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

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(54) **Title**: APPARATUS FOR SPECTROSCOPIC ANALYSIS OF LIGHT FROM A SAMPLE

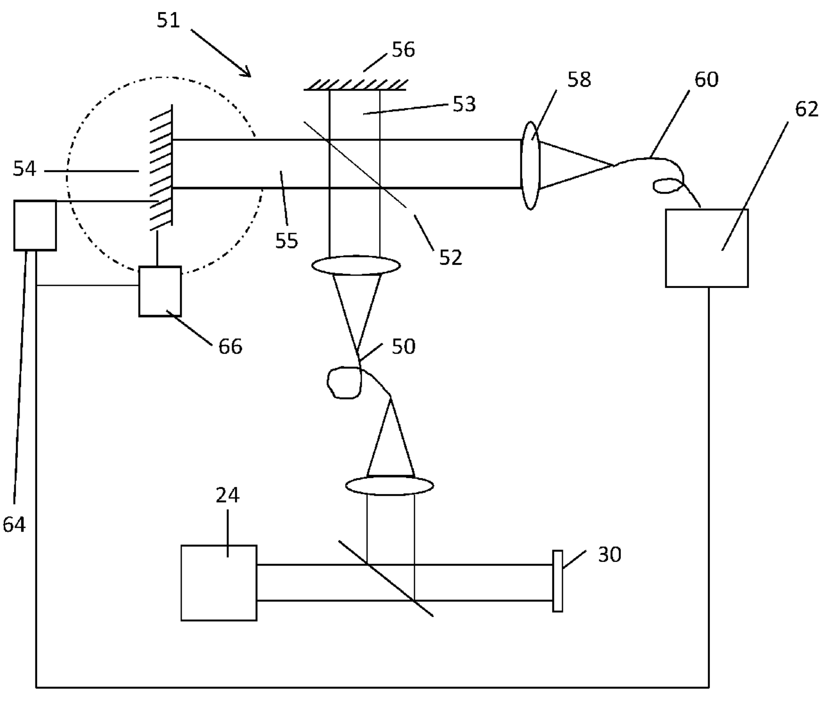

FIG. 4

(57) **Abstract**: Apparatus which has a light source (24) for illuminating a sample. Light from the sample is split into two paths (29, 31) and a path difference between the paths is introduced. Light from the two paths is recombined and the path difference is varied to obtain destructive interference at a particular wavelength in order to improve the sensitivity of the apparatus to weak signals.

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Apparatus for Spectroscopic Analysis of Light from a Sample

The invention relates to an apparatus for conducting spectroscopic analysis of light from a sample. In one embodiment the invention relates to a Brillouin microscope for identifying spectral features created by Brillouin scattering in a sample.

Spectroscopic imaging techniques are becoming increasingly important in biological and biomedical imaging. A common mode of operation entails excitation at one wavelength and recording the resulting spectra for light that is scattered by the sample. In some situations the signal of interest in the spectrum can be positioned very close to the excitation wavelength(s) but several orders of magnitude weaker than the excitation. In one example, Brillouin scattering produces a signal which may be several orders of magnitude weaker than the excitation signal, but separated by only a few GHz. Effective detection of a Brillouin signal presents a considerable technical challenge and typically requires highly sophisticated optical systems that can separate the weak signal from the stronger excitation signal. Previous approaches have included scanning monochromators, interferometers and optical beating. These approaches rely on measuring single spectral components and therefore they can be slow at acquiring complete spectra. This is particularly relevant for biomedical imaging applications where it can be important to minimise optical exposure and scanning time.

Some other techniques use multi-stage VIPA (virtually imaged phase array) etalons to obtain high contrast. These optical systems are technically difficult to set up and can be highly sensitive to misalignment and motion.

Brillouin microscopy (BM) gives information about the mechanical properties of the sample, through acousto-optic interaction with the vibrational modes of the sample (also known as phonons). One application of BM is to measure the mechanical properties of cells and biomechanics, and *in-vivo* biomedical imaging. However, these applications are hampered by the complexity of the current optical systems.

An object of the present invention is to provide an apparatus that overcomes some of these difficulties.

According to an aspect of the invention there is provided an apparatus for conducting spectroscopic analysis of light from a sample, the apparatus comprising: a light source configured to illuminate a sample; an optical path divider configured to separate light from the sample into two optical paths; a path difference control mechanism configured to adjust the path difference between the two optical paths; a re-combiner configured to re-combine light in the two optical paths so that the re-combined light can interfere, wherein destructive interference is selectively achieved for a target wavelength by adjustment of the path difference control mechanism; and a spectrometer configured to perform spectroscopic analysis on light from the re-combiner.

