United States Patent [19]

Sakaide et al.

[11] **3,849,653**

[45] **Nov. 19, 1974**

[54] **MULTICHANNEL BIOLUMINESCENT SENSORS**

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[73] Assignee: **Bausch & Lomb Incorporated**, Rochester, N.Y.

[22] Filed: **Sept. 27, 1973**

[21] Appl. No.: **401,287**

[52] **U.S. Cl.** .... **250/361**, 23/230 B, 23/232 E, 23/254 E, 250/209, 250/458

[51] **Int. Cl.** **G01h 33/00**

[58] **Field of Search**...... 356/179, 218; 195/103.5 R, 195/127; 250/576, 361, 366, 458, 484, 208, 209; 23/230 B, 254 E, 232 E; 340/237 R, 242

[56] **References Cited**

UNITED STATES PATENTS

3,370,175	2/1968	Jordan et al.	356/218 X
3,520,660	7/1970	Webb	356/246 X
3,557,372	1/1971	Ede	250/209 X
3,649,833	3/1972	Leaf	250/458
3,797,999	3/1974	Witz et al.	23/230 B X

Primary Examiner—Walter Stolwein
Attorney, Agent, or Firm—Frank C. Parker; Bernard D. Bogdon

[57] **ABSTRACT**

Two or more strains of bioluminescent microorganisms are used with multichannel luminescence sensors and logic circuitry to enable detection of specific chemical vapors. The organism strains are selected to produce predictable light-output variations when exposed to the specific vapors of interest, and signals analogous to light-output variations are combined in the logic circuitry to generate an alarm signal or other output when the specific vapors are sensed. Light-variation signals not conforming to the predicted pattern are rejected by the logic circuit to suppress an alarm or output when the organisms are exposed to vapors not of interest. Circuitry used with the system enables use of conventional photocells at very low light levels, and provides automatic compensation for long-term drift in organism light output.

