PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : G01N 21/64 | A1

(11) International Publication Number: WO 91/07652

(43) International Publication Date: 30 May 1991 (30.05.91)

(21) International Application Number: PCT/US90/06864

(22) International Filing Date: 21 November 1990 (21.11.90)

(30) Priority data:
439,795 21 November 1989 (21.11.89) US

(60) Parent Application or Grant
(63) Related by Continuation
US 439,795 (CON)
Filed on 21 November 1989 (21.11.89)

(71) Applicant *(for all designated States except US)*: SOURCE SCIENTIFIC SYSTEMS, INC. [US/US]; 7390 Lincoln Way, Garden Grove, CA 92641 (US).

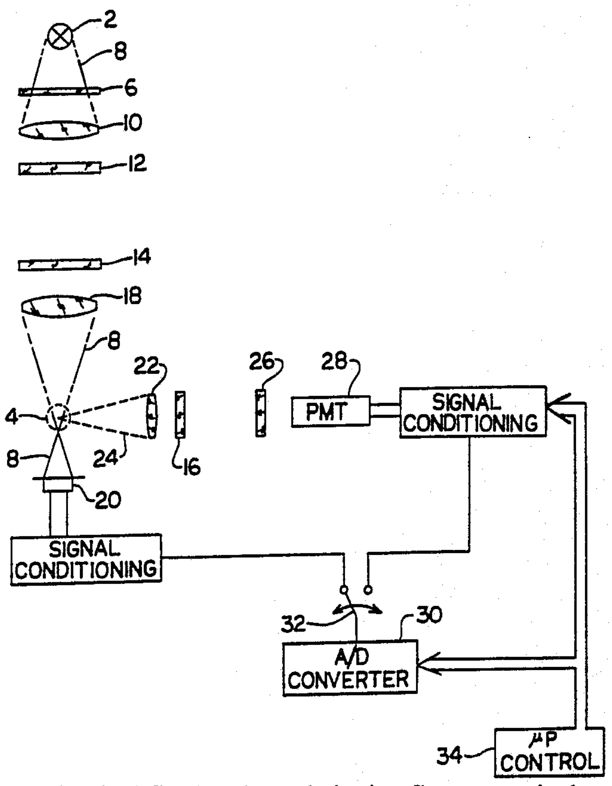
(72) Inventor; and
(75) Inventor/Applicant *(for US only)* : BORUCKI, Ryszard [PL/US]; 11923 Verbena Court, Fountain Valley, CA 92708 (US).

(74) Agent: LEWIS, Donald, G.; 10960 Wilshire Blvd. #1434, Los Angeles, CA 90024 (US).

(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: OPTICS AND METHOD FOR MEASURING FLUORESCENCE POLARIZATION



(57) Abstract

Source corrected fluorescence polarization is defined and a polarization fluorometer is described for measuring such source corrected fluorescence polarization. The polarization fluorometer relies upon a reference photodetector for monitoring the intensity of the light source and for correcting the measured fluorescent intensities. The polarization fluorometer positions the adjustable polarizer within the beam of the source. Mismatch between the transmittances of the adjustable polarizer in its parallel and perpendicular configurations is corrected by the output of the reference photodetector.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MR	Mauritania
BE	Belgium	GA	Gabon	MW	Malawi
BF	Burkina Faso	GB	United Kingdom	NL	Netherlands
BG	Bulgaria	GR	Greece	NO	Norway
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	SD	Sudan
CF	Central African Republic	KP	Democratic People's Republic of Korea	SE	Sweden
CG	Congo			SN	Senegal
CH	Switzerland	KR	Republic of Korea	SU	Soviet Union
CI	Côte d'Ivoire	LI	Liechtenstein	TD	Chad
CM	Cameroon	LK	Sri Lanka	TG	Togo
DE	Germany	LU	Luxembourg	US	United States of America
DK	Denmark	MC	Monaco		

TITLE: Optics and Method for Measuring Fluorescence Polarization

INVENTOR: Ryszard Borucki

RELATED APPLICATIONS

This is a continuation of U.S. application Serial No. 07/439,795, filed November 21, 1989, whose disclosures are incorporated herein by reference.

