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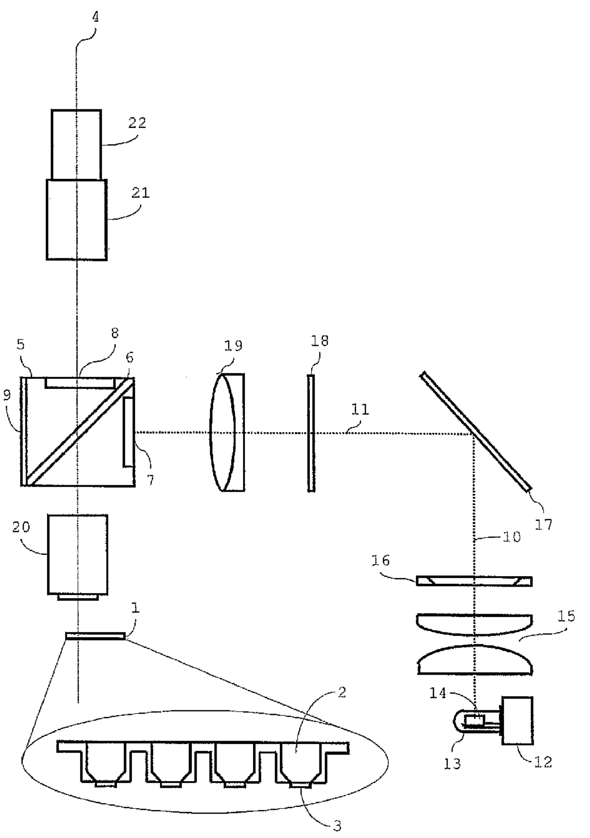
(54) Title: EPIFLUORESCENT MICROSCOPE IMAGING SYSTEM WITH METAL HALIDE LIGHT SOURCE



(57) Abstract: An epifluorescent imaging system is arranged to have an imaging axis (4) along which, in use, light travels between an optical element (5) comprising a dichroic mirror (6) and a sample position of a sample to be analysed. The imaging system comprises an illumination system, comprising a fitting (12) for a metal halide lamp (13). The epifluorescent imaging system comprises a metal halide lamp comprising a gas discharge tube (14), fitted in the fitting (12).

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EPIFLUORESCENT MICROSCOPE IMAGING SYSTEM WITH METAL HALIDE LIGHT SOURCE

Epifluorescent microscope imaging system, system for conducting automated sample analysis and illumination device.

The invention relates generally to epifluorescent imaging systems, to a system for conducting automated sample analysis and to an illumination device for an epifluorescent imaging apparatus, as well as the use of a metal halide lamp.

Examples of such systems and such a device are known. The known system is an integrated microarray system, in which incubator and image-acquisition optics are integrated. It is capable of kinetic signal detection while varying the temperature and other parameters of an array containing the sample to be analysed. To conduct an analysis, probe DNA molecules including fluorescent tags are bound onto a substrate. Light from the illumination system is projected onto a filter cube, which comprises an excitation and a detection filter, as well as a dichroic mirror. The excitation filter lets through light within a frequency range suitable for exciting the fluorescent tags. The detection filter lets through light in a frequency range emitted by the fluorescent tags. The emitted light is captured by a CCD (charge coupled device) camera for automated signal processing. As the intensity of emitted light depends on the amount of bound DNA as well as on the intensity of the excitation light, it is important that a homogeneous bundle of light of a well-defined intensity is projected onto the sample. In the known system, a metal halide light bulb serves as a source of light. The metal halide light bulb lies substantially in a horizontal plane with the filter cube and is an arc lamp working at high pressure.

A problem of the known system is that the light is emitted only from the small arc between the electrodes. Several optical elements (condenser) are needed to make a more homogeneous light bundle. This is of special concern in systems for automated sample analysis, because these systems do not otherwise need many optical elements, as the magnification in such systems is low.

It is an object of the present invention to provide an epifluorescent imaging system, system for conducting automated sample analysis and illumination device for an epifluorescent imaging apparatus that are better suited to conducting bioassays.

