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(54) Title of the Invention: **Apparatus and methods for use in measuring a luminescent property**
Abstract Title: **Measuring a luminescent property of a sample using a dual-modulated excitation beam**

(57) Apparatus for use in measuring a luminescent property such as phosphorescence of a sample comprises a light emitting diode (LED) 18 for emitting radiation to excite the sample 12. Driver circuitry 20 generates a control signal for modulating the intensity of the emitted radiation from the LED 18. The control signal has a first component at a first frequency with a period that is less than, or of the same order as, an expected characteristic time constant, such as a decay constant, of the luminescent property. The control signal has a second component at a second frequency with a period that is greater than the expected characteristic time constant of the luminescent property. A photodiode 22 receives the radiation luminesced from the sample 12 as a result of the excitation and detector circuitry 24 generates a detection signal representing an intensity of the received radiation, and demodulates the detection signal to produce a signal representing the luminescent property of the sample. Measurement of phosphorescence can be made independently of background, reflection and fluorescence effects.

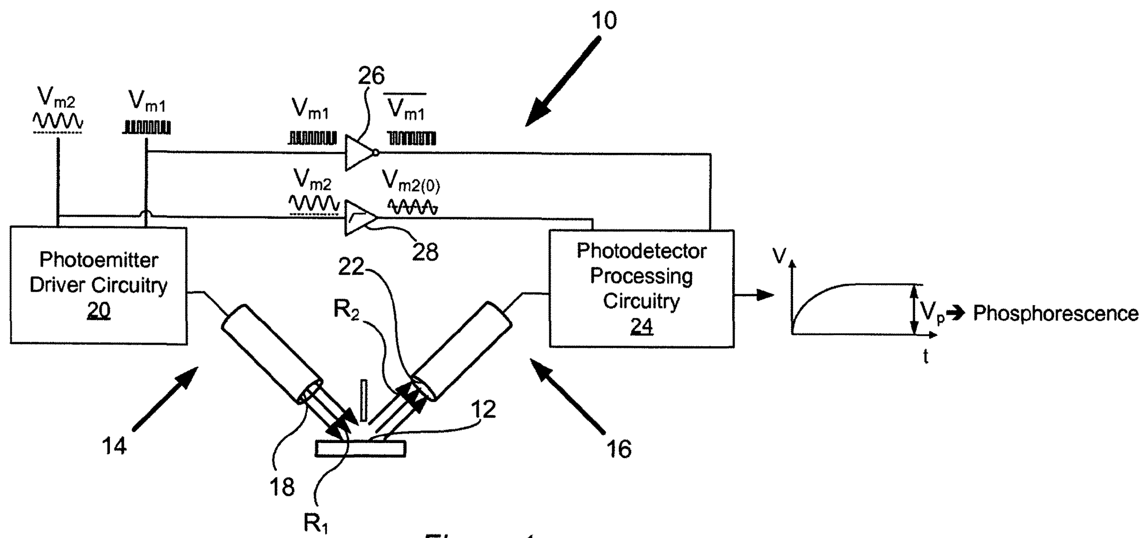

Figure 1

GB 2492950 A

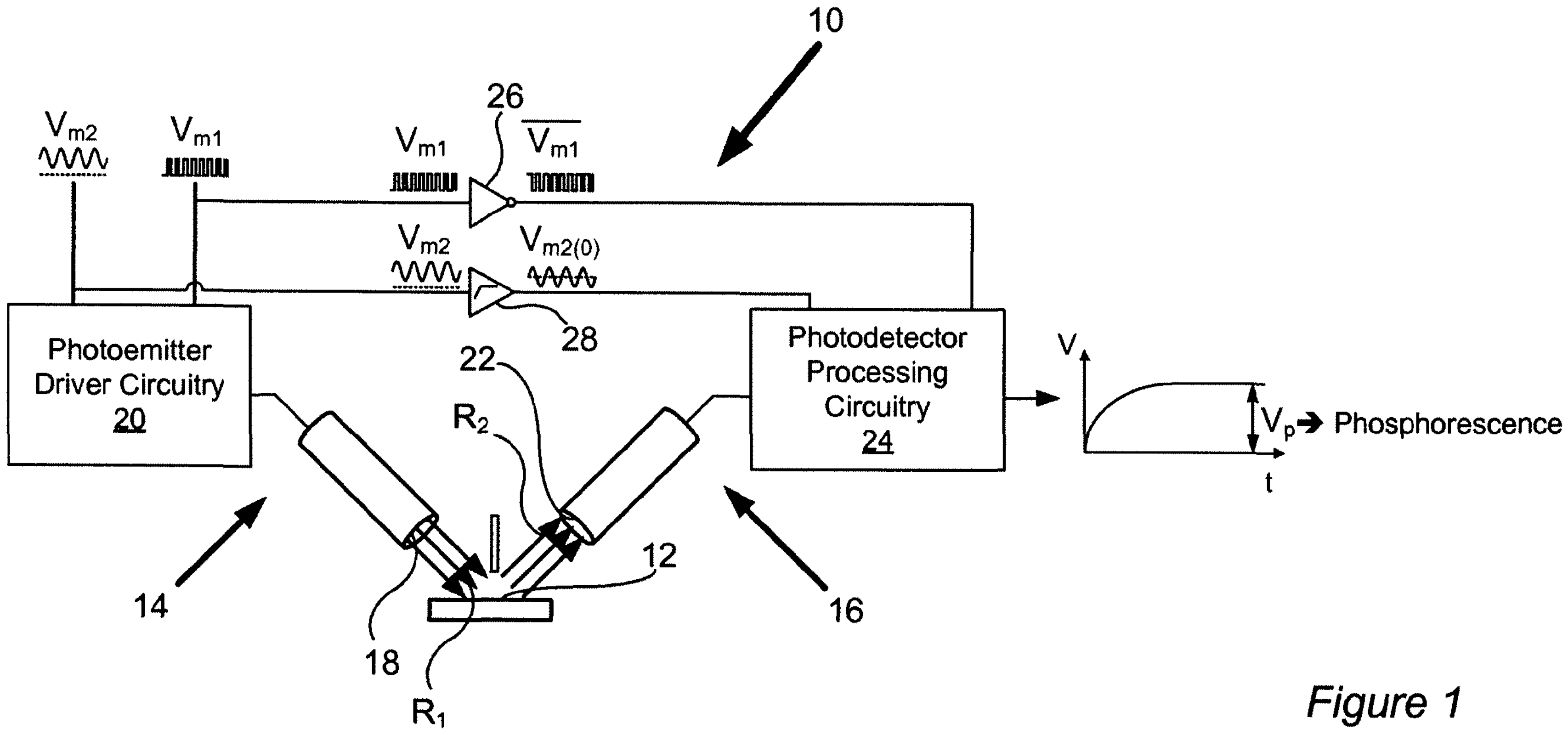
10
26
28
22
R2
R1
12
14
16
18
Vm2
Vm1
Vm1
Vm1
Vm2
Vm2(0)
Photoemitter Driver Circuitry 20
Photodetector Processing Circuitry 24
V
t
Vp → Phosphorescence

Figure 1

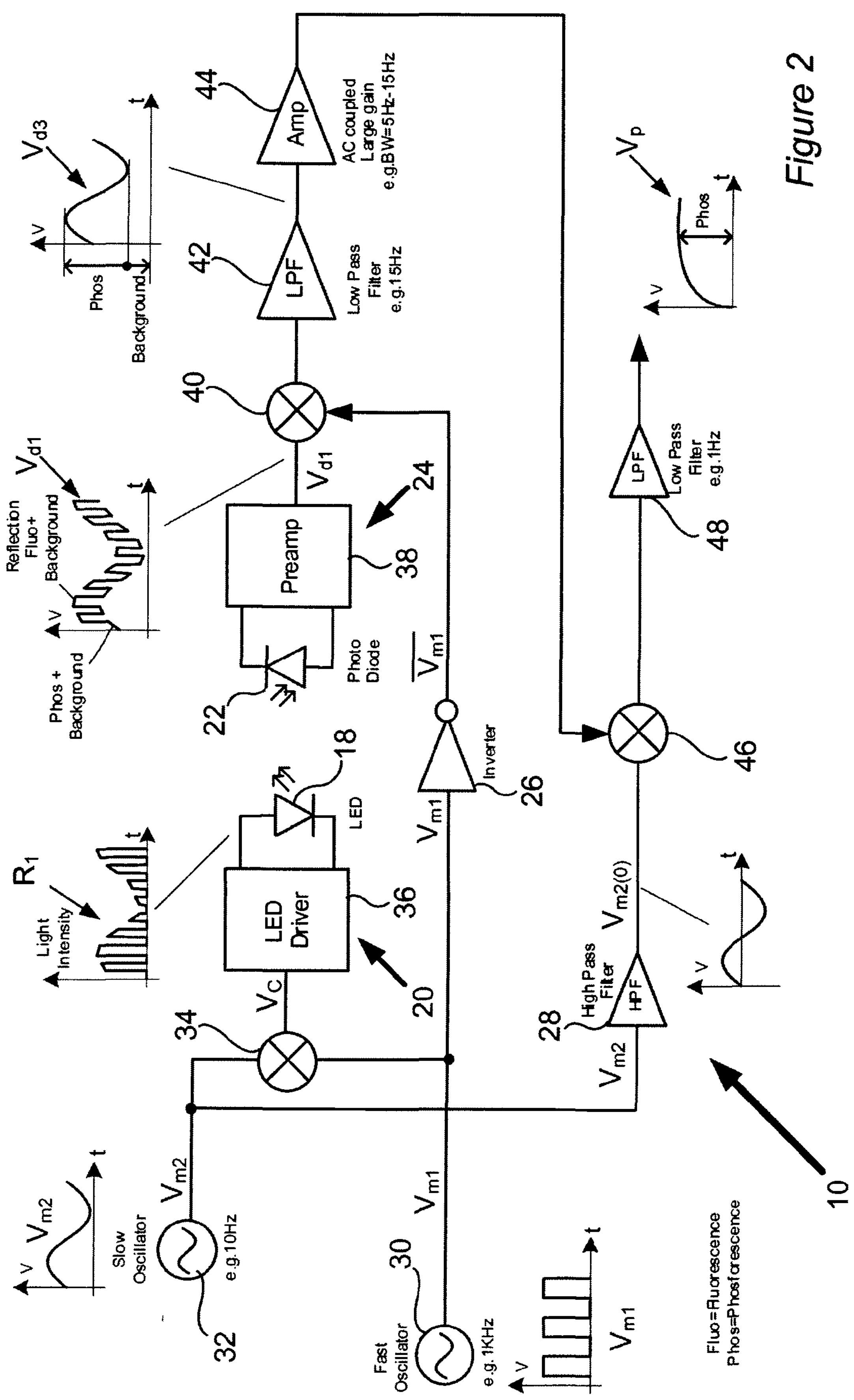
Slow Oscillator
e.g.10Hz
32
V_{m2}
Fast Oscillator
e.g.1KHz
30
V_{m1}
34
V_C
LED Driver
36
20
LED
18
Light Intensity
R_1
Inverter
26
$\overline{V_{m1}}$
Photo Diode
22
Preamp
38
24
V_{d1}
Reflection Fluo+ Background
Phos + Background
40
LPF
Low Pass Filter e.g.1.5Hz
42
Amp
AC coupled Large gain e.g.BW=5Hz-15Hz
44
V_{d3}
Phos
Background
HPF
High Pass Filter
28
$V_{m2(0)}$
46
LPF
Low Pass Filter e.g.1Hz
48
V_p
Phos
V
t
Fluo=Fluorescence
Phos=Phosforescence
10

