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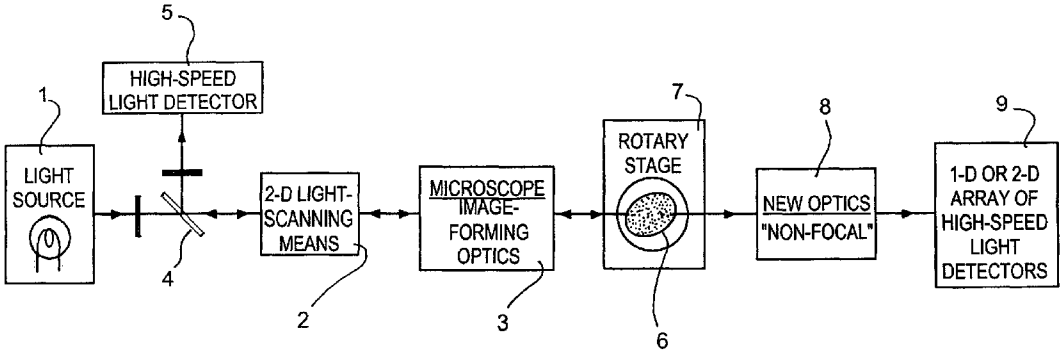
(54) Title: OPTICAL PROJECTION TOMOGRAPHY



(57) Abstract: Apparatus for obtaining an image of a specimen (6) by optical projection tomography comprises a light scanner, such as a light-scanning confocal microscope (1, 2, 3) for subjecting the specimen (6) to a scanning movement of incident light.

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TITLE: OPTICAL PROJECTION TOMOGRAPHY

Field of the Invention

This invention relates to optical projection tomography.

Background to the Invention

Optical projection tomography is a technique for producing three-dimensional images of specimens, one example being disclosed in the applicant's specification WO 02/095476. The invention aims to provide a different way of directing the light onto the specimen, particularly in the case of fluorescent imaging, with a view to reducing noise or interference in the series of images and providing improved depth of focus in the series of images.

Summary of the Invention

According to one aspect of the invention there is provided apparatus for obtaining an image of a specimen by optical projection tomography, the apparatus comprising light-scanning means and a rotary stage for rotating the specimen to indexed positions in each of which the specimen is in use subjected to a scanning movement of incident light by the scanning means.

The incident light may be scanned in a direction perpendicular to an optical axis defined by the light passing through the apparatus.

The light scanning means may form part of a confocal scanning microscope.

According to another aspect of the invention there is provided a method of obtaining an image of a specimen by optical projection tomograpy, the method comprising scanning the specimen with a light beam and detecting light emanating from the specimen to derive the image.

Preferably, the detector detects light which exits or by-passes the specimen parallel to the beam incident on the specimen.

The incident light is preferably scanned in a raster pattern, one complete scan being undertaken at each indexed position of the specimen.

There is also provided use of a method or apparatus as described in any of the aspects as set out above in any one or more of the analyses or procedures listed hereunder.

According to the present invention, the analyses and procedures of the present invention include:

Analysis of the structure of biological tissues.
Analysis of the function of biological tissues.
Analysis of the shapes of biological tissues.
Analysis of the distribution of cell types within biological tissues.
Analysis of the distribution of gene activity within biological tissues,
including the distribution of:

- RNA transcripts
- proteins

Analysis of the distribution of transgenic gene activity within biological tissues,
Analysis of the distribution of cell activities within biological tissues,
including:

- Cell cycle status including arrest
- Cell death
- Cell proliferation
- Cell migration

Analysis of the distribution of physiological states within biological tissues.
Analysis of the results of immunohistochemistry staining techniques.
Analysis of the results of in-situ hybridisation staining techniques.
Analysis of the distribution of molecular markers within biological tissues,
including any coloured or light-absorbing substances, such as:

5,5'-dibromo-4,4'-dichloro-indigo (or other halogenated indigo compounds)
formazan
or other coloured precipitates generated through the catalytic activity of enzymes including: b-galactosidase, alkaline phosphatase or other coloured precipitates formed upon catalytic conversion of staining substrates,
including: Fast Red, Vector Red
And including any light-emitting substances,
Therefore including any fluorescent substances,
such as: Alexa dyes, FITC, rhodamine,
And including any luminescent substances,
such as green fluorescent protein (GFP) or similar proteins,
And including any phosphorescent substances.

Analysis of tissues from all plant species.
Analysis of any tissue for agricultural research,
including:
basic research into all aspects of plant biology (genetics, development, physiology, pathology etc.)
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including:

invertebrates

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amphibians (including the genus Xenopus and axolotls)

reptiles

birds (including chickens and quails)

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Analysis of embryonic tissues for any purpose,

including:

research into any stem cell population

research into developmental biology

research into the causes of abnormal embryo development, including human syndromes

autopsies of human terminated pregnancies (both spontaneous and induced terminations)

Analysis of any tissues for the purpose of genomics research,

including:

the analysis of any tissues for the purpose of genomics research,

including:

the analysis of transgenic, knock-in, knock-down or knock-out organisms

the analysis or discovery of the expression (or activity) of genes including their spatial distribution, and their levels of expression

the analysis of discovery of abnormalities in the structure or morphology of tissues, as a result of interference due to wilful experimentation (such as genetic or physical modifications including a chemical or biochemical genomics approach), and/or spontaneous abnormalities (such as naturally-occurring mutations)

Analysis of any tissue for the purpose of neurobiology research,
including:
the analysis of the morphology of nerves
the analysis of the pathways and connectivity of nerves
the analysis of parts of, or whole, animal brains

Analysis of any tissue for pharmaceutical research,
including:
the analysis of pharmaceutical substances (such as drugs, molecules, proteins, antibodies),
including their spatial distribution within the tissue, and their concentrations
the analysis or discovery of abnormalities in the structure or morphology of tissues.

Analysis of tissues for medical research,
including:
research into the genetics, development, physiology, structure and function of animal tissues
analysis of diseased tissue to further our understanding of all types of diseases
including:
congenital diseases
acquired diseases
including:
infectious
neoplastic
vascular
inflammatory
traumatic
metabolic
endocrine
degenerative

drug-related
iatrogenic or
idiopathic diseases

Analysis of tissues for medical diagnosis, treatment or monitoring,
including:
the diagnosis of cancer patients
including:
searching for cancerous cells and tissues within biopsies
searching for abnormal structure or morphology of tissues within biopsies
the analysis of all biopsies
including the analysis of:
lymph nodes
polyps
liver biopsies
kidney biopsies
prostate biopsies
muscle biopsies
brain tissue
the analysis of tissue removed in the process of extracting a tumour from a patient
including:
determining whether all the tumour has been removed
determining the type of tumour, and the type of cancer.