In this way, spectroscopic nulling can be achieved for a particular wavelength of light using destructive interference. The path difference control mechanism can be adjusted to select a phase path difference of $n\pi/2$ (where n is odd) for the target wavelength. Preferably there is an even amplitude for the target wavelength in the respective optical paths so that complete extinction of the target wavelength is achieved when the optical paths re-combine. By selectively removing a particular wavelength of light the spectrometer can be made more sensitive to weaker signals at closely separated wavelengths. This may be particularly useful for detecting signals due to Brillouin scattering, which would otherwise be difficult to detect due to the presence of a strong signal at a different wavelength due to elastic scattering.

The optical path divider is configured to separate light from the sample into two optical paths. In this way both of the optical paths can include scattered light from the sample. Interference can therefore be achieved in the re-combiner between two common optical paths. This can be advantageous because both paths can exhibit precisely the same amplitude and phase variations due to dynamic behaviour in a sample; dynamic behaviour may be observed for samples *in-vivo*, fluid samples or samples in motion. It has also been found that each point in a sample can scatter light in a different way, resulting in significant amplitude and phase variations from point to point in the sample. By enabling the optical path divider to separate light from the sample into two optical paths, these variations can be common to both paths. This can allow very high attenuation to be achieved for the target wavelength in spite of these variations.

The apparatus may include an amplitude control mechanism configured to adjust the relative amplitude of light in the two optical paths. In this way, selective attenuation of the target wavelength can be achieved through destructive interference by adjustment of the amplitude control mechanism. It may be possible to obtain an even split in the amplitude of light between the two optical paths during the initial setup and therefore a dynamic amplitude control mechanism may not be required in every embodiment.

The optical path divider may comprise a prism disposed in the light from the sample, wherein one of the optical paths comprises light that is transmitted in the prism. Thus, the light that travels through the prism may experience a path difference relative to light in the other optical path. In this context, the prism is preferably a substantially transparent solid body with a refractive index that is different from the refractive index in the other optical path. In one arrangement the other optical path may be in air.

The amplitude control mechanism may be configured to adjust the position of the prism in the light from the sample. This can adjust the proportion of light that is transmitted in the prism along one optical path relative to the light in the other optical path. Precise control of the position of the prism may achieve an even split in amplitude for light in the respective optical paths so that a high level of attenuation can be achieved for a target wavelength following re-combination.

The path difference control mechanism may be configured to adjust the path difference for light that is transmitted in the prism relative to light in the other optical path. In one example this may be achieved by adjusting orientation of the prism. For a generally rectangular prism selective control of the orientation of the prism may be used to control the relative path difference and therefore to select the target wavelength that experiences destructive interference on re-combination.

The prism may comprise a first region with a first refractive index and a second region with a second refractive index. One of the optical paths may comprise light transmitted in the first region and the other optical path may comprise light transmitted in the second region. The prism may be provided as a single solid body or a plurality of solid bodies. By providing both optical paths within the prism the apparatus may have increased resilience to temperature fluctuations. It has been found that optical paths in air can be influenced more by these temperature fluctuations.

The optical path divider and the re-combiner may be provided as one or more beam splitters in an interferometer. A Michelson interferometer is just one example of this type of interferometer where both optical paths are traversed twice and the beam splitter can both separate and re-combine light. In an alternative interferometric set-up (such as in a Mach-Zender interferometer) the re-combiner may be provided separately from the beam splitter, and each optical path may be traversed only once.

The interferometer may comprise two arms, one for each optical path. Each arm may have a mirror, and the path difference control mechanism may be configured to control the position of at least one mirror. In just one example, this may be achieved by mounting one of the mirrors on a piezo-electric transducer.

The amplitude control mechanism may be configured to adjust the amplitude of the target wavelength in one of the arms. This may be achieved using a variable absorber disposed in the optical path. In an alternative, the tilt of the mirror may be adjusted in order to control the coupling efficiency of light into the spectrometer.

The spectrometer may be configured to monitor the amplitude of the target wavelength in the light from the re-combiner and to provide a feedback control signal to the path difference control mechanism and/or the amplitude control mechanism. Environmental changes may cause slight variations in the relative path difference of the two optical paths, which can affect the attenuation achieved by destructive interference for the target wavelength. This can be monitored at the spectrometer in order to create an effective feedback control. Laser drift may also be a factor, and this may result in a shift in the target wavelength for which selective extinction is required. This may be compensated for through adjustments to the path difference control mechanism and/or the amplitude control mechanism.

Feedback signals may be provided periodically, and a preferred period may be around 2 minutes. It has been found that this frequency of feedback can effectively correct variations due to environmental changes and/or laser drift.

The spectrometer may comprise a single mode optical fibre for receiving light from the re-combiner. This technique for spectroscopic nulling may be effective for light in single spatial mode. Destructive interference can therefore be achieved evenly for light at the target wavelength in that spatial mode, allowing effective extinction of light at the target wavelength.