Figure 2

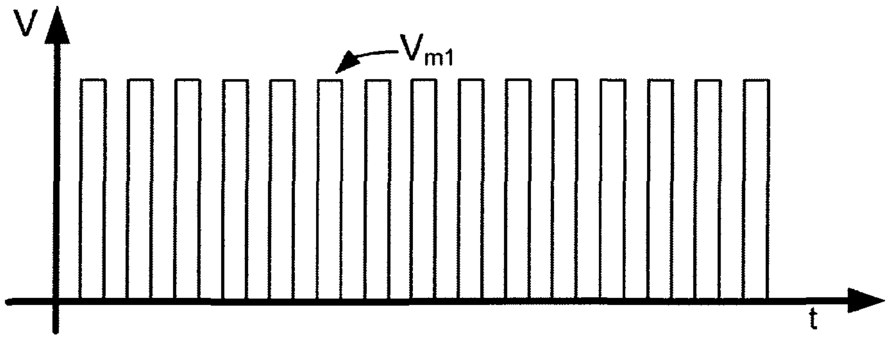

Figure 3a

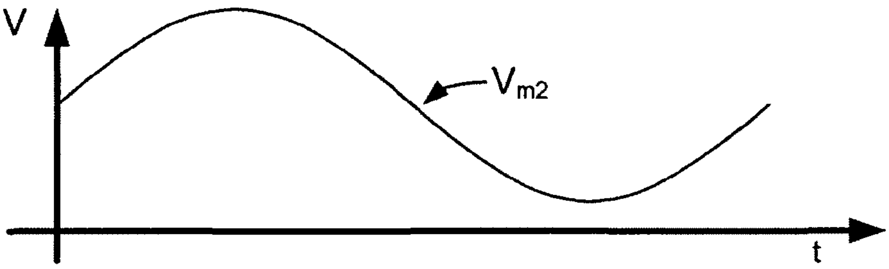

Figure 3b

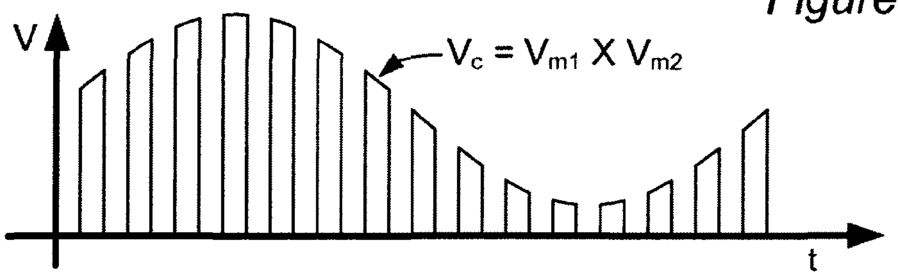

Figure 3c

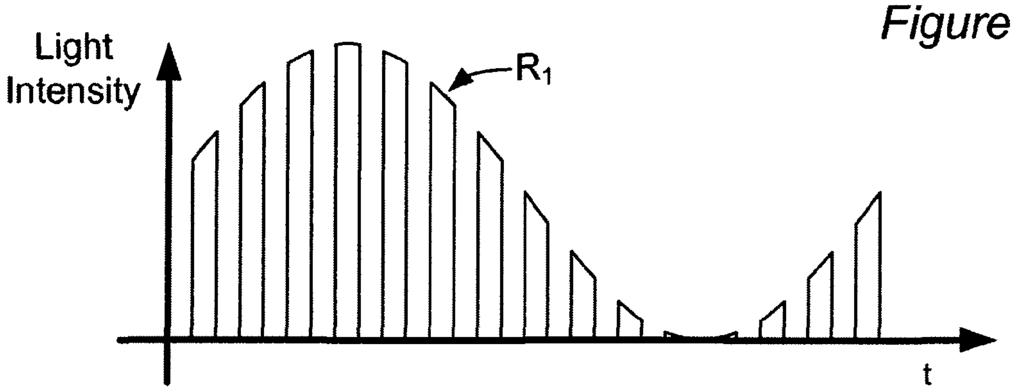

Figure 3d

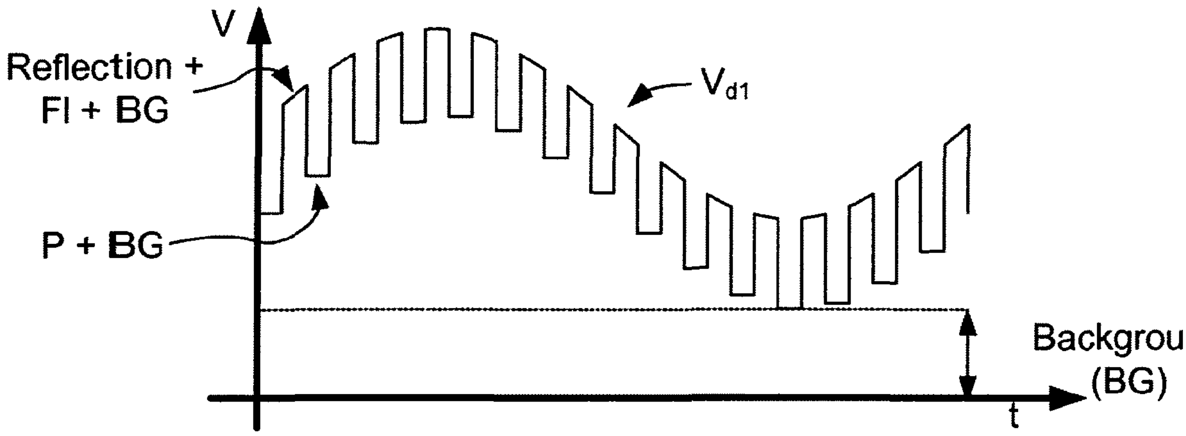

Figure 4a

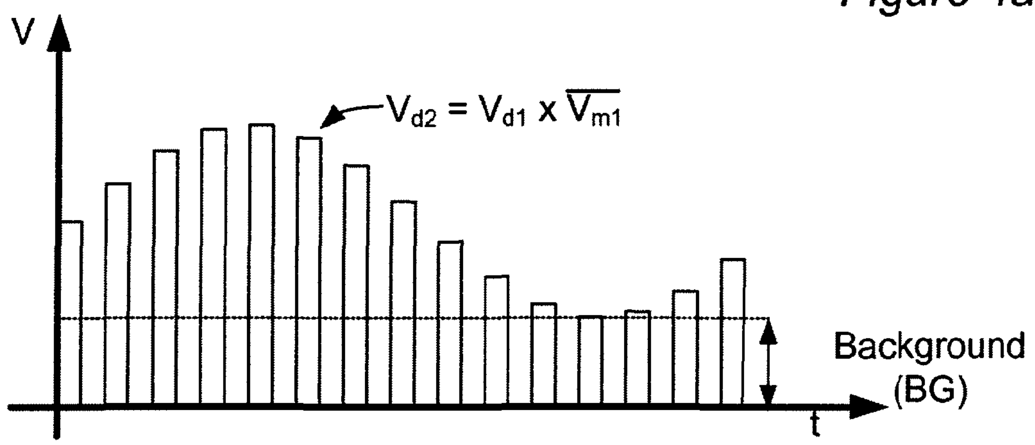

Figure 4b

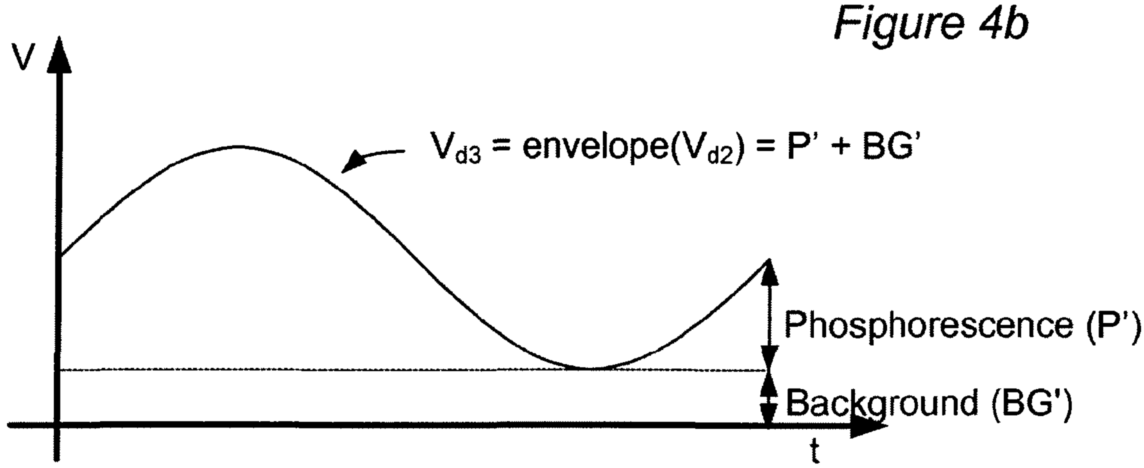

Figure 4c