8 Claims, 3 Drawing Figures

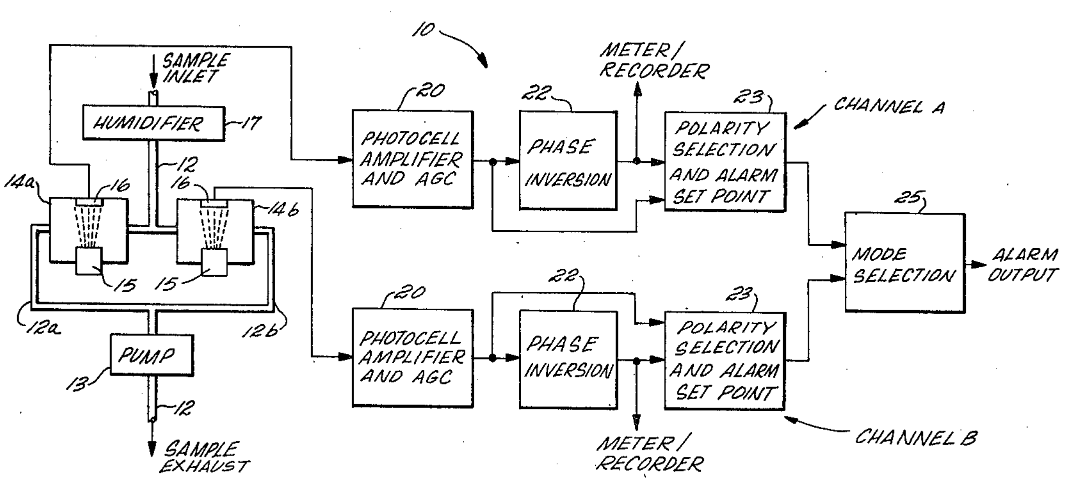
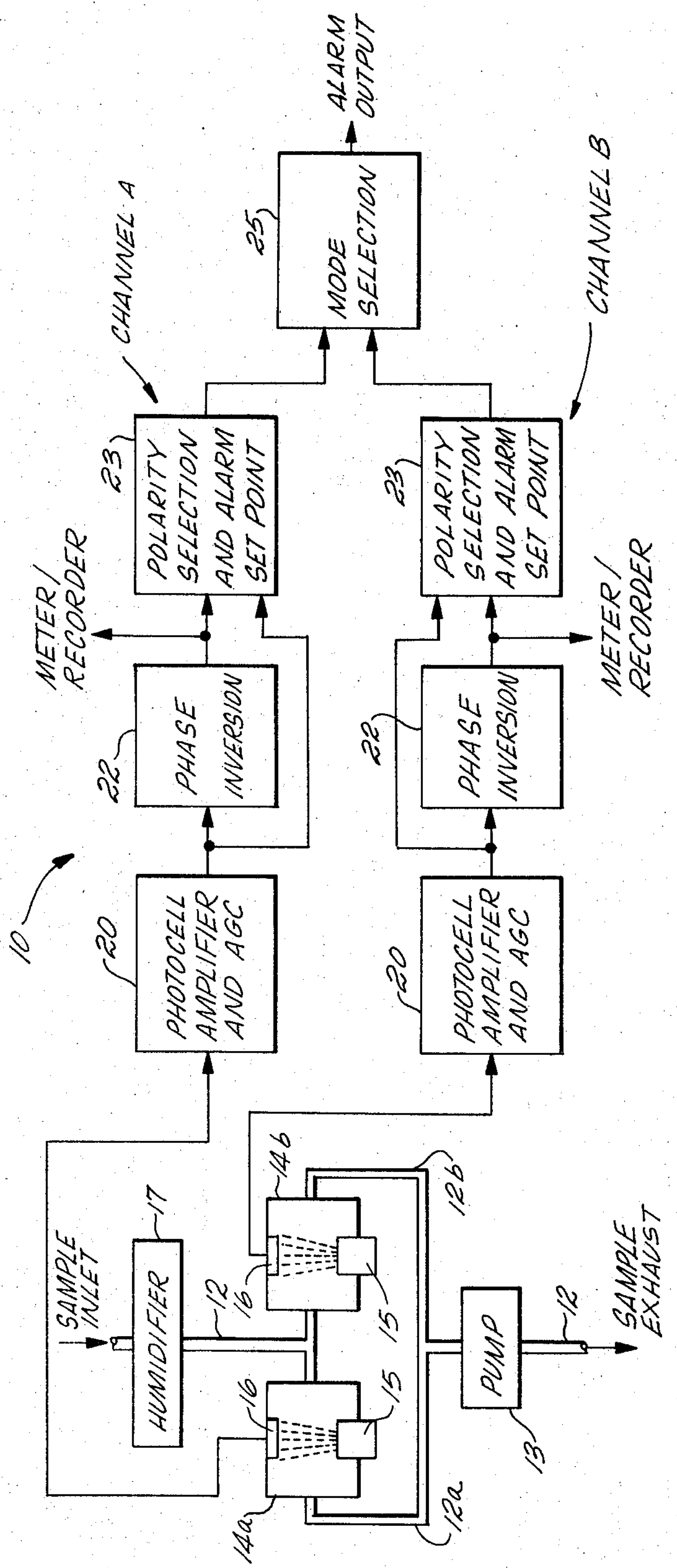


Fig. 1


ALARM OUTPUT
MODE SELECTION
25
CHANNEL A
CHANNEL B
POLARITY SELECTION AND ALARM SET POINT
23
METER/ RECORDER
PHASE INVERSION
22
PHOTOCELL AMPLIFIER AND AGC
20
10
SAMPLE INLET
HUMIDIFIER
17
12
16
15
14a
14b
12a
12b
PUMP
13
SAMPLE EXHAUST

