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(71) Applicant: **PARTICULATE AS** [NO/NO]; Professor Olav Hanssensvei 11, 4068 Stavanger (NO).

(71) Applicant *(for MG only)*: **SAMUELS, Adrian James** [GB/GB]; St Bride's House, 10 Salisbury Square, London EC4Y 8JD (GB).

(72) Inventor: **ANDERSEN, Odd Ketil**; Particulate AS, Professor Olav Hanssensvei 11, 4068 Stavanger (NO).

(74) Agent: **DEHNS**; St Bride's House, 10 Salisbury Square, London Greater London EC4Y 8JD (GB).

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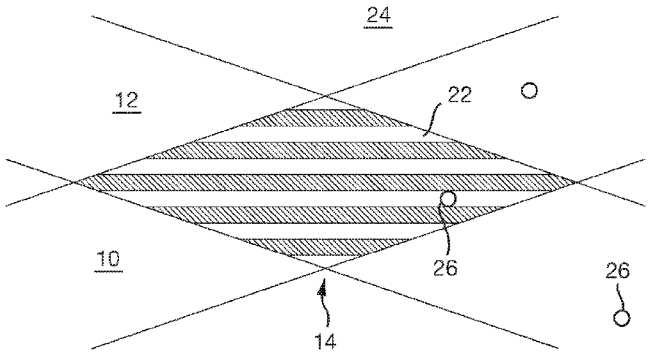
(54) Title: PARTICLE CHARACTERISATION

Fig. 2



(57) Abstract: A method and apparatus for characterising individual particles in an aquatic mass (24) is disclosed. According to a first aspect of the invention, an interference pattern (22) is generated in the aquatic mass (24) by overlapping two beams (10, 12) of electromagnetic radiation. The region of overlap defines a probe volume (14) such that the interference pattern (22) is generated in the probe volume (14). A single particle (26) in the probe volume is illuminated with the interference pattern (22), causing said single particle (26) to emit radiation. The radiation emitted by the single particle (26) is detected, and spectral data derived from the detected radiation is used to determine a property of the single particle. The emitted radiation may be fluorescence. According to a further aspect of the invention, a similar arrangement is used to illuminate a single particle so that the particle scatters radiation via Raman scattering. The Raman-scattered radiation is detected and used to determine a property of the single particle (26).

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Particle characterisation

This invention relates to apparatus and methods for characterising particles in an aquatic mass.

The characterisation of particles is important in many applications. For example, there are many circumstances in which it is necessary to monitor the extent of pollution in an ocean or other body of water. In the event of an oil spill, it is desirable to be able to measure the extent of the spill, and/or to monitor the effect of the use of chemicals (e.g. dispersants) on spills to determine the fate of the oil. It may also be necessary to monitor particles in discharges, for example, drilling mud from drilling operations or produced water from oil production, as well as the discharge of ballast water, which might be a major pathway for introducing species to new environments. Ballast water may be checked to ensure that particles are removed according to protocol, and remaining particles are not alive. This is important to ensure agreement with regulations regarding discharges before water is discharged into an ocean. As another example, there is a need to monitor algae in coastal regions. Many coastal regions are affected by harmful algal blooms (HABs), which are caused by blooms of microscopic algae that may be toxic to humans, fish, birds and other life in and near oceans.

Many particles of interest exhibit fluorescence (i.e. the emission of radiation in response to stimulating radiation). Known methods of monitoring particles include exciting fluorescence in particles in a body of water, and then studying the spectral properties of the emitted radiation to try to characterise the particles. This method can give a reasonable indication of particles in a body of water if the majority of particles emitting radiation are of the same type. However, in many cases, the water contains not only the particles of interest, but many other particle types. For example, in a region where it is desired to monitor oil contamination, there may also be algal particles. It is also possible that the water contains dissolved organic matter. Consequently, the measured fluorescence signal is the sum of many individual particle fluorescence signals, emitted by many different particle types. As a result, it can be difficult to determine from the spectral characteristics of the

measured fluorescence signal what types of particles are present, or whether a particular particle type of interest is present. This is because it may not be easy or possible to separate out the fluorescence signals emitted by each type of particle. For example, data deconvolution may be required to extract information relating to individual particle species.

Accordingly, the Applicant has identified a need for an improved method and apparatus for the characterisation of particles in an aquatic mass which contains a mixture of different particle types.

According to a first aspect of the invention there is provided a method of characterising individual particles in an aquatic mass, the method comprising:

generating an interference pattern in the aquatic mass by overlapping two beams of electromagnetic radiation, wherein the interference pattern is generated in a probe volume defined by a region of overlap of the two beams;

illuminating a single particle in the probe volume with the interference pattern, thereby causing said single particle to emit radiation;

detecting the radiation emitted by the single particle; and

using spectral data derived from the detected radiation to determine a property of the single particle.

The invention extends to an apparatus for characterising individual particles in an aquatic mass, the apparatus comprising:

a source of electromagnetic radiation arranged to generate an interference pattern in the aquatic mass by overlapping two beams of electromagnetic radiation, wherein the interference pattern is generated in a probe volume defined by a region of overlap of the two beams, thereby to illuminate a single particle in the probe volume to cause said single particle to emit radiation;

a detector arranged to detect the radiation emitted by the single particle; and

processing means configured to use spectral data derived from the detected radiation to determine a property of the single particle.

The invention enables more detailed and more useful information to be obtained about particles in an aquatic mass because the particles are characterised individually. In contrast, the prior art only allows the determination of bulk

properties of large numbers of particles in water, e.g. the bulk properties of many thousands of particles, which may provide less information and/or less useful information. In addition, in bulk measurements, the measured signal of radiation emitted from particles is the sum of many individual particle signals, which includes not only signals from the particles of interest, but also signals from other particles, e.g. dissolved organic matter, that may not be of interest. The signals from particles that are not of interest may therefore obscure the signals of interest.

It will be appreciated that, in accordance with the invention, the ability to look at individual particles arises from the use of an interference pattern in a probe volume. Due to, for example, properties of the source used to generate the interference pattern, such as radiation beam size and coherence length, the probe volume may be very small, and the length scale of the interference pattern (e.g. fringe width) may be very small, allowing the interference pattern to illuminate one particle at a time. In a set of embodiments, the probe volume has a maximum dimension that is smaller than a mean separation between particles in the aquatic mass. The maximum dimension of the probe volume may be, for example, less than half of the mean particle separation, less than one fifth of the mean particle separation, or less than one tenth of the mean particle separation.

It will be appreciated that if the probe volume is small compared to the mean particle separation, it is likely that only one particle will be in the probe volume at any one time. Preferably, the method comprises illuminating just one particle of interest at a time. However, there may be occasions when more than one particle is present in the probe volume. In such cases, the detected radiation may be emitted and/or scattered by more than one particle in the probe volume. In a set of embodiments, the method comprises the step of discarding or disregarding spectral data corresponding to radiation detected when more than one particle is simultaneously present in the probe volume. It will be appreciated that when particle density is high, a larger amount of spectral data may be discarded, as the presence of more than one particle in the probe volume may occur with greater frequency. Such data may be discarded automatically by the apparatus.

It will be appreciated that characterising individual particles refers to determining a property of a single particle, i.e. so that individual properties of individual particles

may be determined separately. In a set of embodiments, the method comprises determining at least one property of the particle other than its velocity, speed, or direction of motion. It will be appreciated that a property of the particle may be determined from spectral data that is characteristic of (e.g. unique to) to the single particle that is illuminated.

The spectral data may comprise an emission spectrum, e.g. a fluorescence emission spectrum. The emission spectrum may be a full spectrum (i.e. having sufficient resolution to determine a detailed fluorescence spectrum of the particle). However, alternatively, the spectral data may comprise values indicative of the amount of radiation falling within predefined wavelength bands, e.g. a few wavelength bands. This may be considered equivalent to an emission spectrum having very coarse resolution but which may be sufficient to identify particles of interest in some applications.

It will be appreciated that radiation emitted via fluorescence will be much less intense than the stimulating radiation, or any radiation that is scattered directly by the particle. The spectral data therefore may be acquired by integrating the detected radiation over time, e.g. for the duration of the particle's passage through the probe volume. This allows a more reliable measurement to be obtained than might be achievable using only an instantaneous measurement.

In preferred embodiments, some or all of the radiation emitted by the particle is emitted by fluorescence. Accordingly, the spectrum of the radiation that is emitted by a particle may be indicative of a kind of fluorescence by the particle. For example, the spectrum of fluorescence radiation emitted by a particle may depend on the substance the particle is made from. A fluorescence spectrum may thereby be used to determine what the particle is. Determining a property of the particle may comprise determining one or more of the particle type (i.e. the material or substance the particle is made from), the particle volume, and/or the particle size. Using fluorescence, the particle size can be determined for very small particles, e.g. down to sizes in the region of 3 μm – 5 μm.

In some embodiments, radiation scattered from the particle via Raman scattering is detected. Raman spectroscopy may then be used to determine information about

the particle properties, e.g. what the particle is, what it is made from, etc. Raman scattering involves inelastic scattering of photons when they interact with molecular vibrations or other excitations in the particle, resulting in the energy of the scattered photons being shifted relative to the incident photons. This shift in energy, and thus in wavelength, gives information about the vibrational modes in the particle, and thus provides information about the particle properties. Measurement of radiation from Raman scattering requires a larger particle than fluorescence measurements, but the information obtained can be more diagnostic (i.e. it contains more useful information for determining particle properties and dinstinguishing particles).