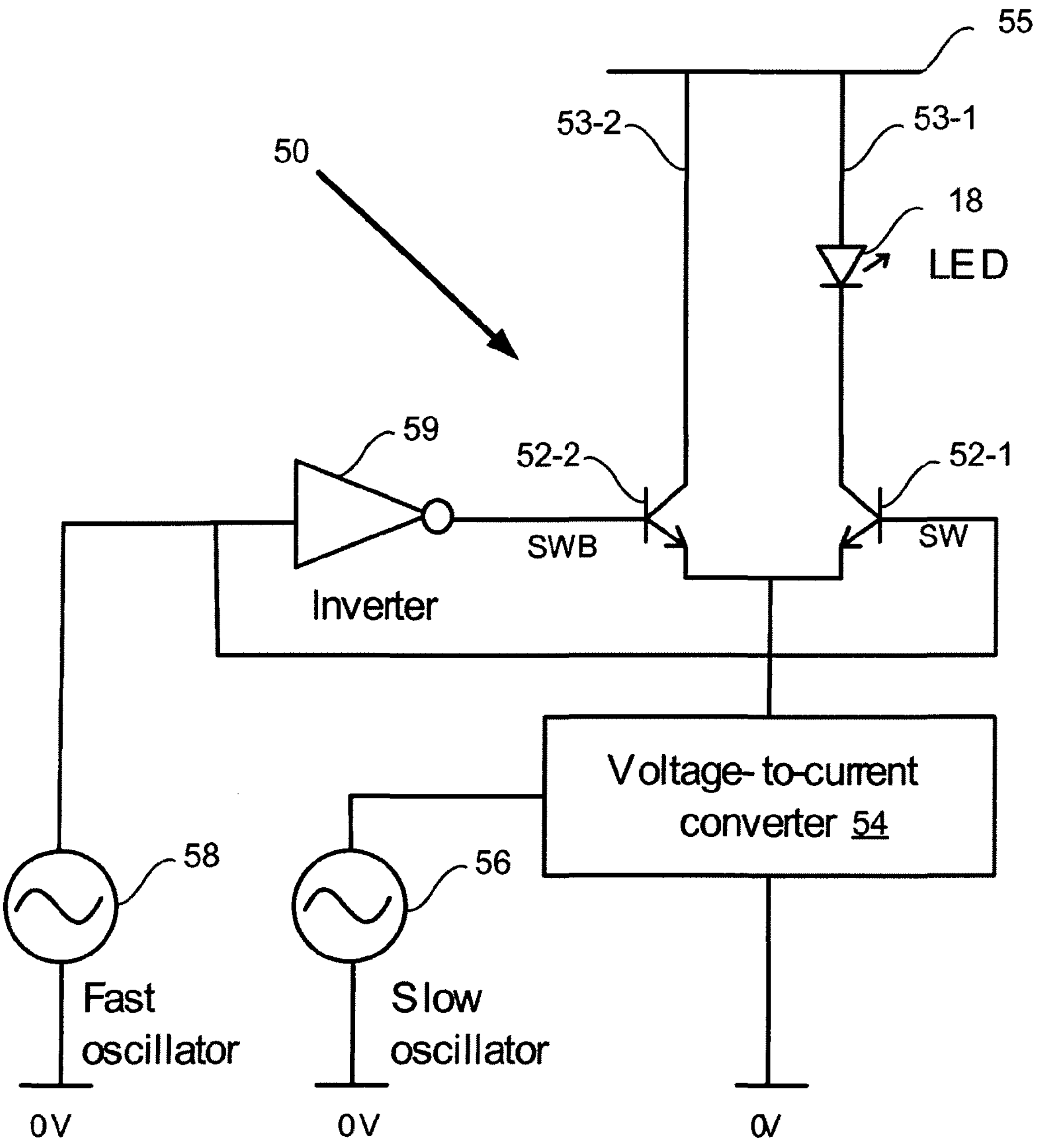
55
53-2
53-1
50
18
LED
59
52-2
52-1
SWB
SW
Inverter
Voltage- to-current
converter 54
58
56
Fast
oscillator
Slow
oscillator
0V
0V
0V

Figure 5

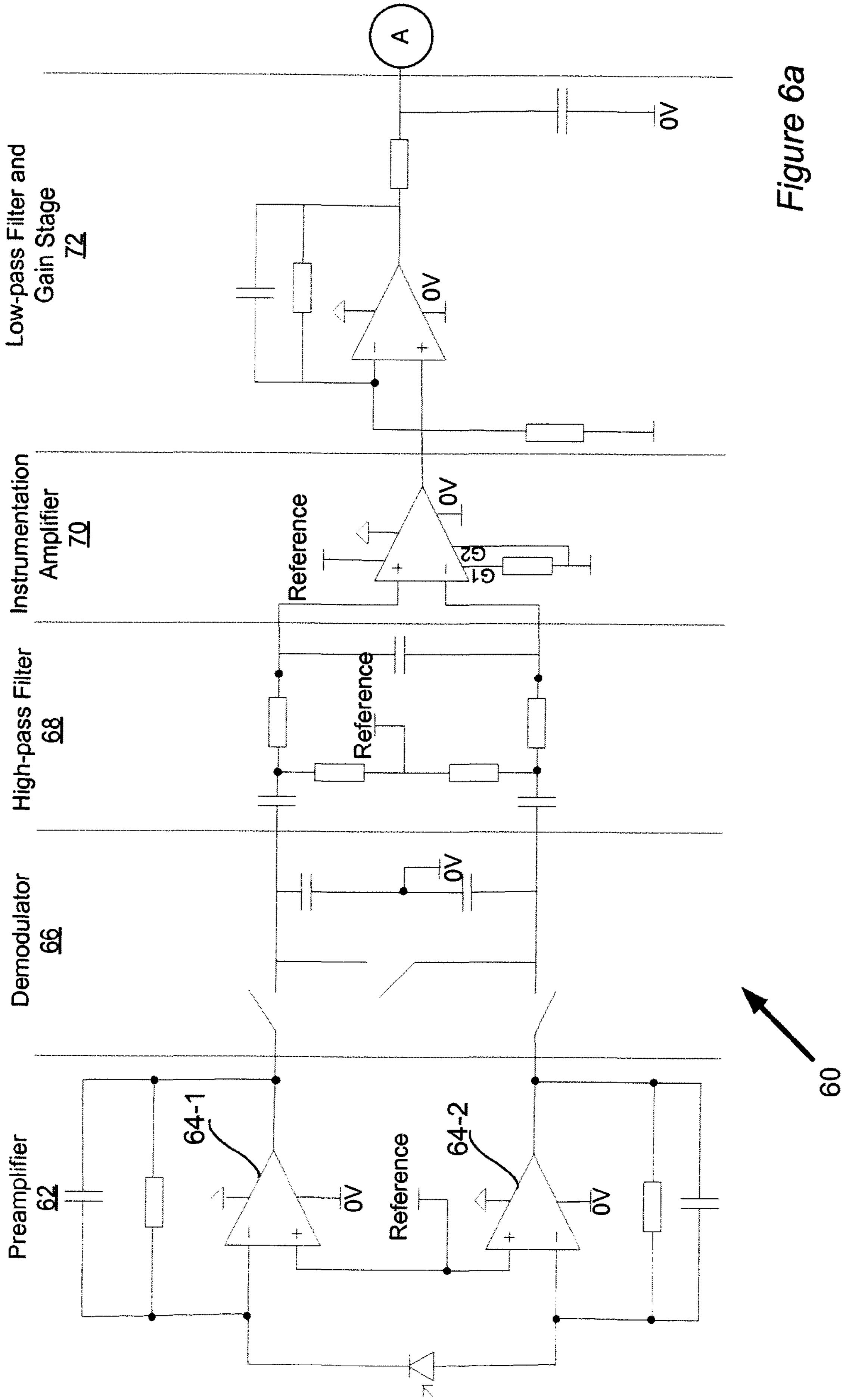
Preamplifier
62
64-1
64-2
Reference
0V
Demodulator
66
High-pass Filter
68
Reference
Instrumentation
Amplifier
70
G1
G2
Low-pass Filter and
Gain Stage
72
A
60