According to one aspect of the invention, this object is achieved by an epifluorescent imaging system, arranged to have an imaging axis along which, in use, light travels between an optical element comprising a dichroic mirror and a sample position of a sample to be analysed, which imaging system comprises an illumination system, comprising a fitting for a metal halide lamp, characterised in that the epifluorescent imaging system comprises a metal halide lamp comprising a gas discharge tube, fitted in the fitting.

A metal halide lamp consisting of a, preferably ceramic, discharge tube does not have a bright arc, but a tube, which gives light as a whole. Therefore no sophisticated optics are needed to create a homogeneous light bundle. An additional advantage is that the lifetime of these kinds of lamps, which are intended for general lighting applications, is relatively long.

According to another aspect, the invention provides an epifluorescent imaging system, arranged to have an imaging axis along which, in use, light travels between an optical element comprising a dichroic mirror and a sample position of a sample to be analysed, which imaging system comprises an illumination system, comprising a fitting for a metal halide lamp and at least one optical element determining an optical path between a location of light emission of a fitted metal halide lamp and the filter element, wherein the optical path comprises a first section closest to the location of light emission characterised in that the fitting is suitable for a metal halide lamp provided with a gas discharge tube at the location of light emission and in that, in an operating position of the system, the first section of the optical path is oriented predominantly opposite the direction of gravitation.

This epifluorescent imaging system enables the attainment of a homogeneous light bundle by use of metal halide lamps with a gas discharge tube. In such lamps, metal halide particles precipitate onto the bottom of the discharge tube. These particles absorb light and lead to inhomogoneities in the emitted bundle of light that is passed through the filter cube and projected onto the sample site.

The system according to the invention is particularly useful for conducting bioassays, as the metal halide lamp with a gas discharge tube has the property of emitting light of a high intensity, which is homogeneously distributed across the spectrum, making it suitable for a wide range of fluorophores. In addition, such lamps have a long lifetime, especially if they are of the low pressure type. Because the first section of the optical path is oriented predominantly opposite the direction of gravitation, light will normally be emitted in an upward direction, thus avoiding inhomogeneities due to deposits at the bottom of the lamp.

Preferably, the optical path determined by the at least one optical element comprises a further section closer to the filter element, the further section being at an angle to the imaging axis.

Thus, the light changes direction at least once, allowing the system to be more compact.

Preferably, the optical elements comprise a cold mirror.

This cold mirror transmits most of the infra-red radiation. This has the advantage of preventing heating of the other optical elements in the imaging system, notably the filter element. The properties of these other elements will thus remain more constant, and their lifetime is extended. In addition, where a CCD camera is used to process the reflected light emitted by the fluorophores, the sensitivity of such a camera to infra-red radiation poses few problems.

Preferably, the system comprises a system of lenses positioned along the imaging axis for projecting an image of a sample at the sample position through an eyepiece, wherein the system of lenses has an optical magnification substantially equal to one, and in the preferred variant, the system of lenses is formed by an objective lens, positioned between the sample position and the filter element, and an imaging lens, positioned between the filter element and the eyepiece, wherein the objective lens and imaging lens have substantially identical properties.

The eyepiece may be an eyepiece for a human observer, but is preferably an eyepiece for attaching a camera, e.g. a CCD camera. By having a magnification substantially equal to one, and using two identical lenses, the system is much simplified. It is easier to select the tube and objective lens. In addition, some aberrations, such as coma, lateral colour and distortion, are cancelled, allowing the use of cheaper lenses.

The system for conducting automated sample analysis according to the invention comprises an epifluorescent imaging system according to the invention.

Preferably, the system for conducting automated sample analysis is adapted for processing at least one sample tray comprising at least one well containing a sample, wherein the illumination system comprises a diaphragm for limiting the diameter of a light beam transmitted between the filter element and the sample to a smallest diameter of the well.

Thus, by correctly positioning the filter element and well in the directions perpendicular to the upright axis, the entire light beam will be reflected by the contents of the well, i.e. the sample situated therein. This means the measurement is not corrupted by light reflected off the tray.

According to another aspect of the invention, the illumination device for an epifluorescent imaging apparatus according to the invention is characterised in that the fitting is suitable for a metal halide lamp provided with a gas discharge tube at the location of light emission and in that, in the operating position, the first section of the optical path is oriented predominantly opposite the direction of gravitation.

