in correspondence with the annexed figures and as an illustrative example, without restricting the object of the invention. In the annexed figures:

- figure 1 shows schematically a sample, sections of the sample and a section of a sample having defects generated when sectioning the soft sample into sections,

- figure 2 shows schematically a device for determining defects in a section of a sample of a subject,
- figure 3 shows a flowchart of an example of carrying out of a method for determining defects in a section of a sample of a subject by using the device of figure 2,
- figure 4 shows schematically an apparatus for correcting defects in a section of a sample of a subject,
- figure 5 shows a flowchart of an example of carrying out of a method for correcting defects in a section of a sample of a subject by using the apparatus of figure 4, and
- figures 6 to 10 show experimental figures obtained when carrying out the method of figure 5.

CONTEXT OF THE RESEARCH CARRIED OUT BY THE APPLICANT

Foreword: This paragraph is devoted to present the context of the research carried out by the Applicant for obtaining the present invention. This paragraph should not be construed as an admitted prior art by the Applicant but rather as preliminary thoughts to reword the general problem and obtain elements of the problematics to be solved by the present invention.

Biological samples are biological tissues comprising diverse cell populations and compounds embedded in an anatomical organization. Therefore, it is desirable to study the distribution of these tissue sub-structures.

Histological analysis remains the gold standard for tissue characterization to verify the significance of changes revealed by non-invasive imaging techniques. A registration of histological sections with in vivo or ex vivo three-dimensional images can potentially provide a more accurate three-dimensional reconstruction of the sample volume after analysis of the sections.

However, accurate registration of histological sections to three-dimensional images is challenging. Ideally, a three-dimensional (3D) histological volume (or, equivalently, a densely sampled set of contiguous two-dimensional slices) would be available to allow true-three-dimensional -to- reconstructed-three-dimensional matching.

However, the spatial partitioning (voxel size versus section thickness) of different imaging modalities is usually highly incongruent, and the information content (histological stains versus signal density) displays a complex relationship.

Different techniques can also lead to the production of imaging artefacts (for instance, signal distortions, heterogeneous staining, optical aberrations) that can affect registration. Histological images commonly suffer from primary (for instance, fixation-related tissue shrinkage) and secondary (e.g., cutting artefacts, missing slices) deformations due to sample processing and sectioning. Using a block-face acquisition method of two-dimensional histological images, where an image is taken prior to each slice being sectioned, avoids secondary deformations.

However, this approach is not readily available in most laboratories and does not account for primary deformations or artefacts due to post-surgical sample distortions (soft tissues are not self-maintained in a formal shape outside the body), embedding and freezing processes (sample volume change), immunohistochemistry (for instance, tissue tears) or others. It is only the post-processing steps that are avoided (cryomicrotomy for example).

To align individual slices after staining, masking of irrelevant information on the image is part of a multi-step procedure to create a three-dimensional volume from two-dimensional slices. As an organ is not a geometrically linear shape, merely aligning the outline of appropriate anatomical sections will not reconstruct an accurate representation of the organ.

Consequently, structural content (in other words, variations in signal intensity) is a valuable source of information to appropriately align individual sections; however, in vivo data present the ultimate geo-metric reference. Whole sample three-dimensional data can therefore be used to refine the three-dimensional histology data set.

One must also consider that most of histological methods require that a tissue or an organ is removed from the body for positioning and sectioning procedures. The organ is thus subjected to shape changes induced both by the removal from the body and by the deposition on flat sample holders. It results that the three-dimensional reconstruction from two-dimensional images obtained from sample sectioning cannot match the three-dimensional image obtained in situ (in vivo or ex vivo). This is making that histological and in situ images cannot be fused or merged for the multimodal analysis of a sample. Therefore, histological images must be corrected to match the shape obtained from in situ images.

Registration of the aligned histological volume can potentially use external surface characteristics; however, this can produce a misalignment of internal structures. A landmark-based registration of the reconstructed histological brain with the 3D images can match internal and external points but is based on user-defined points that at best are sparsely distributed. The selection of landmarks is dependent on anatomical structures;

hence, registration will vary depending on their contrast and consistency. In the case of highly abnormal lesion environments, some of these landmarks may even be absent or shifted. Therefore, a more general intensity-based approach, which can use information from throughout the brain volume to achieve a more accurate registration, is preferable.

Alternatively, two-dimensional histological techniques remain unable to maintain a common signal intensity scale on large series of sections as they are usually not based on a quantitative acquisition of a given signal. Most of histological techniques cannot claim they offer a quantitative analysis as they work only on the surface of the sample section, thus losing information from internal part of the sample section. Other techniques are based on label-derived signals, such as immunohistochemistry, but these are also mostly based on surface analysis, thus prohibiting quantitative analysis of a tissue volume. On the other hand, spectroscopic methods, because they offer a quantitative and global chemical information of the sample while acquiring the signal from transmission measurement, thus crossing the totality of the sample contents, can fulfill the requirements for a quantitative correction of tissue sections allowing further a three-dimensional sample reconstruction matching the true in situ shape of the tissue or organ.

Such preliminary thoughts lead to investigating the defects of a section of a sample.

An example is shown on figure 1 with a sample 10 of a subject. In this case, the sample 10 is a brain and the subject is a mouse.

As schematically shown, the sample 10 is cut in a plurality of sections 12.

When sectioning the sample 10 into sections 12 of the sample 10, defects are generated.

One section to be analyzed 14 is represented and comprises such defects.

Notably, the section 14 has been submitted to a distortion defect 16 and local defects 18.

The distortion defect 16 is emphasized by boundaries 20 which correspond to the reel boundaries of the section to be analyzed.

For this specific example, the section 14 has been impacted by compression, which leads to areas 22 with different dimensions from the real ones.