Figure 6a

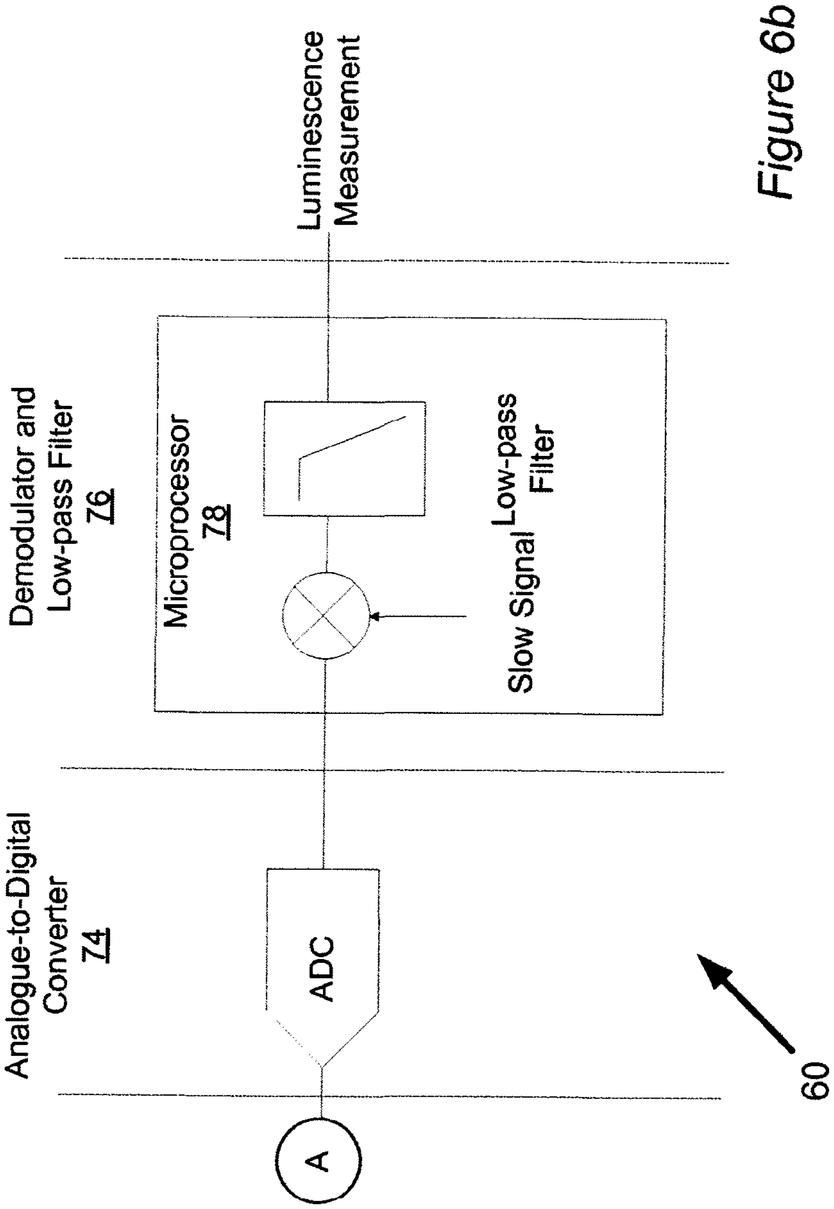
Analogue-to-Digital Converter 74
Demodulator and Low-pass Filter 76
Microprocessor 78
Luminescence Measurement
A
ADC
Slow Signal
Low-pass Filter
60

Figure 6b

Apparatus and methods for use in measuring a luminescent property

The present invention relates to apparatus and associated methods for use in measuring a luminescent property of a sample. In particular, the present invention relates to apparatus and associated methods for use in measuring a time resolved luminescent property of the sample, for example a time resolved fluorescent property such as phosphorescence.

The present invention has particular benefits in, but is not limited to, the measurement of phosphorescent (time-resolved fluorescence / luminescence) properties of samples used in biological assays or the like.

It is known to use biological assays in which optical characteristics of a sample are modified by the presence of an analyte. The change in the optical properties can then be detected by an appropriately calibrated reader. Commonly, for example, a change in colour or absorption properties of the sample occurs in the presence of the analyte. Whilst such changes are relatively straightforward to measure they can lack the required sensitivity. Greater sensitivity may be achieved using assays in which the change in optical properties may be detectable as a change in phosphorescent properties of the sample because the phosphorescence may be distinguished from other optical effects by its temporal characteristics.

The measurement of phosphorescence may be achieved by intermittent optical excitation at one wavelength (for example in the ultra-violet region) and subsequent measurement of the decaying luminescence signal at another wavelength (usually in the visible region). In this way phosphorescence, which has a relatively long decay time (typically anywhere in the region of micro-seconds to seconds), may be distinguished from any fluorescence, which has a relatively short decay time (e.g. typically less than 1 micro-second). However, this requires precise synchronisation between the illumination of the sample and the detection of the resulting luminescence within the reader. Moreover, the intensity of the phosphorescence may be several orders of magnitude less than the intensity measured by absorption-change readers, complicating the electronic design significantly. These practical difficulties have limited the applicability of phosphorescence-inducing assays in the increasing number of applications that require a low-cost reader. Examples include point-of-care and home diagnostic devices based on luminescent immunoassays. These include, amongst others, products to detect cardiovascular conditions and a range of infectious diseases.

Accordingly, the present invention seeks to provide apparatus and associated methods for use in measuring a luminescent property of a sample that overcome or at least partially mitigate the above problems.

According to one aspect of the present invention there is provided apparatus for use in measuring a luminescent property of a sample, the apparatus comprising: an excitation light source for emitting radiation to excite said sample, under the control of a control signal; a signal source for generating said control signal for modulating an intensity of said emitted radiation, said control signal having a first component at a first frequency having a period that is less than, or of the same order as, an expected characteristic time constant of said luminescent property, and a second component at a second frequency having a period that is greater than said expected characteristic time constant of said luminescent property; a photodetector for receiving radiation luminesced from said sample as a result of said excitation, and for generating a detection signal representing an intensity of said received radiation; and a demodulator for demodulating said detection signal whereby to produce a signal representing said luminescent property of said sample.

The luminescent property of the sample may comprise a photoluminescent property of the sample, for example a persistent luminescent property such as a phosphorescent property or the like. The characteristic time constant may be a decay time constant.

The optical filter may be provided between the radiation emitting means and the sample (and/or between the radiation receiving means and the sample), which optical filter may be configured to block radiation having a wavelength associated with the luminescent property to be measured. The optical filter may comprise a UV-pass filter or any other suitable filter.

The radiation may comprise ultraviolet radiation having a wavelength of around 365nm, or around 265nm. The radiation may, however, comprise ultraviolet radiation having a wavelength anywhere in the range of about 400 nm down to about 10 nm (energies of about 3eV to about 124eV). The radiation may, for example be radiation in a 'near' UV spectral region having a wavelength of between about 400nm and about 300nm (~3.10eV to ~4.13 eV), in a 'middle' UV region having a wavelength of between about 300nm and about 200nm (~4.13eV to ~6.20eV), in a 'far' UV region having a wavelength of between about 200nm and about 122nm (~6.20eV to ~10.2eV), and/or in a in a 'extreme' UV region having a wavelength of between about 121 nm and about 10 nm (~10.2eV to ~124eV). The radiation may, for example, be UVA radiation having a wavelength of between about 400nm and about 315nm (~3.10eV to ~3.94eV), UVB radiation having a wavelength of between about 300nm and about 280nm (~3.94eV to ~4.43eV), UVC radiation having a wavelength of between about 280nm and about 100nm (~4.43eV to ~12.4eV), and/or Vacuum UV ('VUV') radiation having a wavelength of between about 200nm and about 10nm (~6.2eV to ~124eV).

According to another aspect of the present invention there is provided apparatus for exciting a sample with radiation whereby to allow measurement of a luminescent property of the sample, the apparatus comprising: an excitation light source for emitting radiation to excite said sample under the control of a control signal; and a signal source for generating a control signal for modulating an intensity of said emitted radiation, said control signal having a first component at a first frequency having a period that is less than, or of the same order as, an expected characteristic time constant of said luminescent property, and a second component at a second frequency having a period that is greater than said expected characteristic time constant of said luminescent property.

The radiation emitting means may have a threshold voltage such that when said control signal exceeds the threshold voltage the radiation emitting means is in an 'ON' state in which it emits radiation, and when the control signal does not exceed said threshold voltage the radiation emitting means is in an 'OFF' state in which it does not emit radiation.

The first component of said control signal may be derived from a first periodic signal. The first periodic signal may oscillate between at least two different discrete voltage levels. In some implementations, a lower magnitude one of said voltage levels of said first periodic signal may have a magnitude below approximately 1V and may be a substantially zero (or near zero) voltage level. The second component of the control signal may be derived from a second periodic signal.