Thus, there is provided an illumination device with the advantageous properties mentioned above in connection with the epifluorescent imaging system according to the invention. The illumination device is preferably adapted to be connected to an imaging apparatus of an existing device. The assembly of imaging apparatus and illumination device then forms an epifluorescent imaging system according to the invention.

According to another aspect of the invention, there is disclosed for the first time the use of a metal halide lamp comprising a gas discharge tube in an epifluorescent imaging system, arranged to have an imaging axis along which, in use, light travels between an optical element comprising a dichroic mirror and a sample position of a sample to be analysed, which imaging system comprises an illumination system, comprising a fitting for the metal halide lamp.

This use enables the attainment of a new effect, namely the provision of a homogeneous bundle of light in an epifluorescent imaging system without requiring elaborate optical systems, such as condensers.

The invention will now be explained with reference to the drawing, which shows schematically the various components in an epifluorescent imaging system.

A preferred use for the system is as part of an integrated device for automated conducting of bioassays. The preferred device receives a substrate plate 1. The substrate plate 1 preferably comprises a number of wells 2, at the bottom of which is positioned a substrate 3. The substrate 3 is preferably of a porous flow-through type. In the preparatory phase of the analysis, DNA probes have been bound to the substrate 3 in certain spots on the top surface of the substrate 3. Prior to insertion into the device for automated conducting of bioassays, a sample fluid, comprising a number of sample molecules which have been tagged with fluorescent markers, is deposited into the well 2.

The integrated device preferably comprises means for incubating the sample in the wells 2, by heating the substrate plate 1 to a desired temperature. The sample fluid is pumped back and forth across the substrate 3 a number of times, during which the sample molecules selectively bind to the DNA probes in the substrate 3. Subsequently, the integrated device washes the substrate 3, so that only the sample molecules that have reacted with specific DNA probes in a hybridisation reaction are left behind.

The imaging system according to the invention is used for quantitative and qualitative analysis of the hybridisation reactions that have taken place, to determine how many sample molecules have bound to which of the DNA probes in a substrate 3. It is noted that a different sample fluid may be placed in each of the wells 2, so that each well 2 is analysed in turn. The substrate plate 1 may comprise any number of wells 2, but preferably comprises four wells, or it may be an industry-standard substrate plate comprising 96 wells.

The substrate plate 1 is placed in a first sample position in which one of the wells 2 is substantially centred on an upright axis 4. Preferably, the integrated device for conducting bioassays has a base, and is positioned on its base in such a manner that the upright axis 4 is substantially vertical, i.e. aligned with the direction of gravitation. In the illustrated example, the substrate plate 1 is at the bottom of the assembly, but it may be at the top, However, the latter mentioned variant is less advisable, as the substrate plate 1, and thus the contents of the wells 2 will be upside down.

It is noted that light is projected into the well 2 from above, because the substrate 3 is generally not very transparent to light. Light within a first frequency range, suitable for exciting the fluorophores of the fluorescent markers is projected onto the substrate 3 at the bottom of the well 2. Bound fluorescent markers will emit light in a second frequency range in response. The source and intensity of light in the second frequency range is a measure of how many tagged sample molecules have bound to which DNA probe, and is therefore to be determined as accurately as possible.

The epifluorescent imaging system in the integrated device for conducting automated bioassays comprises at least one filter cube 5. There may be a number of filter cubes 5 in a carousel, enabling one to be selected from a collection of filter cubes with different properties. In that case, the imaging system according to the invention comprises means for accurately positioning the filter cube 5 in a plane substantially perpendicular to the upright axis 4, such that it is centred on the upright axis 4. The filter cube 5 comprises a dichroic mirror 6, which reflects light in the first frequency range (i.e. light used to excite the fluorophores) and is transparent in the second frequency range (i.e. the frequency range in which the light emitted by the fluorophores lies). The filter cube 5 further comprises an excitation filter 7, which filters out light with a frequency outside the first frequency range. It also comprises a detection filter 8, which only lets through light in the second frequency range. An absorber 9 prevents multiple reflections inside the filter cube 5.