Preferably destructive interference is achieved at the re-combiner for a plurality of wavelengths which are regularly separated from one another. This feature may be used advantageously to remove spectral features that are separated periodically, as may occur if the laser is lasing simultaneously at multiple longitudinal modes. In another arrangement the separation of the plurality of wavelengths may be chosen so that destructive interference is achieved at a first wavelength for light backscattered by Rayleigh scattering and at a second wavelength for light scattered by Brillouin scattering within the optical fibre. Thus, Brillouin scattering from within the optical fibre can be subtracted effectively so that the resultant signal includes only Brillouin scattering from the sample.

According to another aspect of the present invention there is provided a Brillouin microscope for identifying spectral features due to Brillouin scattering of light from a sample, the Brillouin microscope comprising: a light source configured to illuminate a sample; an optical path divider configured to separate light from the sample into two optical paths; a path difference control mechanism configured to adjust the path difference between the two optical paths; a re-combiner configured to re-combine light in the two optical paths so that the light can interfere, wherein destructive interference is selectively achieved for a target wavelength by adjustment of the path difference control mechanism; and a spectrometer configured to identify spectral features due to Brillouin scattering in the sample.

According to yet another aspect of the invention there is provided a method of conducting spectroscopic analysis of light from a sample, the method comprising the steps of: illuminating a sample with a light source; separating light from the sample into two optical paths; re-combining light in the two optical paths so that the light can interfere; selectively achieving destructive interference in the re-combined light for a target wavelength by adjusting the relative path difference between the two optical paths; and performing spectroscopic analysis on the re-combined light.

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

Figure 1 shows an optical setup for an apparatus in an embodiment of the present invention;

Figure 2 is a perspective view of a prism for use in an embodiment of the present invention;

Figure 3 is a perspective view of another prism for use in an embodiment of the present invention;

Figure 4 shows an optical setup for an apparatus in another embodiment of the present invention; and

Figures 5A-5C each show an arm of an interferometer for use in an embodiment of the present invention.

Figure 1 shows an optical setup in which a laser 24 provides an illumination beam with a single longitudinal mode. The laser beam is transmitted through a beam splitter 26 and is focused on a sample 30 by a convex lens 28. Light is scattered from the sample 30 by a variety of mechanisms, and scattered light is returned through the lens 28 and is reflected by the beam splitter 26.

A rectangular glass prism 32 is disposed in the light that is scattered by the sample 30. A portion of the scattered light is captured by the prism 32 and is transmitted within the prism 32 along a first optical path 29. Another portion of the light is transmitted in air, along a second optical path 31. Light from the respective optical paths 29, 31 is focused by another convex lens 34 so that it can be coupled into a single mode optical fibre 36 where it re-combines. The optical fibre 36 is used as the input to a spectrometer 38 which can analyse spectral features in the re-combined light.

A first motor 40 is provided for adjusting the position of the prism 32 in the light from the sample. A second motor 42 is provided for adjusting the orientation of the prism 32 in the light from the sample, and therefore adjusting the path length for light that is transmitted through the prism 32. A feedback signal is provided from the spectrometer 38 to the first motor 40 and the second motor 42. The spectrometer 38 is arranged to analyse the intensity and position of the spectral peak at the wavelength of the laser 24 and to control the first and second motors 40, 42 to achieve spectroscopic nulling for the excitation signal. In some embodiments the first motor 40 may be omitted if the initial setup can evenly split light so that the light has an equal amplitude in the first and second optical paths 29, 31.

Figure 2 is a perspective view of the prism 32. Scattered light 33 from the sample is incident on an end face of the prism 32, is transmitted through the prism, and then emerges from an opposite face. The first motor 40 is arranged to adjust the position of the prism 32 in a direction that is perpendicular to the direction of travel of the beam. Fine control of the position of the prism 32 can control the proportion of light that is transmitted within the prism 32 relative to the proportion of light that travels in free air so that there is an even amplitude for light in the two paths. The second motor 42 is arranged to rotate the prism about an axis that is perpendicular to the direction of travel of the light (and parallel to the direction of movement of the prism 32 by the first motor 40). By adjusting the orientation of the prism 32 in the beam it is possible to adjust the path length for light in the prism 32.

Figure 3 shows a perspective view of another prism 44 having an upper region 46 with a first refractive index, n_1, and a lower region 48 with a second refractive index, n_2. In this arrangement a first optical path is provided for light that is transmitted within the lower region 48 of the prism 44 and a second optical path is provided for light that is transmitted within the upper region 46 of the prism 44. The first motor 40 can be used to adjust the position of the prism 44 in the beam 41 and to adjust the proportion of the beam that travels in the first optical path relative to the second optical path. The orientation of the prism 44 can also be adjusted to control the relative path difference between the two paths. It may be advantageous to use solid bodies for both optical paths because they may be less vulnerable to influence by short term temperature fluctuations.