Background of the Invention

The invention relates to a method and apparatus for measuring fluorescence polarization. More particularly, the invention relates to a method and apparatus for measuring a quantity called "source corrected fluorescence polarization."

When polarized light is employed to electronically excite a fluorophore, the fluorescent light subsequently emitted by the fluorophore may be partially polarized. However, if an excited fluorophore undergoes significant rotational diffusion during the life time of the excited state, the polarization of the emitted light will decline significantly. On the other hand, if the life time of the excited state is particularly short or if the rotational diffusion is relatively slow due to use of a highly viscous medium or due to the large size of the fluorophore, there will be a comparatively lesser decline of the polarization of the fluorescent light. The measure of fluorescence polarization has found wide variety of scientific and clinical applications in several fields of biochemistry.

Fluorescence polarization [P] is defined as follows:

$$[P] = \{I(1) - I(2)\}/\{I(1) + I(2)\}, \quad (1)$$

where [I(1)] is the intensity of fluorescent light which is parallel to the polarization of the source light and I(2)] is the intensity of fluorescent light which is perpendicular to the polarization of the source light.

A variety of polarization fluorometers have been described for measuring fluorescence polarization. An automated polarization fluorometer has been described by Richard Spencer et al. [Clinical Chemistry 19(8), pp 838-844 (1973)]. Spencer's polarization fluorometer splits the source light into two beams and polarizes each beam with perpendicular directions of polarization with respect to one another. The two beams are then spliced by means of a mirrored chopper so that the sample fluorophore may be excited by alternating beam pulses having perpendicular directions of polarization with respect to one another. The polarization of the resultant fluorescent beam will depend upon the sample fluorophore and upon the polarization of the source beam. Accordingly, after the fluorescent beam has passed through a polarizer, its intensity will vary with the same frequency as the rotational frequency of the chopper. The intensity of the polarized fluorescent beam is measured by a photomultiplier tube and analyzed so as to indicate the fluorescence polarization [P] of the particular fluorophore.

A polarization fluorometer with an alternative optical configuration has been described by Mituta et al. (U.S. Pat. No. 4,115,699). Mituta employs only a single polarized source beam. However, Mituta modifies the chopper and moves it from the source beam to the fluorescent beam. Mituta discloses a novel chopper which includes at least one pair of polarizers, each element of the pair having a direction of polarization which is perpendicular to the other element of the pair. After a polarized fluorescent beam passes through Mituta's chopper, the intensity of the resultant beam will vary with a frequency related to the rotational frequency of the chopper. The output and analysis of Mituta's photomultiplier tube is then similar to the output and analysis of the Spencer's photomultiplier tube.

A polarization fluorometer having a constant intensity of source light has been disclosed by Popelka (U.S. Pat. No. 4,516,859). Before passing the polarized source light into the sample fluorophore, Popelka employs a static beam splitter and reference detector to monitor the intensity of the source light. The output of the reference detector is then employed to adjust the power input of the light source so as to maintain a constant output of light. The use of a constant intensity light source significantly enhances the stability of the fluorecence intensity.

Equation (1) indicates that the fluorescence polarization is dependent upon both I(1) and I(2), where I(1) and I(2) correspond respectively to the intensities of fluorescent light polarized parallel and perpendicularly with respect to the polarization of the source light. Equation (1) assumes that the intensity of the source light is constant and that the transmittance of the various optical elements remains constant during the interval which separates the measurements of I(1) and I(2). This assumption may fail if the intensity of the light source is not regulated by feedback as disclosed by Popelka. Alternatively, the assumption may also fail if the transmittance of the various optical elements changes from one measurement to the next. The polarizer is the most likely optical element to change its transmittance during the interval between one measurement and the next.