More generally, histological sections are a prerequisite for the analysis of a tissue sample with its cyto-architectonic areas, layers, and cell networks represented at a microscopic resolution. Sectioning is required as soon as the in vivo / ex vivo imaging techniques cannot offer the necessary resolution deeply enough in the sample for examining tissue microscopic features. It is also due to the fact that some analytical techniques (notably spectroscopies), cannot analyze samples over a certain deepness. The same objective applied to plant samples, where the internal architecture must be

analyzed to confirm sample functions and contents that cannot be defined otherwise. Complex materials, polymers, fabrics... also follow the same rationale where internal structures represent basic information about the quality of the object. Sectioning of the soft sample can be required in these cases, among others, and a microscopic characterization can provide invaluable information about their structure. However, these microscopic details must be interpreted cautiously as soft samples can suffer from morphological distortions while sectioning procedures are applied. The softness of the sample eases these distortions, which can occur while sectioning or depositing the section of a slide for observation.

A typical case is histopathology, the science of biological tissue slides analysis for the diagnostic and research purposes. However, sometimes the presence of certain artefacts in a microscopic section can result in misinterpretations leading to diagnostic pitfalls that can result in increased patient morbidity. Crush artefacts occur due to soft sample distortions resulting from even the most minimal compression of the sample. It occurs most commonly from mutilation of tissues with surgical devices during removal, but can be produced by dull scalpel blades that tear the tissue instead of incising it. It can occur also while depositing sample sections on histological slides. Crushing produces a major type of artefact that rearranges sample morphology, which must be corrected for a correct lecture and interpretation of sample contents.

Sample section defects are usually compressions, stretchings, cracks, tearings, whoever so much the sample material is neither stacked nor totally detached, can be corrected to reconstruct the initial morphology of the sample. It requires that a sample section image is acquired and manipulated for correcting the defects. However, these corrections can be valid only on the basis of tissue section defects induced by sample manipulation and sectioning are limiting the development of digital histology methods, for example for automated pathology or diagnostics, also called sometimes e-pathology. Automatic diagnostic systems based on histological image classification are important for improving therapeutic decisions in clinics. Previous approaches have proposed textural and morphological features for such systems. These features capture patterns in histological images that are useful for both pathology recognition and potential for its staging or grading. However, because many of these features lack a clear biological interpretation, pathologists may be reluctant to adopt these features for clinical diagnosis.

With the recent advent of whole slide digital scanners, tissue histopathology slides can now be digitized and stored in digital image form. Consequently, digitized tissue histopathology has now become amenable to the application of computerized image analysis and machine learning techniques. A systematic computation of tissue section

defects must be developed for making e-pathology viable for automated diagnostics and other biomedical applications. However, visible images of tissue sections do not highlight all tissue defects. If cracks and tearings appear clearly, this is not the case for compressions and stretchings of tissues. A surface visualization of the sample is not likely to reveal all defects. Likewise, a chemical surface analysis (by immunohistochemistry, colorations...) will not offer a quantitative information about tissue contents that might be exploited for such correction. Thus, a correction of section defects to reproduce the initial shape of the sample should be based on a quantitative measurement of sample contents. This can be defined by the thickness of the sample at all points (expressed as volumes, thus voxels) of the section, or alternatively by its atomic mass, considering that a given voxel is a finite space filled by chemical species.

Such quantitative measurement of sample contents can notably be provided by spectroscopic analysis, which can be considered as chemical techniques revealing a global chemical information of the sample. By definition, spectroscopy is the study of the interaction between matter and radiated energy over a broad wavelength region. Thus, multiple experimental techniques are spectroscopic techniques. Infrared spectroscopy, Raman spectroscopy, mass-spectrometry, X-ray fluorescence are major examples of spectroscopic techniques providing quantitative measurement of sample chemical contents.

All these thoughts have led the Applicant to the invention that is now described.

DETAILED DESCRIPTION OF SOME EMBODIMENTS

Figure 2 illustrates a device 24 for determining defects 16 in a section 14 of a sample 10 of a subject.

The device 24 is adapted to carry out a method for determining defects 16, 18 in the section 14 of the sample 10 of the subject.

The device 24 comprises a spectrometer 26, a three-dimensional imager 28 and a calculator 30.

The spectrometer 26 is adapted to obtain a measured signal from radiations at several wavelengths, the radiations being issued from an object.

In the current example, the object is at least one part of a section of the sample 10.

The measured signal corresponds to a signal obtained by spectroscopic analysis, which can be considered as chemical techniques revealing a global chemical information of the sample. By definition, spectroscopy is the study of the interaction between matter and radiated energy over a broad wavelength region. Thus, multiple experimental techniques are spectroscopic techniques. Infrared spectroscopy, Raman spectroscopy,

mass-spectrometry, X-ray fluorescence are major examples of spectroscopic techniques providing quantitative measurement of sample chemical contents.

Therefore, as specific examples, the signal is a signal chosen among an absorption signal, a diffusion signal and a fluorescence signal.

5 The three-dimensional imager 28 is adapted to image a sample in three dimensions.

The three-dimensional imager 28 is adapted to implement a first imaging modality giving access to the anatomy of the sample 10.

The calculator 30 comprises a processor 32 which is adapted to carry out operations on data and a memory 34 adapted to store data.

10 Operating of the device 24 is now described in reference to figure 3 which is a flowchart of an example of carrying out a method for determining defects 16 in the section 14 of the sample 10 of the subject.

The method for determining defects comprises a first phase P1 of constructing a first image I1 of the section 14 (after sectioning) and a second image I2 of the section 14
15 (before sectioning) and a second phase P2 of determining the presence of a distortion defect 16.