Light impinges on the filter cube 5 along an optical path having, in this example, two sections, namely a first, vertical, section 10, and a second, horizontal, section 11. The illumination system comprised in the imaging system includes a fitting 12, accommodating, in use, a metal halide lamp 13. Any type of metal halide lamp may be used, for example a mercury-argon lamp. When the lamp 13 is powered, light originates from a (preferably ceramic) discharge tube 14, comprised in the lamp 13. The discharge tube is disposed in an outer envelope of the lamp 13. Preferably, the outer envelope is made of glass, and the discharge tube is a ceramic discharge tube, rather than quartz. The discharge tube forms an arc chamber. It is observed that the effects of the invention can be achieved through the use of lamps with discharge tubes of both cylindrical and spheroid shape. The type of lamp used has the advantage that the operating temperature can be higher and that more lightgenerating metallic ions are provided, leading to a smoother spectrum, and greater luminous efficacy.

Over the lifetime of the lamp 13, metal deposits accrue at the bottom of the discharge tube 14, which deposits absorb light emitted by the lamp 13. However, this does not affect the intensity or homogeneity of the light emitted, as only the upwardly emitted light is captured.

Thus, in this embodiment of the invention, the further section of the light path is at an angle to the upright axis 4 and defines a reference plane in which the further section lies and which is perpendicular to a plane through the upright axis 4 and the further section. The first section 10 is substantially parallel to the upright axis 4 and located on the same side of the reference plane as a base of the imaging system, in the shown example on the same side as the sample position.

The light emitted by the lamp 13 travels along the first section 10 of the optical path to the filter cube 5 through a condensor 15 and a diaphragm 16. The diaphragm 16 adjusts the beam diameter, to ensure that the diameter of the beam that eventually falls onto the bottom of a well 2, is equal to, or slightly smaller than, the diameter of the bottom of the well 2. Thus, only light originating from the substrate 3 at the bottom of the well 2 is measured.

The light changes direction upon reflection in a cold mirror 17. A cold mirror, as is well-known in the art, has the property of transmitting infra-red radiation. Thus, the cold mirror 17 performs two functions. It firstly changes the direction of the light from vertical to horizontal, and does so in a way that allows the illumination system to be compact. It secondly serves to filter out a large amount of the infra-red radiation from the metal halide lamp 13, thus protecting the optical elements further along the optical path from overheating. The use of a cold mirror 17 as opposed to a filter is advantageous, as the back of the cold mirror 17 can easily be cooled, for example by forced convection or a heat sink attached to the non-reflecting side of the cold mirror 17.

Infra-red radiation may, optionally, be removed further by an infra-red filter 18 positioned after the cold mirror 17 in the second section 11 of the light path. The cold mirror 17 prevents the infra-red filter 18 from overheating and cracking.

A field lens 19 focuses the light onto the filter cube 5, where it first passes through the excitation filter 7, before being reflected in the dichroic mirror 6.

An objective lens 20, positioned along the upright axis 4, focuses the light onto the bottom of the sample well 2 in the sample position. The objective lens 20 forms part of a lens system along the upright axis 4, of which the other component is a tube lens 21. Positioned directly above the tube lens 21 is a CCD camera 22, which captures the image projected onto it by the objective lens 20 and tube lens 21 through an eyepiece (not shown).

Preferably, the objective lens 20 and tube lens 21 form a lens system having a magnification substantially equal to one, which implies that they are identical. Because they are identical, artefacts caused by certain aberrations cancel. Therefore, cheaper lenses than are conventionally used, can be used in the imaging system. For example, it is advantageous to use Single Lens Reflex (SLR) camera objectives, instead of the more expensive microscope objectives usually employed.

It is preferred that the numerical aperture of the objective lens 20 be higher than the effective numerical aperture as determined by the component of the filter cube 5 limiting the diameter of the light beam between the filter cube 5 and the objective lens 20. This prevents vignetting, i.e. darkening of the image around the image, which would affect the measurement of the number of sample molecules bound to DNA probes at the outer edges of the substrate 3.

To increase the depth of focus, a further diaphragm (not shown), limiting the diameter of a bundle of light travelling from the filter cube 5 to the tube lens 21, may be employed. The imaging numerical aperture is thus reduced, so that the effect of any optical aberrations becomes less important. This allows the use of cheaper lenses.