According to the present invention, samples for use in the present invention may be prepared as described in the earlier patent applications and/or employing conventional pathological and histological techniques and procedures well known to persons skilled in the art.

For example, in-situ hybridisation (particularly useful for detecting RNAs):Hammond K L, Hanson I M, Brown A G, Lettice L A, Hill R E "Mammalian and Drosophila dachsund

genes are related to the Ski proto-oncongene and are expressed in eye and limb". Mech Dev. 1998 Jun;74(1-2):121-31.

Immunohistochemistry (particularly useful for detecting proteins and other molecules):
Sharpe J, Ahlgren U, Perry P, Hill B, Ross A, Hecksher-Sorensen J, Baldock R, Davidson D. "Optical projection tomography as a tool for 3D microscopy and gene expression studies" Science. 2002 Apr 19;296(5567):541-5.

It will be appreciated that modification may be made to the invention without departing from the scope of the invention.

Brief Description of the Drawings

The invention will now be described, by way of example, with reference to the accompanying drawings, in which:

Figure 1 is a diagram of the apparatus forming the preferred embodiment of the invention,

Figures 2a and 2b show how the microscope optics of the apparatus can be arranged to have low numerical aperture or high numerical aperture,

Figure 3 shows known image-forming optics,

Figures 4 and 5 show the image-forming optics of an optical system of the inventive apparatus,

Figures 6a, 6b, 6c and 6d show representative light paths for the optical system of the inventive apparatus,

Figures 7a, 7b and 7c illustrate how different degrees of refraction affect operation of the optical system,

Figure 8 illustrates how refraction is measured using a one-dimensional array of detectors, and

Figures 9 to 12 illustrate, in three dimensions, the operation of the optical system.

Detailed Description of the Drawings

Referring to Figure 1, the apparatus comprises a light source 1 (in the form of a laser) which supplies light to a two-dimensional light scanning means 2, the scanning mechanism of which has a dual mirror system. Light with a scanning motion is fed through image-forming optics 3. A dichroic mirror 4 interposed between the light source 1 and the scanning means 2 directs returned light to a high speed light detector 5. The components 1 to 5 may be provided by a confocal light-scanning microscope.

Light from the optics 3 passes through a specimen 6 which is rotated within, and supported by, a rotary stage 7 which in structure corresponds to the rotary stage disclosed in the applicant's co-pending International Patent Application No. PCT/GB02/02373. The rotary stage 7 rotates the specimen 6 to successive indexed positions at each of which one complete scan of the excitation light is undertaken whilst the specimen is stationary. After passing through the specimen 6, the light is processed by an optical system 8 which directs the light to a one-dimensional or two-dimensional array of high speed light detectors 9.

In fluorescence mode, light from the specimen 6 is returned through the optics 3 and the scanning means 2 and thence, via the mirror 4, to the high speed light detector 5. In this method of fluorescence imaging, the excitation light enters one side of the specimen and leaves the specimen from the same side thereof before being detected. It is in the

transmission mode, to be described, that the components shown to the right of the stage 7 in Figure 1 are used.

The microscope optics 3 may have a high numerical aperture (Figure 2a) or may be adapted to have a low numerical aperture (Figure 2b) which is useful for some specimens to be imaged.

Figure 3 illustrates a known image-forming system. The light from any point on the focal plane 12 (within the specimen) is collected and refracted by a lens 13 towards a single point in the image plane 14. There exists a symmetry such that any point on the image plane 14 maps to a point in the focal plane 12 and *vice versa*.

By contrast, the need for an *image-forming* optical arrangement is removed in the inventive "non-focal" optics of Figures 4 and 5 which displays no such symmetry. The non-focal optical system 8 is represented by a convex lens 15. The light from a single point on the focal plane 12 is not focussed onto a single light detector. It is diverged such that only the light which exits or by-passes the specimen 6 parallel to the incident beam reaches the single light detector 9a positioned on the optical axis. The purpose of the lens 15 in Figures 4 and 5 is different from Figure 3. It functions in a light-scanning situation. The light beam is scanned (e.g. in a raster pattern) across the specimen through a multitude of different positions (five of which are illustrated as the black arrows in Figure 5). The purpose of the non-focal optical system 8 (i.e. the lens 15) is to direct onto the single light detector 9a, light which exits or by-passes the specimen parallel to the incident beam, irrespective of the scanning position of the light beam. In specimens which cause significant scattering of light the system allows a higher signal-to-noise ratio to be obtained by limiting detection of scattering light.

Figures 6a to 6d, which illustrate scattering as an example to show deviation from the original beam position, illustrate some representative light paths for rays (derived from a laser beam) emitted from the specimen 6 while passing through the non-focal optical

system. The beam approaching the specimen from the left is the beam incident on the specimen.

In Figure 6a rays scattered from a point in the centre of the specimen 6 are diverged away from the light detector 9a. The proportion of scattered rays which are detected can be adjusted by changing the effective size of the detector. An adjustable iris allows this control (which is very similar to the pin-hole in a scanning confocal microscope). Alternatively, the position of the lens can be adjusted to cause more or less divergence of the scattered rays. In optical image-forming systems, an airy disc is the interference pattern produced by the light emitted from a single point within the specimen. Optical systems which produce larger airy discs have lower resolving power, as airy discs from neighbouring points within the specimen will overlap. The concept of the airy disc is not strictly relevant to a projection-measuring system like this, however a similar concept does exist. In the case of the non-focal optics described here, light from each projection creates a very broad distribution of intensities (at the position of the detector) similar to a broad airy disc, which might suggest low resolving power. However, as only a single projection is measured at any one time even very broad distributions cannot interfere with each other.

In Figure 6b rays scattered from other points along the same line sampled in Figure 6a, are also diverged away from the light detector 9a.

In Figure 6c unscattered light from a different scanned position (black arrow) is emitted from the specimen 6 substantially parallel to the optical axis, and is therefore refracted towards the light detector 9a. As in Figures 6a and 6b, scattered light is directed away from the detector 9a.