According to the specific example described, the first phase P1 of constructing comprises three steps labelled S10, S12 and S14.

For the first image I1, the three first steps are a step S10 of providing, a step S12 of
20 dividing and a step S14 of assigning.

At the step S10 of providing, it is provided a measured signal of radiations at several wavelengths by at least one part of the section 14, for obtaining a global signal S_{global} .

As an example, the measurement is carried out independently and only the results are provided to the calculator 30.

25 In the present embodiment, the measurement is carried out by the spectrometer 26.

The wavelengths of the radiations are included in a wavelength range. The extent of the wavelength range is chosen sufficiently large enough to determine the whole sample matter for every pixel.

Applied to the case of infrared spectroscopy, this results in an extent superior or
30 equal to 100 nanometers and inferior to 700 nanometers.

In such case, the number of wavelengths is comprised between 20 wavelengths and 30 wavelengths.

In the specific example which is detailed, the step S10 of providing comprises calculating the integral of the measured signal over the wavelength range.

35 The result of the integral is the global signal S_{global} .

Such calculation is achieved by the processor 32.

At the end of the step S10 of providing, the memory 34 of the calculator 30 stores the global signal S_{global} of the section 14.

During the step S12 of dividing, the section 14 is divided in pixels to be analyzed.

At the end of the step S12 of dividing, the memory 34 of the calculator 30 stores a
5 division of the sections 14 in pixels.

The division is achieved by using the physical division made by the spectrometer 26.

During the step S14 of assigning, an expected signal S_{expected} is assigned for each pixel.

The expected signal S_{expected} for each pixel is obtained by distributing the global
10 signal S_{global} over each pixel.

According to the specific example which is described, at the step S14 of assigning, the expected signal S_{expected} is the same for each pixel.

In other words, this means that the following formula is applicable:

$$S_{\text{expected}} = \frac{S_{\text{global}}}{\text{number of pixels}}$$

The association of the expected signal S_{expected} to each pixel defines the first
15 image I1 of the section 14.

At the end of the step S14 of assigning, the memory 34 of the calculator 30 stores the first image I1 of the section 14.

For the second image I2, the three first steps are a step S10 of providing images, a step S12 of storing and a step S14 of extracting.

At the step S10 of providing, an image of the sample 10 is provided.
20

The image of the sample is a three-dimensional image.

For instance, the image is obtained by using the first imaging modality provided by three-dimensional imager 28.

The first imaging modality is chosen in the group consisting notably of magnetic
25 resonance imaging (MRI), computed tomography (CT) techniques, X-ray imaging, positron emission tomography (PET), single photon emission computed tomography (SPECT), optical coherence tomography (OCT) and ultrasound imaging (U.S.).

At the end of the step S10 of providing, a three-dimensional of the sample 10 is provided,

Such image is then stored in the memory 34 of the calculator 30 during the step S12
30 of storing.

During the step S14 of extracting, it is extracted from the three-dimensional image of the sample 10 a two-dimensional image of the section 14.

For this, it is used data relative to the position of the section 14 with relation to the sample 10.

It is then applied a cutting operation to extract from the three-dimensional image the image of the section 14.

5 This two-dimensional image of the section 14 is the second image I2.

At the end of the step S14 of extracting, the second image I2 of the section 14 is stored in the memory 34 of the calculator 30.

10 In the specific example of figure 3, the second phase P2 of determining the presence of a distortion defect 16 comprises a first step S20 of obtaining, a second step S22 of obtaining and a step S24 of comparing.

At the first step S20 of obtaining, the first boundaries 36 of the section 14 in the first image I1 are obtained.

For this, boundaries are extracted thanks to a boundary detecting technique.

15 An example of such technique is an automated segmentation method, which can be assisted by machine-learning techniques.

At the end of the first step S20 of obtaining, the first boundaries 36 are stored in the memory 34 of the calculator 30.

The second step S22 of obtaining is similar to the first step S20 of obtaining except that it is applied to the second image I2 instead of the first image I1.

20 At the end of the second step S22 of obtaining, the second boundaries 38 are stored in the memory 34 of the calculator 30.

During the step S24 of comparing, the distances of the boundaries 36 and 38 with relation to a reference point are compared.

For instance, the ratio of the distances is calculated by the processor 32.

25 A distortion defect is determined as present if distances are spotted between the reference and the tissue slice boundaries with values superior to a predefined threshold. Typically, the acceptable distance between boundaries will be defined by the pixel resolution of images providing the boundary lines. The threshold normally set at 1% of the total size of the sample (for instance, height and width of the tissue section), or 1% of the pixels covering these distances. The threshold level can be adjusted according to the expected accuracy. In other words, 5% might be acceptable for global analyses not requiring specific accuracy while less than 1% might be required for three-dimensional reconstruction of very thin details of the tissue structure.

35 In other words, the difference in absolute value between 1 and the ratio of the compared distances is the magnitude of the distortion defect and a distortion defect is

detected if the magnitude of this defect is sufficient compared with the precision of the measurement.

At the end of the step S24 of comparing, the presence of a distortion defect is determined as well as the magnitude of this distortion defect.

5 At the end of the second phase P2, the presence of each distortion defect 16 is known by the calculator 30 of the device 10.

Such information may be advantageously used in a correcting method.

For this, as illustrated in figure 4, an apparatus 50 for correcting defects in the section 14 is illustrated.

10 The apparatus 50 comprises the same elements than the device 10.

Thus, the apparatus 50 comprises the spectrometer 26, the three-dimensional imager 28 and the calculator 30.

The calculator 30 is further adapted to carry out a phase of correcting the distortion defect 16.

