Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2080691 C 2002/02/26

(11)(21) 2 080 691

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 1991/04/17
(87) Date publication PCT/PCT Publication Date: 1991/10/31
(45) Date de délivrance/Issue Date: 2002/02/26
(85) Entrée phase nationale/National Entry: 1992/10/15
(86) N° demande PCT/PCT Application No.: US 1991/002633
(87) N° publication PCT/PCT Publication No.: 1991/015992
(30) Priorité/Priority: 1990/04/19 (511,341) US

(51) Cl.Int.^{5}/Int.Cl.^{5} G01N 21/59

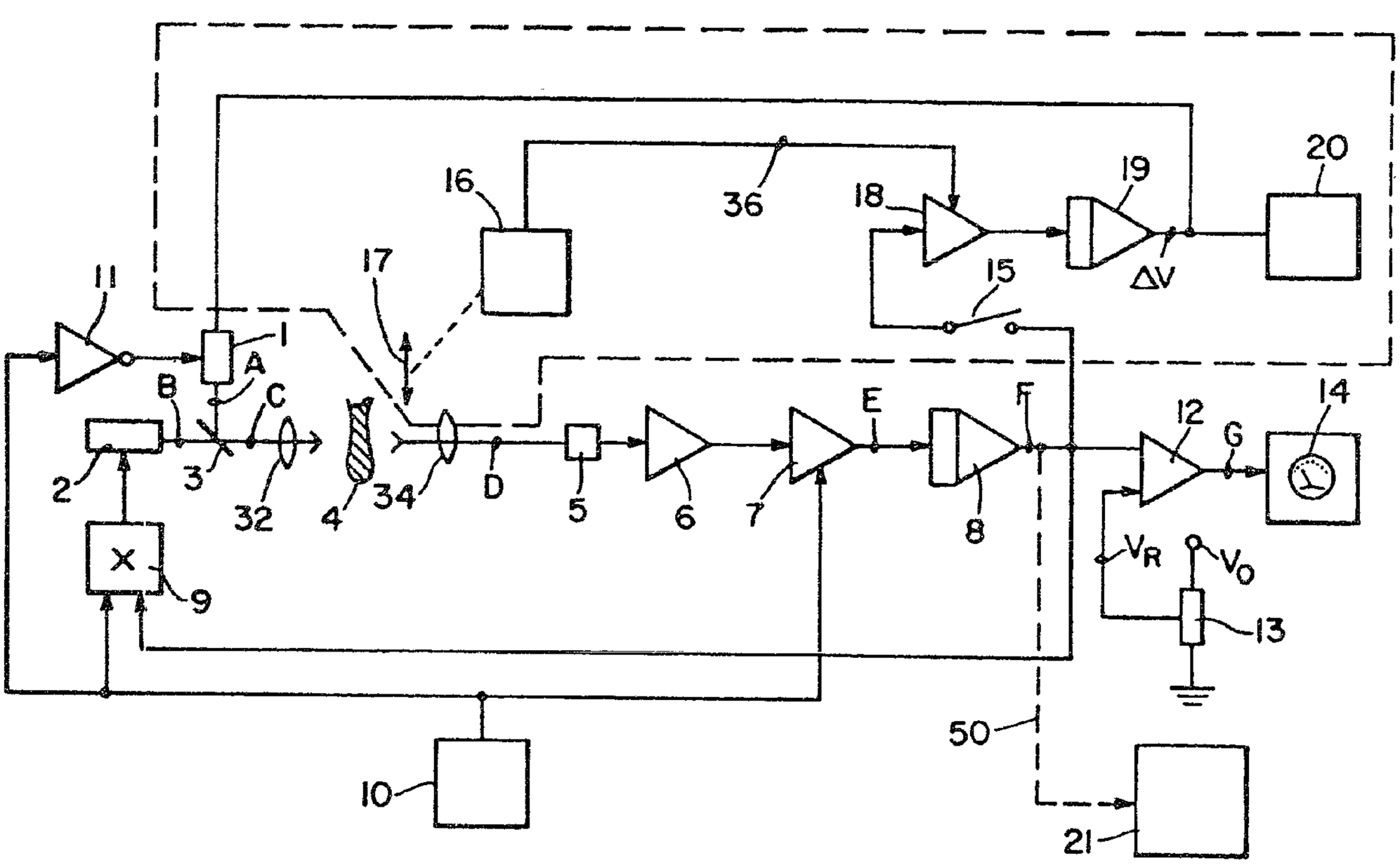
(72) Inventeurs/Inventors:
Mendelson, Yitzhak, US;
Peura, Robert A., US;
Harjunmaa, Hannu, CH