In Figure 6d unscattered rays from any scanned position are directed onto the light detector 6. The arrows represent successive positions of the laser beam as it is scanned across the specimen 6 in a direction perpendicular to the optical axis.

All experiments done so far with optical projection tomography have had to assume that although some of the light is scattered, the refractive index of the specimen is uniform. Recent experiments have demonstrated that a number of important specimens (including medical imaging of biopsies) display non-uniform refractive indexes. This means that the current algorithms are not accurately imaging the specimen – distortions and artefacts are introduced. The apparatus described reduces this problem by measuring information not previously available relating to the angle at which a light beam exits from the specimen. In general, in specimens with low scattering but non-uniform distribution of refractive index the system allows this non-uniform distribution to be calculated by measuring the degree of refraction experienced by each projection.

In the use of the present apparatus a clearing agent (such as BABB) is used such that the majority of the light is not scattered. It is however subject to a different form of disruption – refraction. In Figure 7, scattered light is indicated by broken lines, while the main path of light is shown as a solid line. In the first example of Figure 7a this path is not bent as it passes through the specimen 6 (it is only refracted on passing through the lens). The main path does pass through a region of the specimen with a higher refractive index than the rest (grey disc), however both the interfaces it encounters between regions of differing refractive index are perpendicular to the light path, so no refraction occurs.

In the second case of Figure 7b, the illumination beam is slightly higher and therefore the interfaces it encounters between the grey region and the white region of the specimen (different refractive indexes) are slightly displaced from perpendicular. This causes two slight refractions of the main path such that when the light emerges from the specimen it is no longer parallel to the incident beam and is directed slightly to the side of the original central light detector 9a. If auxiliary light detectors 9b are positioned on either side of the central detector 9a, these can measure the degree of refraction. Any projection will give a certain distribution of intensities along the array of light detectors. The distribution of intensities can be used to determine the angle at which the main light path emerged from the specimen. The system need only determine where the centre of this distribution is (usually the strongest intensity) to measure the angle at which the main light path emerged

from the specimen. In the last case of Figure 7c, a different scanned position has caused greater refraction of the beam, which is reflected in a further shift along the array of detectors.

In Figure 8, an oblong region of the specimen 6 has a higher refractive index (grey shape) than the rest. Rays passing around the specimen are not refracted and so are directed to the central light detector 9a. Rays passing through the middle of the specimen (middle two rays 11 in Figure 8) are refracted twice. The two interfaces which the light passes through (white-to-grey and then grey-to-white) are parallel with each other, and the light rays therefore exit the specimen at the same angle that they entered it. These rays are also directed onto the central detector 9a. Rays passing through other parts of the grey region are also refracted twice but do not pass through parallel interfaces, so these rays are detected by the adjacent light detectors 9b.

The fact that some rays will be refracted and still exit the specimen 6 parallel to the incident beam is not a problem. The example of Figure 8 shows only one of the many sets of projections taken through this section. Full imaging involves capturing such a data set for many orientations through the section, and the combination of all this data allows a full reconstruction of the distribution.

Figures 9 to 12 show three-dimensional views of the apparatus. In Figure 9, all un-refracted (and unscattered) rays through a two-dimensional section of the specimen are focused onto the central light detector of the array. The specimen 6 is rotated about a vertical axis between indexed positions in each of which a complete scan is undertaken.

Figure 10 shows the path of scattered or refracted light onto auxiliary light detectors.

Figure 11 illustrates that the lens (or optical system) allows the one-dimensional array of detectors 9 to capture data from a full two-dimensional raster-scan of the specimen. A row of scanned positions is always directed down or up to the row of detectors, irrespective of the vertical height of the scan.

A two-dimensional array of light detectors 9 may be used instead of a one-dimensional array, as shown in Figure 12. This would be able to measure light which is scattered or refracted above or below the plane occupied by the light rays shown in Figure 12.

In prior-art wide-field optical projection tomography, each pixel of the CCD should record the information from an approximate projection through the specimen. Wide-field fluorescence optical projection tomography suffers a problem due to the fact that illumination/excitation of the specimen must also be wide-field. If the optical properties of the specimen cause internal scattering of light, then many photons exit the specimen along trajectories which cause them to be detected by pixels which do not represent the projection from which the photon originated. This adds significant noise to the image. The light-scanning invention described here avoids this problem because only the fluorescent particles within the approximate projection are excited at any one time.

The data derived from the detector array 9 optics is interpreted by an algorithm.

Many different algorithmic approaches already exist for performing back-projection calculations. One approach is to use a standard linear filtered back-projection algorithm (as in US Patent 5680484). Other approaches include iterative, maximum entropy and algebraic reconstruction technique. (R. Gordon et al., "Three-Dimensional Reconstruction form Projections: A Review of Algorithms".

The algorithm works as follows:

1. The data is used as if it were parallel (or fan-beam) data to perform back-projection. This produces a "fuzzy" estimation of the distribution of absorption characteristics of the specimen, or alternatively a fuzzy distribution of the fluorescence of the specimen.

2. A first approximation of the distribution of refractive index is estimated. This can be done in a number of ways. One useful method is to assume that the absorption or fluorescent distribution will reflect the distribution of refractive index. Within each section a 2-D gradient vector is calculated for each voxel. An alternative is to start with a uniform or a random distribution.

3. The estimated refraction distribution is used to perform a forward-projection, i.e. a prediction of what the projection data should look like if the initial estimate of the refraction distribution was correct.

4. The predicted projections and the actual projections are compared.

5. The estimated refraction distribution is modified. The projections with a greater difference between predicted and actual, pin-point which regions of the distribution need more modification. For example, in the case of the grey shape shown in Figure 8, projections from the curved ends of the oblong will differ greatly from the predictions due to the large amount of refraction. Voxels in the regions therefore have their predicted refraction indexes changed more than other regions.

6. The loop from 3 to 6 is repeated until no further improvements to the predicted projections can be made.

The algorithm approach above can also be used to interpret other optical signals, for example fluorescence or scattering.

The apparatus and methods can be used in various analyses and procedures, as set out below:

Analysis of the structure of biological tissues.
Analysis of the function of biological tissues.
Analysis of the shapes of biological tissues.

Analysis of the distribution of cell types within biological tissues.