15 Operating of the apparatus 50 is now described in reference to figure 5 which is a flowchart of an example of carrying out a method for correcting defects 16 in the section 14 of the sample 10 of the subject.

The method for correcting comprises a phase P3 of correcting the distortion defect 16 which follows the first phase P1 and the second phase P2 of the determining method as previously described.

The phase P3 only comprises a step S52 of applying.

During the step S52 of applying, a redistribution is applied to the first image I1 by using the calculated ratio.

25 By "redistribution", it is meant that missing pixels are filled with signal coming from neighbouring pixels with high signal while pixels to eliminate are eliminated and their signal are distributed to neighbouring pixels with low signal.

The method for correcting enables to obtain corrected sections.

Figures 6 to 10 illustrates the results obtained when using micro-computed tomography.

30 Figures 6 and 7 show the three-dimension imaging of a brain of a mouse obtained by micro-computed tomography.

Figure 8 shows the image of a section obtained with infrared spectroscopy.

On figure 8, the boundaries of the section obtained with infrared spectroscopy and the boundaries of the section obtained with micro-computed tomography are represented.

35 The two boundaries are distinct which is the sign of a distortion defect.

In the case of figure 8, the surface of the section obtained with infrared spectroscopy encompasses an area of 153.9 mm² with a signal intensity of $0.824 \cdot 10^{23}$ in arbitrary units.

Figure 9 illustrates the corrected image of the section obtained after carrying out the method for correcting.

5 The case of figure 8, the surface of the section obtained after correction encompasses an area of 153.9 mm² with a signal intensity of $0.821 \cdot 10^{23}$ in arbitrary units.

This shows that with a nearly constant signal intensity, the boundaries have been modified to fit the boundaries of the section obtained with micro-computed tomography.

In addition, the chemical information is correctly redistributed.

10 By correcting each section, the three-dimensional image of the sample 10 in figure 10 is obtained. This image is to be compared with the image obtained in figure 7.

It is apparent that the image of figure 10 contains more information than the image of figure 7

Such method for correcting is able to operate in an automatic way without the
15 intervention of an operator because it is based on the digital information of the image. It is also allowing to cross-match in vivo images and two-dimensional and three-dimensional histology results, thus providing accurate co-localization of related events (anatomical and chemical or functional, depending on the in vivo imaging method(s) used).

Such method for correcting enables to obtain corrected sections 14 for which the
20 information of the chemical quantity is reliable.

This opens the way to an imaging method which enables to reconstructing a three-dimensional image based on the corrected first images of each section, the three-dimensional image corresponding to a quantitative chemical image of the sample.

Furthermore, such imaging method is applicable to any kind of sample and notably
25 soft samples and biological samples.

Other examples can also be considered, such as fabrics or substrates, vegetal species, polymers, and any other organic material, which global chemical information can be analyzed by a spectrometry method.

In addition, any device or apparatus or method enabling to obtain at least one of the
30 previous mentioned results is to be considered.

In addition, the previously presented methods may be implemented by a server to which the measurement data are provided.

In such case, a system and a computer program product whose interaction enables to carry out a method related to defects in sections of the sample can be considered.

35 A method for determining defects in a sample and a method for correcting defects in a sample are specific examples of methods related to defects of a sample.

System is a computer. In the present case, system is a laptop.

More generally, system is a computer or computing system, or similar electronic computing device adapted to manipulate and/or transform data represented as physical, such as electronic, quantities within the computing system's registers and/or memories into other data similarly represented as physical quantities within the computing system's memories, registers or other such information storage, transmission or display devices.

System comprises a processor, a keyboard and a display unit.

The processor comprises a data-processing unit, memories and a reader. The reader is adapted to read a computer readable medium.

The computer program product comprises a computer readable medium.

The computer readable medium is a medium that can be read by the reader of the processor. The computer readable medium is a medium suitable for storing electronic instructions, and capable of being coupled to a computer system bus.

Such computer readable storage medium is, for instance, a disk, a floppy disks, optical disks, CD-ROMs, magnetic-optical disks, read-only memories (ROMs), random access memories (RAMs) electrically programmable read-only memories (EPROMs), electrically erasable and programmable read only memories (EEPROMs), magnetic or optical cards, or any other type of media suitable for storing electronic instructions, and capable of being coupled to a computer system bus.

A computer program is stored in the computer readable storage medium. The computer program comprises one or more stored sequence of program instructions.

The computer program is loadable into the data-processing unit and adapted to cause execution of a method related to defects in sections of a soft sample when the computer program is run by the data-processing unit.

More generally, the embodiments and alternative embodiments considered here-above can be combined to generate further embodiments of the invention.

CLAIMS

1.- A method for determining defects (16) in sections (12) of a sample (10) of a subject, the defects (16) being generated when sectioning the sample (10) into sections (12) of the sample (10), the method for determining comprising at least a phase of:

- constructing a first image (I1) of a section (14), the phase of constructing the first image (I1) comprising at least a step of :

- providing a measurement of the signal of radiations at several wavelengths by at least one part of a section of the sample, for obtaining a global signal (S_{global}),

- dividing the at least one part in pixels to be analyzed, and

- assigning an expected signal (S_{expected}) for each pixel, the expected signal (S_{expected}) for each pixel being obtained by distributing the global signal (S_{global}) over each pixel, the association of the expected signal (S_{expected}) to each pixel defining a first image (I1) of the section (14),

- constructing a second image (I2) of the same section (14), the phase of constructing the second image (I2) comprising at least a step of :

- providing an image of the sample (10), the image of the sample (10) being a three-dimensional image and being obtained by using a first imaging modality, and

- extracting from the image of the sample (10), a two-dimensional image of the section (14) by using data relative to the position of the section (14) with relation to the sample (10), the two-dimensional image being the second image (I2), and

- determining the presence of a distortion defect, the phase of determining comprising at least a step of:

- obtaining the first boundaries (36) of the section (14) in the first image (I1),

- obtaining the second boundaries (38) of the section (14) in the second image (I2), and

- comparing the distances of the boundaries (36, 38) with relation to a reference point, a distortion defect (16) being determined as present if the difference in absolute value between 1 and the ratio of the compared distances is superior to a predefined threshold.