(73) Propriétaire/Owner:
WORCESTER POLYTECHNIC INSTITUTE, US

(74) Agent: SWABEY OGILVY RENAULT

(54) Titre : METHODE ET INSTALLATION POUR MESURER LA CONCENTRATION DE SUBSTANCES ABSORBANTES

(54) Title: METHOD AND APPARATUS FOR MEASURING THE CONCENTRATION OF ABSORBING SUBSTANCES



(57) Abrégé/Abstract:
A non-invasive system for measuring the concentration of an analyte in an absorbing matrix is described. The system directs a beam of radiation at the matrix. The beam consists of a series of successive alternate pulses of electro-magnetic radiation, one of which is highly absorbed by the analyte and the other of which is non-absorbed. The transmitted or reflected beam is optically detected and an electrical signal proportional to beam intensity is used to adjust the beam intensity and as a measure of analyte concentration.

2080691

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A61B 5/00

A1

(11) International Publication Number: WO 91/15992

(43) International Publication Date: 31 October 1991 (31.10.91)

(21) International Application Number: PCT/US91/02633

(22) International Filing Date: 17 April 1991 (17.04.91)

(30) Priority data:
511,341 19 April 1990 (19.04.90) US

(71) Applicant: WORCESTER POLYTECHNIC INSTITUTE [US/US]; 100 Institute Road, Worcester, MA 01609 (US).