Analysis of the distribution of gene activity within biological tissues,

including the distribution of:

- RNA transcripts
- proteins

Analysis of the distribution of transgenic gene activity within biological tissues,

Analysis of the distribution of cell activities within biological tissues,

including:

- Cell cycle status including arrest
- Cell death
- Cell proliferation
- Cell migration

Analysis of the distribution of physiological states within biological tissues.

Analysis of the results of immunohistochemistry staining techniques.

Analysis of the results of in-situ hybridisation staining techniques.

Analysis of the distribution of molecular markers within biological tissues,

including any coloured or light-absorbing substances,

such as:

5,5'-dibromo-4,4'-dichloro-indigo (or other halogenated indigo compounds)

formazan

or other coloured precipitates generated through the catalytic activity of enzymes

including: b-galactosidase, alkaline phosphatase or other coloured precipitates formed upon catalytic conversion of staining substrates,

including: Fast Red, Vector Red

And including any light-emitting substances,

Therefore including any fluorescent substances,

such as: Alexa dyes, FITC, rhodamine,

And including any luminescent substances,

such as green fluorescent protein (GFP) or similar proteins,

And including any phosphorescent substances.

Analysis of tissues from all plant species.

Analysis of any tissue for agricultural research,

including:

basic research into all aspects of plant biology (genetics, development, physiology, pathology etc.)

analysis of tissues which have been genetically altered.

Analysis of tissues from all animal species,

including:

invertebrates

nematode worms

vertebrates

all types of fish

(including teleosts, such as zebrafish, and chondrycthes including sharks)

amphibians (including the genus Xenopus and axolotls)

reptiles

birds (including chickens and quails)

all mammals (including all rodents, dogs, cats and all primates, including human)

Analysis of embryonic tissues for any purpose,

including:

research into any stem cell population

research into developmental biology

research into the causes of abnormal embryo development, including human syndromes

autopsies of human terminated pregnancies (both spontaneous and induced terminations)

Analysis of any tissues for the purpose of genomics research,

including:

the analysis of transgenic, knock-in, knock-down or knock-out organisms

the analysis or discovery of the expression (or activity) of genes including their spatial distribution, and their levels of expression

the analysis of discovery of abnormalities in the structure or morphology of tissues, as a result of interference due to wilful experimentation (such as genetic or physical modifications including a chemical or biochemical genomics approach),

and/or spontaneous abnormalities (such as naturally-occurring mutations)

Analysis of any tissue for the purpose of neurobiology research,

including:

the analysis of the morphology of nerves

the analysis of the pathways and connectivity of nerves

the analysis of parts of, or whole, animal brains

Analysis of any tissue for pharmaceutical research,

including:

the analysis of pharmaceutical substances (such as drugs, molecules, proteins, antibodies),

including their spatial distribution within the tissue, and their concentrations

the analysis or discovery of abnormalities in the structure or morphology of tissues.

Analysis of tissues for medical research,

including:

research into the genetics, development, physiology, structure and function of animal tissues

analysis of diseased tissue to further our understanding of all types of diseases

including:

congenital diseases

acquired diseases

including:

infectious

neoplastic

vascular
inflammatory
traumatic
metabolic
endocrine
degenerative
drug-related
iatrogenic or
idiopathic diseases

Analysis of tissues for medical diagnosis, treatment or monitoring,
including:
the diagnosis of cancer patients
including:
searching for cancerous cells and tissues within biopsies
searching for abnormal structure or morphology of tissues within biopsies
the analysis of all biopsies
including the analysis of:
lymph nodes
polyps
liver biopsies
kidney biopsies
prostate biopsies
muscle biopsies
brain tissue
the analysis of tissue removed in the process of extracting a tumour from a patient
including:
determining whether all the tumour has been removed
determining the type of tumour, and the type of cancer.

It will be appreciated that modification may be made to the invention without departing from the scope of the invention.

CLAIMS

1. Apparatus for obtaining an image of a specimen by optical projection tomography, the apparatus comprising light scanning means and a rotary stage for rotating the specimen to indexed positions in each of which the specimen is in use subjected to a scanning movement of incident light by the scanning means.

2. Apparatus according to claim 1, wherein the incident light is scanned in a direction perpendicular to an optical axis followed by the light passing through the apparatus.

3. Apparatus according to claim 1 or 2, wherein the incident light is scanned in a raster pattern, one complete scan being undertaken at each indexed position of the specimen.

4. Apparatus according to any of the preceding claims, wherein the light scanning means form part of a confocal scanning microscope.

5. A method of obtaining an image of a specimen by optical projection tomography, comprising scanning the specimen with a light beam and detecting light emanating from the specimen to derive the image.

6. A method according to claim 5, wherein the light passes through the specimen prior to being detected.

7. A method according to claim 5, wherein the light enters from one side of the specimen and leaves the specimen from the same side thereof.

8. A method according to any of claims 5 to 7, wherein the specimen is rotated to indexed positions and one complete scan is undertaken at each indexed position of the specimen.

9. A method according to any of claims 5 to 7, wherein the detector detects light which exits or by-passes the specimen parallel to the beam incident on the specimen.

10. A method according to any of claims 5 to 9, wherein the light is laser light.

11. A method of performing any one or more of the analyses or procedures listed hereunder comprising use of a method or apparatus according to any of claims 1 to 10:

Analysis of the structure of biological tissues.
Analysis of the function of biological tissues.
Analysis of the shapes of biological tissues.
Analysis of the distribution of cell types within biological tissues.
Analysis of the distribution of gene activity within biological tissues,
including the distribution of:

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including:

- Cell cycle status including arrest
- Cell death
- Cell proliferation
- Cell migration

Analysis of the distribution of physiological states within biological tissues.
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Analysis of the results of in-situ hybridisation staining techniques.
Analysis of the distribution of molecular markers within biological tissues,
including any coloured or light-absorbing substances, such as:

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formazan

or other coloured precipitates generated through the catalytic activity of enzymes including: b-galactosidase, alkaline phosphatase or other coloured precipitates formed upon catalytic conversion of staining substrates,

including: Fast Red, Vector Red

And including any light-emitting substances,

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such as: Alexa dyes, FITC, rhodamine,

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such as green fluorescent protein (GFP) or similar proteins,

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invertebrates

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amphibians (including the genus Xenopus and axolotls)

reptiles

birds (including chickens and quails)

all mammals (including all rodents, dogs, cats and all primates, including human)

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including:

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including their spatial distribution within the tissue, and their concentrations
the analysis or discovery of abnormalities in the structure or morphology of tissues.