The prior art discloses that polarization fluorometry requires the use of a two polarizers, i.e. a first polarizer having a constant direction of polarization and a second polarizer which is adjustable between parallel and perpendicular configurations with respect to the first polarizer. The first polarizer may be positioned in either the source beam or the fluorescent beam; the second polarizer is then postioned in the remaining beam. As indicated in equation (1), the fluorescence polarization is then determined by taking a first measurement of the fluorescence intensity with the adjustable polarizer in one configuration and a second measurement of the fluorescence intensity with the adjustable polarizer in the remaining configuration. However, prior art polarization fluorometers have relied upon the use of adjustable polarizers having matched or reproducible transmittances with respect to each configuration. The use of an adjustable polarizer having unmatched transmittances in the prior art would have resulted in measurements having an artifactual bias. Furthermore, if the mismatch of transmittances drifts from one measurement to the next, the magnitude and/or direction of the bias becomes unpredictable.

Summary

A method and apparatus for measuring source corrected fluorescence polarization, i.e. [P(c)], is disclosed. The invention employs a reference photodetector to monitor the intensity of the source beam and employs the output of the reference photodetector to correct the fluorescent intensity with respect to each direction of polarization. The invention also positions the adjustable polarizer within the source beam. Accordingly, if the transmittances of the two configurations of the adjustable polarizer differ from one another, the reference photodetector will detect the change of source intensity.

The apparatus and technique of the invention enhances the accuracy and reproducibility of the measurement and dispenses with the need for stabilizing the intensity of the light source by means of electronic feedback and/or the need for employing adjustable polarizers having well matched transmittances in each their configurations.

The invention relies on the concept of a quantity called "source corrected fluorescence polarization," i.e. [P(c)]. In analogy with expression for the fluorescence polarization given in equation (1), source corrected fluorescence polarization [P(c)] is defined by the following equation:

[P(c)] =
{[I(c)(1)] - [I(c)(2)]}/{[I(c)(1)] + [I(c)(2)]}, (2)

where the terms [I(c)(1)] and [I(c)(2)] are the source corrected intensities of the fluorescent beam with respect to parallel and perpendicularly polarized light respectively. Measurement of the term [I(c)(1)] requires that the source and fluorescent beam polarizers be aligned parallel to one another. Measurement of the term [I(c)(2)] requires that the source and fluorescent beam polarizers be aligned perpendicularly with respect to one another.

The source corrected intensities of parallel and perpendicularly polarized fluorescent light, i.e. [I(c)(1)] and [I(c)(2)] respectively, are provided by the following equations:

[I(c)(1)] = k[I(m)(1)]/[F(1)] and (3)

[I(c)(2)] = k[I(m)(2)]/[F(2)], (4)

where the measured fluorescence intensity terms, i.e. [I(m)(1)] and [I(m)(2)], are the measured signals from the fluorescence photodetector with the adjustable polarizer set in its parallel and perpendicular configurations respectively and where the reference intensity terms, i.e. [F(1)] and [F(2)], are the corresponding signals from the reference photodetector for the source beam. The term "k" is a scaling factor.

In effect, the use of the reference intensity terms, i.e. [F(1)] and [F(2)], in equations (3) and (4) serves to correct the measured fluorescence intensity terms, i.e. [I(m)(1)] and [I(m)(2)], so as to provide the terms for the source corrected intensities, i.e. [I(c)(1)] and [I(c)(2)]. The reference intensity terms reflect any change of light flux within the source beam between the measurement of the fluorescent photodetector terms, i.e. [I(m)(1)] and [I(m)(2)].

The fluorometer of the present invention employs a reference photodetector in the path of the source beam for monitoring its flux or intensity. Furthermore, the fluorometer of the present invention positions the adjustable polarizer within the path of the source beam. Accordingly, the variability of the intensity the source beam includes any variability caused by mismatch or drift with respect to the transmittances of the two configurations of the adjustable polarizer. In particular, the variability of the intensity the source beam is monitored as the adjustable polarizer changes from one configuration to another.