Any suitable radiation of any suitable wavelength may be used to induce Raman scattering, e.g. laser radiation. The radiation may be the same radiation that is used to generate the interference pattern. In that case, the wavelength of the exciting radiation would be the same as the radiation used for inducing fluorescence.

Alternatively, a different wavelength of radiation may be used, in which case an additional source of electromagnetic radiation is used. An additional detector may be provided, e.g. to detect the Raman scattered radiation if a different wavelength radiation is used. Using an additional source having a different wavelength from that used for inducing fluorescence is advantageous as it can prevent the fluorescence signal masking the Raman scattering signal.

Where the radiation used to induce Raman scattering is the same radiation that is used to generate the interference pattern, as discussed previously the length scale of the probe volume and the interference fringes means that typically only one particle will be illuminated at a time. This means that it is possible to detect Raman-scattered radiation from one particle at a time, thus allowing the determination of the properties of a single particle via Raman spectroscopy.

If an additional radiation source is provided, the radiation from the additional source may be used to illuminate a probe volume by overlapping two beams of the radiation. A coherent (e.g. monochromatic) additional light source may be used so that an additional interference pattern is generated in the probe volume. Thus, as explained above, the length scale of the probe volume and (where present) the

interference fringes provides the above-mentioned advantage of being able to illuminate (and thus detect Raman scattered radiation from) one particle at a time.

Alternatively, the two beams may be non-coherent such that a further fringe pattern is not produced.

This is novel and inventive in its own right, and thus when viewed from a second aspect, the invention provides a method of characterising individual particles in an aquatic mass, the method comprising:

overlapping two beams of electromagnetic radiation to illuminate a probe volume defined by a region of overlap of the two beams;

illuminating a single particle in the probe volume, thereby causing said single particle to scatter radiation via Raman scattering;

detecting the radiation scattered via Raman scattering by the single particle; and

using spectral data derived from the detected radiation to determine a property of the single particle.

The invention extends to an apparatus for characterising individual particles in an aquatic mass, the apparatus comprising:

a source of electromagnetic radiation arranged to overlap two beams of electromagnetic radiation to illuminate a probe volume defined by a region of overlap of the two beams, thereby to illuminate a single particle in the probe volume to cause said single particle to scatter radiation via Raman scattering;

a detector arranged to detect the radiation scattered via Raman scattering by the single particle; and

processing means configured to use spectral data derived from the detected radiation to determine a property of the single particle.

The beams of radiation may be coherent so that an interference pattern is generated in the probe volume.

Features of the first aspect discussed above may also be features of the second aspect. Similarly, features discussed below may be features of the first and second aspects.

In some embodiments, radiation scattered by the particle is detected (other than or in addition to radiation scattered by Raman scattering). The scattered radiation may be used to determine a speed of the particle. The apparatus preferably comprises a Laser Doppler Velocimeter arranged to determine a speed of the particle using Laser Doppler Velocimetry (LDV). For example, as a particle passes through the interference pattern, the amount of light it scatters will depend on whether the particle is in a region of constructive or destructive interference. In a region of constructive interference, i.e. a bright region of the interference pattern, the particle will tend to scatter more light to the detector than when it is in a region of destructive interference, i.e. a dark region of the interference pattern. As the particle moves through the interference pattern, the detected radiation will vary in intensity. For example, if the interference pattern is a sinusoidally varying fringe pattern, the intensity of detected radiation will also be sinusoidally varying. The frequency of variation in intensity will depend on the speed of the particle and the fringe separation. The speed of the particle perpendicular to the fringes may thus be related to the fringe spacing and the frequency of variation in the intensity of the detected radiation.

In some embodiments, the apparatus may be mounted on a moving vehicle, e.g. an autonomous underwater vehicle (AUV), or a remotely operated vehicle (ROV). In such embodiments, the apparatus may be advantageously positioned and configured so that the interference pattern comprises fringes aligned substantially perpendicular to a direction of propulsion of the vehicle. It is also preferred that the probe volume is sufficiently far from the vehicle to be in a region of laminar flow of the water. In that situation, the flow of particles relative to the vehicle will be substantially perpendicular to the fringe direction, allowing the particle speed relative to the vehicle to be more accurately determined.

The method may also comprise counting particles, e.g. counting the number of particles illuminated by the interference pattern in a time period. Particle counting may be used to determine a particle density and thereby to determine a particle flow rate in the aquatic mass.

The variation in the signal corresponding to the intensity of the detected radiation may be also be used to determine the reliability of the data collected. For example, as discussed above, the recorded intensity of the scattered radiation may vary according to the brightness of the fringes as the particle moves through the interference pattern, e.g. exhibiting a number of peaks corresponding to the number of fringes that the particle has moved through. The interference pattern may have, for example, between five and ten fringes. If a particle passes close to the centre of the probe volume, the recorded scattered radiation may exhibit several peaks, e.g. five or more peaks. In contrast, if the particle passes close to the edge of the probe volume, there might be only one or two peaks. The reliability of the data may be greater if the particle has passed near the centre of the probe volume, as there is more scattered and/or emitted radiation that can be collected and used. Accordingly, data corresponding to a particle may be discarded if the recorded intensity of the scattered radiation exhibits fewer than a threshold number of peaks. The threshold number may be, for example, three, four, five, six, or more than six. It will be understood, therefore, that the actual sampling volume in which particles may be characterised may be smaller than the probe volume, as data corresponding to particles passing through the probe volume extrema may be rejected. It will therefore also be appreciated that the size and shape of the actual sampling volume may be dynamically determined depending on, for example, particle and flow characteristics, the laser properties, and the threshold number of peaks.

As used herein, particle means any individual piece of matter or material, e.g. a piece of particulate matter or a droplet. For example, the particle may be an algae particle. The algae particle may emit radiation by fluorescence due to the presence of chlorophyll a in the algae. As another example, an algae particle may emit radiation by fluorescence due to phycocyanine, for example in blue-green algae. As another example, an algae particle may emit radiation by fluorescence due to the presence of phycoerythrin, e.g. in red algae. The particle may be a droplet. For example, the particle may be an oil droplet. The oil droplet may emit radiation by fluorescence due to the presence of polycyclic aromatic hydrocarbons (PAH) in the oil droplet. The particle may be coated in oil or organic matter that emits fluorescence radiation, thereby causing the particle (e.g. which may be a particle comprising inert matter coated by organic matter) to emit fluorescence radiation.

The interference pattern may be generated by any source of electromagnetic radiation but a laser is used. Preferably the interference pattern is created using two beams from a single electromagnetic radiation source. For example, the electromagnetic radiation source may be arranged (e.g. using optical components) to provide two separate radiation beams directed so that they overlap to define the probe volume, thereby generating an interference pattern.

The distance of the probe volume from the apparatus may be chosen depending on the type of particles to be characterised and the situation in which the apparatus is used. The distance may also be chosen to optimise the amount of light received by the detector. For example, if the beams are directed so that they overlap close to the apparatus, more of the scattered and/or emitted radiation may be received by the detector. This is because of two factors. First, light from the probe volume will be incident on the detector over a greater area which allows for more sensitive measurements from a detector having a given sensitivity per unit detection surface area. Second, there is a reduced amount of absorption by the water (e.g. sea water) if the light only travels a short distance through the water. Accordingly, if the probe volume is far away from the apparatus, there may be reduced sensitivity.

However, there are additional factors that may be used to determine the distance of the probe volume from the apparatus. For example, if the probe volume is close to the apparatus (e.g. close to an optical arrangement for directing light onto the detector), good spatial resolution may be obtained for small particles. If the probe volume is farther away, good spatial resolution may be obtained for medium and large particles. If the apparatus is mounted on a moving under water vehicle (e.g. an AUV), there may be turbulence in the water around the vehicle when it is moving, with a region of laminar flow farther away from the vehicle. In the region of laminar flow, the particles may all move with approximately the same speed, while in the turbulence, the particle may have many different speeds. The distance to the probe beam may be advantageously chosen so that it is outside the turbulence and in the laminar flow, which may yield better results for the particle characterisation.

In embodiments comprising a Laser Doppler Velocimeter, the source of electromagnetic radiation may comprise the Laser Doppler Velocimeter laser. It will

be appreciated that this advantageously obviates the need to provide two separate electromagnetic sources, e.g. two separate lasers, as the LDV laser can be used for both purposes. However, in some embodiments, in addition to the source of electromagnetic radiation which illuminates the particle to cause it to emit radiation, the apparatus comprises a further electromagnetic radiation source. The source of electromagnetic radiation and the further electromagnetic radiation source preferably have different wavelengths. This is advantageous as the wavelengths may, for example, be chosen so that the source of electromagnetic radiation has a wavelength that is optimal for inducing the particle to emit light, while the further electromagnetic radiation source has a wavelength optimal for scattering by the particle.