Analysis of tissues for medical research,
including:
research into the genetics, development, physiology, structure and function of animal tissues
analysis of diseased tissue to further our understanding of all types of diseases
including:
congenital diseases
acquired diseases
including:
infectious
neoplastic
vascular
inflammatory
traumatic
metabolic
endocrine
degenerative
drug-related
iatrogenic or
idiopathic diseases

Analysis of tissues for medical diagnosis, treatment or monitoring,
including:
the diagnosis of cancer patients
including:
searching for cancerous cells and tissues within biopsies
searching for abnormal structure or morphology of tissues within biopsies
the analysis of all biopsies

including the analysis of:

lymph nodes

polyps

liver biopsies

kidney biopsies

prostate biopsies

muscle biopsies

brain tissue

the analysis of tissue removed in the process of extracting a tumour from a patient including:

determining whether all the tumour has been removed

determining the type of tumour, and the type of cancer.

12. Use of a method or apparatus as described in any of claims 1 to 10 in any one or more of the analyses or procedures listed hereunder:

Analysis of the structure of biological tissues.

Analysis of the function of biological tissues.

Analysis of the shapes of biological tissues.

Analysis of the distribution of cell types within biological tissues.

Analysis of the distribution of gene activity within biological tissues,

including the distribution of:

- RNA transcripts
- proteins

Analysis of the distribution of transgenic gene activity within biological tissues,

Analysis of the distribution of cell activities within biological tissues,

including:

- Cell cycle status including arrest
- Cell death
- Cell proliferation
- Cell migration

Analysis of the distribution of physiological states within biological tissues.

Analysis of the results of immunohistochemistry staining techniques.

Analysis of the results of in-situ hybridisation staining techniques.

Analysis of the distribution of molecular markers within biological tissues,

including any coloured or light-absorbing substances, such as:

5,5'-dibromo-4,4'-dichloro-indigo (or other halogenated indigo compounds)

formazan

or other coloured precipitates generated through the catalytic activity of enzymes including: b-galactosidase, alkaline phosphatase or other coloured precipitates formed upon catalytic conversion of staining substrates,

including: Fast Red, Vector Red

And including any light-emitting substances,

Therefore including any fluorescent substances,

such as: Alexa dyes, FITC, rhodamine,

And including any luminescent substances,

such as green fluorescent protein (GFP) or similar proteins,

And including any phosphorescent substances.

Analysis of tissues from all plant species.

Analysis of any tissue for agricultural research,

including:

basic research into all aspects of plant biology (genetics, development, physiology, pathology etc.)

analysis of tissues which have been genetically altered.

Analysis of tissues from all animal species.

including:

invertebrates

nematode worms

vertebrates

all types of fish (including teleosts, such as zebrafish, and chondrycthes including sharks)

amphibians (including the genus Xenopus and axolotls)

reptiles

birds (including chickens and quails)

all mammals (including all rodents, dogs, cats and all primates, including human)

Analysis of embryonic tissues for any purpose,

including:

research into any stem cell population

research into developmental biology

research into the causes of abnormal embryo development, including human syndromes

autopsies of human terminated pregnancies (both spontaneous and induced terminations)

Analysis of any tissues for the purpose of genomics research,

including:

the analysis of any tissues for the purpose of genomics research,

including:

the analysis of transgenic, knock-in, knock-down or knock-out organisms

the analysis or discovery of the expression (or activity) of genes including their spatial distribution, and their levels of expression

the analysis of discovery of abnormalities in the structure or morphology of tissues, as a result of interference due to wilful experimentation (such as genetic or physical modifications including a chemical or biochemical genomics approach), and/or spontaneous abnormalities (such as naturally-occurring mutations)

Analysis of any tissue for the purpose of neurobiology research,

including:

the analysis of the morphology of nerves
the analysis of the pathways and connectivity of nerves
the analysis of parts of, or whole, animal brains

Analysis of any tissue for pharmaceutical research,
including:
the analysis of pharmaceutical substances (such as drugs, molecules, proteins, antibodies),
including their spatial distribution within the tissue, and their concentrations
the analysis or discovery of abnormalities in the structure or morphology of tissues.

Analysis of tissues for medical research,
including:
research into the genetics, development, physiology, structure and function of animal tissues
analysis of diseased tissue to further our understanding of all types of diseases
including:
congenital diseases
acquired diseases
including:
infectious
neoplastic
vascular
inflammatory
traumatic
metabolic
endocrine
degenerative
drug-related
iatrogenic or
idiopathic diseases

Analysis of tissues for medical diagnosis, treatment or monitoring,
including:
the diagnosis of cancer patients
including:
searching for cancerous cells and tissues within biopsies
searching for abnormal structure or morphology of tissues within biopsies
the analysis of all biopsies
including the analysis of:
lymph nodes
polyps
liver biopsies
kidney biopsies
prostate biopsies
muscle biopsies
brain tissue
the analysis of tissue removed in the process of extracting a tumour from a patient
including:
determining whether all the tumour has been removed
determining the type of tumour, and the type of cancer.