The measurement of the intensity of the source light obviates the need to precisely regulate its intensity and enables the use of an adjustable polarizer having unmatched transmittances with respect to its two configurations.

Brief Description of the Drawings

Fig. 1 illustrates a schematic view of an example polarization fluorometer of the invention.

Detailed Description

Example Apparatus:

As indicated in Fig. 1, the apparatus requires a light source (2) for exciting the fluorophore (4). Preferred source lights (2) include tungsten halogen lamps of the type employed with prior art polarization fluorometers. However, other conventional source lights (2) may also be employed.

In the preferred embodiment, a heat glass (6) separates the source light from the remainder of the apparatus. The heat glass (6) serves to block a major portion of the infra-red radiation eminating from the source light.

In the preferred embodiment, the source light is then be collimated to form a source beam (8). The source light (8) may be collimated by means of a first lens (10).

In the preferred embodiment, the collimated source beam (8) may then be filtered by a first filter (12) so as to pass a source beam of monochromatic light. The wavelength of the monochromatic light should correspond to the excitation energy of the particular fluorophore (4) which is targeted for excitation. Preferred filters may be obtained from Corion, Inc. (Holliston, Massachusetts), e.g. P/N CFS-001564. Alternatively, a monochromator may be employed in lieu of the filter.

The collimated source beam (8) is also passed through an adjustable polarizer (14). The adjustable polarizer (14) has two configurations, i.e. a parallel configuration and a perpendicular configuration. When the adjustable polarizer (14) is set in its parallel configuration, it passes polarized light having a direction of polarization which is parallel with respect to the direction of polarization of a second polarizer (16), described below, which serves to polarize the emitted fluorescent light. When the adjustable polarizer (14) is set in its perpendicular configuration, it passes polarized light having a direction of polarization which is perpendicular with respect to the parallel polarized light. Preferred adjustable polarizers may be obtained from Melles Griot, Inc. (Irvine, California), e.g. P/N 03 FPG-001. The polarization configuration of adjustable polarizers (14) may be changed mechanically or electronically.

In the preferred embodiment, the collimated source beam (8) is focused by a second lens (18) upon the sample fluorophore (4). Under certain circumstances, the sequence and/or relative positions of the first (10) and second lens (18), the first filter (12), and the adjustable polarizer (14) may be altered. However, after passing through an appropriate combination of these optical elements or their equivalent, a source beam (8) of appropriately polarized light having a wavelength which substantially corresponds to the excitation energy of the sample fluorophore (4), will impinge upon a cuvette or other appropriate sample holder for containing such sample fluorophore (4) or blank.

After passing through the cuvette or blank, the source beam (8) then impinges upon a reference photodetector (20). Silicon photodiodes may serve as perferred reference photodectors. Appropriate silicone photodiodes may be obtained from Hamamatsu (Bridgewater, New Jersey). The reference photodector serves to monitor the intensity of the light flux which passes through the sample holder. The reference photodetector (20) is energized so as to produce a signal which is proportional to the light flux which impinges it. The signal from the reference photodetector (20) is conditioned and amplified. The conditioned signal from the reference photodetector (20) is then fed to an analog to digital converter (30) or to an equivalent device for reading electronic signals.

After the source beam (8) impinges the sample fluorophore (4), the sample fluorophore (4) becomes electronically excited. The excited fluorophore (4) may then relax by emitting fluorescent light. A portion of the emitted fluorescent light is collimated by a third lens (22) to form a fluorescent beam (24). It is preferred that the third lens (22) not be aligned with the source beam (8). In the preferred embodiment, the fluorescent beam (24) forms an angle which is substantially normal to the source beam (8).

The fluorescent beam (24) is then passed through a second polarizer (16) having a fixed direction of polarization. In particular, the direction of polarization of the second polarizer (16) serves as the standard by which the parallel direction of the adjustable polarizer (14) is estabished. Preferred polarizers may be obtained from Melles Griot, Inc. (Irvine, California).