The type of electromagnetic radiation source, e.g. the type of laser, may be selected so as to provide a wavelength suitable for exciting fluorescence in a particle. For example, the radiation source may be selected to have a wavelength to excite fluorescence in a particular particle type of interest, or a particular particle type that is expected to be seen in an aquatic mass. For example, the radiation source may emit radiation in the blue-violet frequency region of the visible spectrum; for example, the radiation emitted by the electromagnetic radiation source may have a wavelength between 300 nm and 500 nm. In some embodiments, the radiation source comprises a blue laser. It will be understood by one skilled in the art that a blue laser may refer to a laser having a wavelength spectrum covering part of the short wavelength end of the visible spectrum, e.g. part of the blue to violet spectrum. For example, a 405 nm laser may be used. A 405nm laser is particularly advantageous for several reasons. Suitable 405nm lasers produce a strong (i.e. intense) beam which is suitable for use in the sea. The wavelength spectrum of a 405nm laser falls within the range of wavelength absorbed by many different of particles of interest (e.g. different algae species) to produce detectable fluorescence. In particular, chlorophyll a absorbs 405nm radiation strongly. In addition, suitable dichroic mirrors are readily available to separate to the LDV Doppler signal and the fluorescence signal. However, other wavelengths may be used. The radiation source's wavelength may fall outside of the visible spectrum. However, preferably the wavelength is a visible wavelength. This may be better from a health and safety viewpoint, e.g. to satisfy safety regulations relating to open radiation sources. In addition, it facilitates the

alignment of the apparatus (e.g. optical components) which may be more easily achieved if the radiation is visible. It will be appreciated that the wavelength of a radiation source may refer to a peak or central wavelength of the spectrum of the radiation emitted by the source.

Additional radiation sources (e.g. additional lasers) may be used to induce the particle to emit or scatter radiation, e.g. to enhance fluorescence from the particle, or to characterise the particle using Raman scattering. The additional radiation sources may have a different wavelength. For example, if a main laser of 405 nm is used to excite fluorescence in polycyclic aromatic hydrocarbons (PAH) at 405 nm, normally PAH structures comprising four or more ring structures will be excited. Naphthalene absorbs predominantly at 266 nm, so an additional laser may be provided to excite smaller ring structures. Similarly, other particles will have characteristic absorption frequencies and so additional lasers may be selected with suitable frequencies to excite the desired particles.

In preferred embodiments, the detected radiation is separated according to its wavelength. For example, the detected radiation may be separated into two portions. A first portion of the separated radiation may comprise radiation that is below a threshold wavelength. The first portion may correspond to scattered radiation, which is likely to be of approximately the same wavelength as the incident radiation. A second portion of the separated radiation may comprise radiation that is above the threshold wavelength. The second portion may correspond to emitted radiation, as emitted radiation is typically of lower energy (i.e. longer wavelength) than the radiation that excites the emission. The threshold may be chosen so that the detected radiation is separated into (i) predominantly emitted radiation and (ii) predominantly scattered radiation. The detected radiation may thereby be split into a portion for spectral analysis (e.g. emitted radiation) and a portion for velocity, speed or direction of motion (e.g. LDV Doppler signal) analysis (e.g. scattered radiation). The detected radiation may be split into more than two portions according to more than one threshold wavelength, e.g. into scattered radiation, emission from a first particle type having a characteristic range of frequencies, and emission from a second particle type having a different characteristic range of frequencies.

Any suitable means, e.g. optical component(s), may be used to separate the radiation. For example, a dichroic mirror or filter may be used. The radiation may be separated so as to send emitted radiation (e.g. fluorescence signals) to a spectrometer and to send scattered radiation an LDV processing engine, or other speed, velocity, and/or direction analysis engine.

A threshold wavelength (e.g. a single threshold wavelength in a case where the radiation is separated into two portions) may be slightly above the wavelength of the electromagnetic source. For example, it may be between 5 and 10 nm above. As an example, for a 405 nm radiation source, the threshold may be 410 nm. This may help to avoid any of the 405nm radiation entering into the spectrometer. It is undesirable for 405nm radiation to enter the spectrometer as it may cause backscattering and obscure the radiation of interest (i.e. the fluorescence radiation), as the 405nm radiation may be significantly more intense than the fluorescence radiation.

It will be appreciated that the size of the probe volume will be determined by the characteristics of the radiation sources or lasers and/or any optical components used to focus the radiation, e.g. laser beam width, radiation wavelength, focal length of optical components. For example, the size of the probe volume may be on the scale of tens of microns, e.g. between 50 μm and 100 μm, or between 20 μm and 200 μm. As a non-limiting specific example, the probe volume may be around 200μm in length, around 50 μm in width and around 50 μm in depth. However, it will be understood that larger and smaller probe volume sizes may be used. The characteristic length scale of the interference pattern, e.g. the fringe spacing, depends on the wavelength of the radiation. The characteristic length scale, e.g. fringe spacing, may be on the order of microns, e.g. between 5 μm and 10 μm, or between 2 μm and 20 μm, although it will be understood that the length scale/fringe spacing may be larger or smaller than this.

Any suitable detector may be used to detect the emitted and/or scattered radiation. For example, photomultiplier tubes may be used. Alternatively a charge coupled device may be used. A linear photo diode array may be used.

A high resolution (e.g. 1-2 nm) is preferable for achieving the characterisation of the particle, e.g. identifying the particle by its material, but high resolution arrangements may have lower sensitivity. Lower resolution but higher sensitivity improves the chance of detecting fluorescence radiation for a particle. Accordingly, it may be necessary to balance resolution and sensitivity. For example, a charge coupled device may give better resolution, but may have reduced sensitivity. If its sensitivity is sufficiently high, a charge coupled device is preferable for the detector.

The method of the invention may be applied on test samples that have been collected from an aquatic mass, e.g. in a laboratory setting. However, preferably the method is applied in situ in ambient water, e.g. in an aquatic mass where particles of interest are found. As mentioned above, the apparatus may be mounted on a moving vehicle. In other embodiments, the apparatus is fixed at a location in an aquatic mass, e.g. at a monitoring station in an ocean. The method may be applied “in line”, for example, in discharge outlets. For example, oil droplet concentration or type may be measured in produced water discharges. The method may be used for characterisation of ballast water, for example to determine the size of particles present to ensure agreement with regulations before discharging the water.

In situ, the method may be used to detect changes in water mass characteristics (e.g. which may be characterised by changes in algal composition). As another example, it may be used to detect oil droplets from leaks or spills, e.g. to measure the extent of a spill. It may be used to document the effects of the use of chemicals on spills, for example during clean-up operations. It may be used to validate distribution models for produced water and drilling mud.

The method may comprise carrying out a spectral analysis of the radiation to obtain the spectral data. For example, the method may comprise determining the spectral components of detected radiation.

The method may comprise comparing the spectral data with recorded spectral data in a database, and/or reviewing of the spectral data by an operator. This may enable the processing means or an operator to identify or characterise a particle based on the comparison of the spectral data with recorded spectral data. The

spectral data may be used to create a record, e.g. database files, of spectral data corresponding to particular particles or particle types. These records may then be used subsequently to identify particles. It will be understood that different algal species have different fluorescence spectra, and that some spectra are easier to distinguish/identify than others. The recorded data may added to the database to build up a library of different recorded spectra, irrespective of whether a particle or particle type has been identified as corresponding to the data. The data added to the database may include and/or be grouped according to the results of data analysis, e.g. multivariate analysis. Such data may be used subsequently to help distinguish spectra and/or to identify particles.

The apparatus may comprise a user interface. The user interface may allow an operator to view spectral data or data relating to particles in the aquatic mass. For example, the user interface may allow the operator to view data embodying the characterisation of particles, e.g. data indicating a particle's material or size. It may also allow the operator to view data indicating to a particle's velocity, speed or direction of motion, or data indicating a particle density or flow rate in the aquatic mass.

The user interface may allow an operator to select what data to display. The user interface may be capable of displaying data in real time, e.g. spectral data or data indicating the properties of particles, as it is received, or shortly after it is received. It may also allow an operator to view stored data, e.g. previously recorded data, and/or reference data, e.g. library data indicating known spectral signatures of certain types of particles. For example, data collected on an AUV may be stored for later retrieval and analysis, while data on an ROV following a ship may transmit recorded data in real time for real time analysis.

Where optional features are described with reference to the method of the invention, corresponding optional features may be provided for the apparatus, and vice versa.

Certain preferred embodiments will now be described, by way of example only, with reference to the accompanying drawings, in which:

Figure 1 shows a Laser Doppler Velocimeter used in embodiments of the present invention;

Figure 2 shows a close-up view of a probe volume formed by an intersection of two laser beams produced by the Laser Doppler Velocimeter of Figure 1;

Figure 3 shows an absorption spectrum of chlorophyll a;

Figure 4 shows a fluorescence emission spectrum of chlorophyll a;

Figure 5 shows four detected radiation spectra 68, 70, 72, 74 obtained in accordance with the method of the present invention.

Figure 6 shows a graph of fluorescence signals observed from single algae cells in a sample of tetraselmis sp in water.

Figure 7 shows the Laser Doppler Velocimeter of Figure 1 with a dichroic mirror for separating the received radiation that is scattered or emitted from particles in the probe volume according to its wavelength;

Figure 8 shows the variation with time of the light intensity scattered from a particle in a probe volume of the Laser Doppler Velocimeter of Figure 1;

Figure 9 shows the light intensity of Figure 8 filtered using a bandpass filter to remove the Gaussian envelope; and

Figure 10 shows a schematic representation of signal and data handling according to embodiments of the present invention.