2.- The method for determining according to claim 1, wherein the first imaging modality is chosen in the group consisting of magnetic resonance imaging, X-ray imaging and positron emission tomography.

5 3.- The method for determining according to claim 1 or 2, wherein the wavelengths of the radiations are included in a wavelength range, the providing step comprising calculating the integral of the measured signal over the wavelength range, the global signal (S_{global}) being the result of the integral.

10 4.- A method for correcting defects (16) in a section (14) of a sample (10) of a subject, the defects (16) being generated when sectioning the sample (10) into sections (12) of the sample (10), the method for correcting comprising at least a phase of correcting the distortion defect (16), the phase of correcting the distortion defect (16) comprising at least a step of :

- 15 - determining the presence of a distortion defect (16) by carrying out a method for determining defects (16) in the section (14) of the sample (10) according to any one of claims 1 to 3, and
- applying a transformation to the first image (I1) by using the ratio of the compared distances.

20 5.- An imaging method comprising at least a step of:

- for each section (14) of a sample (10) of a subject, obtaining a corrected first image (I1) by using the method for correcting according to claim 4, and
- reconstructing a three-dimensional image based on the corrected first images (I1)
- 25 of each section (14), the three-dimensional image corresponding to a quantitative chemical image of the sample (10).

 6.- A computer program product comprising instructions for carrying out at least one step of a method according to any one of claims 1 to 5 when said computer program

30 product is executed on a suitable computer device.

 7.- A computer readable medium having encoded thereon a computer program product according to claim 6.

35 8.- A device (24) for determining defects (16) in a section (14) of a sample (10) of a subject, the defects (16) being generated when sectioning the sample (10) into sections

(14) of the sample (10), the device (24) for determining comprising a spectrometer (26), a three-dimensional imager (28) and a calculator (30), the device (24) for determining being adapted to carry out a method for determining defects (16) in sections (12) of the sample (10) of the subject, the method for determining comprising at least a phase of:

5 - constructing a first image (I1) of a section (14), the phase of constructing the first image (I1) comprising at least a step of:

 - providing a measured signal of radiations at several wavelengths by at least one part of a section of the sample, for obtaining a global signal (S_{global}),

10 - dividing the at least one part in pixels to be analyzed, and

 - assigning an expected signal (S_{expected}) for each pixel, the expected signal (S_{expected}) for each pixel being obtained by distributing the global signal (S_{global}) over each pixel, the association of the expected signal (S_{expected}) to each pixel defining a first image (I1) of the section (14),

15 - constructing a second image (I2) of the same section (14), the phase of constructing the second image (I2) comprising at least a step of:

 - providing an image of the sample (10), the image of the sample (10) being a three-dimensional image and being obtained by using a first imaging modality, and

20 - extracting from the image of the sample (10), a two-dimensional image of the section (14) by using data relative to the position of the section (14) with relation to the sample (10), the two-dimensional image being the second image (I2), and

25 - determining the presence of a distortion defect, the phase of determining comprising at least a step of:

 - obtaining the first boundaries (36) of the section (14) in the first image (I1),

 - obtaining the second boundaries (38) of the section (14) in the second image (I2), and

30 - comparing the distances between the boundaries (36, 38) with relation to a reference point, a distortion defect (16) being determined as present if the difference in absolute value between 1 and the ratio of the compared distances is superior to a predefined threshold.