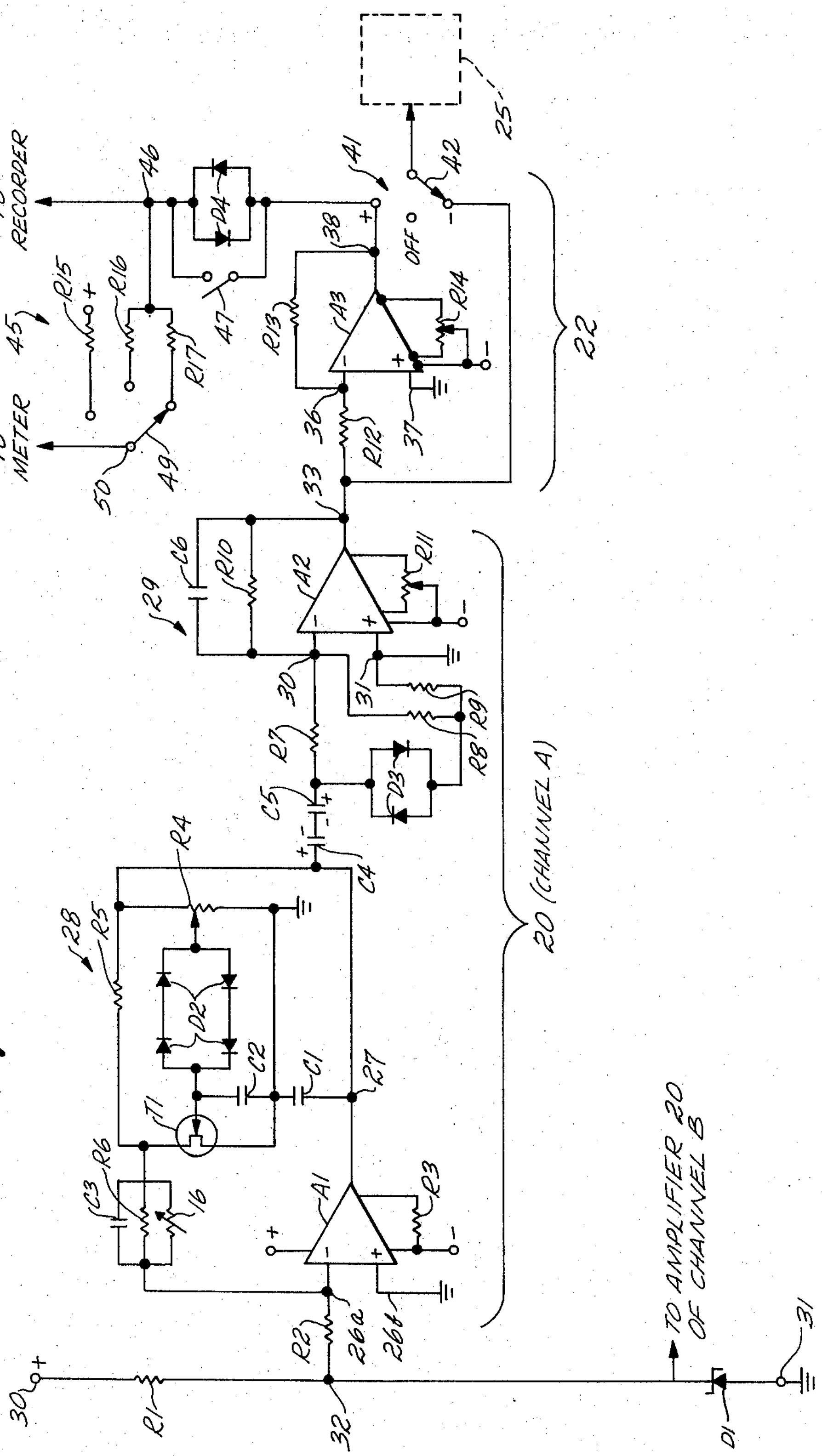

TO RECORDER
TO METER
TO AMPLIFIER 20 OF CHANNEL B
20 (CHANNEL A)
OFF

Fig. 2A

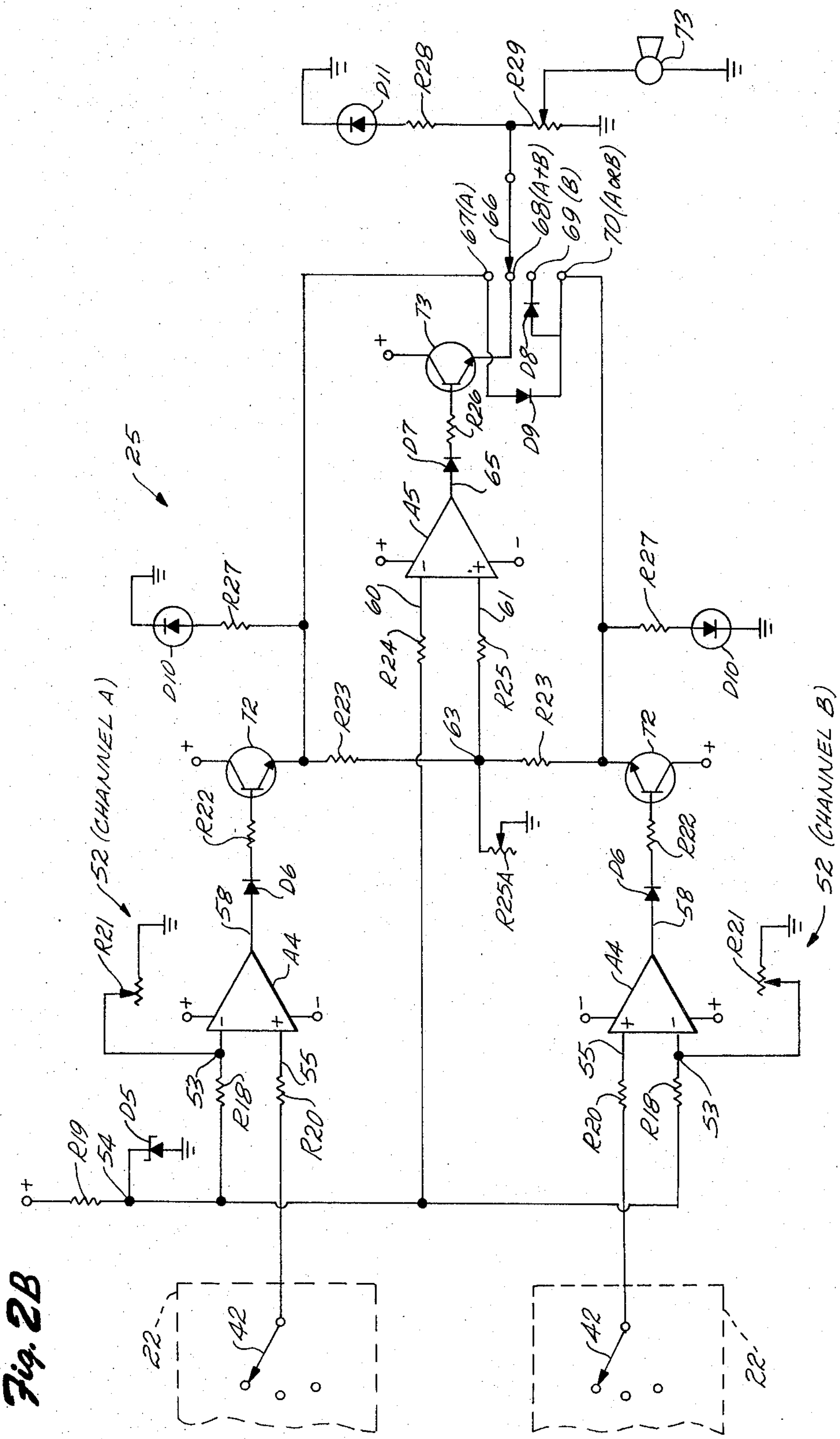

25
52 (CHANNEL A)
52 (CHANNEL B)
R19
54
D5
R18
R20
53
55
A4
58
D6
R21
R22
72
R23
R27
D10
R24
R25
R25A
63
60
61
A5
65
D7
R26
73
D8
D9
67(A)
66
68(A+B)
69(B)
70(A or B)
R28
R29
D11
42
22

Fig. 2B

MULTICHANNEL BIOLUMINESCENT SENSORS

BACKGROUND OF THE INVENTION

The use of bioluminescent micro-organisms (such as bacteria or fungi) as sensors for toxic materials in gaseous (including aerosol) form is described in U.S. Pat. No. 3,370,175. The use of these organisms is not limited to detection of toxic materials, and a variety of gases not usually considered toxic will produce a change in bioluminescence which can be measured and used to generate an output signal or activate an alarm. Any given strain of organisms may, however, respond with a light-output change upon exposure to a number of different materials, and false outputs may be generated by a detection system which is intended to respond to the presence of only a specific material.

We have found that each bioluminescent organism strain has its own predictable and repeatable response characteristics upon exposure to any given gas. Broadly speaking, the light output upon such exposure may initially increase, decrease, or remain unchanged. When an increase or decrease occurs, finer distinctions may sometimes be noted which can enhance the selective-response properties of the strain. For example, the magnitude of the change, the time required to return to a stable level of luminescence, and the waveform of the variation are all characteristics which may aid in identifying the presence of a specific gas of interest.

Two or more organism strains are selected to produce patterns of light-output variations which occur only in the presence of specific materials of interest. For example, strain X may respond positively (increased light output) to gases A, B, C and D, and have no response or a negative response to other gases. Strain Y may respond negatively (decreased light output) to gas A and certain other gases, but respond positively (or not at all) to gases B, D and D. A logic circuit set to recognize the combination of a positive strain-X response and a negative strain-Y response will generate the desired output or alarm only when the strains are exposed to gas A.