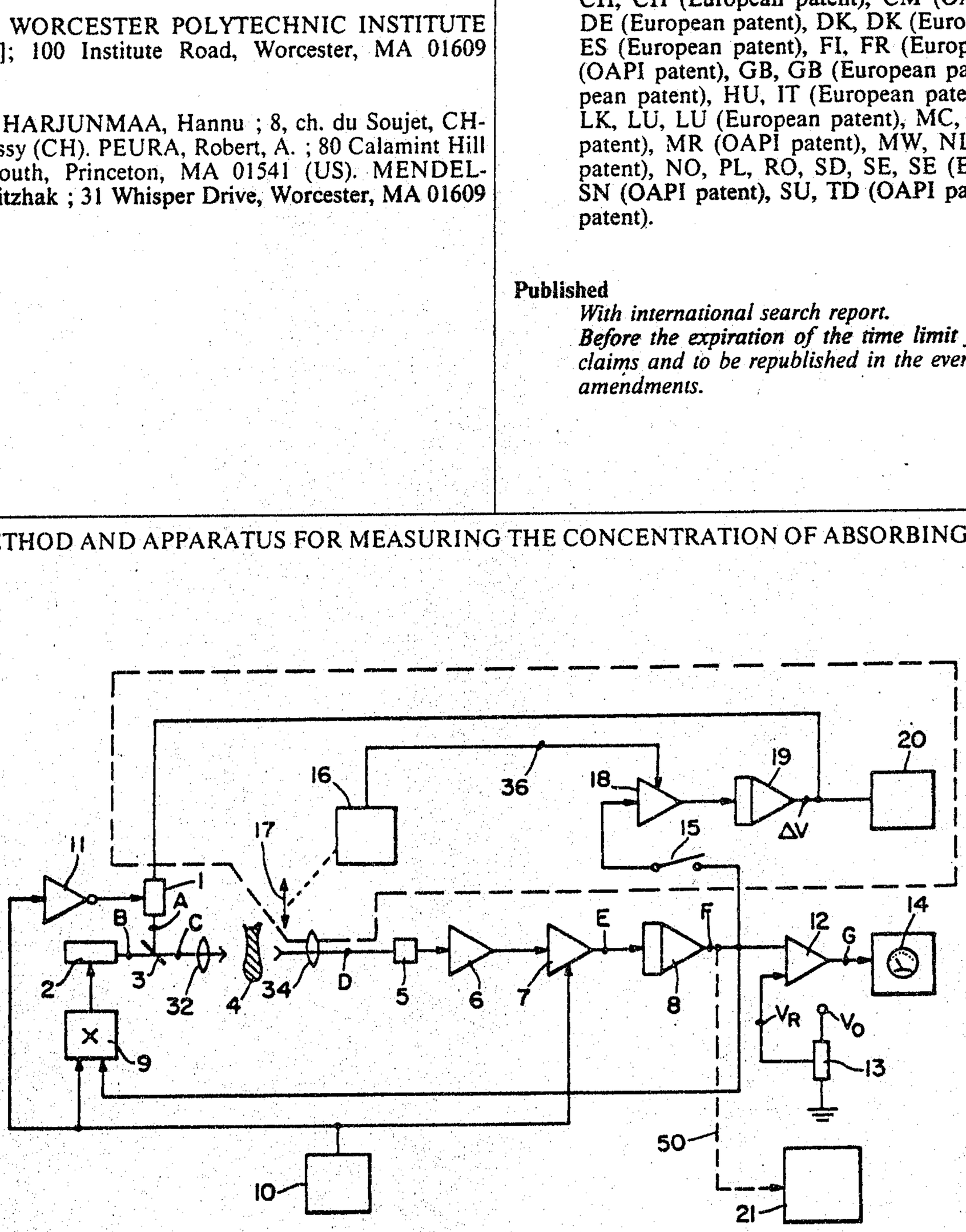
(72) Inventors: HARJUNMAA, Hannu ; 8, ch. du Soujet, CH-1234 Vessy (CH). PEURA, Robert, A. ; 80 Calamint Hill Road South, Princeton, MA 01541 (US). MENDELSON, Yitzhak ; 31 Whisper Drive, Worcester, MA 01609 (US).

(74) Agents: REYNOLDS, Leo, R. et al.; Hamilton, Brook, Smith & Reynolds, Two Militia Drive, Lexington, MA 02173 (US).

(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CM (OAPI patent), DE, DE (European patent), DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL, NL (European patent), NO, PL, RO, SD, SE, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent).

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: METHOD AND APPARATUS FOR MEASURING THE CONCENTRATION OF ABSORBING SUBSTANCES



(57) Abstract

A non-invasive system for measuring the concentration of an analyte in an absorbing matrix is described. The system directs a beam of radiation at the matrix. The beam consists of a series of successive alternate pulses of electro-magnetic radiation, one of which is highly absorbed by the analyte and the other of which is non-absorbed. The transmitted or reflected beam is optically detected and an electrical signal proportional to beam intensity is used to adjust the beam intensity and as a measure of analyte concentration.

METHOD AND APPARATUS FOR MEASURING THE CONCENTRATION OF ABSORBING SUBSTANCES

Description

Background of the Invention

This invention relates to the non-invasive measurement of the concentration of substances that absorb electromagnetic radiation, such as light or infrared radiation, in absorbing and turbid matrices. In particular, the invention is directed to substances, such as glucose, found in the blood of absorbing and turbid matrices, such as human or animal body tissue.

Numerous techniques have been proposed for the determination of glucose by non-invasive optical monitoring methods. (See "Blood Glucose Sensors: An Overview", by Peura, R.A. and Mendelson, Y., Proceedings of the IEEE/NSF Symposium on Biosensors, Los Angeles, CA, 1984.) Many of the proposed methods rely on transmissive and diffuse reflective absorption measurements using infrared radiation.