In one embodiment of the invention, the illumination system is comprises in a separate illumination device, which is detachable from the other parts of the epifluorescent imaging apparatus. The imaging apparatus comprises an inlet port (not shown), through which light from the illumination device is let in. The illumination device comprises an outlet port through which light is emitted. The inlet and outlet port are aligned when the illumination device is connected to the imaging apparatus by means of appropriately shaped fittings, which also determine the orientation of the illumination device. The illumination device comprises at least the fitting 12 and the cold mirror 17 of the illumination system shown in the drawing. The fittings determine the orientation of the illumination device such that the first section 10 of the optical path from the metal halide lamp 13 is vertical and the second section 11 is horizontal, when the illumination device is connected to the imaging apparatus.

The invention is not limited to the above-described embodiment which can be varied within the scope of the attached claims. For instance, various means can be used to determine the position of the filter cube 5, such as a Maltese cross or a servo motor.

CLAIMS

1. Epifluorescent imaging system, arranged to have an imaging axis (4) along which, in use, light travels between an optical element (5) comprising a dichroic mirror (6) and a sample position of a sample to be analysed, which imaging system comprises an illumination system, comprising a fitting (12) for a metal halide lamp (13), **characterised in that** the epifluorescent imaging system comprises a metal halide lamp comprising a gas discharge tube (14), fitted in the fitting (12).

2. Epifluorescent imaging system, arranged to have an imaging axis (4) along which, in use, light travels between an optical element (5) comprising a dichroic mirror (6) and a sample position of a sample to be analysed, which imaging system comprises an illumination system, comprising a fitting (12) for a metal halide lamp (13) and at least one optical element determining an optical path between a location of light emission of a fitted metal halide lamp (13) and the filter element (5), wherein the optical path comprises a first section (10) closest to the location of light emission **characterised in that**
the fitting is suitable for a metal halide lamp (13) provided with a gas discharge tube (14) at the location of light emission and in that, in an operating position of the system, the first section (10) of the optical path is oriented predominantly opposite the direction of gravitation.

3. System according to claim 2, wherein the optical path determined by the at least one optical element comprises a further section (11) closer to the filter element (5), the further section (11) being at an angle to the imaging axis (4).

4. System according to claim 2 or 3, wherein the optical elements comprise a cold mirror (17).

5. System according to any one of claims 2-4, wherein the optical elements comprise an infrared filter (18).

6. System according to any one of claims 2-5, comprising a system of lenses positioned along the imaging axis for projecting an image of a sample at the sample position through an eyepiece, wherein the system of lenses has an optical magnification substantially equal to one.

7. System according to claim 6, wherein the system of lenses is formed by an objective lens (20), positioned between the sample position and the filter element (5), and an imaging lens (21), positioned between the filter element and the eyepiece, wherein the objective lens (20) and imaging lens (21) have substantially identical properties.

8. System for conducting automated sample analysis comprising an epifluorescent imaging system according to any one of claims 1-7.

9. System for conducting automated sample analysis according to claim 8, adapted for processing at least one sample tray (1) comprising at least one well (2) containing a sample, wherein the illumination system comprises a diaphragm (16) for limiting the diameter of a light beam transmitted between the optical element (5) comprising the dichroic mirror and the sample to a smallest diameter of the well (2).

10. Illumination device for an epifluorescent imaging apparatus having an imaging axis (4) along which, in use, light travels between an optical element (5) comprising a dichroic mirror (6) and a sample position of a sample to be analysed, which illumination device comprises a fitting (12) for a metal halide lamp (13) and at least one optical element determining an optical path between a location of light emission of a fitted metal halide lamp (13) and a light outlet port adapted to be connected to a light inlet port of the imaging apparatus, wherein the optical path comprises a first section (10) closest to the location of light emission , wherein the illumination device is adapted to be connected to the imaging apparatus in an operating position, **characterised in that**
the fitting is suitable for a metal halide lamp (13) provided with a gas discharge tube (14) at the location of light emis-

sion and in that, in the operating position, the first section (10) of the optical path is oriented predominantly opposite the direction of gravitation.

11. Use of a metal halide lamp comprising a gas discharge tube in an epifluorescent imaging system, arranged to have an imaging axis (4) along which, in use, light travels between an optical element (5) comprising a dichroic mirror (6) and a sample position of a sample to be analysed, which imaging system comprises an illumination system, comprising a fitting (12) for the metal halide lamp (13).