Figure 4 shows an optical setup in which a laser 24 is provided for illuminating a sample and the back-scattered light is focused into a single-mode optical fibre 50. This fibre 50 is used as the input for a Michelson interferometer 51. The interferometer 51 comprises a beam splitter 52 that divides the input light with approximately even amplitudes into first and second optical paths 53, 55. In the first optical path 53 light is transmitted by the beam splitter 52 and is incident on a first mirror 56. In a second optical path 55 light is reflected by the beam splitter 52 and is incident on a second mirror 54. A motor 64 is provided for adjusting the position of the second mirror 54 in order to control the relative path difference between the first and second optical paths 53, 55.

In the arrangement shown in Figure 4 the second mirror 54 is a gradient reflectivity mirror, which has a continuously variable reflectivity across its surface. A controller 66 is provided for adjusting the position of the second mirror 54 in the beam in order to control attenuation of the signal. Careful control of the position of the second mirror 54 can therefore be used to adjust the relative amplitude of light between the first and second optical paths 53, 55.

Figures 5A-5C show alternative arrangements for controlling attenuation of light in the second optical path 55. Figure 5A shows a moveable beam block 68 attached to the controller 66. The controller 66 can adjust the position of the beam block 68 within the beam to control attenuation of the signal in the second optical path. Figure

5B shows a variable absorber 70 provided in the beam and connected to the controller 66. The variable absorber 70 may be provided as a liquid crystal device with controllable absorbance. In an alternative the variable absorber 70 may be a block with a controllable aperture for selectively permitting transmission of light. Figure 5C shows the controller 66 attached to the second mirror 54 for controlling its tilt. By adjusting the angle of tilt of the second mirror 54 the controller 66 can control the coupling efficiency of light into the single mode fibre 60.

In the embodiments shown in Figures 1 and 4 the first and second optical paths both include scattered light from the sample 30. In the embodiments shown in Figures 1 and 4, when light from the optical paths is re-combined interference occurs between two common optical paths. This can be advantageous because both paths can exhibit precisely the same amplitude and phase variations due to dynamic behaviour in a sample; dynamic behaviour may be observed for samples *in-vivo*, fluid samples or samples in motion. It has also been found that each point in a sample can scatter light in a different way, resulting in significant amplitude and phase variations from point to point in the sample. In the embodiments shown in Figures 1 and 4 these variations are common to both optical paths. Thus, very high attenuation can be achieved for the target wavelength in spite of these variations. In addition, since both optical paths have identical light from the sample, the path difference control mechanism can be used to select the target wavelength that will experience destructive interference on re-combination. The target wavelength could be the wavelength of the illuminating laser 2 or any other wavelength, as required by the circumstances of the relevant setup.

The attenuation provided by the apparatus is sinusoidal with the wavelength of light. Thus, the same device could be used to remove spectral features that are separated periodically, as may occur if the laser is lasing simultaneously at multiple longitudinal modes. This sinusoidal behaviour of the attenuation can also be used to remove simultaneously light backscattered by Rayleigh scattering and the Brillouin peaks that arise in an optical fibre by choosing the sinusoidal period to match the Brillouin shift of the optical fibre material.

In another specific setup the high attenuation could be used to remove the fundamental mode in a laser output. This may permit observation and measurement of much weaker laser sidebands.

Claims

1. An apparatus for conducting spectroscopic analysis of light from a sample, the apparatus comprising:

a light source configured to illuminate a sample;

an optical path divider configured to separate light from the sample into two optical paths;

a path difference control mechanism configured to adjust the path difference between the two optical paths;

a re-combiner configured to re-combine light in the two optical paths so that the re-combined light can interfere, wherein destructive interference is selectively achieved for a target wavelength by adjustment of the path difference control mechanism; and

a spectrometer configured to perform spectroscopic analysis on light from the re-combiner.

2. The apparatus of claim 1 further comprising an amplitude control mechanism configured to adjust the relative amplitude of light in the two optical paths, wherein selective attenuation of the target wavelength is achieved through destructive interference by adjustment of the amplitude control mechanism.

3. The apparatus of claim 2 wherein the optical path divider comprises a prism disposed in the light from the sample, wherein one of the optical paths comprises light that is transmitted in the prism.

4. The apparatus of claim 3 wherein the amplitude control mechanism is configured to adjust the position of the prism in the light from the sample.

5. The apparatus of claim 3 or claim 4 wherein the path difference control mechanism is configured to adjust the path difference for light that is transmitted in the prism relative to light in the other optical path.

6. The apparatus of any of claims 3 to 5 wherein the prism comprises a first region with a first refractive index and a second region with a second refractive index, and

wherein one of the optical path comprises light transmitted in the first region and the other optical path comprises light transmitted in the second region.