United States Patent No. 4,863,265 discloses an apparatus and method for non-invasive determination of constituent concentrations in blood. In particular, the device determines the concentration of saturated oxygen in blood. The apparatus utilizes two LEDs, one emitting red light (660 nm) and the other emitting infrared light (940 nm) to

transmit light through tissue. At each wavelength, the device measures the variations in the opacity of blood using the minimum and maximum light level passing through the tissue for each pulse cycle. If the light level received is not of a sufficient amplitude, the LED output is increased.

The infrared measurement methods known in the art are not well adapted to the problem of quantifying an analyte dissolved in a strongly absorbing solvent. The known methods include separate or directly alternating measurements using radiations at a "glucose" wavelength and at a "reference" wavelength, where glucose does not absorb, as well as differential wavelength modulation about a glucose absorption band (C. Dahne, D. Gross, European Patent 0 160 768 and

references therein). In the known methods, the signal is easily lost into the strong background presented by the water in tissues and in the capillary blood flow. The normal concentration range of glucose in blood for male adults is 4 to 6 mmol/l (70 to 110 mg/dl).

A need still exists, therefore, for a non-invasive method and apparatus having sufficient long-term sensitivity to accurately measure the concentration of light absorbing substances in absorbing and turbid matrices found in the human or animal body.

Summary of the Invention

The present invention comprises a system which periodically or continuously directs two beams of electromagnetic radiation of different wavelengths λ_1 and λ_2, respectively, at a radiation absorbing body. The radiation at one wavelength λ_1 is alternated at equal successive intervals in time with radiation at the other wavelength λ_2.

One of the wavelengths, say λ_1, is chosen to be highly absorbent by the analyte to be measured, while the other, λ_2, is selected to be substantially non-absorbed by the analyte. In this manner, a single beam is formed of a series of successive alternate pulses of equal duration and amplitude of radiation λ_1 and λ_2. The transmissive/reflective beam is optically detected and an electrical signal is generated proportional to the detected beam

intensity. With no analyte present, the system is calibrated so that the intensity of the detected beam is a constant D.C. signal during the pulse periods.

The variation of the detected signal when the analyte is present is integrated over time to produce a control signal. This control signal is used to constantly adjust the intensity of one of the laser beams in a direction which tends to bring the detected signal to zero or null. The control signal is also a measure of the analyte concentration and is calibrated against a reference voltage and displayed as an analyte concentration value.

An important difference between the present method and those known in the prior art is that, in contrast to the known methods, the method of this invention forms the difference of the signals obtained at an analyte wavelength λ_1 (where the analyte absorbs), and a reference wavelength λ_2 (where the analyte essentially does not absorb) directly at the optical level (i.e., by optical means), instead of comparing electronically the two signals at the analog or digital level (i.e., by electronic circuitry means). The difference of the intensity signals at λ_1 and λ_2 is used in an optical null arrangement which, as its output, gives a control signal proportional to the concentration of the analyte.

The two wavelengths λ_1 and λ_2 are selected so the radiation has exactly the same degree of matrix

extinction at these wavelengths. Note: The matrix extinction is the sum of the absorption and scattering experienced by the beam in a matrix sample without the analyte. With the method of this invention, one can detect lower glucose concentrations in human tissue than with the currently known methods.

Detailed Description of the Drawings

Fig. 1 is a block diagram of the system of the invention.

Fig. 2 is a timing diagram showing Rows A-G of waveforms at different locations in the block diagram of Fig. 1. The three columns show the waveforms under different analyte conditions, i.e., Column 1 is the calibrated condition with no analyte present in the sample matrix, Column 2 the transient condition after introduction of the analyte into the sample matrix and Column 3 with analyte after steady state is obtained.

Fig. 3 is a schematic representation of an optical device for use in maintaining the optical probe in fixed position on the subject of measurement.

Detailed Description of the Invention

The invention will now be described in connection with the drawings.