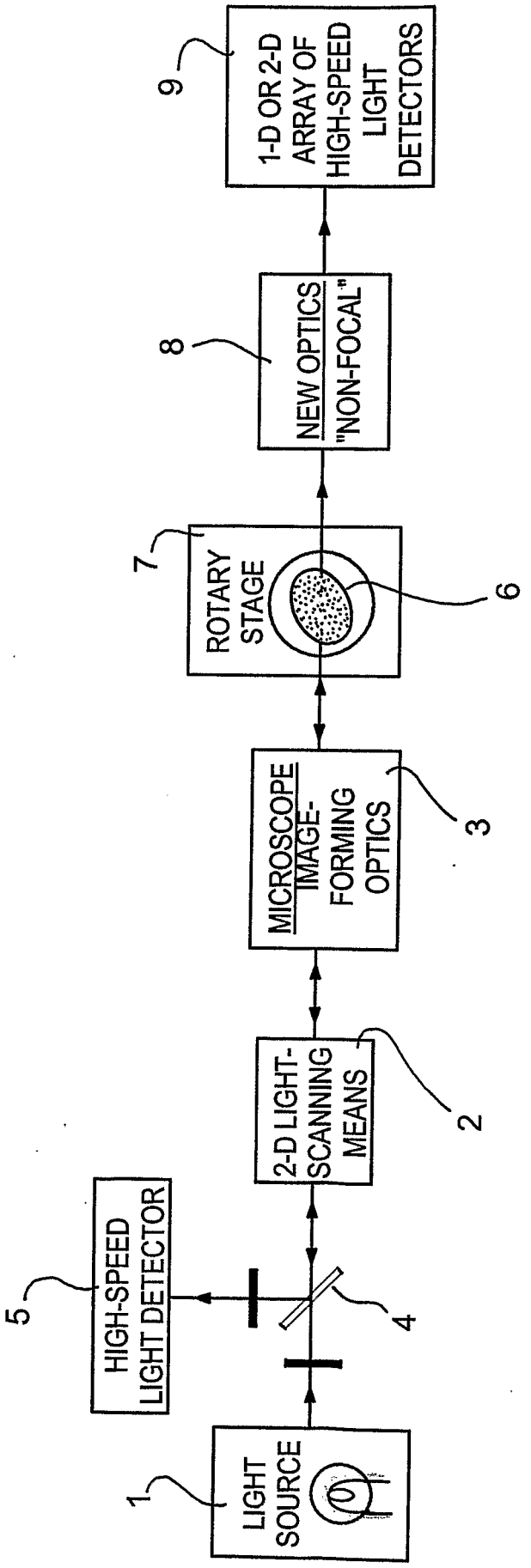
1-D OR 2-D ARRAY OF HIGH-SPEED LIGHT DETECTORS
9
NEW OPTICS "NON-FOCAL"
8
ROTARY STAGE
7
6
MICROSCOPE IMAGE-FORMING OPTICS
3
2-D LIGHT-SCANNING MEANS
2
HIGH-SPEED LIGHT DETECTOR
5
4
LIGHT SOURCE
1