The first and second periodic signals may be such that said control signal may cause the radiation emitting means to switch between an 'ON' state in which it emits light and an 'OFF' state in which it does not emit light at a frequency approximately equal to a frequency of said first periodic signal. It will be appreciated, however, that whilst the switching frequency may be substantially equal to that at which the radiation emitting means switches it need not be exactly equal to that of the first periodic signal. For example, the switching frequency may be a function of both the 'fast' first periodic frequency 'f_{fast}' signal and the 'slow' second periodic signal frequency 'f_{slow}'. In the case of fast and slow sinusoidal periodic signals, for example, the radiation emitting means will switch at a frequency having components at the frequencies $f_{fast} \pm f_{slow}$.

The second periodic signal may be a sinusoidal signal. The second periodic signal may oscillate between at least two different discrete voltage levels. The second periodic signal may be offset such that the signal remains at the same polarity throughout its cycle, which offset may be such that the minimum magnitude of the signal is non-zero.

In some implementations, where the second periodic signal comprises a plurality of discrete voltage levels, one of the voltage levels of said second periodic signal (e.g. a minimum magnitude level) may have a magnitude below approximately 1V, for example a substantially zero (or near zero) voltage level.

The radiation emitting means may comprise a light emitting diode, for example a light emitting diode that is operable to emit radiation in ultra violet region.

According to another aspect of the present invention there is provided apparatus for detecting radiation luminesced from a sample whereby to allow measurement of a luminescent property of the sample, the apparatus comprising: a photodetector for receiving radiation luminesced from said sample as a result of excitation of said sample with radiation from a radiation source, and for generating a detection signal representing an intensity of said received radiation, wherein: said radiation from said radiation source is modulated by a control signal having a first component at a first frequency having a period that is less than, or of the same order as an expected characteristic time constant of said luminescent property, and a second component at a second frequency having a period which is greater than the expected characteristic time constant of said luminescent property; and a demodulator for demodulating said detection signal whereby to produce a signal representing said luminescent property of said sample.

The demodulator may comprise a first (e.g. 'fast') demodulation arrangement for demodulating a first (e.g. 'high') frequency component of said detection signal, which first frequency component may be associated with said first component of the control signal. The demodulator may comprise a second (e.g. 'slow') demodulation arrangement for demodulating a second (e.g. 'low') frequency component of said detection signal, which second frequency component may be associated with said second component of the control signal.

The first demodulation arrangement may be operable to suppress (e.g. inhibit or 'switch out') a first component of said detection signal arising when said sample is being excited (e.g. when the radiation source is in an 'ON' state / emitting radiation). The first component of said detection signal may arise, at least in part, from fluorescence from said sample. The first component of said detection signal may arise, at least in part, from short time-constant phosphorescence.

The first demodulation arrangement may be operable not to suppress, inhibit, or switch out, a second component of said detection signal which may arise when said sample is not being excited (e.g. when the radiation source is in an 'OFF' state / not emitting radiation). The second component of the detection signal may arise, at least in part, from persistent luminescence (e.g. phosphorescence) from said sample.

The first demodulation arrangement may comprise a switched demodulator arrangement arranged to switch said detection signal 'on' and 'off' whereby to demodulate said signal. The first demodulation arrangement may comprise means for switching the gain of the generating means between a high gain and a low gain whereby to demodulate said signal. The first demodulation arrangement may further comprise a filter for filtering out a remaining component at the first frequency from the detection signal. The filter comprises a low-pass filter.

The second demodulation arrangement may be operable to demodulate the second frequency component of the detection signal as demodulated by said first demodulation arrangement (e.g. a version of the detection signal that has been demodulated to suppress the first frequency component(s)).

The demodulating means may further comprise an amplifier for amplifying the detection signal as demodulated by said first demodulation arrangement. The second demodulation arrangement may be operable to demodulate the second frequency component of the detection signal as demodulated by said first demodulation arrangement and/or as amplified by said amplifier. The amplifier may comprise a large gain (and/or ac coupled) amplifier.

The second demodulation arrangement may comprise a multiplier (e.g. a mixer) for multiplying the detection signal with a signal having a frequency substantially equal to that of the second frequency component. The second demodulation arrangement may comprise an envelope detector operable to suppress the second frequency component of the detection signal. The second demodulation arrangement may comprise a filter for filtering out a remaining component at the second frequency from the detection signal. The filter for filtering out the remaining component at the second frequency may comprise a low-pass filter.

Where the detection signal is mentioned herein, it will be appreciated that the detection signal referred to may be the detection signal as detected (e.g. prior to an demodulation / filtering / amplification or the like) or as processed or partially processed by a demodulator, filter, amplifier and/or the like

According to another aspect of the present invention there is provided a method of for generating a signal representing a luminescent property of a sample for use in measuring said luminescent property of the sample, the method comprising: generating a control signal having a first component at a first frequency having a period that is less than, or of the same order as, an expected characteristic time constant of said luminescent property, and a second component at a second frequency having a period that is greater than said expected characteristic time constant of said luminescent property; emitting radiation to excite said sample, under the control of said control signal, such that said emitted radiation is modulated by the control signal; receiving radiation luminesced from said sample as a result of said excitation, and generating a detection signal representing an intensity of said received radiation; and demodulating said detection signal whereby to produce a signal representing said luminescent property of said sample.

According to another aspect of the present invention there is provided a method of exciting a sample with radiation whereby to allow measurement of a luminescent property of the sample, the method comprising: generating a control signal having a first component at a first frequency having a period that is less than, or of the same order as, an expected characteristic time constant of said luminescent property, and a second component at a second frequency having a period that is greater than said expected characteristic time constant of said luminescent property; and emitting radiation to excite said sample, under the control of said control signal, such that said emitted radiation is modulated by the control signal.

According to another aspect of the present invention there is provided a method of generating a signal representing a luminescent property of a sample for use in measuring said luminescent property of the sample, the method comprising: receiving radiation luminesced from said sample as a result of excitation of said sample with radiation from a radiation source and generating a detection signal representing an intensity of said received radiation, wherein: said radiation from said radiation source is modulated by a control signal having a first component at a first frequency having a period that is less than, or of the same order as, an expected characteristic time constant of said luminescent property, and a second component at a second frequency having a period which is greater than the expected characteristic time constant of said luminescent property; and demodulating said detection signal whereby to produce a signal representing said luminescent property of said sample.

In one embodiment of the invention, there is provided a detector for the measurement of the phosphorescence of a sample. The detector comprises: an ultra-violet LED suitable for optical excitation, an electronic circuit that is able to modulate the brightness of the LED in response to a control signal, a controller that generates a modulating signal that is the product of two oscillations, one fast (or possibly of the same order) and one slow relative to the phosphorescence decay time, a photodiode detector that measures the brightness of the luminescence, and a processor that demodulates the detector signal with respect to first high and then low frequency control signals, resulting in an output that depends only upon the phosphorescence of the sample.

This detector has the potential to overcome or at least mitigate problems with the known detectors. Firstly, the use of fast modulation of the source helps to ensure that the intensity of the phosphorescence is maximised. Secondly, the use of a second slow modulation helps to simplify the electronic circuitry required to measure the signal associated with phosphorescence even when it is relatively small. Thirdly, the dual-modulation approach can help to enable a measurement of phosphorescence to be made independently of background and other parasitic effects such as ambient lighting, photodiode leakage and/or amplifier offsets.

The fast component of the modulation signal may oscillate between two levels, one of which may be zero or near-zero. Advantageously, this has the potential to simplify isolation of the phosphorescence information, typically with a switched demodulator.

The slow modulation signal may be sinusoidal in nature and is demodulated with a linear multiplier. Advantageously, this has the potential to enable high sensitivity without excessively long measurement times.

The slow component of the modulation signal may oscillate between two levels, one of which may be zero or near-zero. Advantageously this has the potential to allow a simplified switched demodulation to be used when the required measurement bandwidth is low.

The slow modulation frequency may advantageously be selected so as to be distinct from dynamic sources of interfering light, for example, interfering light such as can be produced by incandescent bulbs or fluorescent tubes, thereby providing the potential to minimise sensitivity of the apparatus to ambient light.

The invention will now be described by way of example only with reference to the attached figures in which:

Figure 1 shows a simplified overview of measurement apparatus for measuring phosphorescent properties of a sample;

Figure 2 shows a simplified circuit schematic illustrating key components of the measurement apparatus of Figure 1;

Figures 3a to 3d and 4a to 4c show simplified and idealised illustrations of electronic/light waveforms that may be observed at different parts of the circuit of Figure 2;

Figure 5 shows a simplified circuit schematic illustrating photoemitter drive circuitry for an embodiment of the invention; and

Figures 6a and 6b show a simplified circuit schematic illustrating photoreceiver circuitry for an embodiment of the invention.

Overview

Figure 1 shows a simplified overview of measurement apparatus 10 for measuring phosphorescent (time-resolved fluorescence/luminescence) properties of a sample 12.

As seen in Figure 1 the measurement apparatus 10 comprises photo-emitter apparatus 14 for exciting the sample 12 with radiation of a desired wavelength, and photodetector apparatus 16 for detecting photoluminescence arising as a result of the excitation.

The photo-emitter apparatus 14 comprises a radiation emitting device 18 for emitting radiation R_1 to excite the sample 12. In this embodiment the radiation emitting device 18 comprises a light emitting diode ('LED') that emits light in the ultraviolet ('UV') region.

The UV LED 18 is driven by driver circuitry 20 that is arranged to modulate the intensity of the radiation emitted by the LED 18. The driver circuitry 20 receives a high frequency input signal (V_{m1}) and a low frequency input signal (V_{m2}), which the circuitry 20 multiplies together to generate a control signal for controlling the intensity of the UV radiation emitted by the LED 18.