The invention is described as applied to the special case of glucose measurement in human tissue using near-infrared radiation. This should in no

way detract from the general applicability of the invention to measure the concentration of any species that absorbs electromagnetic radiation, especially in strongly absorbing and turbid matrices.

A. GENERAL OPERATION

In the method of this invention, the measurement is made by combining into a single beam (curve C), the alternate pulses curve A and curve B defined as "half-periods" of radiation at two wavelengths λ_1 and λ_2. The single beam is directed against the sample 4, i.e., an ear lobe, thus providing a response beam (curve D) to be detected by a detector 5. With no analyte present, the optical intensity should be constant, as shown in curve D, row 1. The electrical response generated in the detector 5 by the constant intensity λ_1 and λ_2 half-period response beam is calibrated to be zero, or null (curve E row 1). When there is a nonzero concentration of the analyte, the intensity of the beam is no longer constant. The intensity of one of the half-periods changes with respect to the other, as shown in curve D row 2. This change is detected by detector 5 and the amplitude of the alternating-current (AC) signal given by the detector 5 is representative of the analyte concentration (curve E row 2). This signal is not used directly to quantify the analyte concentration, but is used, instead, in an optical null arrangement

to change the relative intensity of the two half-periods. The AC signal is amplified and rectified in the lock-in amplifier 7. The resulting DC signal is integrated in integrator 8 to produce a control signal (curve F row 3). The value of the control signal needed to restore the signal from the detector 5 to zero (curve E row 3) is used as the indicator of the analyte concentration.

The measurement geometry may be either direct transmission, transflection or attenuated total reflection. Direct transmission is shown herein by way of example.

B. THEORY

The principles governing the method of the present invention are briefly outlined below with the assumption that the Beer-Lambert law, $P = P_o e^{-kx}$ is valid.

In the above relation, P_o is the power of the incident collimated beam falling on the sample, k is the absorption coefficient (usually in 1/cm) and x is the length (in cm) of the sample in which interaction occurs. To simplify the equations, only essential quantities are retained and the signal is considered radiative only; scattering can be included in k, and, if its contribution is desired explicitly, it is a straightforward operation to replace k by the sum of absorption and scattering effects.

In view of the above, the powers collected at

wavelengths λ_1 and λ_2 are $P_{\lambda_1} = P_{01} e^{-k1x}$ and and $P_{\lambda_2} = P_{02} e^{-k2x}$, respectively.

Since provision is made that the absorption of the background is the same at λ_1 and λ_2, the difference

$$S = \Delta P = P_{\lambda 1} - P_{\lambda 2} = 0$$

if no analyte is present. This difference is hereafter called the error signal. The electrical signal produced in the detector is assumed to be proportional to the optical power.

When analyte is present, it absorbs at one of the wavelengths, but not at the other, which means that for the first wavelength, say λ_2, the absorption coefficient has changed by, say, Δk. Hence now,

$$S \neq 0 = P_0 [e^{-(k-\Delta k)x} - e^{-kx}] \text{ or}$$

$$= P_0 e^{-kx} [e^{\Delta kx} - 1].$$

Now for Δk small, i.e, < 0.1, the known approximation $e^{\Delta kx} = 1 + \Delta kx$ holds; so $S = P_0 \Delta kx e^{-kx}$, i.e., the error signal is proportional to Δk, that is, to the analyte concentration. Also, it can be seen that the error signal has a maximum with respect to path length, for a given analyte concentration. This maximum can be obtained by

taking the derivative of the above equation. It occurs at the path length of 1/k.