Figure 1 shows a Laser Doppler Velocimeter 2 used in embodiments of the present invention for characterising individual particles in an aquatic mass. The Laser Doppler Velocimeter 2 comprises a housing 4 containing a laser 6. On the front of the housing 4 there is a lens 8 for coupling radiation in and out of the housing 4. The laser 6 is used to produce two beams 10, 12 of coherent laser light at 405 nm. The beams 10, 12 are directed by additional optical components (not shown)

through holes 13 at the edge of the lens 8 so that the beams overlap to define a probe volume 14. The overlap of the beams in the probe volume 14 is shown in Figure 2, which magnifies the region in the dotted circle 16.

In Figure 2, the first beam 10 and second beam 12 are directed to overlap to define the probe volume 14. As the beams 10, 12 are coherent, at the overlap in the probe volume 14, an interference pattern 22 is created. The interference pattern 22 consists of interference fringes localised in the probe volume.

In use, the Laser Doppler Velocimeter 2 is disposed in an aquatic mass 24, e.g. an ocean. Particles 26 in the aquatic mass move through the probe volume 14, due to the flow of the aquatic mass 24 in which the particles 26 are suspended. The particles 26 therefore move through the probe volume 14. Due to the size of the probe volume 14, typically only one particle 26 is in the probe volume at a time. If data should be recorded indicating that more than one particle 26 is in the probe volume 14, that data may be discarded. However, due to the small size of the probe volume 14, for typical particle densities, the presence of more than one particle 26 in the probe volume 14 occurs very infrequently.

When a particle enters the probe volume 14, it is illuminated by the interference fringes. When a particle is illuminated by a fringe, depending on the type of particle, the particle may scatter and/or emit radiation.

Returning to Figure 1, radiation 28 that is emitted and/or scattered by a particle 26 is coupled in to one or more optical fibres 30 by the centre portion of the lens 8 and optical components 31. The optical components 31 are represented schematically by a lens, but it will be appreciated that the optical components may comprise any number of optical components possibly including, but not limited to, one or more lenses, diffraction gratings, mirrors, etc. The radiation 28 is then transmitted via the optical fibre(s) 30 to one or more detectors, as described further below with reference to Figure 7.

As discussed above with reference to Figure 2, a particle may scatter and/or emit radiation. The radiation emitted or scattered will depend on the type of particle. In the present embodiment, algae particles containing chlorophyll a are described.

However in other embodiments, other particles may be characterised. For example, other types of algae particles or oil droplets containing polycyclic aromatic hydrocarbons may be characterised.

Figure 3 shows an absorption spectrum of chlorophyll a. There is an absorption peak 32 at approximately 400 nm. When radiation from the laser 6 is incident on a particle 26, energy from the radiation is absorbed by the particle 26. This is because the wavelength of the laser beams 10, 12 coincides with the absorption band 34 of chlorophyll a. The absorbed energy is them re-emitted via fluorescence.

Figure 4 shows a fluorescence emission spectrum of chlorophyll a. The emitted fluorescence radiation falls within a band 36 of approximately 600 nm to 750 nm, i.e. the emitted radiation is generally of a longer wavelength than the incident radiation from laser beams 10, 12. In contrast, radiation that is scattered from the particle 26 is approximately the same wavelength as the incident radiation from laser beams 10, 12.

Figure 5 shows four detected radiation spectra 68, 70, 72, 74 obtained in accordance with the method of the present invention. The first spectrum 68 is a spectrum obtained for a sample of crude oil droplets in water. The spectrum 68 exhibits a peak 76 at around 560 nm resulting from fluorescence by polycyclic aromatic hydrocarbons (PAH). The second spectrum 72 was obtained for a sample of isochrysis galbana (T. Iso) in water. The third spectrum 74 was obtained for a sample of rhodomonas lens in water. The fourth spectrum 76 was obtained for a sample of tetraselmis sp in water. The second, third, and fourth spectra 70, 72, 74 each contain a peak 78 at approximately 680 nm resulting from fluorescence by chlorophyll A in the samples. All four of the spectra 68, 70, 72, 74 exhibit a peak 80 at approximately 460 nm, resulting from Raman scattering by the water molecules in the samples. It can thus be seen that the radiation detected in accordance with the method of the invention can be used to characterise particles according to fluorescence peaks present in the spectra.

Figure 6 shows a graph of fluorescence signals observed from single algae cells in a sample of tetraselmis sp in water. Tetraselmis sp is an alga with particle size in the range of approximately 14 μm to 23 μm. The graph shows the fluorescence

signals obtain from 100 scans, where each scan records the radiation detected at the detector over a period of 1 ms. For ten of the scans, there is a peak in the detected radiation at 680 nm. This coincides with the wavelength of fluorescence from Chlorophyll A present in algae. The measurement from a single particle is in the range of 50 to 80 counts, which corresponds to an energy range of 10 nW to 16 nW. As a peak is observed in only 10 of the 100 scans, it can be inferred that each of these peaks corresponds to a single algae cell passing through the probe volume. It can thus be seen that the method of the present invention allows the detection of fluorescence from individual particles such as single cells, thus allowing those particles to be characterised individually.

Figure 7 shows the Laser Doppler Velocimeter 2 of Figure 1, and shows how the received radiation 28 is directed via one or more optical fibres 30 to the dichroic mirror 38. The dichroic mirror 38 (here represented simply by a functional block) separates the radiation according to wavelength. Radiation that is above 410 nm is directed along a first optical fibre 40 towards a spectrometer 42.

The longer wavelength portion which passes along the fibre 40 contains predominantly radiation that is emitted from the particle 26, e.g. by fluorescence. The spectrometer 42 is used to measure the spectral components of this radiation. The spectrum of emitted radiation 28 can be used to determine the type of particle 26 that was in the probe volume 14. For example, if the florescence spectrum matches the characteristic fluorescence spectrum of chlorophyll a, it can be determined that an algae particle containing chlorophyll a is in the probe volume 14. Further information may be obtained from the spectrum. For example, depending on the intensity of the spectrum, the size of the particle 26 may be inferred.

The component of the received radiation that is below 410 nm is directed along a second optical fibre 44 towards an LDV processing engine 46, where the intensity of the radiation is measured and the speed of the particle 26 is inferred. Such shorter wavelength component (i.e. comprising wavelengths that are generally close to the wavelength of the incident radiation) comprises predominantly radiation that was scattered from the particle 26. These can be used to infer speed as explained below.

Figure 8 shows the time variation in the intensity of radiation scattered from a particle moving through the probe volume 14. As the particle 26 moves through the probe volume 14, it is periodically illuminated by the interference fringes arising from constructive interference of the laser beams. This gives rise to a sinusoidal variation in the intensity of scattered light. The profile of the light intensity has a Gaussian envelope, which arises because of the Gaussian profile of the laser beams 10, 12. Between the peaks in Figure 8, the light intensity does not fall close to zero. This is because, in the example shown, the particle size is comparable to the fringe width. In such a case, as the particle moves through the fringes, it is partially illuminated by the next fringe before it has completely moved out of the previous fringe. If the particle were much smaller than the fringe width, the intensity would fall much closer to zero as there would be times when the small particle is almost entirely within a region of destructive interference. Nonetheless the variation in intensity can still be used to determine the speed of the particle.

Figure 9 shows the light intensity profile of Figure 8 filtered using a bandpass filter to remove the Gaussian profile. As the intensity of scattered light depends on whether the particle is in a region of constructive interference or destructive interference, the frequency of the sinusoidal variation of measured scattered light intensity will depend on how quickly the particle is moving through the fringes.

The component of the particle velocity perpendicular to the fringes is related to the fringe spacing and the frequency f of the sinusoidal profile showed in Figure 8 by

Perpendicular component of velocity = fringe spacing x f.

The frequency f is calculated from the bandpass filtered light intensity profile (such as shown in Figure 9). The frequency may be obtained from the sinusoidal profile using, for example, a Fast Fourier Transform.

It is thus possible to obtain information regarding the speed of the particle moving through the probe volume, in addition to information characterising the particle.

It is particularly advantageous, as shown in the present embodiment, for the same laser to be used to provide the light scattered by the particle as well as to excite

fluorescence in the particle. This means that information relating to both the speed of the particle and the fluorescence spectrum of the particle can be obtained using one laser.

However, in some other embodiments, two different lasers may be used as described earlier. In such embodiments, the lasers may be arranged so that they both focus on the same probe volume and produce separate interference patterns. In such embodiments, a different dichroic mirror may be used to separate the wavelength components into suitable bands.

Figure 10 shows a schematic representation of the signal and data handling carried out in accordance with embodiments of the present invention.

The radiation 28 emitted and/or scattered from particles is separated by a dichroic mirror 38 into a first radiation component 48 (comprising radiation that is less than 410 nm in wavelength) and a second radiation component 50 (comprising radiation that is greater than 410 nm in wavelength) as previously described.

The first component 48 is detected by a detector measuring intensity, such as a photomultiplier tube, and the measured intensity is processed by the LDV processing engine 46 as described above to determine the speed of the particle. The second component is directed to a spectrometer 42 which measures the spectrum of the radiation.