In accordance with the invention, the luminescence or emitted light of the several strains is sensed with photocells or similar transducers to generate electrical signals analogous to organism light output. These signals, suitably amplified, are combined in a logic circuit which generates an output signal only when a predetermined pattern of variations occurs. The use of multiple sensors greatly reduces the risk of an undesired alarm upon exposure of the organisms to gases not of interest, and substantially limits alarms to those cases where a preselected gas of interest is detected.

The circuits here disclosed permit use of simple photo-cells (rather than more complex and expensive photomultipliers or other sensors) to monitor luminescence, while preserving adequate sensitivity to detect minute variations about an initially low level of light output. The system is thus useful to detect trace quantities of gases which produce relatively small variations in the average light output of the organisms. Amplifiers used with the photocells include automatic gain control to compensate for long-term variations in average light output as the organisms progress through their lift cycles.

SUMMARY OF THE INVENTION

Briefly stated, the system of this invention is arranged

for detection of a specific vapor in an atmosphere being sampled, and includes a plurality of bioluminescent sensors using different strains of micro-organisms having predetermined light-output characteristics upon exposure to the vapor. A transducer means, preferably a plurality of photo-resistive cells, is disposed to receive light from the sensors and generate electrical signals analogous to the luminescence of each sensor. A circuit means is connected to the transducer means to receive the transducer signals, and to generate an output signal for activating an alarm device when the transducer signals represent the predetermined luminescence characteristics arising from exposure of the micro-organisms to the specific vapor.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a block diagram of a system according to the invention;

FIG. **2A** is a schematic diagram of a first portion of the system circuit; and

FIG. **2B** is a continuation of FIG. **2A** showing the balance of the system circuit.

DESCRIPTION OF THE PREFERRED EMBODIMENT

FIG. **1** is a block diagram of a multi-channel bioluminescent sensor system **10** according to the invention. The system is shown in a two-channel configuration, but a larger number of channels may be used if the material to be detected requires more than two strains of bioluminescent organisms to insure an appropriately selective response of the instrument.

System **10** includes a sample manifold **12** having a pump **13** at its exhaust end. A central part of the manifold is divided into two parallel lines **12*a*** and **12*b*** each connected in series with a respective light-tight exposure chamber **14*a*** and **14*b***. A housing or cartridge **15** is removably mounted in each exposure chamber, and supports a body of nutrient material (such as conventional agar) and bioluminescent micro-organisms. Light emitted by the organisms is sensed by a photosensitive transducer **16** mounted in each chamber **14**. The transducer is preferably a cadmium-sulfide photoresistive cell.

A gas sample to be monitored for presence of a specific material is admitted to the inlet of manifold **12**, and drawn through the manifold and exposure chambers by pump **13**. Preferably, a humidifier **17** is inserted in series with the manifold inlet line (or within the exposure chambers) to insure that the gas being sampled is sufficiently humid to avoid drying of the organisms and nutrient material. The manifold and exposure chambers should preferably be maintained at a relatively constant temperature, and these assemblies may be mounted in a constant-temperature enclosure (not shown) if the instrument is used in a varying temperature environment.

Each photo-sensitive transducer **16** is connected to a photocell amplifier **20** having an automatic-gain-control circuit to compensate for long-term variations in average light level as the organisms progress through their life cycles. As will be explained in greater detail below, photo-cell amplifier **20** includes a pair of capacitively coupled operational amplifiers, the photosensitive transducer acting as a variable resistance in the feedback loop around the first-stage amplifier. The output of amplifier **20** is fed to a unity-gain phase inverter 22, and also directly to a circuit 23 which provides polarity selection and adjustment of an alarm set point. Output of phase inverter 22 is also connected to circuit 23, so the output signal of amplifier 20 may be fed to circuit 23 in either true polarity or inverted polarity.

The output signals from the several circuits 23 in the channels of the system are then fed to a mode-selection circuit 25 which drives a lamp, warning buzzer, or other alarm device. The mode-selection circuit includes switching and logical circuitry enabling the operator to establish a particular variation of light output variations from the organisms which will produce an alarm output.