The photodetector apparatus 16 comprises a radiation receiving device 22 arranged to receive radiation R_2 from the sample 12 and to generate a signal representing the received radiation. In this embodiment, the radiation receiving device 22 comprises a photodiode in the form of a P-type/intrinsic/N-type (PiN) diode. The received radiation of this embodiment is in the visible spectral region and typically comprises a plurality of components including, for example, light that is luminesced by the sample 12 as a result of the excitation by radiation from the LED 18 (e.g. fluorescence and/or phosphorescence), light reflected by the sample 12, and/or background/ambient (parasitic) light.

The signal representing the radiation received by the photodiode 22 is processed by *photodetector processing circuitry* 24. The *photodetector processing circuitry* 24 demodulates the signal representing the received radiation using an inverted version ($\overline{V_{m1}}$) of the high frequency input V_{m1} (produced by an inverter 26) and a zero centred version ($V_{m2(0)}$) of the low frequency input V_{m2} (produced by a high pass filter 28), to remove components of the signal associated with fluorescence, reflection and background/ambient light. Thus, as will be described in more detail below, the photodetector processing circuitry 24 extracts a signal 'V_P' representing the magnitude of the phosphorescent component of the light received by the photodiode 22, excluding components of the received light associated with fluorescence, reflection and background/ambient light.

Circuit Implementation

Figure 2 shows a simplified circuit schematic illustrating key components of the photoemitter driver circuitry 20 and photodetector processing circuitry 24 of Figure 1 according to one possible implementation. Figures 3a to 3d show, by way of illustration only, electronic signals at different points in the photoemitter driver circuitry 20, and the time varying intensity of the radiation emitted by the LED 18. Figures 4a to 4c show, by way of illustration only, signals at different points in the photodetector processing circuitry 24.

As seen in Figure 2, a pair of oscillators 30 and 32 is provided for respectively generating the high frequency input signal (V_{m1}) and a low frequency input signal (V_{m2}).

The high frequency, or 'fast', oscillator 32 of this embodiment is configured to generate a high frequency input signal (V_{m1}), as illustrated in Figure 3a, comprising a two-level waveform (for example a square-wave). In this embodiment the high frequency waveform is at a frequency that is significantly higher than the reciprocal of the expected phosphorescence equivalent time constant for the sample 12 being tested. However, it will be appreciated that in some embodiments the high frequency waveform may have a frequency that is comparable to the reciprocal of the expected phosphorescence equivalent time constant for the sample 12 being tested. In this embodiment, V_{m1} has an exemplary frequency of 1kHz. The high frequency two-level wave, V_{m1}, produced by the fast oscillator 30 has a lower level that is substantially zero volts (or sufficiently low so to avoid the LED 18 becoming illuminated) and a high level that is sufficient, when V_{m1} and V_{m2} are multiplied, to ensure that the LED 18 is illuminated when V_{m1} is high.

The low frequency, or 'slow', oscillator 32 of this embodiment is configured to generate a low frequency input signal (V_{m2}), as illustrated in Figure 3b, comprising a sinusoidal waveform with a frequency that is significantly lower than the reciprocal of the expected phosphorescence equivalent time constant for the sample 12 being tested. Advantageously, the frequency is selected to be distinct from the frequency of any sources of interfering light (commonly harmonics of the mains supply frequency). In this embodiment, V_{m2} has an exemplary frequency of 10Hz. The low frequency sinusoid, V_{m2}, produced by the slow oscillator 32 has an offset such that the waveform remains positive throughout its wavelength. Typically, the magnitude of the offset is sufficiently high to ensure that, when V_{m1} and V_{m2} are multiplied, the LED 18 will be illuminated when V_{m1} is high (although this need not always be the case).

The signals V_{m1} and V_{m2} are inputted to a mixer 34 that is configured to produce an output signal (V_C), as illustrated in Figure 3c, comprising the product of V_{m1} and V_{m2}. The output signal, V_C, from the mixer 34 is provided as an input to a driver circuit 36 that is configured to generate an output current that is proportional to its input signal, V_C, for driving the LED 18.

The LED 18 emits light R_1, onto the sample 12, which has an intensity that is substantially proportional to the current that it is driven with, as illustrated in Figure 3d. Accordingly, the LED 18 emits light R_1 having a generally sinusoidal intensity 'envelope' that varies slowly, relative to the reciprocal of the expected phosphorescence equivalent time constant for the sample 12 being tested, at the frequency of V_{m2}. Within this sinusoidal 'envelope' the emitted light R_1 switches on and off intermittently at the high frequency.

The sample 12 photoluminesces, as a result of excitation by the incident light R_1, to produce a time-varying photoluminescence waveform, which is detected by the photodiode 22 along with light from other, parasitic, sources (e.g. as a result of reflection and/or background/ambient light). The photodiode 22 generates a time-varying current that is input to a photodetector circuit, comprising a preamplifier 38, that produces a time-varying signal (V_{d1}) that is substantially proportional to the light R_2 detected by the photodiode 22 (as illustrated in Figure 4a).

The intensity waveform detected by the photodiode detector circuit has components due to several physical effects. During the short time periods when V_{m1} is zero (or at least sufficiently below the threshold voltage of the LED 18 to avoid illumination), the LED 18 is not illuminated and, accordingly, the sample 12 is not excited. Any fluorescence resulting from the previous excitation while V_{m1} was high is very short-lived, decaying almost instantaneously compared to the time during which V_{m1} remains low. Accordingly, the light R_2 emitted from the sample, and detected by the photodiode 22, is associated with phosphorescence, and any background light arising, for example, from ambient light. During the short time periods when V_{m1} is zero, therefore, the time-varying signal V_{d1}, includes a component (P) arising from the phosphorescence and a component (BG) arising from the background light and other parasitic effects such as leakage of the photodiode 22. The phosphorescence component of V_{d1}, P, remains approximately constant because the period of V_{m1} is short by comparison to the phosphorescence decay time, but is modulated by the excitation intensity when the LED 18 is illuminated.

During the short time periods when V_{m1} is high, the LED 18 is illuminated and, accordingly, the sample 12 is excited. During these periods, therefore, the light R_2 emitted from the sample, and detected by the photodiode 22, is associated with fluorescence, reflection, and any background light. Accordingly, during the short time periods when V_{m1} is high, the time-varying signal V_{d1}, includes: a component (FL) arising from the fluorescence; a component (BG) arising from the background light and other parasitic effects such as leakage of the photodiode 22; and a component associated with reflection of the incident light R_1. The response of the photodiode 22 when the LED 18 is illuminated is relatively large and, because the fluorescence decay time is relatively short compared to the period of V_{m1}, tracks the sinusoidal envelope of the light waveform emitted by the LED 18.

In order to demodulate the high frequency component of the time-varying signal V_{d1}, a high frequency switched demodulator arrangement is used. In the switched demodulator arrangement, the high frequency input signal V_{m1} is inverted by the inverter 26, and the resulting inverted signal, $\overline{V_{m1}}$, fed to mixer 40 where it is multiplied by the output signal V_{d1} from the preamplifier circuit 38 to produce an output signal (V_{d2}) in which the components (FL and BG) arising when the LED 18 is illuminated are removed (as illustrated in Figure 4b). It will be appreciated that appropriate delay components may be added to the circuit to ensure that the 'lows' (typically zeros) of the inverted signal, $\overline{V_{m1}}$, are synchronised with the 'highs' of the preamplifier output signal V_{d1} to ensure that the components arising when the LED 18 is illuminated are suppressed/inhibited successfully whilst the components arising when the LED 18 is not illuminated are not suppressed/inhibited but are instead passed to the next part of the circuit.

The output signal, V_{d2}, from the mixer 40 is then filtered (or 'averaged') by a low pass filter 42 to remove the switching component, effectively extracting the envelope of the mixer output signal, V_{d2}. The resulting wave-form (V_{d3}) includes a component (P') arising from the phosphorescence and a component (BG') arising from the background light and the other parasitic effects, but no components associated with fluorescence or reflection of the incident light R_1 (as illustrated in Figure 4c). Accordingly, the signal components due to phosphorescence and parasitic effects have effectively been isolated. The low pass filter 42 may comprise any suitable filter circuitry to give an appropriate filter characteristic. Typically, however, the cut-off frequency of the low pass filter 42 would be at least an order of magnitude or so smaller than the frequency of V_{m1} (in this embodiment an exemplary value of 15Hz is used).

The component, BG', of the low pass filter output, V_{d3}, due to any background radiation and other parasitic effects is thus substantially static, whilst the component, P', arising from the phosphorescence is dynamic (in this case sinusoidal) oscillating at the slow oscillation frequency of V_{m2}. The magnitude of the dynamic component, P', arising from the phosphorescence therefore represents the phosphorescence of the sample 12.

The low pass filter output, V_{d3}, is amplified using an amplifier 44. The amplifier 44 is arranged to amplify signals having a frequency in a region corresponding to that of the low frequency input signal, V_{m2}, with a bandwidth set by the overall measurement bandwidth. In this embodiment the amplifier 44 comprises an AC coupled, large gain, amplifier having an exemplary bandwidth of 5Hz to 15Hz (i.e. centred on the exemplary 10Hz frequency of V_{m2}). Beneficially, because the desired information representing the sample phosphorescence is contained in the magnitude of a sinusoid of known frequency, it is possible to apply a very large gain to the signal without encountering problems associated, for example, with amplifier offset or background magnification.