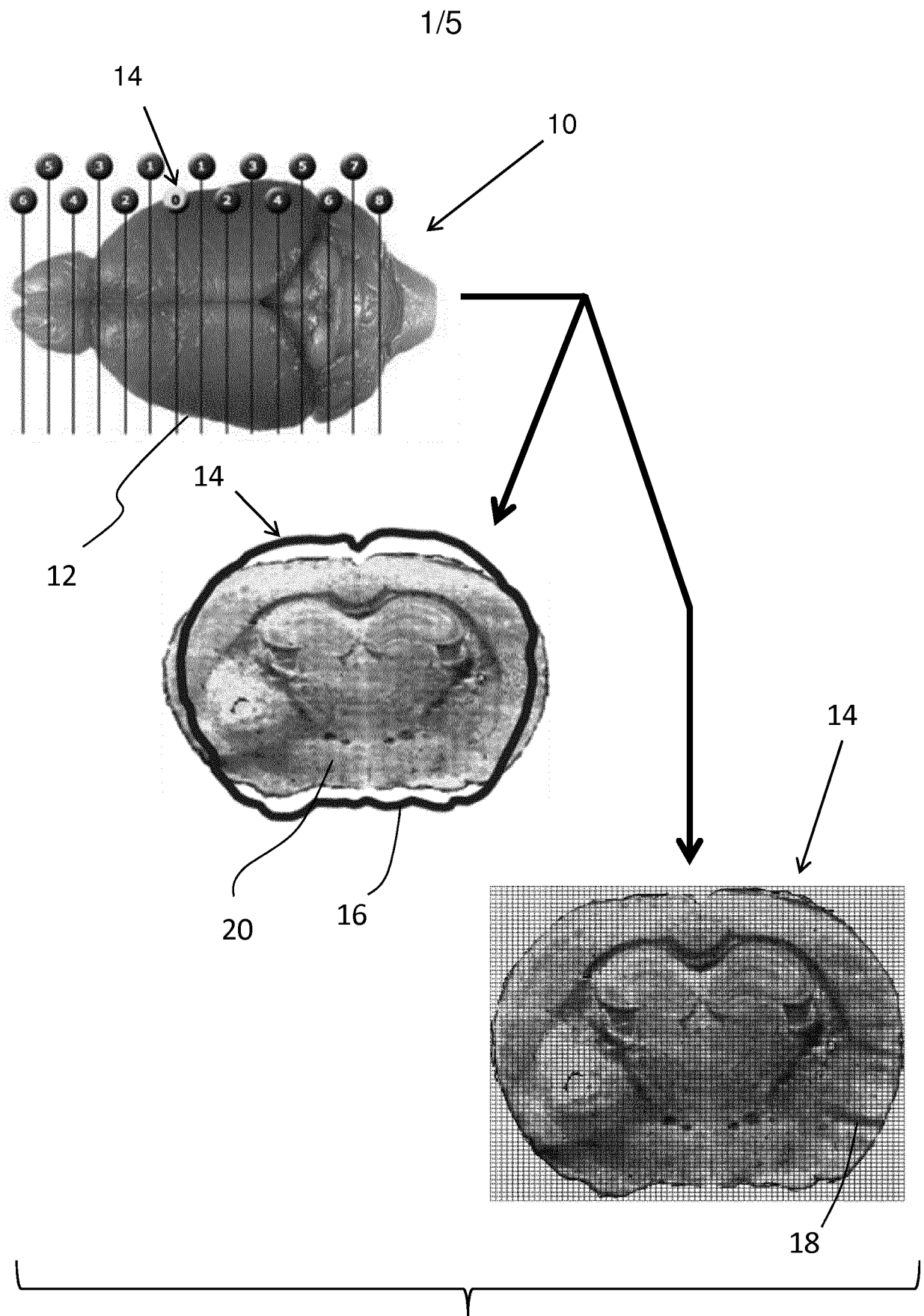
35 9.- An apparatus (50) for correcting defects (16) in a section (14) of a sample (10) of a subject, the defects (16) being generated when sectioning the sample (10) into sections (12) of the sample (10), the apparatus (50) for correcting comprising a device (24) for

determining according to claim 8, the device (24) determining the presence of a distortion defect (16), the apparatus (50) being further adapted to carry out a method for correcting defects (16, 18) in the section (14) of the sample (10) of the subject, the method for correcting comprising at least a phase of correcting the distortion defect (16), the phase of
5 correcting the distortion defect (16) comprising at least a step of applying a transformation to the first image (I1) by using the ratio of the compared distances.

10.- An imaging system comprising the apparatus (50) of claim 9, the imaging system being further adapted to carry out an imaging method comprising at least a step
10 of:

- for each section of a sample of a subject, obtaining a corrected first image by using the apparatus, and

- reconstructing a three-dimensional image based on the corrected first images (I1) of each section (14), the three-dimensional image corresponding to a quantitative
15 chemical image of the sample (10).



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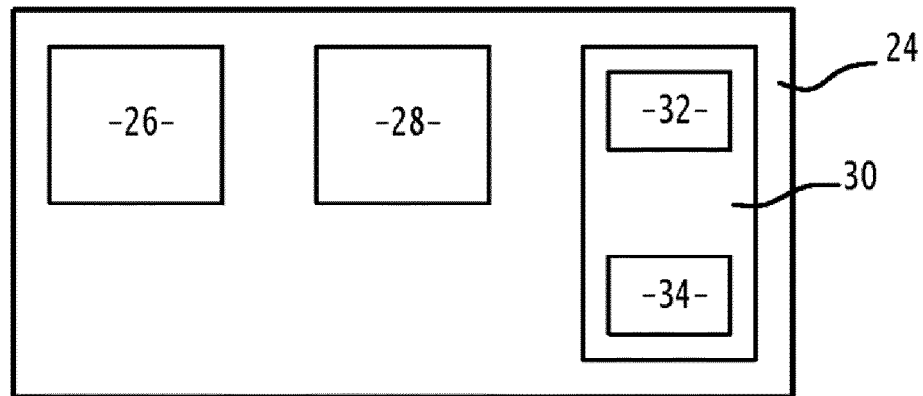


FIG.2

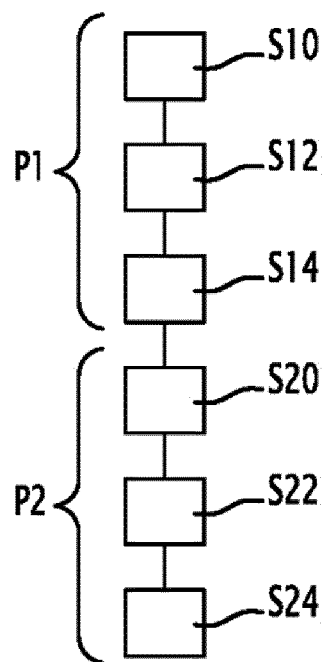


FIG.3

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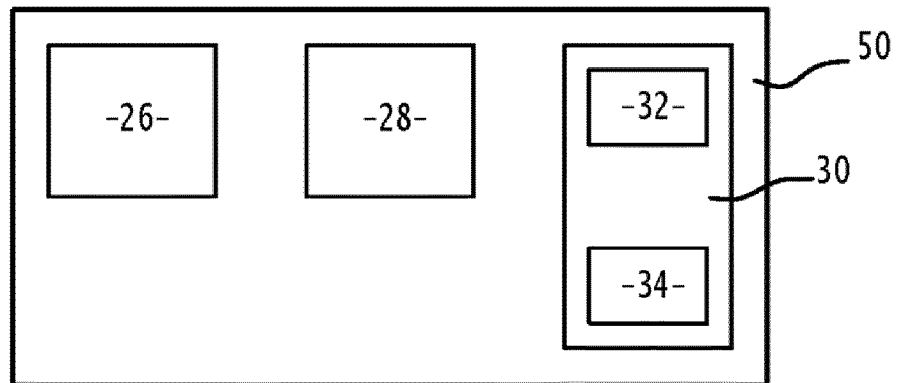