7. The apparatus of any of the preceding claims wherein the optical path divider and the re-combiner are provided as one or more beam splitters in an interferometer.

8. The apparatus of claim 7 wherein the interferometer comprises two arms, one for each optical path, wherein each arm has a mirror, and wherein the path difference control mechanism is configured to control the position of at least one mirror.

9. The apparatus of claim 7 or claim 8 when dependent on claim 2 wherein the amplitude control mechanism is configured to adjust the amplitude of light at the target wavelength in one of the arms.

10. The apparatus of any of the preceding claims wherein the spectrometer is configured to monitor the amplitude of light at the target wavelength in the light from the re-combiner and to provide a feedback control signal to the path difference control mechanism and/or the amplitude control mechanism.

11. The apparatus of any of the preceding claims wherein the spectrometer comprises a single mode optical fibre for receiving light from the re-combiner.

12. The apparatus of any of the preceding claims wherein the spectrometer is configured to identify spectral features created by Brillouin scattering in the sample.

13. A Brillouin microscope for identifying spectral features due to Brillouin scattering of light from a sample, the Brillouin microscope comprising:

a light source configured to illuminate a sample;

an optical path divider configured to separate light from the sample into two optical paths;

a path difference control mechanism configured to adjust the path difference between the two optical paths;

a re-combiner configured to re-combine light in the two optical paths so that the light can interfere, wherein destructive interference is selectively achieved for a target wavelength by adjustment of the path difference control mechanism; and

a spectrometer configured to identify spectral features due to Brillouin scattering in the sample.

14. A method of conducting spectroscopic analysis of light from a sample, the method comprising the steps of:

illuminating a sample with a light source;

separating light from the sample into two optical paths;

re-combining light in the two optical paths so that the light can interfere;

selectively achieving destructive interference in the re-combined light for a target wavelength by adjusting the relative path difference between the two optical paths; and

performing spectroscopic analysis on the re-combined light.