The low frequency sinusoid, V_{m2}, produced by the slow oscillator 32 is also input to the high-pass filter 28, to remove the static offset component and thereby zero centre the low frequency input signal, V_{m2}, thereby producing a bipolar version, $V_{m2(0)}$. The resulting bipolar version, $V_{m2(0)}$, of the low frequency input signal, V_{m2}, and the output from the large gain amplifier 44 are multiplied together using a mixer comprising a linear multiplier 46. The resultant output signal from the linear multiplier 46 is then filtered, by a further low pass filter 48, using an appropriate measurement bandwidth, to produce a filtered final output signal (V_p) from which the low frequency sinusoidal components of the linear multiplier output signal have, effectively, been eliminated. Accordingly, the magnitude of the final output signal, V_p, output by circuit of Figure 2 represents a measurement of the amplified sinusoid from which information about the phosphorescence of the sample 12 can be extracted independently of any signals associated with the background/ambient light, other parasitic effects, and the fluorescence of the sample. This measurement technique can be made highly sensitive due to the large amplification that may be applied by the large gain amplifier 44.

Figure 5 shows a simplified circuit schematic of an example of photoemitter drive circuitry 50, for another embodiment of the invention, in more detail.

As seen in Figure 5, the driver circuitry comprises a pair of semiconductor switches 52-1, 52-2 which, in this example, comprise bipolar junction transistors (BJTs). The BJTs 52 in this example are NPN type BJTs although it will be appreciated a similar circuit could be adapted to use PNP type BJTs. Each BJT 52 has an emitter that is coupled to the emitter of the other BJT 52, in a manner similar to a ‘long-tailed pair’ arrangement. The emitters of the BJT 52 are coupled to ground (or possibly a negative power rail) via a voltage to current converter 54.

The radiation emitting device 18 being driven by the circuit 50, is provided in a first current branch 53-1 of the circuit 50 between a collector of one of the BJTs 52-1 (the ‘driver’ BJT) and a high voltage rail 55. A collector of the other BJT 52-1 is connected directly to the high voltage rail 55 to provide a second current branch 53-2 of the circuit.

An oscillator 56, operating as a ‘slow’ oscillator as described previously, provides the low frequency input signal V_{m2} to the voltage to current converter 54 to modulate the current through the emitters of the BJTs 52.

An oscillator 58, operating as a ‘fast’ oscillator as described previously, provides the high frequency input signal V_{m1} to a base of one of the BJTs 52-1 and to an inverter 59 to generate an inverted version of the high frequency input signal. The inverted version of the high frequency input signal is provided to the base of the other of the BJTs 52-2. Accordingly, when the signal from the fast oscillator 58 is high, the driver BJT 52-1 is in an ‘ON’ state and the other BJT 52-2 is in an ‘OFF’ state, and the current flows through the first current branch 53-1 thereby driving the radiation emitting device 18. When the signal from the fast oscillator 58 is low, the driver BJT 52-1 is in an ‘OFF’ state and the other BJT 52-2 is in an ‘ON’ state, and the current flows through the second current branch 53-2. Thus, the fast oscillator 58 effectively switches current flow through the emitter coupled ‘tail’ of the BJT pair 52 between one current branch 53-1 to the other current branch 53-2 thereby modulating the current flow through the radiation emitting device 18.

Figures 6a and 6b show a simplified circuit schematic of an example of photoreceiver circuitry 60, for another embodiment of the invention, in more detail.

As seen in Figure 6a, the photoreceiver circuitry 60 comprises a preamplifier circuit stage 62 comprising a pair of operational amplifier circuits 64 arranged to generate a time varying output signal in dependence on the light detected by the photodiode 22. A switched demodulator stage 66, demodulates the signal output from the preamplifier 62 with respect to the high frequency modulation signal.

When the LED 18 is turned off, the two switches at the outputs of the operational amplifiers 64-1 and 64-2 are closed, and the switch connecting the two outputs remains open. In this state, the signal coming from the photodiode is amplified and filtered by the subsequent electronics. When LED 18 is turned on, the two switches at the outputs of the operational amplifiers 64-1 and 64-2 are open and the switch connecting the two outputs is closed. In this state, the signal coming from the photodiode is not propagated further down the signal chain. The two switches at the outputs of the operational amplifiers 64-1 and 64-2 are driven by an inverted version of the fast oscillator, whereas the switch across the two lines is driven by a non-inverted version of the same oscillator.

The demodulated signal from the demodulator 66 is filtered, using a high pass filter stage 68, to remove low frequency and semi-static components (e.g. components below the frequency of the slow oscillator signal such as components associated with background radiation). The filtered output of the high pass filter 68 is then amplified by an amplifier stage 70 and the resulting amplified signal is filtered, by a low pass filter and gain stage 72, to remove remaining high frequency components of the signal (e.g. components above the frequency of the slow oscillator signal). At this stage, the signal is equivalent to the output of the AC coupled, large gain, amplifier 44 described previously.

Referring now to Figure 6b, the output from the low pass filter 72, in this embodiment, is converted into a digital signal using an analogue to digital converter (ADC) 74, and further processing of the signal, to extract the luminescence characteristic of interest, is achieved using a demodulation and low-pass filter stage 76, comprising a microprocessor 78.

In this embodiment, the microprocessor 78 is programmed to multiply the digitised output of the low pass filter 72 together with a zeroed version of the slow input signal. The microprocessor 78 filters the resulting signal product to remove the low frequency sinusoidal components, in a similar manner to that described previously, to produce a result that is representative of the amplitude of the sinusoidal input to the ADC 74. From this resultant signal, the phosphorescence of the sample 12 can be extracted independently of any signals associated with the background/ambient light, other parasitic effects, and the fluorescence of the sample. This measurement technique can be made highly sensitive due to the large amplification that may be applied by the amplifier stage 70.

Modifications and Alternatives

Detailed embodiments have been described above. As those skilled in the art will appreciate, a number of modifications and alternatives can be made to the above embodiment whilst still benefiting from the inventions embodied therein.

An optical filter may, for example, be placed between the LED 18 and the sample 12 (and/or between the photodiode 22 and the sample) such that it blocks light of a similar wavelength to that expected for the phosphorescence. The filter may, for example comprise a UV-pass filter or the like. This arrangement beneficially thereby inhibits luminescence from the radiation emitting side of the apparatus from contaminating the measurement and can therefore help to enhance sensitivity.

In another variation on the described embodiment, the demodulation of the high frequency component of the light detected by the detection circuit may advantageously be achieved by use of a photodiode detector circuit whose gain can be switched between a high mode (when the LED 18 is off) and a low mode (when the LED 18 is on), for example under the control of the inverted high frequency signal. This can be advantageous over the use of the fixed-gain detector circuit, and switched demodulator, described for the above embodiment because it can reduce the detection dynamic range and simplify the implementation.

It will be appreciated that whilst the signals have been described as having frequencies of 10Hz and 1kHz, the frequencies may be any suitable value. For example, the lower frequency signal will generally be 10Hz or lower, although in some cases it may be higher. Similarly, the higher frequency signal will generally be 1kHz or higher, although in some cases it may be lower.

In one exemplary embodiment, for example, a slow square wave signal with a frequency of 5Hz (a period of 200ms) and a fast modulation signal of 2.5 kHz (a period of 400μs) are used. In this exemplary embodiment, a dye with a phosphorescence lifetime of approximately 640 μs is used in the sample. In other exemplary embodiments, however, dyes are used that have time constants in the order of 1200μs and 2400μs and for which the fast modulation frequency is commensurately lower (in these the slow modulation frequency of 5Hz may remain unaffected).

It will also be appreciated that whilst a sinusoidal low frequency input signal V_{m2} has advantages, V_{m2} may be any suitable waveform such as a two-level (e.g. square) or other shape waveform.

Whilst the final demodulation step comprising multiplication of a high-pass filtered version, $V_{m2(0)}$, of the low frequency input signal, V_{m2}, with the amplified version of V_{d3} is particularly advantageous, it will be appreciated that the final demodulation step may be carried out using other appropriate circuitry. For example, an envelope detector may beneficially be used if the measurement bandwidth and the frequency of the low frequency input signal, V_{m2}, are such that the final low-pass filter can have a cut-off frequency that is significantly lower than the slow oscillation frequency. Alternatively, where a two-level low frequency input signal, V_{m2}, is used instead of a sinusoidal signal, the linear multiplier 46 can be replaced by a simple switched demodulator.

It will be appreciated that the incident radiation R_1 may comprise any radiation suitable for exciting the sample to luminesce, although ultraviolet (UV) radiation is particularly beneficial. Where the radiation is UV it may have a wavelength in the range of about 400 nm down to about 10 nm (energies of about 3eV to about 124eV). The radiation may, for example be radiation in a 'near' UV spectral region having a wavelength of between about 400nm and about 300nm (~3.10eV to ~4.13 eV), in a 'middle' UV region having a wavelength of between about 300nm and about 200nm (~4.13eV to ~6.20eV), in a 'far' UV region having a wavelength of between about 200nm and about 122nm (~6.20eV to ~10.2eV), and/or in a in a 'extreme' UV region having a wavelength of between about 121 nm and about 10 nm (~10.2eV to ~124eV). The radiation may, for example, be UVA radiation having a wavelength of between about 400nm and about 315nm (~3.10eV to ~3.94eV), UVB radiation having a wavelength of between about 300nm and about 280nm (~3.94eV to ~4.43eV), UVC radiation having a wavelength of between about 280nm and about 100nm (~4.43eV to ~12.4eV), and/or Vacuum UV ('VUV') radiation having a wavelength of between about

200nm and about 10nm (~6.2eV to ~124eV). UV having a wavelength of around 365nm or around 265nm has been of particular benefit.