FIG. 4

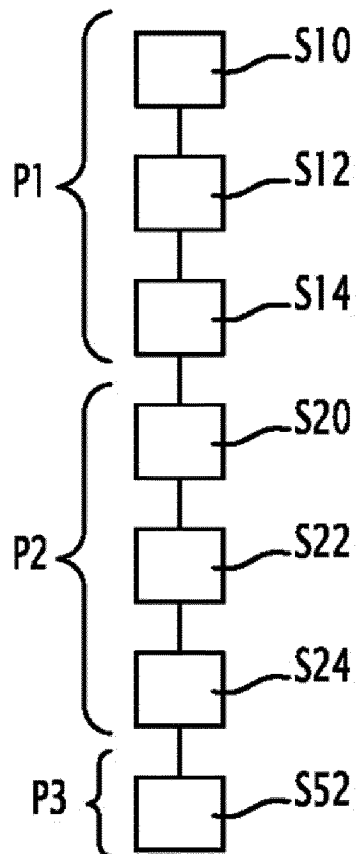


FIG. 5

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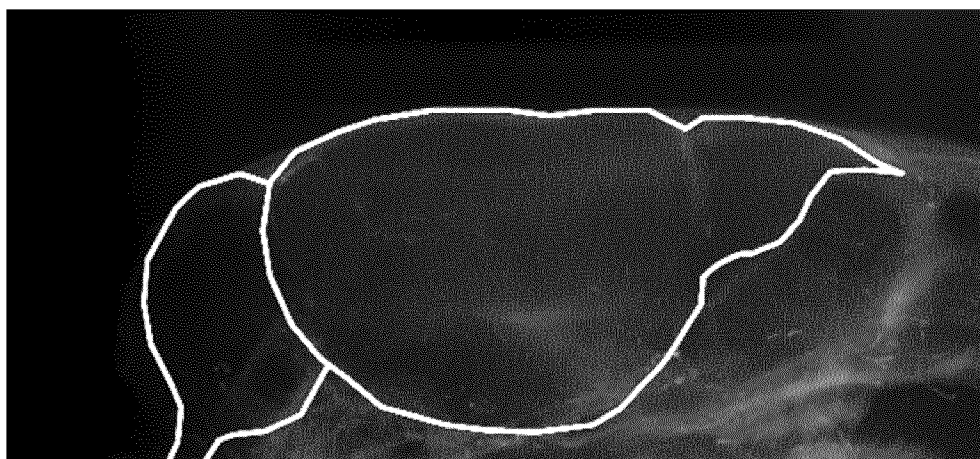