15. An apparatus substantially as herein described with reference to and/or as illustrated in the accompanying drawings.

16. A method substantially as herein described with reference to the accompanying drawings.

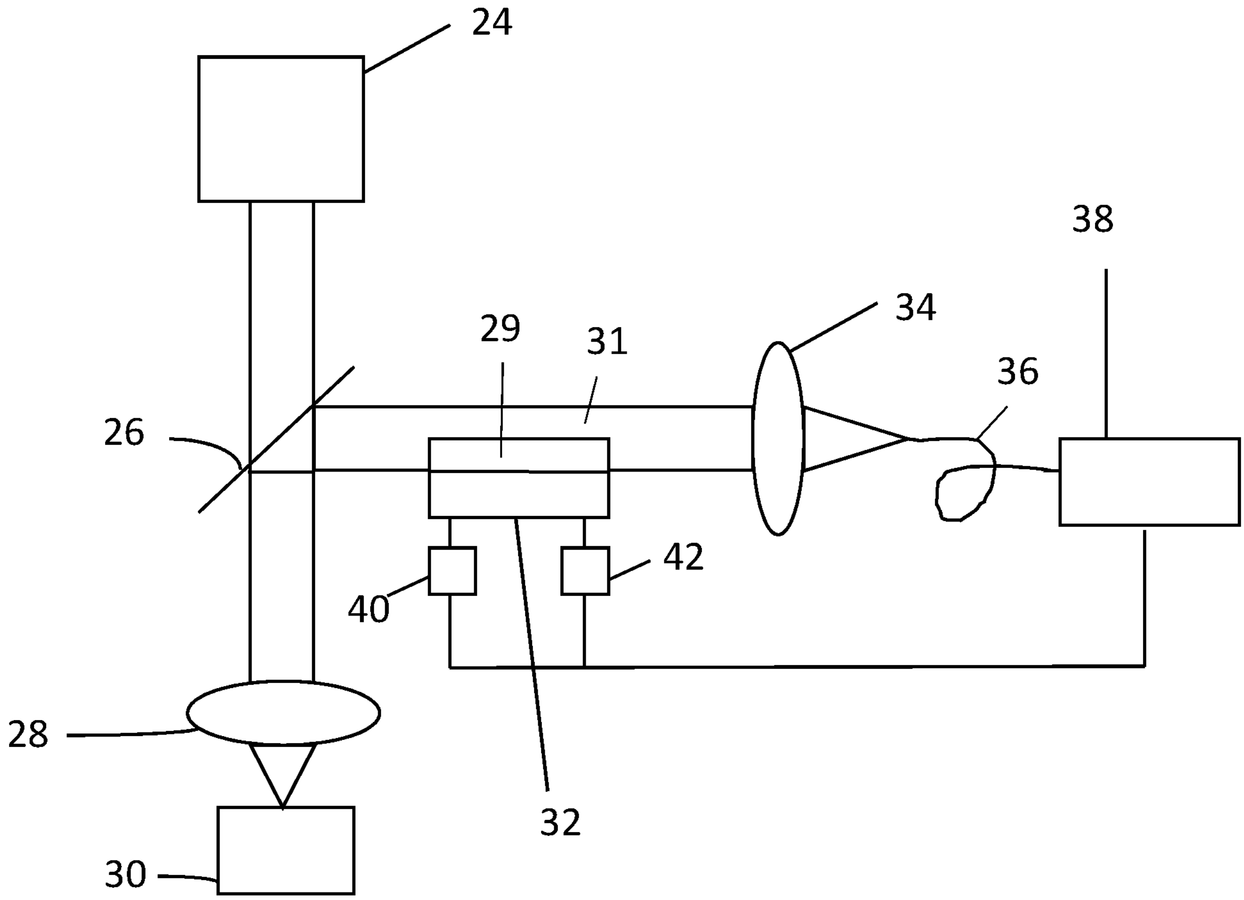

FIG. 1

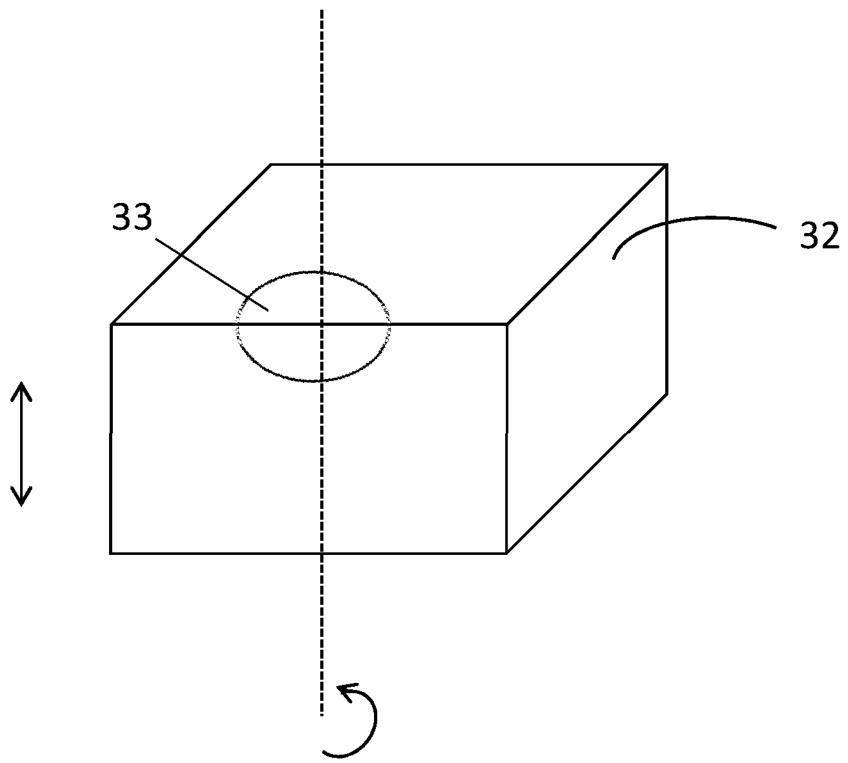

FIG. 2

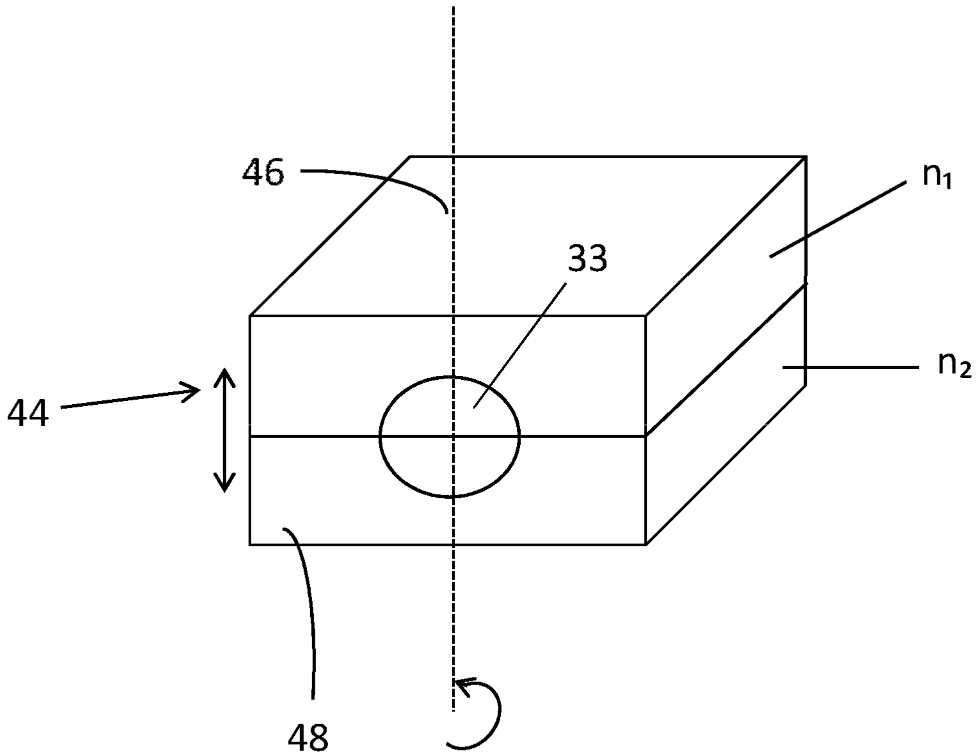

FIG. 3

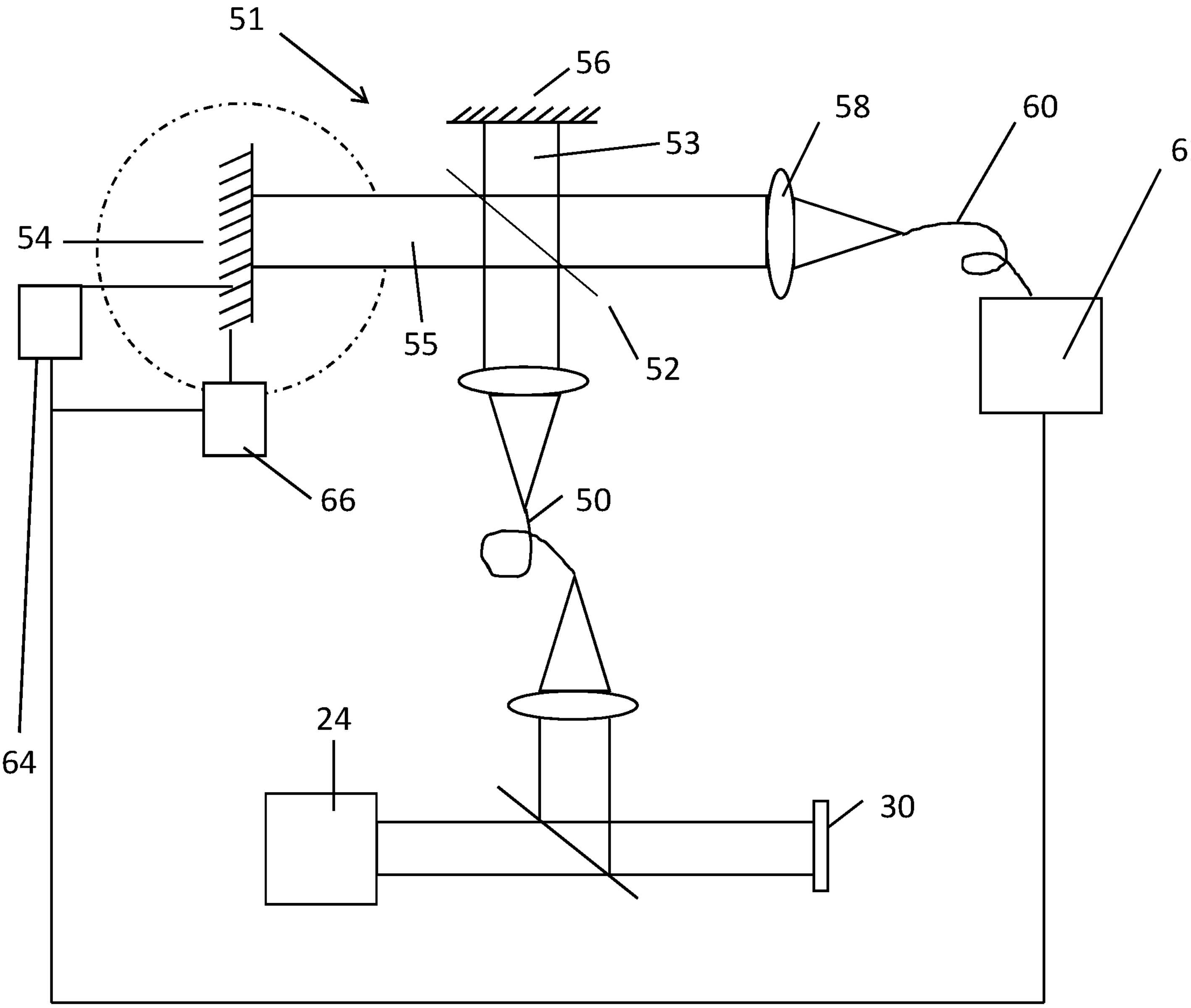

FIG. 4

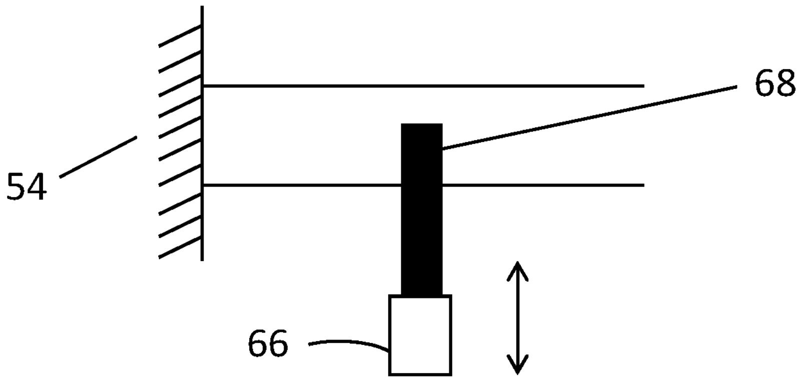

FIG. 5A

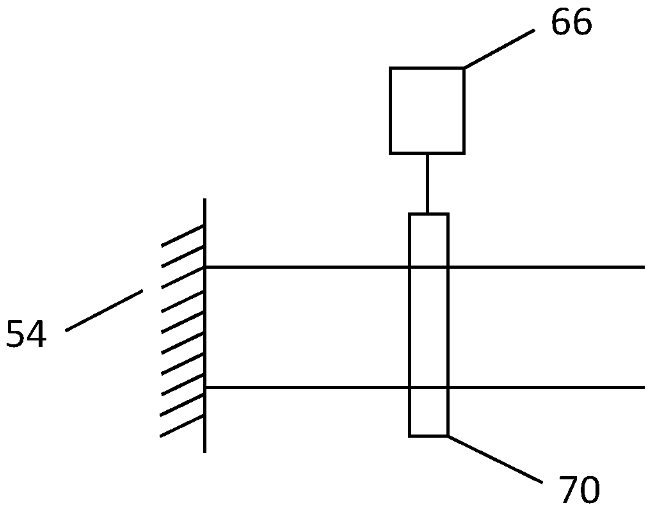

FIG. 5B

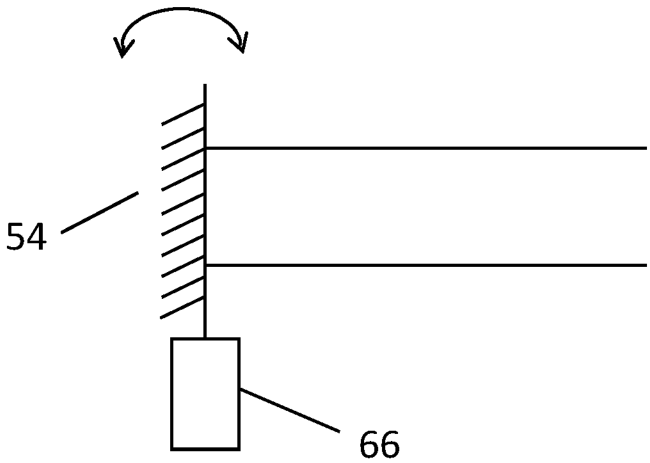

FIG. 5C

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2016/052354

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01J3/44 G01J3/02 G01J3/28 G02B26/02 G01N21/63
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)