Claims

1. Apparatus for use in measuring a luminescent property of a sample, the apparatus comprising:

   means for emitting radiation to excite said sample, under the control of a control signal;

   means for generating said control signal for modulating an intensity of said emitted radiation, said control signal having a first component at a first frequency having a period that is less than, or of the same order as, an expected characteristic time constant of said luminescent property, and a second component at a second frequency having a period that is greater than said expected characteristic time constant of said luminescent property;

   means for receiving radiation luminesced from said sample as a result of said excitation, and for generating a detection signal representing an intensity of said received radiation; and

   means for demodulating said detection signal whereby to produce a signal representing said luminescent property of said sample.

2. Apparatus as claimed in claim 1 wherein the luminescent property of the sample comprises a persistent luminescent property, for example phosphorescent property.

3. Apparatus as claimed in claim 1 or 2 wherein an optical filter is provided between the radiation emitting means and the sample, which optical filter is configured to block radiation having a wavelength associated with the luminescent property to be measured.

4. Apparatus as claimed in claim 3 wherein the optical filter comprises a UV-pass filter.

5. Apparatus as claimed in any of claims 1 to 4 wherein an optical filter is provided between the sample and the radiation receiving means, which optical filter is configured to block radiation having wavelengths different

from the wavelengths associated with the luminescent property to be measured.

6. Apparatus for exciting a sample with radiation whereby to allow measurement of a luminescent property of the sample, the apparatus comprising:

means for emitting radiation to excite said sample under the control of a control signal; and

means for generating a control signal for modulating an intensity of said emitted radiation, said control signal having a first component at a first frequency having a period that is less than, or of the same order as, an expected characteristic time constant of said luminescent property, and a second component at a second frequency having a period that is greater than said expected characteristic time constant of said luminescent property.

7. Apparatus as claimed in claim 1 or 6 wherein said first component of said control signal is derived from a first periodic signal.

8. Apparatus as claimed in claim 7 wherein said first periodic signal oscillates between at least two different voltage levels.

9. Apparatus as claimed in claim 8 wherein a lower magnitude one of said voltage levels is a substantially zero (or near zero) voltage level.

10. Apparatus as claimed in claim 7 wherein said second component of said control signal is derived from a second periodic signal.

11. Apparatus as claimed in claim 10 wherein said first and second periodic signals are such that said control signal causes the radiation emitting means to switch between an 'ON' state in which it emits light and an 'OFF' state in which it does not emit light at a frequency approximately equal to a frequency of said first periodic signal.

12. Apparatus as claimed in any of claim 1 or 6 wherein said second component of said control signal is derived from a second periodic signal.

13. Apparatus as claimed in claim 11 or 12 wherein said second periodic signal oscillates between at least two different voltage levels.

14. Apparatus as claimed in claim 13 wherein said second periodic signal is a sinusoidal signal.

15. Apparatus as claimed in claim 13 or 14 wherein one of said voltage levels of said second periodic signal is a substantially zero (or near zero) voltage level.

16. Apparatus as claimed in any of claims 11 to 15 wherein said second periodic signal is offset such that the signal remains at the same polarity throughout its cycle.

17. Apparatus as claimed in any of claims 11 to 14 wherein said second periodic signal is offset such that the minimum magnitude of the signal is non-zero.

18. Apparatus as claimed in any preceding claim wherein said radiation emitting means comprises a light emitting diode.

19. Apparatus as claimed in claim 18 wherein said light emitting diode is operable to emit radiation in ultra violet region.

20. Apparatus for detecting radiation luminesced from a sample whereby to allow measurement of a luminescent property of the sample, the apparatus comprising:

means for receiving radiation luminesced from said sample as a result of excitation of said sample with radiation from a radiation source and means for generating a detection signal representing an intensity of said received radiation, wherein:

said radiation from said radiation source is modulated by a control signal having a first component at a first frequency having a period that is less than, or of the same order as, an expected characteristic time constant of said luminescent property, and a second component at a second frequency having a period which is greater than the expected characteristic time constant of said luminescent property;

and means for demodulating said detection signal whereby to produce a signal representing said luminescent property of said sample.

21. Apparatus as claimed in any of claims 1 to 4 or 20 wherein said demodulating means comprises:

a first demodulation arrangement for demodulating a first frequency component of said detection signal, which first frequency component is associated with said first component of the control signal; and

a second demodulation arrangement for demodulating a second frequency component of said detection signal, which second frequency component is associated with said second component of the control signal.

22. Apparatus as claimed in claim 21 wherein the first demodulation arrangement is operable to suppress a first component of said detection signal arising when said sample is being excited.

23. Apparatus as claimed in claim 22 wherein said first component of said detection signal arises, at least in part, from fluorescence or short time-constant phosphorescence from said sample.

24. Apparatus as claimed in any of claims 21 to 23 wherein the first demodulation arrangement is operable not to suppress a second component of said detection signal arising when said sample is not being excited.

25. Apparatus as claimed in claim 24 wherein said second component of said detection signal arises, at least in part, from phosphorescence from said sample.

26. Apparatus as claimed in any of claims 21 to 25 wherein the first demodulation arrangement comprises a switched demodulator arrangement arranged to switch said detection signal on and off whereby to demodulate said signal.

27. Apparatus as claimed in any of claims 21 to 25 wherein the first demodulation arrangement comprises means for switching the gain of the generating means between a high gain and a low gain whereby to demodulate said signal.

28. Apparatus as claimed in any of claims 21 to 27 wherein the first demodulation arrangement further comprises a filter for filtering out any remaining components at the first frequency from the detection signal.

29. Apparatus as claimed in claim 28 wherein the filter comprises a low-pass filter.

30. Apparatus as claimed in any of claims 21 to 29 wherein the second demodulation arrangement is operable to demodulate the second frequency component of the detection signal as demodulated by said first demodulation arrangement.

31. Apparatus as claimed in any of claims 21 to 29 wherein the demodulating means further comprises an amplifier for amplifying the detection signal as demodulated by said first demodulation arrangement.

32. Apparatus as claimed in claim 31 wherein the second demodulation arrangement is operable to demodulate the second frequency component of the detection signal as demodulated by said first demodulation arrangement and amplified by said amplifier.

33. Apparatus as claimed in claim 31 or 32 wherein the amplifier comprises a large gain (and/or ac coupled) amplifier.

34. Apparatus as claimed in any of claims 21 to 33 wherein the second demodulation arrangement comprises a multiplier (e.g. a mixer) for multiplying the detection signal with a signal having a frequency substantially equal to that of the second frequency component.

35. Apparatus as claimed in any of claims 21 to 33 wherein the second demodulation arrangement comprises an envelope detector operable to suppress the second frequency component of the detection signal.

36. Apparatus as claimed in any of claims 21 to 35 wherein the second demodulation arrangement comprises a filter for filtering out any remaining components at the second frequency from the detection signal.

37. Apparatus as claimed in claim 35 wherein the filter for filtering out the remaining component(s) at the second frequency comprises a low-pass filter.

38. A method of generating a signal representing a luminescent property of a sample for use in measuring said luminescent property of the sample, the method comprising:

generating a control signal having a first component at a first frequency having a period that is less than, or of the same order as, an expected characteristic time constant of said luminescent property, and a second component at a second frequency having a period that is greater than said expected characteristic time constant of said luminescent property;

emitting radiation to excite said sample, under the control of said control signal, such that said emitted radiation is modulated by the control signal;

receiving radiation luminesced from said sample as a result of said excitation, and generating a detection signal representing an intensity of said received radiation; and

demodulating said detection signal whereby to produce a signal representing said luminescent property of said sample.

39. A method of exciting a sample with radiation whereby to allow measurement of a luminescent property of the sample, the method comprising:

generating a control signal having a first component at a first frequency having a period that is less than, or of the same order as, an expected characteristic time constant of said luminescent property, and a second component at a second frequency having a period that is greater than said expected characteristic time constant of said luminescent property; and emitting radiation to excite said sample, under the control of said control signal, such that said emitted radiation is modulated by the control signal.

40. A method of generating a signal representing a luminescent property of a sample for use in measuring said luminescent property of the sample, the method comprising:

receiving radiation luminesced from said sample as a result of excitation of said sample with radiation from a radiation source and generating a detection signal representing an intensity of said received radiation, wherein:

said radiation from said radiation source is modulated by a control signal having a first component at a first frequency having a period that is less than, or of the same order as, an expected characteristic time constant of said luminescent property, and a second component at a second frequency having a period which is greater than the expected characteristic time constant of said luminescent property;

and demodulating said detection signal whereby to produce a signal representing said luminescent property of said sample.

Application No: GB1111855.1 **Examiner:** Dr Susan Dewar
Claims searched: 1-40 **Date of search:** 5 October 2011

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	6, 39 at least	US 6297509 A (LIPKOWITSCH et al) See column 3 line 10 - column 4 line 21 and Fig 2
X	6, 39 at least	GB 2404013 A (ISIS INNOVATION) See in particular page 9 lines 7-15 and Fig. 5

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
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G01J; G01N

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Online: EPODOC, WPI

International Classification:

Subclass	Subgroup	Valid From
G01J	0003/44	01/01/2006
G01N	0021/64	01/01/2006