The fluorescent beam (24) should also preferrably pass through a second filter (26) which blocks light having an energy corresponding to the monochromatic source beam (8). The second filter (26) serves to block out scattered source light while passing fluorescent light emitted by the sample fluorophore (4). Preferred second filters (26) may be obtained from Corion, Inc. (Holliston, Massachusetts), e.g. P/N CFS-001565.

The sequence and relative positions of the third lens (22), the second polarizer (16), and the second filter (26) may be altered. However, after passing through these optical elements, the fluorescent beam (24) should include only polarized light having a wavelength corresponding to the fluorescent light only.

The resultant fluorescent light is then allowed to impinge upon a second photodetector (28). In the preferred embodiment, the second photodetector (28) is a high gain photomultiplier tube. Appropriate high gain photomultiplier tubes may be obtain from Hamamatsu (Bridgewater, New Jersey). However, other photodetectors conventionally employed with polarization fluorometers of the prior art may also be employed.

The output of the second photodetector (28) is amplified and conditions per manufacturer's suggestions and connected to an analog to digital converter (30) or an equivalent device for reading eletctronic signals. In the preferred embodiment, a 16 bit precision analog to digital converter (30) is employed. An appropriate 16 bit precision analog to digital converter (30) may be obtained from Burr-Brown (Tucson, Arizona), e.g. P/N ACD 700. With appropriate switching (32), a common analog to digital converter (30) may be employed for reading the signals of both the reference photodetector (20) and the second photodetector (28).

A microprocessor (34) connected to the analog to digital converter (30) may serve to control the signal conditioning of the second photodetector (28).

Example of the Method

Each determination of fluorescence polarization requires four measurements, viz. I(m)(1), I(m)(2), F(1) and F(2). Measurements of I(m)(1) and F(1) are performed with the adjustable polarizer (14) set in its parallel configuration. Measurements of I(m)(2) and F(2) are performed with the adjustable polarizer (14) set in its perpendicular configuration. Measurements of I(m)(1) and F(1) are made in conjunction with one another; while measurements of I(m)(2) and F(2) are made in conjunction with one another. The measurements of I(m)(1) and F(1) may be performed in either sequence, i.e. I(m)(1) before F(1) or F(1) before I(m)(1); similarly measurements of I(m)(2) and F(2) may be performed in either sequence. However, it is preferred that each pair of measurements me performed as close to one another in time as possible.

Measurment of F(1) and F(2) may be performed with a blank cuvette or with no cuvette. The blank may be empty or may contain solvent without fluorophore (4). Measurment of I(m)(1) and I(m)(2) are made with a cuvette or other container loaded with a sample fluorophore (4).

In a preferred sequence, F(1) is first measured with the adjustable polarizer (14) in its parallel configuration and without any cuvette in the sample holder. Next, a cuvette containing a sample fluorophore (4) is loaded into the sample holder and the measurement of I(m)(1) is taken. The configuration of the adjustable polarizer (14) is then reset to its perpendicular configuration without disturbing the cuvette. The measurement of I(m)(2) is then taken. Finally, the cuvette is removed from the sample holder without disturbing the adjustable polarizer (14) and the measurement of F(2) is taken.

When measuring I(m)(1) and I(m)(2), the signal from the fluorescence photodetector is fed to the analog to digital converter (30) where it is amplified and read.

Similarly, when measuring F(1) and F(2), the input to the analog to digital converter (30) is switched to output of the reference photodetector (20). Hence, F(1) and F(2) may be read from the same analog to digital converter (30) as is employed to read I(m)(1) and I(m)(2).

A microprocessor (34) may be employed for controlling the fluorescence photodetector and the analog to digital converter (30).