Referring now to the detailed schematic diagram in FIGS. 2A and 2B, a resistor R1 and zener diode D1 are connected in series between a positive power input terminal 30 and a ground terminal 31. A battery power source (not shown) of about 15 volts is suitable for operating the instrument. Zener diode D1 establishes a constant potential of about 2.4 volts at a junction 32 of the diode and resistor R1.

Photocell amplifier 20 includes an operational amplifier A1 having a first input terminal 26a connected to junction 32 through an input resistor R2. A second input terminal 26b of the operational amplifier is connected to ground. The operational amplifier has an output terminal 27. An automatic gain control (AGC) circuit 28 is connected in series with photo-sensitive transducer 16 to form a feedback loop between output terminal 27 and first input terminal 26a of the operational amplifier.

The AGC circuit includes a capacitor C1 and a potentiometer R4 (a sensitivity matching control for the several channels) connected in parallel between output terminal 27 and ground. The arm of the potentiometer is connected to one end of a diode circuit having two sets of series connected diodes D2 connected in back-to-back relationship. The other terminal of the diode circuit is coupled through a capacitor C2 to ground, and is also connected to the gate electrode of a field-effect transistor T1. One of the conductive electrodes of transistor T1 is connected to ground, and the other electrode to the junction of transducer 16 and a resistor R5 which is in turn connected to amplifier output terminal 27.

Photocell amplifier 20 further includes a low-pass AC amplifier 29 with a frequency range of about 0.05 to 2.0 HERTZ. Amplifier 29 includes an operational amplifier A2 having a first input terminal 30 connected through a resistor R7 and a pair of series-connected capacitors C4 and C5 to output terminal 27. Amplifier A2 has a second input terminal 31 connected to ground, and also to first input terminal 30 through series-connected resistors R8 and R9. A pair of paralleled back-to-back connected diodes D3 (effecting rapid charging of capacitors C4 and C5 during large signal operation) are connected to the junction of capacitor C5 and resistor R7, and to the junction of resistors R8 and R9. A capacitor C6 and resistor R10 are connected in parallel between first input terminal 30 and an output terminal 33 of amplifier A2.

Phase inverter 22 includes an operational amplifier A3 having a first input terminal 36 connected through an input resistor R12 to output terminal 33 of the low-pass AC amplifier. A second input terminal 37 of amplifier A3 is grounded. A feedback resistor R13 is connected between an output terminal 38 and first input terminal 36 of amplifier A3.

A three-position polarity-selection switch 41 has a positive terminal connected to output terminal 38, an open-circuit off terminal, and a negative terminal connected to output terminal 33. An arm 42 of switch 41 is connected to mode-selection circuit 25 as described below.

A recorder and meter circuit 45 includes a pair of paralleled back-to-back connected diodes D4 connected between terminal 38 and a recorder output terminal 46. A single-pole single-throw shorting switch 47 is connected across diodes D4. A meter selector switch 49 has a first terminal connected to the positive power source of the circuit through a resistor R15. Two other terminals of the switch are connected to recorder output terminal 46 through respective ranging resistors R16 and R17. The arm of switch 49 is connected to a meter output terminal 50.

System 10 of this invention uses at least two channels of the type just described, and a second channel is schematically indicated at the lower part of FIG. 2A. There may be three or more channels in an instrument using three different strains of organisms for highly selective detection of a particular material. These additional channels are preferably identical to the circuit described above.

Referring to FIG. 2B which is a continuation of FIG. 2A, mode-selection circuit 25 includes a pair of identical trigger circuits 52 each having an operational amplifier A4 arranged to act as a binary or two-state switch. Each amplifier has a first input terminal 53 connected through an input resistor R18 to a junction 54 of a series-connected resistor R19 and zener diode D5 coupled between the positive power input terminal and ground. A second input terminal 55 is connected through a resistor R20 to a respective switch arm 42 at the output of phase inverters 22. Terminal 53 of each operational amplifier is also connected through an alarm set-point rheostat R21 to ground.

An output terminal 58 of each amplifier A4 is connected through a series-connected diode D6 and resistor R22 to the base electrode of a transistor I2. The collectors of the two transistors are connected to the positive power input terminal, and the emitters of the transistors are connected together through a pair of series-connected resistors R23.

Circuit 25 also includes an operational amplifier A5 which acts as an "and" gate to generate an output signal when receiving inputs from both of the trigger circuits just described. Amplifier A5 has a first input terminal 60 connected through a resistor R24 to junction 54, and a second input terminal 61 connected through a resistor R25 to a junction 63 of the two resistors R23. Junction 63 is also connected to ground through a level-setting rheostat R25A.