The data obtained from the LDV processing engine 46 and the spectrometer 42 are sent to a computer 52 for analysis and display. The computer 52 comprises a processor 54 and a memory 56. Stored in the memory is a database 58. In other embodiments the memory 56 (and therefore the database 58) may be remote from the computer 52 (for example, accessed via a network). The speed data and spectral data obtained by the LDV processing engine 46 and the spectrometer 42 are written to the database 58. Positioning information 59 is also provided to the database 58, e.g. for mapping purposes. Positioning data may include GPS (global positioning system) data and/or depth data (e.g. depth of an underwater vehicle below sea level). For example, an AUV may record GPS and depth data. An ROV

following a ship may record depth data, and acquire GPS data recorded on the ship.

The spectral data are compared with recorded spectral data in the database 58 to allow the particle 26 to be identified according to its emission spectrum. If the particle cannot be positively identified, the spectrum and/or a multivariate data analysis thereof may be recorded in the database for use in subsequent analysis or comparison. The computer 52 provides a possibility for the comparison to be automatic, although the comparison may be initiated by an operator via a user interface 60 provided on the computer 52, e.g. by inputting an instruction to activate a comparison with data stored in the database (e.g. via a search of stored reference spectra).

The computer 52 also provides the option to display the recorded spectral data along with spectral data from the database 58 for a visual comparison by the operator. The processor 54 is configured to carry out multivariate pattern recognition 62 to facilitate the comparison of the spectral data with recorded spectral data in the database 58. Once the corresponding spectrum or spectra have been identified in the database, information related to the identified spectrum is used in a further data analysis step 64 to present the data in a useful format (e.g. including a visual representation) in a 3D graphical information system (GIS) presentation 66 for the operator to study. The user interface 60 can be used to control the output on the 3D GIS presentation 66. For example, the user interface can get the GIS presentation 62 to display a continuous log of data obtained, or it can retrieve previously recorded data and/or reference data from the database for display, e.g. for comparison purposes.

It will be appreciated that only one possible embodiment has been described herein, and that variations are possible within the scope of the present invention.

Claims:

1. A method of characterising individual particles in an aquatic mass, the method comprising:

generating an interference pattern in the aquatic mass by overlapping two beams of electromagnetic radiation, wherein the interference pattern is generated in a probe volume defined by a region of overlap of the two beams;

illuminating a single particle in the probe volume with the interference pattern, thereby causing said single particle to emit radiation;

detecting the radiation emitted by the single particle; and

using spectral data derived from the detected radiation to determine a property of the single particle.

2. The method as claimed in claim 1, wherein the spectral data comprises an emission spectrum.

3. The method as claimed in claim 1 or 2, wherein some or all of the radiation emitted by the particle is emitted by fluorescence.

4. The method as claimed in claim 1, 2 or 3, comprising detecting radiation scattered from the particle via Raman scattering.

5. The method as claimed in claim 4, comprising using an additional source of electromagnetic radiation to induce Raman scattering by the particle.

6. The method as claimed in claim 5, comprising using the radiation from the additional source to illuminate a probe volume by overlapping two beams of said radiation.

7. A method of characterising individual particles in an aquatic mass, the method comprising:

overlapping two beams of electromagnetic radiation to illuminate a probe volume defined by a region of overlap of the two beams;

illuminating a single particle in the probe volume, thereby causing said single particle to scatter radiation via Raman scattering;

detecting the radiation scattered via Raman scattering by the single particle; and

using spectral data derived from the detected radiation to determine a property of the single particle.

8. The method as claimed in claim 7 wherein the beams of radiation are coherent so that an interference pattern is generated in the probe volume.

9. The method as claimed in any preceding claim, wherein the probe volume has a maximum dimension that is smaller than a mean separation between particles in the aquatic mass.

10. The method as claimed in any preceding claim, wherein the size of the probe volume is between 20 μm and 200 μm.

11. The method as claimed in any preceding claim, wherein a characteristic length scale of the interference pattern is between 2 μm and 20 μm.

12. The method as claimed in any preceding claim, comprising illuminating just one particle of interest at a time.

13. The method as claimed in any preceding claim, comprising the step of discarding or disregarding spectral data corresponding to radiation detected when more than one particle is simultaneously present in the probe volume.

14. The method as claimed in any preceding claim, comprising determining at least one property of the particle other than its velocity, speed, or direction of motion.

15. The method as claimed in any preceding claims comprising determining a speed of the particle using Laser Doppler Velocimetry.

16. The method as claimed in any preceding claim, wherein the method is applied in situ in the monitored environment.

17. The method of any preceding claim wherein the apparatus is mounted on a moving vehicle, and wherein the apparatus is positioned and configured so that the interference pattern comprises fringes aligned substantially perpendicular to a direction of propulsion of the vehicle.

18. The method as claimed in any preceding claim, further comprising counting particles.

19. The method as claimed in any preceding claim, comprising discarding data corresponding to a particle if a recorded intensity of scattered radiation exhibits fewer than a threshold number of peaks.

20. The method as claimed in any preceding claim, wherein the source of electromagnetic radiation comprises a laser.

21. The method as claimed in any preceding claim, wherein the radiation emitted by the electromagnetic radiation source has a wavelength between 300 nm and 500 nm.

22. The method as claimed in any preceding claim, comprising separating the detected radiation according to its wavelength.

23. The method as claimed in any preceding claim, comprising separating the detected radiation into a portion for spectral analysis and a portion for velocity, speed or direction of motion analysis.

24. The method as claimed in any preceding claim, comprising displaying data on a user interface in real time.

25. An apparatus for characterising individual particles in an aquatic mass, the apparatus comprising:

a source of electromagnetic radiation arranged to generate an interference pattern in the aquatic mass by overlapping two beams of electromagnetic radiation, wherein the interference pattern is generated in a probe volume defined by a region

of overlap of the two beams, thereby to illuminate a single particle in the probe volume to cause said single particle to emit radiation;

a detector arranged to detect the radiation emitted by the single particle; and

processing means configured to use spectral data derived from the detected radiation to determine a property of the single particle.

26. The apparatus as claimed in claim 25, wherein the spectral data comprises an emission spectrum.

27. The apparatus as claimed in claim 25 or 26, wherein some or all of the radiation emitted by the particle is emitted by fluorescence.

28. The apparatus as claimed in claim 25, 26 or 27, wherein the apparatus is arranged to detect radiation scattered from the particle via Raman scattering.

29. The apparatus as claimed in claim 28, comprising an additional source of electromagnetic radiation for inducing Raman scattering by the particle.

30. The apparatus as claimed in claim 29, wherein the additional source is arranged to illuminate a probe volume by overlapping two beams of radiation from said additional source.

31. An apparatus for characterising individual particles in an aquatic mass, the apparatus comprising:

a source of electromagnetic radiation arranged to overlap two beams of electromagnetic radiation to illuminate a probe volume defined by a region of overlap of the two beams, thereby to illuminate a single particle in the probe volume to cause said single particle to scatter radiation via Raman scattering;

a detector arranged to detect the radiation scattered via Raman scattering by the single particle; and

processing means configured to use spectral data derived from the detected radiation to determine a property of the single particle.

32. The apparatus as claimed in claim 31 wherein the beams of radiation are coherent so that an interference pattern is generated in the probe volume.

34. The apparatus as claimed in any of claims 25 to 33, wherein the probe volume has a maximum dimension that is smaller than a mean separation between particles in the aquatic mass.

35. The apparatus as claimed in any of claims 25 to 34, wherein the size of the probe volume is between 20 μm and 200 μm.

36. The apparatus as claimed in any of claims 25 to 35, wherein a characteristic length scale of the interference pattern is between 2 μm and 20 μm.

37. The apparatus as claimed in any of claims 25 to 36, wherein the apparatus is configured to discard or disregard spectral data corresponding to radiation detected when more than one particle is simultaneously present in the probe volume.

38. The apparatus as claimed in any of claims 25 to 37, configured to determine at least one property of the particle other than its velocity, speed, or direction of motion.

39. The apparatus as claimed in any of claims 25 to 38, comprising a Laser Doppler Velocimeter arranged to determine a speed of the particle using Laser Doppler Velocimetry.

40. The apparatus as claimed in any of claims 25 to 39, wherein the apparatus is operable in situ in the monitored environment.

41. The apparatus of any of claims 25 to 40, wherein the apparatus is mounted on a moving vehicle, and wherein the apparatus is positioned and configured so that the interference pattern comprises fringes aligned substantially perpendicular to a direction of propulsion of the vehicle.

42. The apparatus as claimed in any of claims 25 to 41, further arranged to count particles.

43. The apparatus as claimed in any of claims 25 to 42, configured to discard data corresponding to a particle if a recorded intensity of scattered radiation exhibits fewer than a threshold number of peaks.

44. The apparatus as claimed in any of claims 25 to 43, wherein the source of electromagnetic radiation comprises a laser.

45. The apparatus as claimed in any of claims 25 to 44, wherein the radiation emitted by the electromagnetic radiation source has a wavelength between 300 nm and 500 nm.

46. The apparatus as claimed in any of claims 25 to 45, arranged to separate the detected radiation according to its wavelength.

47. The apparatus as claimed in any of claims 25 to 46, arranged to separate the detected radiation into a portion for spectral analysis and a portion for velocity, speed or direction of motion analysis.