What is claimed is:

1. A method for measuring a source corrected intensity [I(c)(1)] of parallel polarized fluorescent light emitted from a sample fluorophore, the method comprising the following steps:

Step A: measuring the relative intensity [F(1)] of a source beam incident upon a sample blank, the source beam having a first direction of polarization and a first wavelength for exciting the sample fluorophore;

Step B: measuring the intensity [I(m)(1)] of a fluorescent beam emitted by the sample fluorophore upon excitation by the source beam of said Step A, the fluorescent beam having a direction of polarization which is parallel to the first direction of polarization of the source beam and a second wavelength corresponding to the fluorescent emission of the sample fluorophore; then

Step C: determining the source corrected intensity [I(c)(1)] of parallel polarized fluorescent light emitted from the sample fluorophore having the second wavelength as measured in said Step B according to the formula:

[I(c)(1)] = k[I(m)(1)]/[F(1)],

where k is a scaling factor.

2. A method for measuring a source corrected intensity [I(c)(2)] of perpendicularly polarized fluorescent light emitted from a sample fluorophore, the method comprising the following steps:

Step A: measuring the relative intensity [F(2)] of a source beam incident upon a sample blank, the source beam having a second direction of polarization and a first wavelength for exciting the sample fluorophore;

Step B: measuring the intensity [I(m)(2)] of a fluorescent beam emitted by the sample fluorophore upon excitation by the source beam of said Step A, the fluorescent beam having a direction of polarization which is perpendicular to the second direction of polarization of the source beam and a second wavelength corresponding to the fluorescent emission of the sample fluorophore; then

Step C: determining the source corrected intensity [I(c)(2)] of parallel polarized fluorescent light emitted from the sample fluorophore having the second wavelength as measured in said Step B according to the formula:

[I(c)(2)] = k[I(m)(2)]/[F(2)],

where k is a scaling factor.

3. A method for measuring a source corrected fluorescence polarization [P(c)] of fluorescent light emitted from a sample fluorophore, the method comprising the following steps:

Step A: measuring a source corrected intensity [I(c)(1)] of parallel polarized fluorescent light emitted from the sample fluorophore after excitation with a source light having a first polarization;

Step B: measuring a source corrected intensity [I(c)(2)] of perpendicular polarized fluorescent light emitted from the sample fluorophore after excitation with a source light having a second polarization, the second polarization being perpendicular to the first polarization; then

Step C: determining the source corrected fluorescence polarization [P(c)] of fluorescent light emitted from the sample fluorophore using the source corrected intensity [I(c)(1)] of parallel polarized fluorescent light as measured in said Step A and the source corrected intensity [I(c)(2)] of perpendicular polarized fluorescent light as measured in said Step B according to the formula:

[P(c)] =

{[I(c)(1)] - [I(c)(2)]}/{[I(c)(1)] + [I(c)(2)]}.

4. An apparatus for measuring a source corrected polarization [P(c)] of fluorescent light emitted from a sample fluorophore, the apparatus comprising:

a light source for providing source light,

a first filter optically aligned with the source light for producing monochromatic source light having a first wave length,

an adjustable polarizer optically aligned with the source light for producing polarized source light, said adjustable polarizer having a first position for producing polarized source light having a first polarization and a second position for producing polarized source light having a second polarization, the first polarization being perpendicular to the second polarization,

said first filter and said adjustable polarizer being optically aligned with respect to one another for producing polarized monochromatic source light,

the polarized monochromatic source light having a relative intensity [F(1)] with respect to the first polarization and having a relative intensity [F(2)] with respect to the second polarization,

a first photodetector optically aligned with the polarized monochromatic source light for measuring the relative intensities [F(1)] and [F(2)],

means for positioning the sample fluorphore within the path of the polarized monochromatic source light for optically exciting the sample fluorophore and causing the sample fluorphore to emit fluorescent light,

a second polarizer having a fixed polarization and optically aligned with the fluorescent light for producing polarized fluorescent light having a polarization which is parallel with respect to the first polarization and perpendicular with respect to the second polarization of the polarized source light, a second filter optically aligned with the fluorescent light for producing monochromatic fluorescent light having a second wave length, the first wave length being shorter than the second wave length,