When the concentration of analyte is nonzero, an error signal is generated, but the system strives to keep it at zero by changing the intensity of one component wavelength:

$$P_{O2} = (1 + f)\ P_{O1}.$$

Here, f is the relative change in the intensity at λ_2 with respect to the equilibrium state.

$$S = P_{O1}\ e^{-k1x} - P_{O2}\ e^{-k2x} = 0$$

$$P_{O1}\ e^{-k1x} - (1 + f)\ P_{O1}\ e^{-k2x} = 0$$

$$e^{-k1x} = (1 + f)\ e^{-k2x}$$

$$1 + f = e^{\Delta kx}.$$

If Δkx is small, which is to be expected, the approximation $e^{\Delta kx} = 1 + \Delta kx$ is valid, which leads to

$$f = \Delta kx,$$

or, the relative deviation from equilibrium intensity is proportional to analyte concentration and to path length.

If there is some analyte absorption at the reference wavelength, the signal diminishes in proportion to the difference of the analyte absorptions at the analyte wavelength and the reference wavelength.

In order to account correctly for scattering, the wavelength choice must be made on the basis of the sum spectrum of absorption and scattering in the sample matrix (that is, extinction spectrum), with due consideration to the measuring geometry, which affects the relative importance of scattering.

Table 1, below, indicates a few wavelengths at which glucose absorbs which can be used to practice the invention in combination with the background absorption values on the same line of the Table. Water absorption coefficients at the indicated wavelengths are also in the Table.

TABLE I

Wavelength in (μm)	Glucose Absorption (μm)	Background Absorption (μm)	kH_2O (1/cm)
1.57	1.75 (gl)*,	1.38 (st)*	9
1.77	1.55 (gl),	1.39 (st)	7
2.10	2.29 (gl),	1.87 (st), 1.48 (pk)*	30
2.17	1.86 (st)	1.49 (st), 1.41 (st)	25
2.27	2.15 (gl)	1.86 (st), 1.48 (pk), 1.40 (st)	30

where: *st = steep; pk = peaking; gl = glucose absorption.

For fine tuning the wavelengths, one keeps a member of the pair constant while the other is adjusted. Preferably, the glucose wavelength is kept constant in order to have a constant sensitivity for glucose. The reference wavelength is preferably situated on a moderate or shallow slope of the water absorption spectrum: with a steep slope, accurate control is more difficult. In Table 1, some reference wavelengths are situated on a steep slope; others are at or near a peak; some reference wavelengths have glucose absorption.

The fine tuning can be achieved automatically, as will be described in the alternate embodiment, shown in dotted lines in Fig. 1.

C. PREFERRED EMBODIMENT

The following example illustrates the invention with reference to the annexed drawings. This invention can be carried out using many other embodiments not specifically exemplified here but which should not be excluded from protection.

Because of the strength of glucose absorption at 2.1 μm, the present embodiment has been devised for the wavelength pair 2.10/1.48 μm. This wavelengths selection is only one example, no other suitable wavelength pairs being excluded from the scope of this application.

Referring to Figs. 1 and 2, the radiation source of this example consists of two pulsed lasers 1,2 operating at the wavelengths λ_1 and λ_2, respectively. In the timing diagram of Fig. 2, the

optical intensity of these beams and the resulting detected voltages at various points in the system are plotted versus time for different conditions. That is, Column 1 shows the various waveforms for a calibrated system in which no analyte is present in the matrix, Column 2 shows the transient condition after introduction of analyte into the sample matrix, and Column 3 shows the steady state condition with analyte present in the sample matrix. The following is a summary of the timing diagram waveforms:

A. Relative optical power in the constituent beam marked A.

B. Relative optical power in the constituent beam marked B, which is modulated in antiphase to A. The on-value of this power is adjustable and proportional to the signal F.

C. Relative optical power in the combined beam before the sample. In a calibrated system without sample, this has no AC component.

D. Relative optical power after the sample. The system seeks to keep the AC component of D to zero.

E. Lock-in amplifier 7 output, proportional to the AC component of D. This is the error signal for the servo loop. The system seeks to keep E to zero.

F. Integrated error signal equals the intensity control voltage for one of the constituent beams.

G. Deviation of the F voltage from its initial value. Obtained using a zero-shift circuit (difference amplifier). As the last step of the calibration process, potentiometer 13 is adjusted to give a displayed value (G) of zero units. Thereafter, G is proportional to the analyte concentration.