An output terminal 65 of amplifier A5 is connected to the base electrode of a transistor T3 through a series-connected diode D7 and resistor R26. The collector electrode of transistor T3 is connected to the positive input power terminal.

A mode selector switch 66 has a first terminal 67 connected to the emitter of transistor T2 (channel A), and a second terminal 68 connected to the emitter of transistor T3. A third terminal 69 is connected to the anode of a diode D8 having a cathode electrode connected to a fourth terminal 70 of switch 66. Another diode D9 has its anode connected to terminal **67** and its cathode connected to terminal **70.** Terminal **70** is also connected to the emitter of transistor T2 in the trigger circuit associated with the second channel (channel A) of the system.

Connected between the emitter electrode of each transistor T2 and ground is a series-connected resistor R27 and light-emitting diode D10. Another light emitting diode D11 is connected between ground and the arm of switch **66** through a resistor R28. A potentiometer R29 is connected between the arm of switch **66** and ground. A warning horn or similar audio alarm **73** is connected between the arm of potentiometer R29 and ground.

Component types and typical values for the system just described are as follows:

R1 - 8.2K ohms
R2 - approx. 1–5 megohms, depending on photocell

R3, 10, 20, 25 – 22 megohms
R4, 5, 7, 8, 11, 14, 15, 18, 24 - 100K ohms
R6 - approx. 1–10 megohms, depending on photocell

R9, 17, 22, 26 – 5.1K ohms
R12, 13 – 47K ohms
R16 - 120K ohms
R19, 23, 29 – 10K ohms
R21 - 25K ohms
R25A - 20K ohms
R27 - 1.3K ohms
R28 - 600 ohms
C1 - 0.1 mfd.
C2 - 2 mfd.
C3 - 0.001 mfd.
C4, 5 – 22 mfd.
C6 - 0.01 mfd.
T1 - 2N5,486
T2 - 2N4,400
T3 - 2N4,400
A1 - 4,250
A2-5 - 741

The operation of system **10** will now be described in terms of a two-channel system having channels A and B as shown in the drawings. The amplifiers and other components in each channel provide a D-C voltage level of several volts which is proportional to the light output of an associated body of bioluminescent organisms in a respective cartridge **15.** These organisms typically have a luminosity level in the range of 0.01 to 0.0001 foot candles (approximately 10^{-11} to 10^{-13} watts per centimeter^2). The circuit is capable of linearly detecting changes of about 0.1 percent to 5.0 percent from the average steady-state light output of the organisms, while maintaining a signal-to-noise ratio of about 100 to 1 for a signal stemming from a 1 percent intensity change.

Photo-sensitive transducer **16** is preferably a cadmium-sulfide photo-resistive cell which is capable of detecting these low-level light outputs, and of providing acceptable signals for further processing and amplification by the electronic circuitry. The signal from the photo-resistive cell is conditioned by the circuitry just described to provide desired output levels, while maintaining an acceptable signal-to-noise ratio, linearity, and AGC compensation for long-term variations in steady-state light output as the organisms progress through their life cycles.

AGC circuit **28** provides a dual-mode time constant, and uses field-effect transistor T1 as a variable resistance to control the amount of feedback necessary to maintain constant output from amplifier A1. This dual-mode time constant is provided by series-parallel back-to-back diodes D2 which act as a voltage-dependent resistance of an RC time-constant network driving the gate of the field-effect transistor. At detector balance, the voltage across diodes D2 is essentially zero, and the resulting high resistance of the diodes effects a long time constant for the circuit.

Short-term signals resulting from slight light-output changes of the organisms are thus transmitted directly to the output of the circuit without being appreciably affected by the AGC circuit. Cumulative signals resulting from long-term drift in organism light output (or from power turn-on or other large transient conditions) continually reset the AGC circuit to constant operating conditions without requiring an undesirable long stabilization time for the circuit.

The input to each amplifier A1 is a D-C reference signal applied through resistor R2. Gain of this amplifier is established by the ratio of the combined resistance of transducer **16** and calibrating resistor R6 to the resistance of input resistor R2. This arrangement is useful as it compensates for the proportionate response time characteristic of cadmium-sulfide photocells to various light levels.