48. The method as claimed in any of claims 25 to 47, comprising a user interface arranged to display data in real time.

Fig. 1

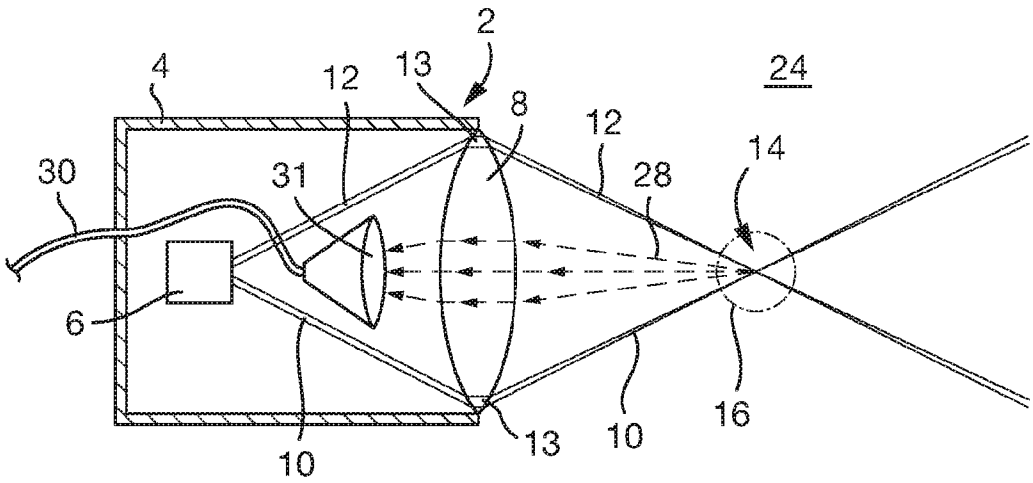


Fig. 2

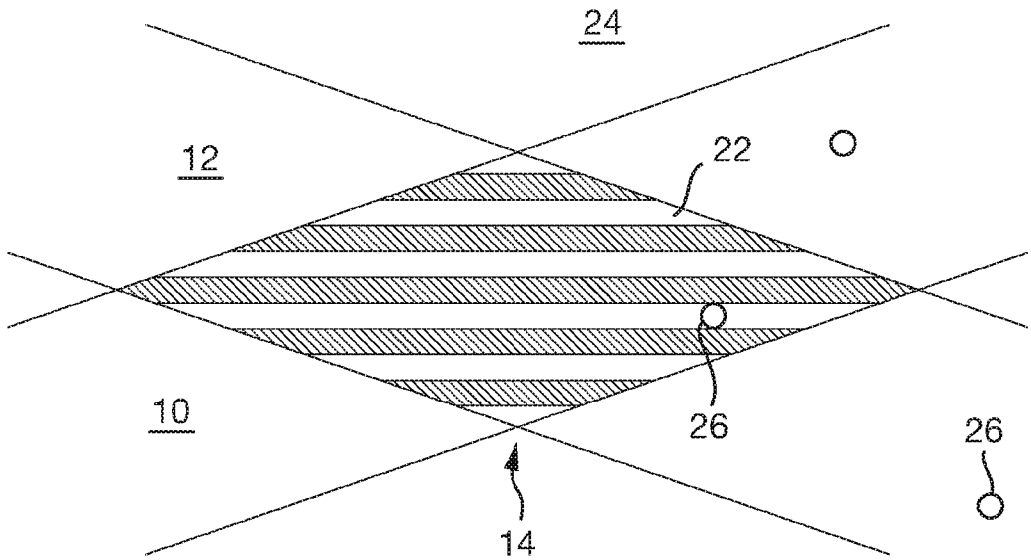


Fig. 3

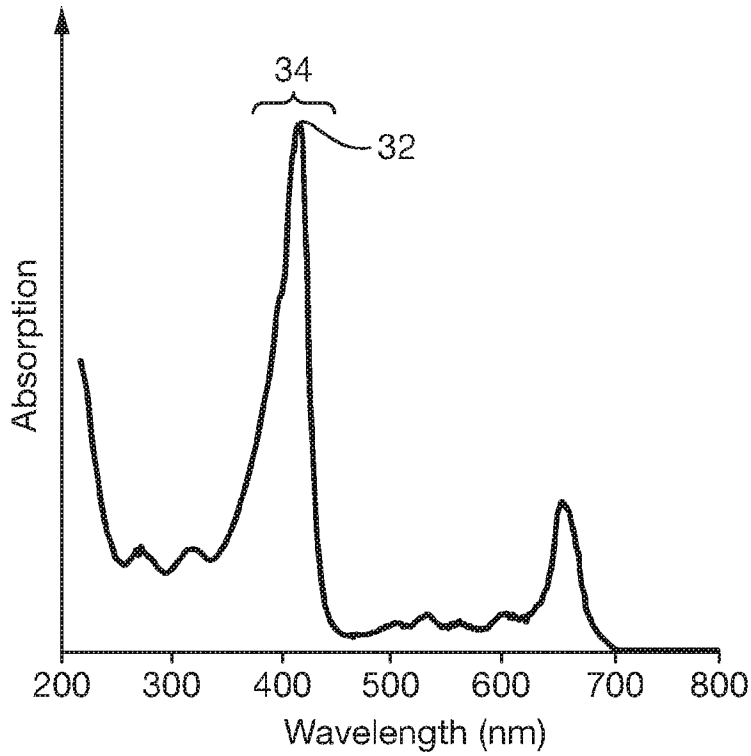

34
32
Absorption
200
300
400
500
600
700
800
Wavelength (nm)

Fig. 4

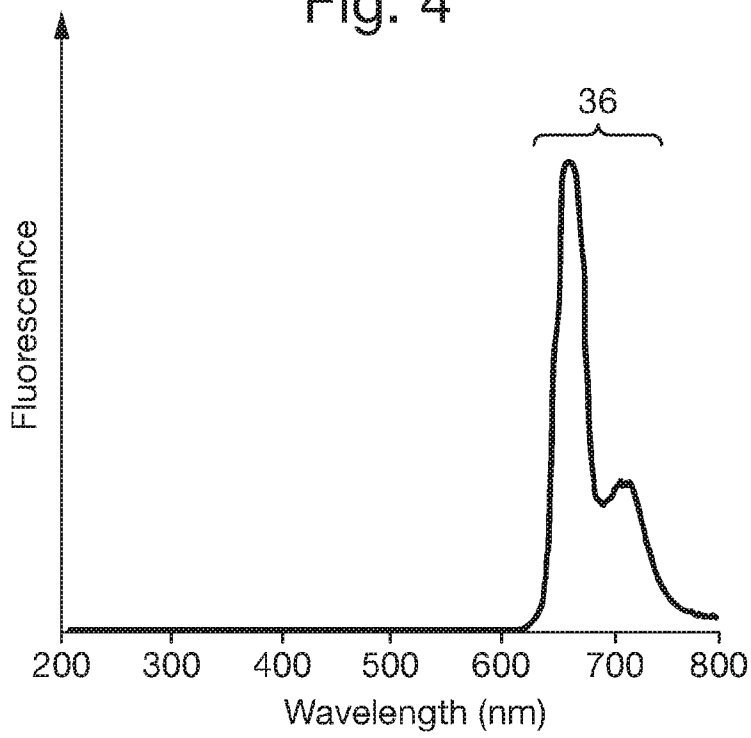
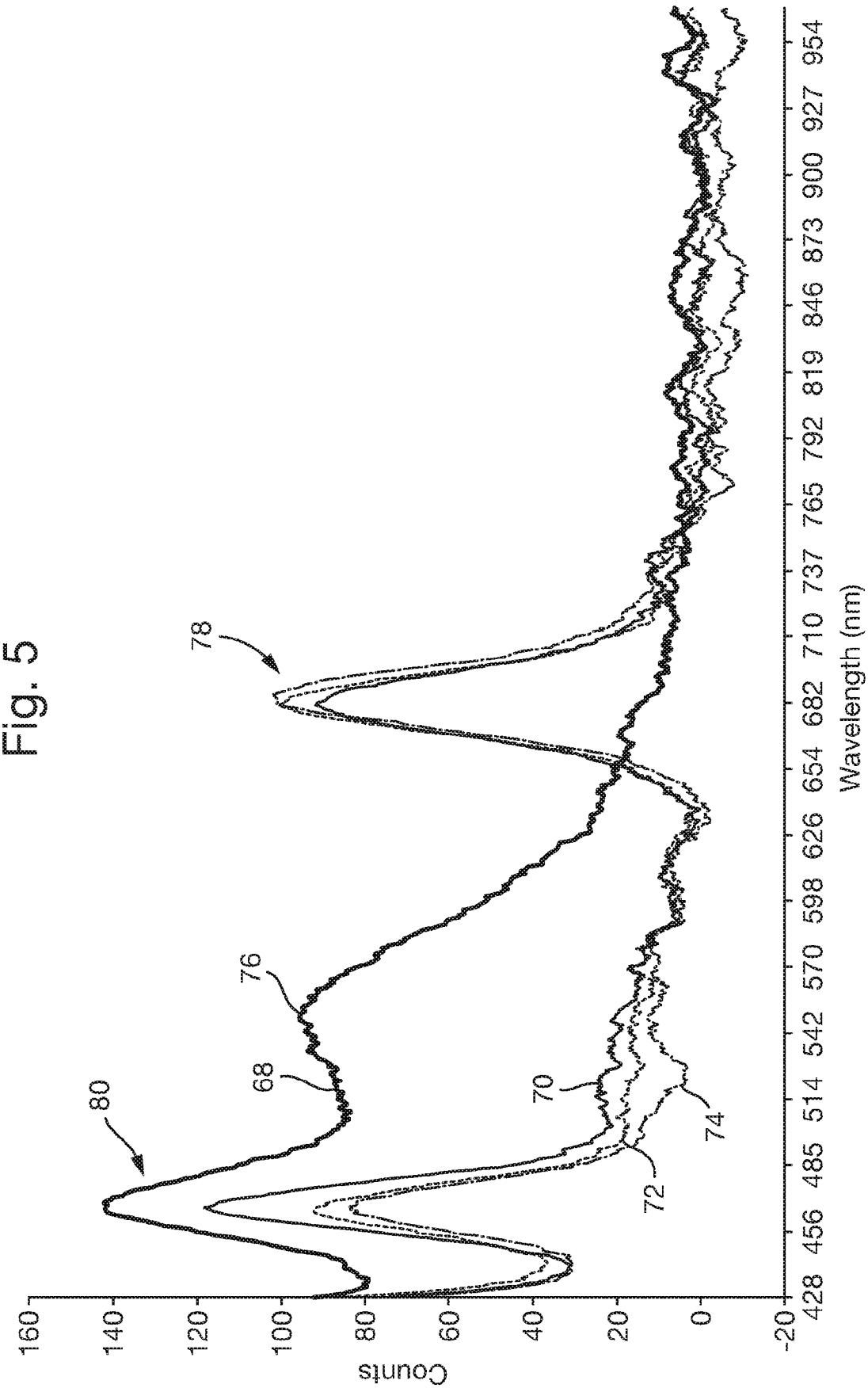