Note: The term "relative optical power" above refers to the optical power as measured with the particular detection system used in this application. Generally, the sensitivity of the detection system will not be the same at the two different wavelengths, and, in reality, the "relative optical powers" are not absolutely equal at the point C. They produce, however, equal responses in the detection circuit of this system. The fact that the powers are not absolutely equal has no significance to the operation of the system of this invention.

Referring back to Fig. 1, the output beams "A" and "B" of the lasers are combined in the beam combiner 3. The combined beam "C" is directed into a sample 4, such as an ear lobe. After reflection or transmission, the optical power is as shown at curve "D".

The optical system includes collimating means 32 and 34, respectively, i.e., lenses or mirrors to

direct the sample channel beam "C" into the sample 4 and from the sample 4 beam "D" to the sample channel detectors 5.

The system uses a photoconductive PbS infrared detector 5 operating at room temperature. Its spectral sensitivity peaks at about 2.0 to 2.5 μm. The PbS detector 5 is operated in the classical bolometer circuit, and AC-coupled to a preamplifier 6. Other detectors sensitive in the relevant wavelength range could be used, with the appropriate coupling and amplifying method.

The output of the PbS detector 5 is quantified using a lock-in amplifier 7 that uses the signal produced by a square wave generator 10 as its reference signal. The output "E" of the lock-in amplifier 7 is a rectified direct-current signal proportional to the alternating-current signal produced by the detector 5. It is important to preserve the sign (phase) of the AC signal, because these signals are used for closed-loop control. For this reason, simple rectification where the phase is lost cannot be used. The present circuitry takes care of this phase selection requirement. The error signal "E" from lock-in amplifier 7 is integrated over time in integrator 8 to produce a control signal "F".

The operation of the system is governed by the square wave generator 10 operating at a frequency of typically between 10 Hz and 100 kHz, and using the techniques of this example, 1 Khz. The generator 10 signal determines which one of the two wavelengths

and which one of the two corresponding intensity levels is to be used at any given moment.

It is assumed that the output of the lasers 1 and 2 are proportional to intensity control voltages (if the control voltage is zero, then the laser beam is off). If in a particular embodiment the lasers should be of a type whose intensity cannot be controlled by a voltage, then an appropriate modulator is used to the same effect. The inverter 11 ensures that the lasers operate in antiphase, or that one of them is "off" while the other is "on". The analog multiplier 9 changes the intensity of the beam between the two intensity values and adjusts one of the intensities according to the output of the integrator 8. As long as that output is non-zero, the intensity is constantly adjusted to zero the output of laser 2.

During operation, the error signal servoes itself to zero. This establishes the basic equisensitivity of the channels at the wavelengths initially selected. The intensity control signal from integrator 8 is also used as the basis for the glucose concentration display. The zero point of this signal is set by comparison with a reference voltage V_R in the difference amplifier 12 established by DC reference voltage V_o across precision potentiometer 13. The resulting voltage "G" is scaled and displayed in the display unit 14 to show the glucose concentration.

D. AUTOMATIC WAVELENGTH TUNING

An automatic calibration system option is shown in dotted lines in Fig. 1 as part of the present invention. The reference wavelength selected for measurement depends on the calibration of the subject to be tested. The calibration and the subsequent measurements are performed at a well-defined and easily available test site 4, such as the ear lobe or the skin of the fingerwebs, where the glucose concentration in blood is known. This glucose in blood concentration should preferably be low. To perform the calibration, the switch 15 is closed and the sample 4 is moved In and Out of the beam, or the beam is moved In and Out of the sample, by actuator 16 and beamshifter mechanism 17 at a low frequency, for instance 1 Hz. The intensity control signal, that is to say, the output of the integrator 8, will vary at the same frequency. If the matrix extinction of the sample 4 is not exactly the same at the two wavelengths, the amplitude of this variation is obtained at the output of lock-in amplifier 18, using the timing signal of the actuator 16 on line 36 as the reference. A wavelength control signal ΔV is obtained by integrating the output of the lock-in amplifier 18 in the integrator 19. It is assumed that the laser 1 can be tuned using a control voltage.