G01J G02B 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 A	W. PROFFITT ET AL: "An Interferometric Rejection Filter for Raman Spectroscopy", APPLIED OPTICS, vol. 10, no. 3, 1 March 1971 (1971-03-01), page 531, XP055312800, WASHINGTON, DC; US ISSN: 0003-6935, DOI: 10.1364/AO.10.000531 introduction; page 533, columns 1,2; figures 2,3 ----- -/--	1-3,5, 7-16 4,6

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

[X] See patent family annex.

* Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
20 October 2016	10/11/2016

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

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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2016/052354

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	K. H. LANGLEY ET AL: "Attenuation of the Rayleigh Component in Brillouin Spectroscopy Using Interferometric Filtering*", JOURNAL OF THE OPTICAL SOCIETY OF AMERICA, vol. 59, no. 3, 1 March 1969 (1969-03-01), page 281, XP055312533, US ISSN: 0030-3941, DOI: 10.1364/JOSA.59.000281 introductory section, Experimental; pages 281-283; figure 1 -----	1,13-16
A	JP 2006 250826 A (YAMAGATA PREFECTURE) 21 September 2006 (2006-09-21) paragraph [0023]; figures 1,2 -----	1-16
A	JP 2013 205231 A (SUMITOMO OSAKA CEMENT CO LTD) 7 October 2013 (2013-10-07) abstract; figures 1,2 -----	1-16

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2016/052354

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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