With a constant-gain amplifier, the result of this slow-response characteristic would be smaller signals as organism luminosity decreased during the later stages of organism life. The circuit herein disclosed, however, includes the photocell resistance (which is inversely proportional to incident light) as part of the operational-amplifier feedback gain-determining resistance. The circuit thus increases operational-amplifier gain with lower organism luminosity, thereby maintaining relatively constant output signals. By compensating for the recognized characteristics of cadmium-sulfide photocells, the circuit thus responds uniformly to bioluminescent organisms having a wide range of ambient or steady-state luminosities.

Switches **41** and **66** provide a logical arrangement capable of detecting twelve different states of organism luminosity outputs. That is, an alarm can be generated for any of the following specific conditions of channels A and B.

Plus A Alone	Plus A or Plus B	Plus A and Plus B
Minus A Alone	Plus A or Minus B	Plus A and Minus B
Plus B Alone	Minus A or Plus B	Minus B and Plus B
Minus B Alone	Minus A or Minus B	Minus B and Minus B

Rheostats R21 set the triggering point of each channel, and the output state of each channel is independently indicated by light-emitting diodes D10. The total system output received from mode-selector switch **66** is indicated on light-emitting diode D11 and by audio alarm **73.** Meter and recorder outputs for each channel are provided by circuits **45,** and switch **47** is used as a noise compression adjustment.

What is claimed is:

1. A system for detecting presence of a specific vapor in an atmosphere being sampled, comprising:

a plurality of bioluminescent sensors utilizing different strains of micro-organisms which have predetermined luminescence characteristics upon exposure to the vapor;

transducer means for monitoring the sensors and generating a plurality of signals representing luminescence of each sensor;

circuit means connected to the transducer means to receive the transducer signals and to generate an output signal when the transducer signals represent the predetermined luminescence characteristics produced by exposure of the micro-organisms to the specific vapor.

2. The system defined in claim **1** wherein the circuit means comprises an amplifier for each transducer means, a mode-selection means for sensing predetermined and selectable combinations of outputs from the amplifiers and generating an alarm output upon sensing a selected combination, indicating means connected to the mode-selection means to receive the alarm output, and coupling means connected between the amplifiers and mode-selection means.

3. The system defined in claim **2** wherein the coupling means comprises a phase inverter for each amplifier connected to receive an output from the amplifier, polarity-selection means connected to the mode-selection means, and means connecting the amplifiers, polarity-selection means and mode-selection means whereby the output from each amplifier is delivered to

the mode-selection means in a selectable polarity.

4. The system defined in claim **3** wherein the mode-selection means is arranged to generate an alarm output upon receiving an amplifier output of predetermined polarity, and also upon receiving selected combinations of several such amplifier outputs.

5. The system defined in claim **4** wherein transducer means comprises a photo-resistive cell for each bioluminescent sensor, each cell being connected in circuit with a respective amplifier.

6. The system defined in claim **5** wherein each amplifier has input and output terminals, and the photo-resistive cell is connected between the terminals to constitute a portion of a feedback loop for the amplifier.

7. The system defined in claim **5** wherein each amplifier has input and output terminals, and each amplifier includes an automatic-gain-control circuit connected in series with the associated photo-resistive cell between the terminals in feedback relationship.

8. The system defined in claim **7** wherein the automatic-gain-control circuit is arranged to adjust amplifier gain to compensate for long-term variations in steady-state luminescence emitted by the associated micro-organisms.

* * * * *

UNITED STATES PATENT OFFICE

CERTIFICATE OF CORRECTION

Patent No. 3,849,653 Dated November 19, 1974

Inventor(s) Roy R. Sakaida et al.

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Page 1, in both places change the inventor's name from "Sakaide" to --Sakaida--.

Col. 1, line 64, delete "lift" and substitute therefor --life--.

Signed and Sealed this

thirteenth Day of *April 1976*

[SEAL]

Attest:

RUTH C. MASON
Attesting Officer

C. MARSHALL DANN
Commissioner of Patents and Trademarks

UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 3,849,653 Dated November 19, 1974

Inventor(s) Roy R. Sakaida & Clifford A. Shank

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Col. 4, line 43, after "transistor" delete "12" and substitute therefor --T2--.

Signed and sealed this 18th day of February 1975.

(SEAL)
Attest:

RUTH C. MASON
Attesting Officer

C. MARSHALL DANN
Commissioner of Patents
and Trademarks