Fig.1

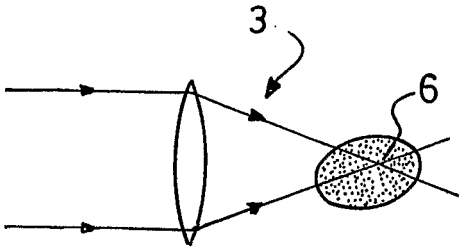

Fig. 2a

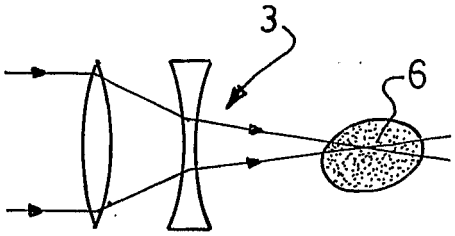

Fig. 2b

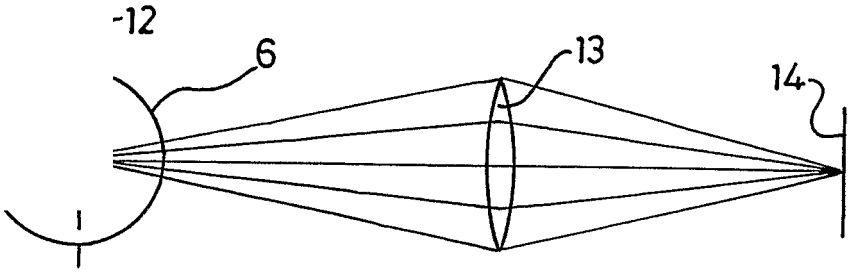

Fig. 3

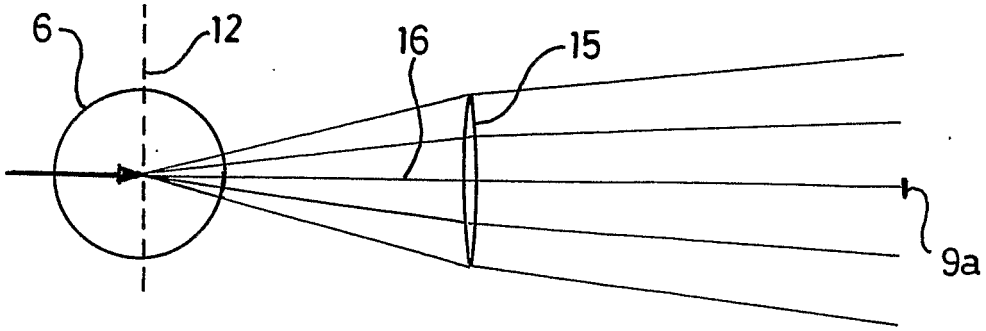

Fig. 4

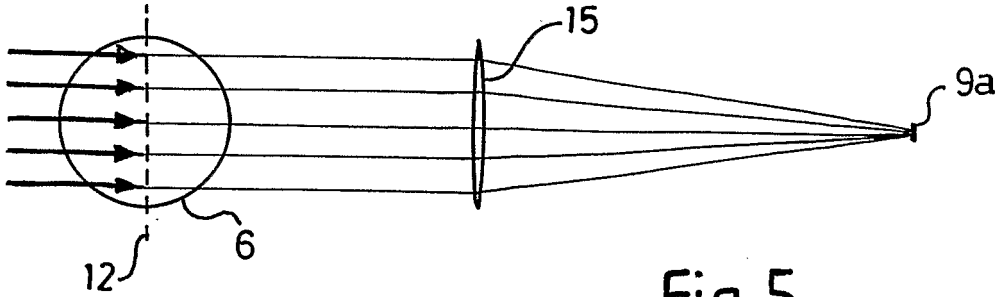

Fig. 5

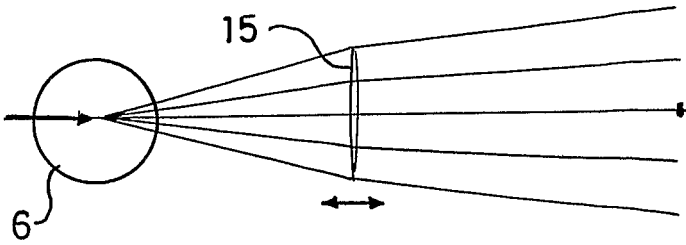

Fig. 6a

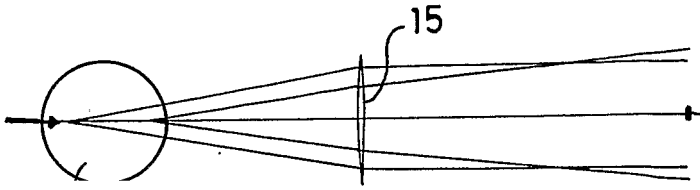

Fig. 6b

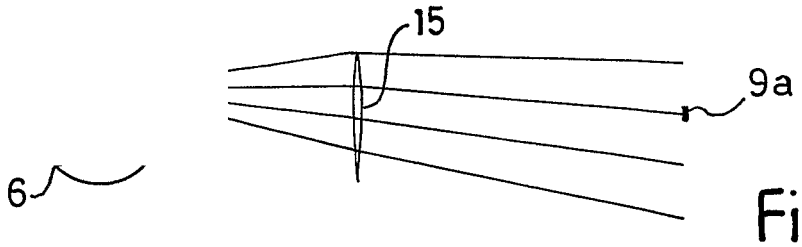

Fig. 6c

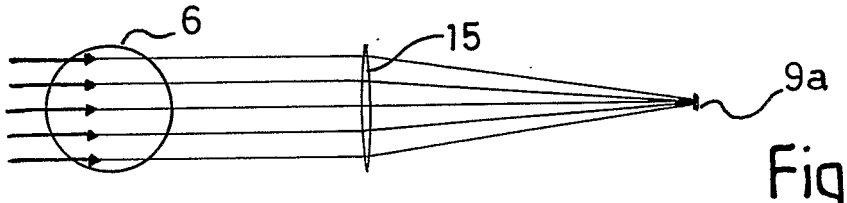

Fig. 6d

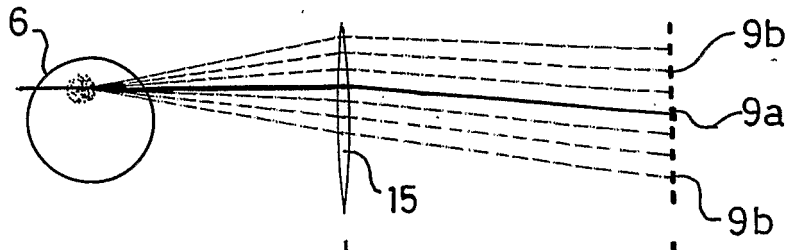

Fig. 7a

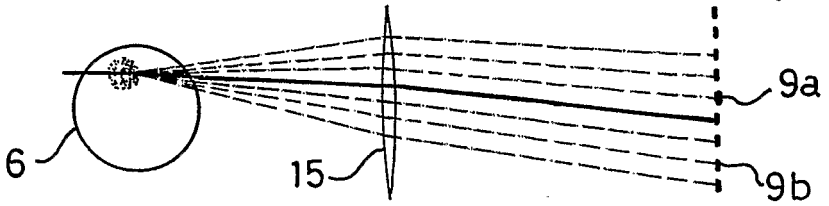

Fig. 7b

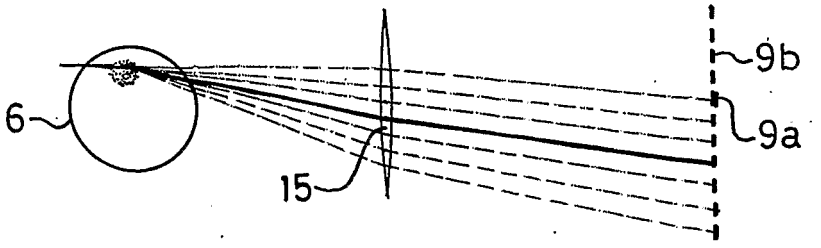

Fig. 7c

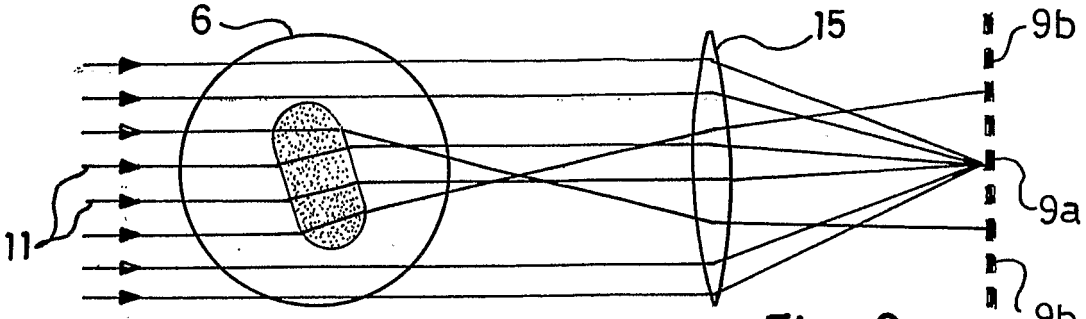

Fig. 8

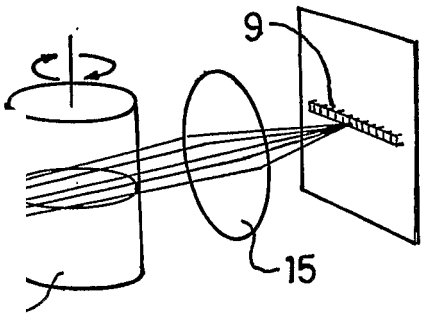

Fig. 9

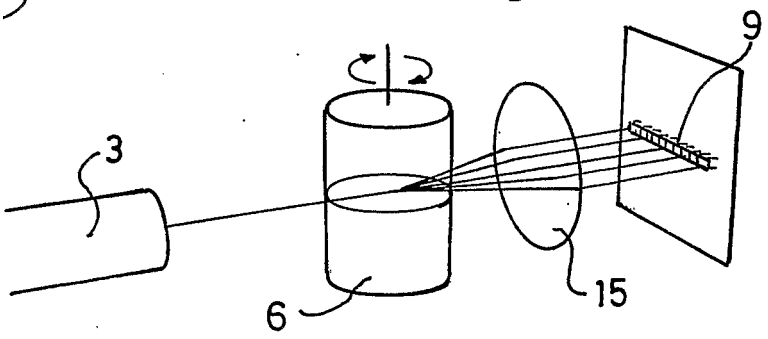

Fig. 10

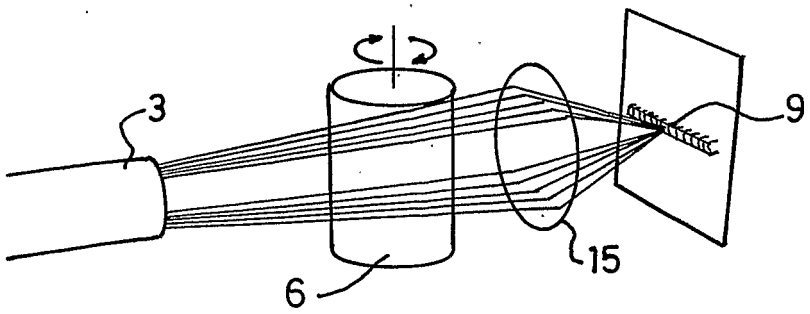

Fig. 11

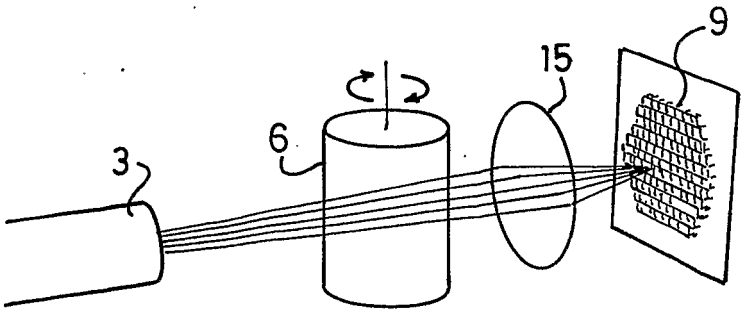

Fig. 12

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 03/03726

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/50 G01N1/31 G01N21/17 G02B21/00

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)
IPC 7 G01N G02B

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 practical, search terms used)
EPO-Internal, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 02 095476 A (MEDICAL RES COUNCIL ;PERRY PAUL ERNEST (GB); SHARPE JAMES ALEXANDE) 28 November 2002 (2002-11-28) page 2 -page 20; claims; figures --- -/--	1-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in 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 document 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
21 November 2003	19/12/2003

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, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	GONCALVES M L F C

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	SHARPE J ET AL: "OPTICAL PROJECTION TOMOGRAPHY AS A TOOL FOR 3D MICROSCOPY AND GENE EXPRESSION STUDIES" SCIENCE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, US, vol. 296, 19 April 2002 (2002-04-19), pages 541-545, XP001152115 ISSN: 0036-8075 cited in the application *see also the supplemental data part of this artricle relating to "specimen staining " and "imaging" at www.sciencemag.org/cgi/content/full/296/5567/541/DC1* page 543; figure 1 ---	1-10
P,A	SHARPE J: "Optical projection tomography as a new tool for studying embryo anatomy" JOURNAL OF ANATOMY 01 FEB 2003 UNITED KINGDOM, vol. 202, no. 2, 1 February 2003 (2003-02-01), pages 175-181, XP002262076 ISSN: 0021-8782 the whole document ---	1-10