FIG.6

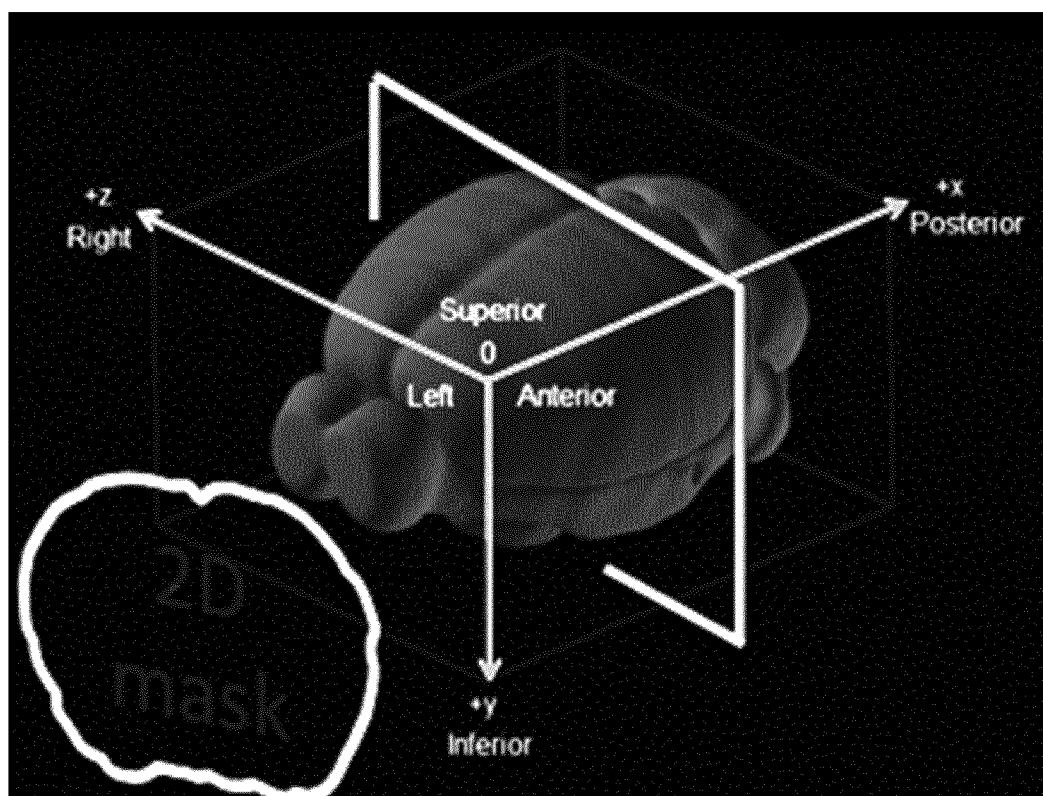


FIG.7

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FIG.8

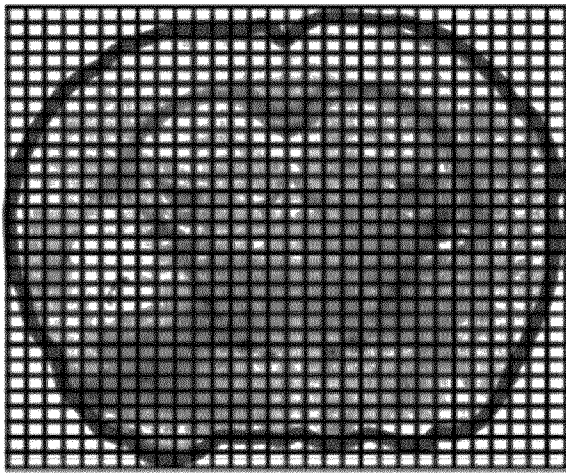
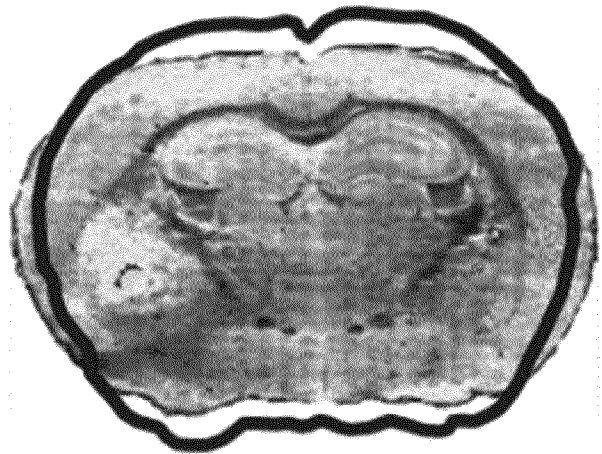
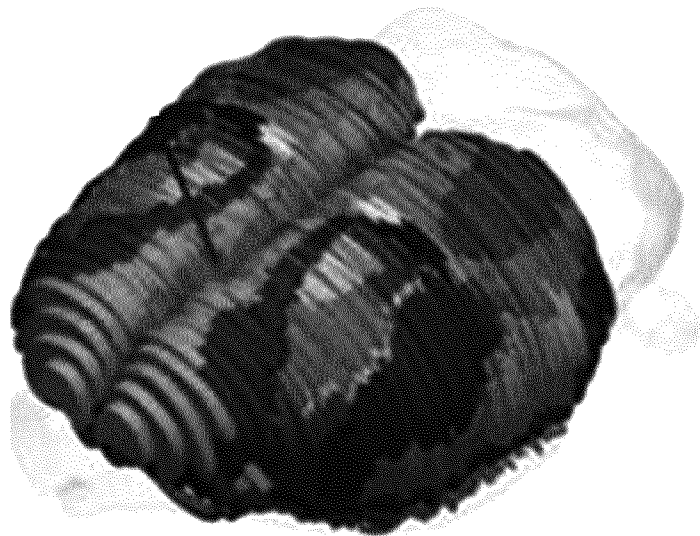


FIG.9

FIG.10



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/081216

A. CLASSIFICATION OF SUBJECT MATTER

INV. G06T7/00 G01J3/00 G01N21/31
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)

G06T G01J 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, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2007/091428 A1 (WILSON DAVID L [US] ET AL) 26 April 2007 (2007-04-26) abstract paragraph [0010] - paragraph [0012] paragraph [0024] - paragraph [0026] paragraph [0037] - paragraph [0038] paragraph [0052] - paragraph [0053] ----- -/--	1-10



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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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

30 January 2018

Date of mailing of the international search report

08/02/2018

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Katsoulas, Dimitrios

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/081216

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	YUTONG LIU ET AL: "An image warping technique for rodent brain MRI-histology registration based on thin-plate splines with landmark optimization", SPIE - INTERNATIONAL SOCIETY FOR OPTICAL ENGINEERING. PROCEEDINGS, vol. 7259, 26 February 2009 (2009-02-26), page 72592K, XP055374546, US ISSN: 0277-786X, DOI: 10.1117/12.812045 ISBN: 978-1-5106-0753-8 abstract page 1 - page 3; figures 1-2 -----	1,6-8
Y	Michael Jackson ET AL: "Infrared Spectroscopy, Ex Vivo Tissue Analysis by : Applications, Theory and Instrumentation" In: "Encyclopedia of analytical chemistry : applications, theory and instrumentation", 15 September 2006 (2006-09-15), John Wiley & Sons Ltd., Chichester [u.a.], XP055374548, ISBN: 978-0-470-02731-8 DOI: 10.1002/9780470027318.a0107, page 4 - page 5 -----	1,6-8
A	GURCAN M N ET AL: "Histopathological Image Analysis: A Review", IEEE REVIEWS IN BIOMEDICAL ENGINEERING, IEEE, USA, vol. 2, 1 January 2009 (2009-01-01), pages 147-171, XP011507549, ISSN: 1937-3333, DOI: 10.1109/RBME.2009.2034865 page 150 -----	1-10
A	Z Yang ET AL: "Registration of Histology and MR Images using Local Rigid Registration and Differential Evolution", 1 January 2010 (2010-01-01), page 1, XP055374550, Retrieved from the Internet: URL: http://cds.ismrm.org/protected/10MProceedings/files/5100_4321.pdf [retrieved on 2017-05-19] the whole document -----	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2017/081216

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		US 2011091088 A1	21-04-2011