said second polarizer and said second filter being optically aligned with respect to one another for producing polarized monochromatic fluorescent light,

the polarized monochromatic fluorescent light having a relative intensity [I(m)(1)] with respect to source light having the first polarization and having a relative intensity [I(m)(2)] with respect to source light having the the second polarization, and

a second photodetector optically aligned with the polarized monochromatic fluorescent light for measuring relative fluorescent intensities [I(m)(1)] and [I(m)(2)],

whereby the source corrected polarization [P(c)] of fluorescent light emitted from the sample fluorophore may be determined by the measurments of [F(1)], [F(2)], [I(m)(1)], and [I(m)(2)] by the following formula:

$$[P(c)] = \{[I(m)(1)]/[F(1)] - [I(m)(2)]/[F(2)]\}/\{[I(m)(1)]/[F(1)] + [I(m)(2)]/[F(2)]\}.$$

5. An apparatus for measuring a source corrected polarization [P(c)] of fluorescent light emitted from a sample fluorophore as described in claim 4 wherein:

said second polarizer and said second filter being optically non-aligned with respect to the source light.

6. An apparatus for measuring a source corrected polarization [P(c)] of fluorescent light emitted from a sample fluorophore as described in claim 4 wherein:

said second filter is interposed between said second polarizer and said second photodetector.

7. An apparatus for measuring a source corrected polarization [P(c)] of fluorescent light emitted from a sample fluorophore as described in claim 4 wherein:

said second polarizer is interposed between said second filter and said second photodetector.

8. An apparatus for measuring a source corrected polarization [P(c)] of fluorescent light emitted from a sample fluorophore as described in claim 4 wherein:

said first filter is interposed between said light source and said adjustable polarizer.

9. An apparatus for measuring a source corrected polarization [P(c)] of fluorescent light emitted from a sample fluorophore as described in claim 4 wherein:

said adjustable polarizer is interposed between said light source and said first filter.

10. An improved polarization polarimeter to the type having a source beam polarized by a polarimeter,

wherein the improvement allows the measurement of the source corrected fluorescence polarization and comprises:

a reference photodector for monitoring the intensity of the polarized source beam.

11. An improved polarization polarimeter as described in claim 10 wherein the improvement further comprises:

said polarimeter being an adjustable polarimeter for polarizing the source beam in either of two mutually perpendicular configurations.