A	SHARPE JAMES: "OPT microscopy: A new approach for 3D microscopy and gene expression studies" FASEB JOURNAL, vol. 16, no. 5, 22 March 2002 (2002-03-22), page A1092 XP009021394 Annual Meeting of Professional Research Scientists on Experimental Biology;New Orleans, Louisiana, USA; April 20-24, 2002 ISSN: 0892-6638 abstract ---	1-10
A,P	KENG WEE TEIK., SHARPE J., FITZPATRICK D. R.: "Optical Projection Tomographic Examination of Miscarried Human Embryos" JOURNAL OF MEDICAL GENETICS (BRITISH HUMAJM GENETICS CONFERENCE. YORK, ENGLAND, UK. SEPTEMBER 23-25), vol. 39, no. supplement 1, September 2002 (2002-09), page s23 XP009021392 ISSN 0022-2593 abstract ---	1-10
X	US 5 680 484 A (KIKUCHI SUSUMU ET AL) 21 October 1997 (1997-10-21) cited in the application abstract; claims ---	1-10
	-/--	

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 03/03726

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 30167 A (MEDICAL RES COUNCIL ;WHITE JOHN GRAHAM (US); AMOS WILLIAM BRADSHAW) 9 November 1995 (1995-11-09) abstract; claims; figures	1-10
A	US 5 724 171 A (AMOS WILLIAM BRADSHAW ET AL) 3 March 1998 (1998-03-03) abstract; claims	1-10
A	US 5 032 720 A (WHITE JOHN G) 16 July 1991 (1991-07-16) abstract; claims	1-10

INTERNATIONAL SEARCH REPORT

Inte nal application No.
PCT/GB 03/03726

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 11, 12
because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(iv) PCT - Diagnostic method practised on the human or animal body

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 03/03726

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 02095476 A	28-11-2002	WO 02095476 A2	28-11-2002
US 5680484 A	21-10-1997	JP 3327948 B2	24-09-2002
		JP 5341195 A	24-12-1993
WO 9530167 A	09-11-1995	WO 9530167 A1	09-11-1995
US 5724171 A	03-03-1998	DE 69415519 D1	04-02-1999
		DE 69415519 T2	27-05-1999
		EP 0699308 A1	06-03-1996
		WO 9427167 A1	24-11-1994
		JP 3403408 B2	06-05-2003
		JP 8510550 T	05-11-1996
US 5032720 A	16-07-1991	CA 1329255 C	03-05-1994
		US 5144477 A	01-09-1992

专利名称(译)	光学投影断层扫描		
公开(公告)号	EP1532443A1	公开(公告)日	2005-05-25
申请号	EP2003791037	申请日	2003-08-29
[标]申请(专利权)人(译)	医药研究委员会		
申请(专利权)人(译)	医学研究理事会		
当前申请(专利权)人(译)	医学研究理事会		
[标]发明人	SHARPE JAMES ALEXANDER		
发明人	SHARPE, JAMES, ALEXANDER		
IPC分类号	G01N15/14 G01N21/47 G01N21/64 G01N33/48 G01N33/53 G01N33/50 G01N1/31 G01N21/17 G02B21/00		
CPC分类号	G01N21/6456 G01N15/1468 G01N21/4795 G01N2015/1472		
优先权	2002027649 2002-11-27 GB 2002020157 2002-08-30 GB		
外部链接	Espacenet		

摘要(译)

用于通过光学投影断层摄影获得样本（6）的图像的设备包括光扫描仪，例如光扫描共焦显微镜（1,2,3），用于使样本（6）经受入射光的扫描移动。