36
Fluorescence
200
300
400
500
600
700
800
Wavelength (nm)

Fig. 5


Counts
160
140
120
100
80
60
40
20
0
-20
Wavelength (nm)
428
456
485
514
542
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598
626
654
682
710
737
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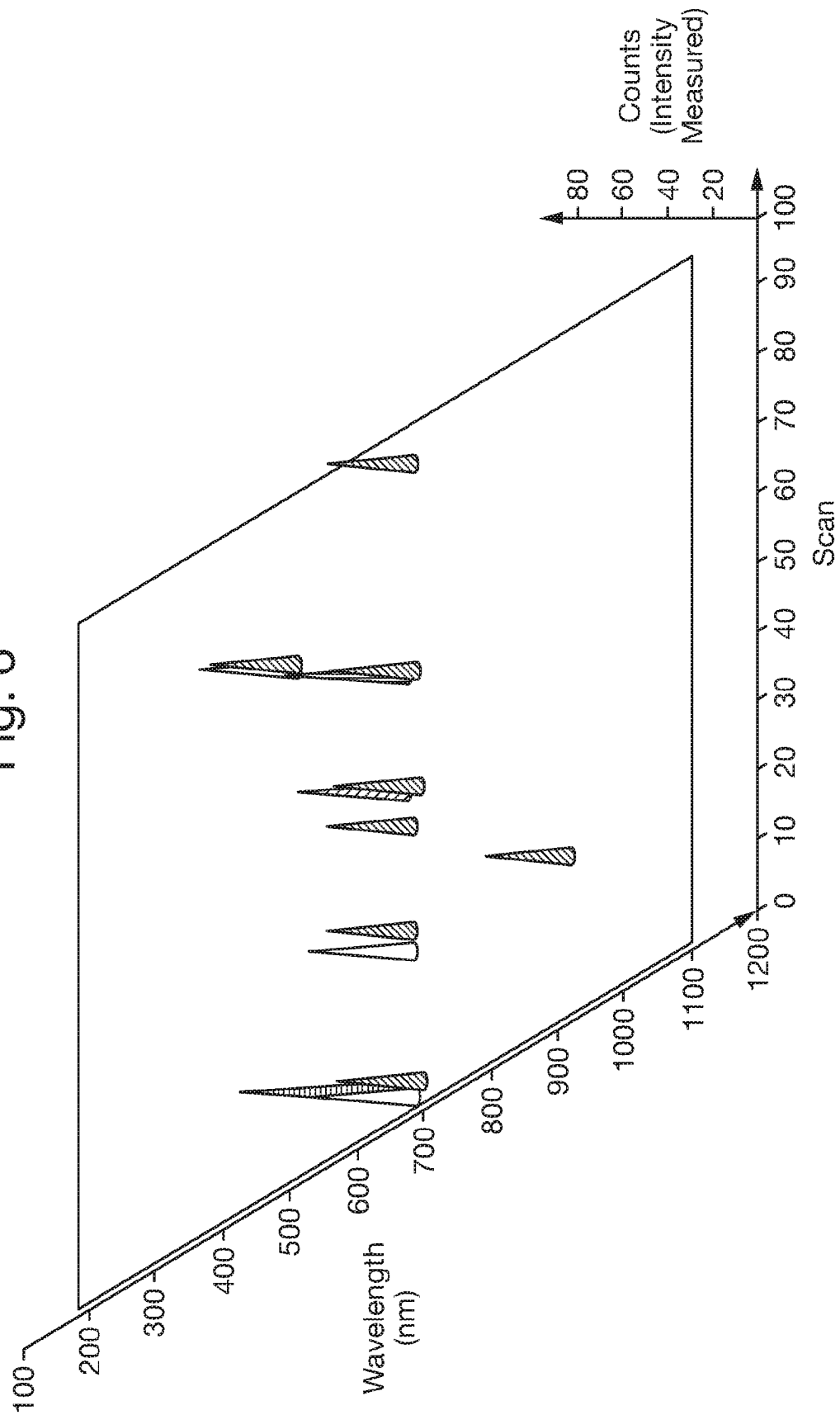
792
819
846
873
900
927
954
78
76
68
80
70
74
72

Fig. 6


Counts (Intensity Measured)
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60
40
20
Scan
0
10
20
30
40
50
60
70
80
90
100
1200
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1000
900
800
700
600
500
400
300
200
100
Wavelength (nm)