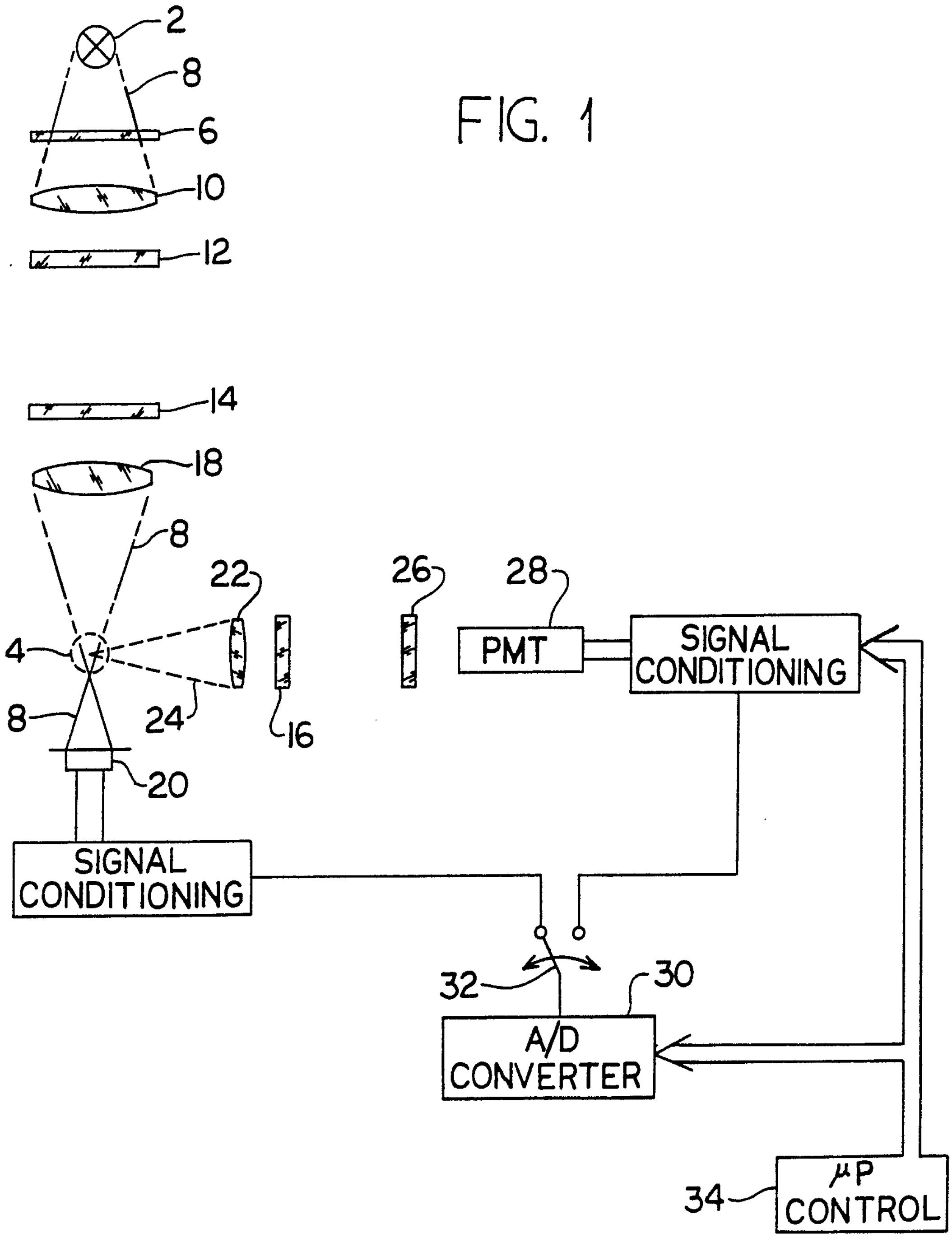

2
8
6
10
12
14
18
8
22
26
28
PMT
SIGNAL
CONDITIONING
4
24
16
8
20
SIGNAL
CONDITIONING
32
30
A/D
CONVERTER
34
μP
CONTROL

FIG. 1

INTERNATIONAL SEARCH REPOR

International Application No PCT/US 90/06864

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC^5: G 01 N 21/64

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC^5	G 01 N 21/64

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	GB, A, 2090971 (ABBOTT LABORATORIES) 21 July 1982 see page 1; page 2, down to line 4 (cited in the application)	1-4,10
	--	
A	US, A, 4299486 (T. NOGAMI et al.) 10 November 1981 see column 3, from line 54; columns 4-6; column 7, down to line 53	1-4,10
	----------	

* Special categories of cited documents: [10]

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

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

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

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

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

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

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

"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

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14th March 1991	- 3. 04. 91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	p. MISS D. S. KOWALCZYK

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9006864 SA 42545

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 27/03/91
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB-A- 2090971	21-07-82	AU-B- 544788	13-06-85
		AU-A- 7919482	15-07-82
		BE-A- 891736	08-07-82
		CA-A- 1165585	17-04-84
		DE-A,C 3200391	29-07-82
		FR-A,B 2497951	16-07-82
		JP-A- 57136142	23-08-82
		US-A- 4516856	14-05-85
US-A- 4299486	10-11-81	JP-C- 1411125	24-11-87
		JP-A- 55109949	23-08-80
		JP-B- 62017695	18-04-87
		DE-A,C 3005749	28-08-80
		GB-A,B 2044924	22-10-80

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82