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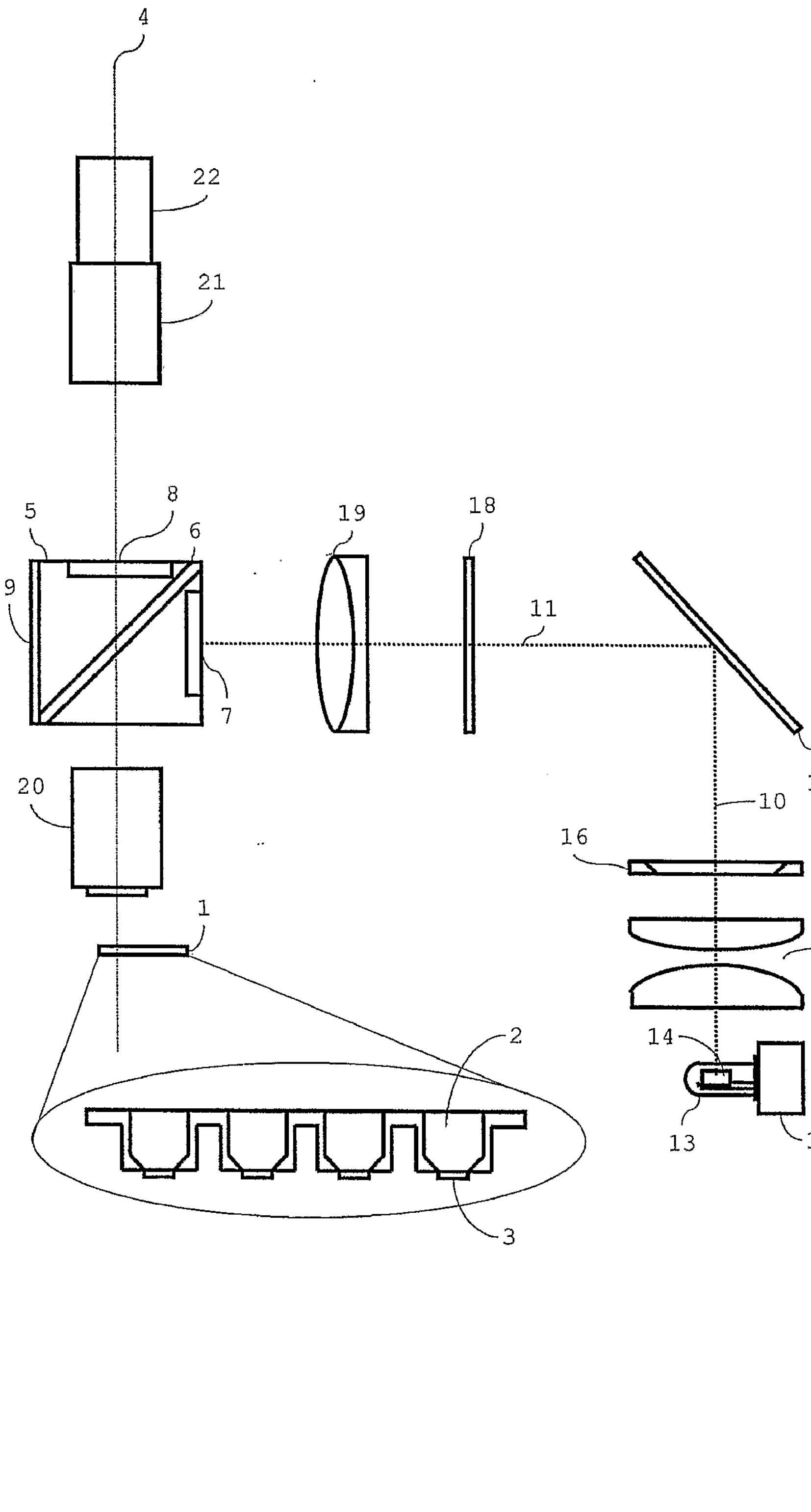

INTERNATIONAL SEARCH REPORT

International Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G02B21/16 G02B21/24

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 G02B G01N H01J

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, INSPEC, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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☒ Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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