Fig. 7

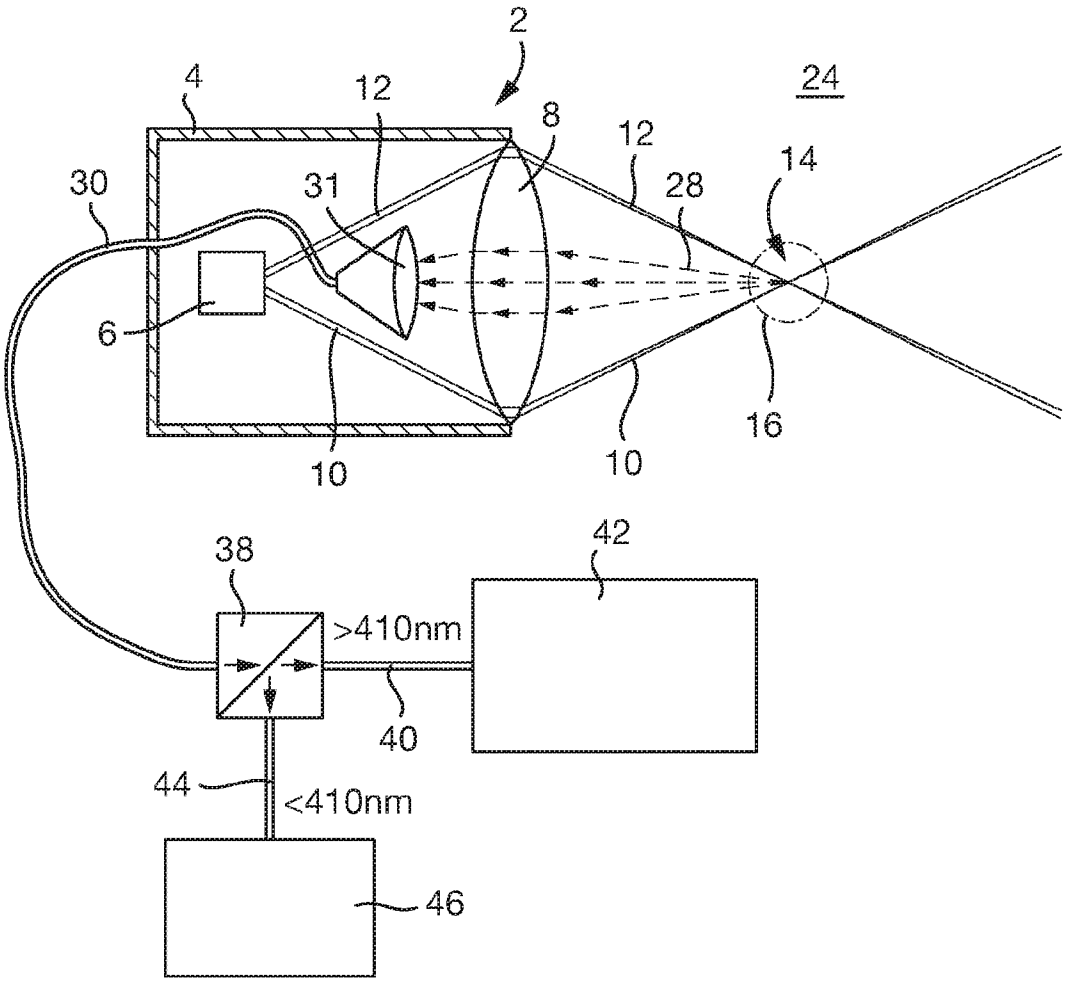


Fig. 8

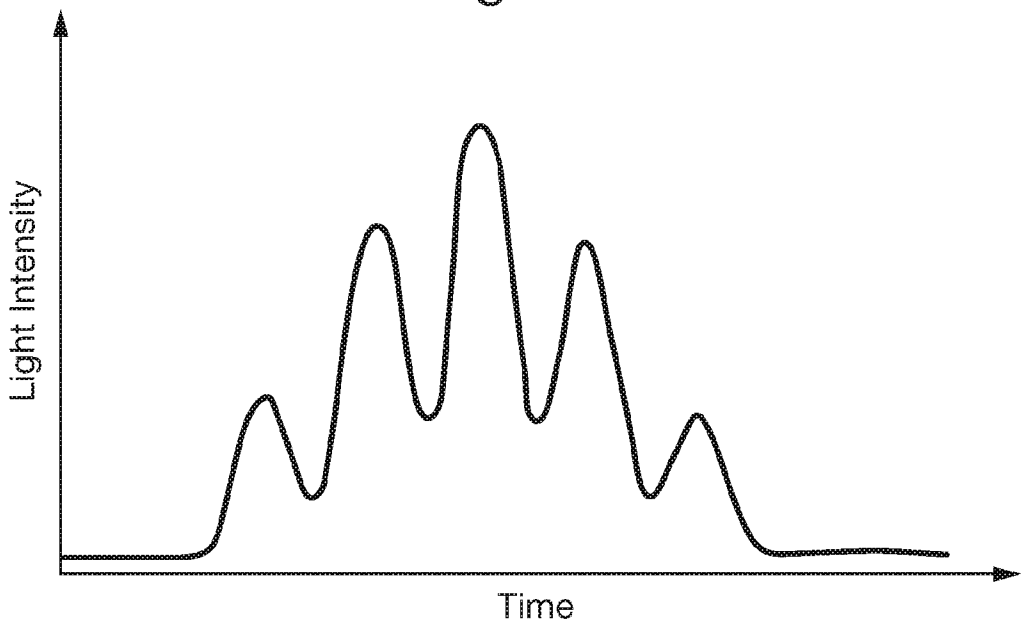

Light Intensity
Time

Fig. 9

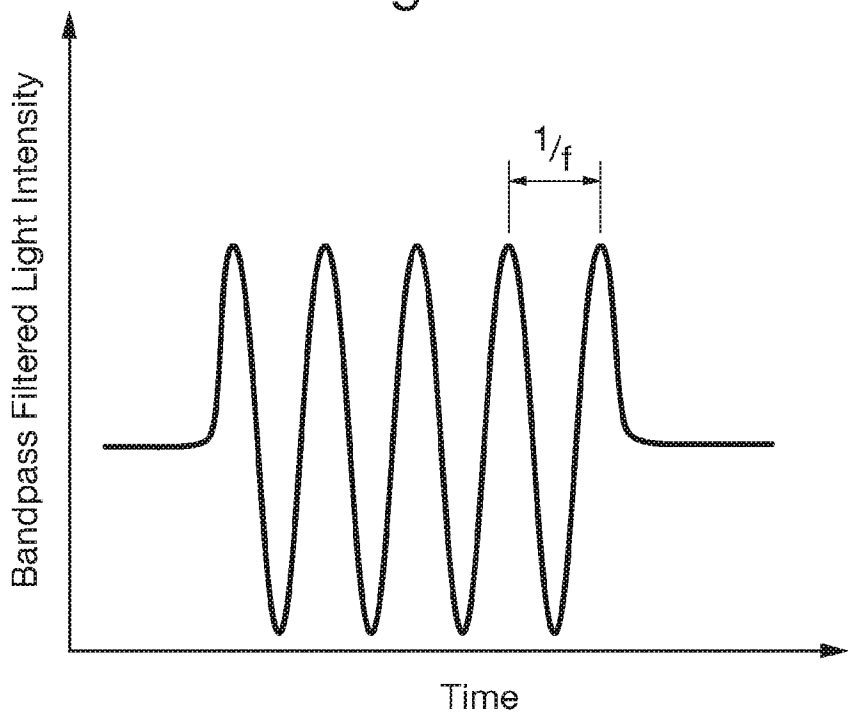
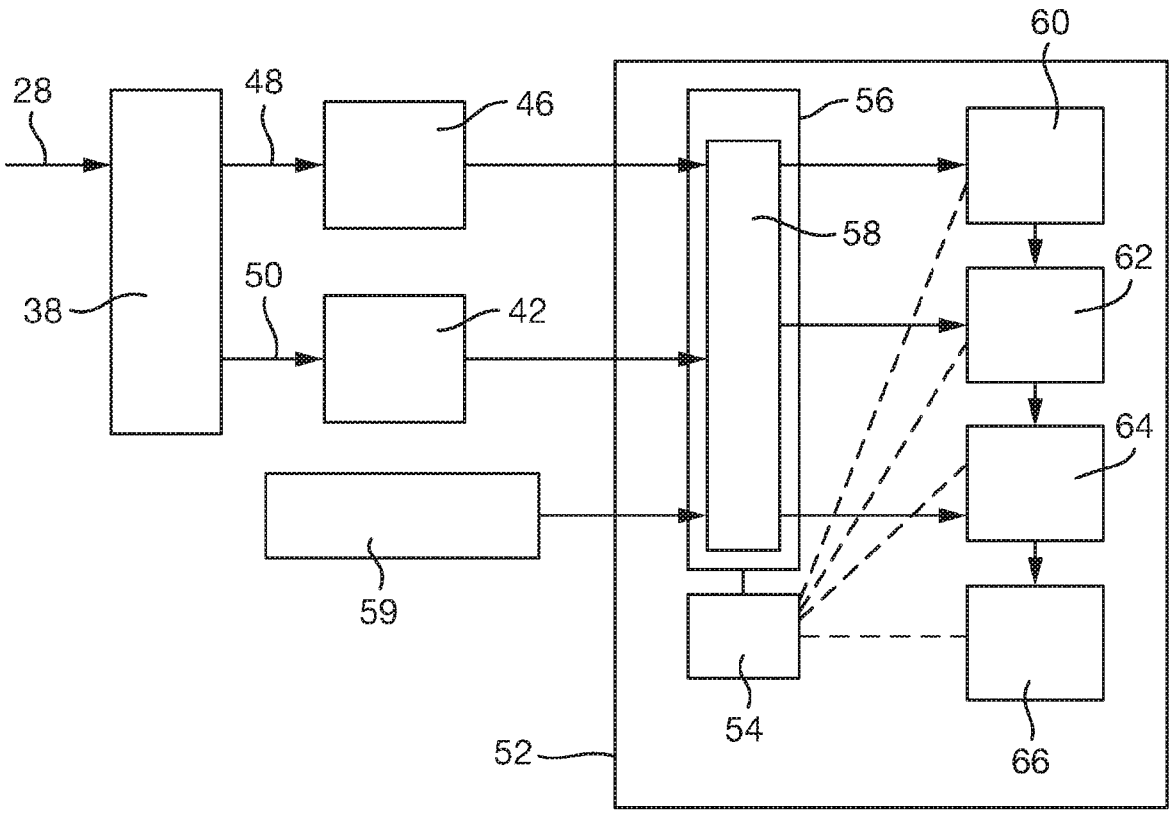

Bandpass Filtered Light Intensity
1/f
Time

Fig. 10



INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2017/050943

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/18
ADD. G01N21/64 G01N21/65 G01P5/26 G01N15/14

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N G01P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 596 036 A (NORGREN RICHARD M [US] ET AL) 17 June 1986 (1986-06-17) column 5, line 33 - column 6, line 8; figure 1	1-4, 8-16, 18-21, 24-28, 31-40, 42-45,48
X	DE 198 36 183 A1 (GIMSA JAN PRIV DOZ DR [DE]) 18 March 1999 (1999-03-18) column 5, line 24 - line 30 column 5, line 56 - column 6, line 35; figure 1	1-48

-/--

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 July 2017	21/07/2017

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	Mauritz, Jakob

3

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

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 2004/025261 A2 (WELLDOG INC [US]; POPE JOHN [US]; COX RICK [US]; BUTTRY DANIEL [US]) 25 March 2004 (2004-03-25) paragraph [0021]; figure 1	17,41
A	LEMOINE F ET AL: "SIMULTANEOUS CONCENTRATION AND VELOCITY MEASUREMENTS USING COMBINED LASER-INDUCED FLUORESCENCE AND LASER DOPPLER VELOCIMETRY: APPLICATION TO TURBULENT TRANSPORT", EXPERIMENTS IN FLUIDS, SPRINGER, HEIDELBERG, DE, vol. 20, no. 5, 1 March 1996 (1996-03-01), pages 319-327, XP000689340, ISSN: 0723-4864, DOI: 10.1007/BF00191013 abstract	1-48
A	US 4 483 614 A (ROGERS PHILIP L [US]) 20 November 1984 (1984-11-20) column 15, line 1 - line 5 column 13, line 45 - column 14, line 65; figures 8,9 column 7, line 20 - line 33; figure 3	1-48

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2017/050943

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4596036 A	17-06-1986	NONE	
DE 19836183 A1	18-03-1999	NONE	
WO 2004025261 A2	25-03-2004	AU 2003272357 A1	30-04-2004
		WO 2004025261 A2	25-03-2004
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