The exact reference wavelength obtained is noted and kept on record in a digital memory or computer 20 for that particular patient.

Potentiometer 13 is used to set the display to show a concentration value equal to the known concentration (zero or non-zero) of the calibration sample. The sensitivity of the intensity control signal to glucose concentration, known on the basis of previous tests and substantially constant at constant path length in the sample, is used to establish the complete response function of the system.

The measurement must always be done exactly at the same test site for a particular patient in order to preserve the validity of the calibration. To that effect, an optical device, interfacing with the basic optical system, may be semi-permanently attached to the test subject at a suitable test site. This is depicted schematically in Fig. 3. The optical device may, for example, take the appearance of an earring 24, having an optical input element 22 on one side of the ear lobe 4 and an optical output element 23 on the other side of the ear lobe, both transparent at the measurement wavelengths. The device 24 has the property of maintaining the probe at a fixed position on the ear lobe.

In a living test subject, the dependence of the signal on path length may cause the pulsing of the blood circulation to be seen, depending on the measuring geometry. This can be used to select the exact moment of recording the signal to occur at a constant phase of the pulse.

The pulsing effect is only seen on the signal if the system is fast enough. This will require the wavelength-alternating frequency to be preferably at least of the order of 1 kHz, and the servo loop cutoff frequency (essentially the lock-in cutoff frequency or the inverse of the output time constant divided by 2π) at least of the order of 10 Hz. The system can also be deliberately made slow so that the pulsing is not seen and does not affect the accuracy.

Accordingly, an optional provision, shown by dotted line 50, is provided in Fig. 1, wherein an analog computing circuit or, preferably, a digital computer 21, may be used to control the taking of the reading at a constant phase of the pulse cycle, be it at the systolic or diastolic extremes or somewhere else, and coupling this control signal back to one of the lasers for intensity control. It is not important which phase is used, as long as it is always the same.

Equivalents

Those skilled in the art will know, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. For example, it may be possible to produce more than one wavelength from a given laser, so that switching between two widely spaced wavelengths of one laser may be used in place of the two laser sources 1 and 2. Light

sources, other than lasers, may be used and filtered to produce monochromatic light.

These and all other equivalents are intended to be encompassed by the following claims.

CLAIMS

1. A method for determining and measuring in a sample matrix being tested (4) the concentration of an analyte which absorbs electromagnetic radiation of wavelength λ_1, this method comprising the steps of:

a) generating said radiation of wavelength λ_1 and another wavelength of electromagnetic radiation λ_2 at which the absorption coefficient of the analyte is different from the one at λ_1;

wherein the improvement comprises:

b) adjusting the intensities of the two wavelengths so that the extinction of both wavelengths is equal when the concentration of analyte in the sample matrix being tested (4) is low or zero;

c) combining said radiations into a probing beam which alternates in time at wavelengths λ_1 and λ_2, the intensity of the beam being controllable in at least one of the wavelength durations, and directing this beam at the sample matrix being tested (4) to produce an incident beam;

d) detecting the incident beam after it has traversed a path in the sample matrix being tested (4) and producing an alternating signal corresponding to the

alternations of the two wavelength radiations in the incident beam;

e) generating an intensity control signal from said alternating signal;

f) using the said intensity control signal to control the intensity ratio of one of the two wavelength radiations of the probing beam to reduce the alternating signal substantially to zero; and

g) comparing the intensity control signal to a reference signal and using the result of the comparison as a measure of the concentration of the analyte in the sample matrix being tested (4).

2. The method according to Claim 1 wherein one of the wavelengths λ_1 and λ_2 is tuned by the steps of:

a) changing periodically the relation of a calibration sample (4) to the probing beam at a frequency substantially lower than the wavelength frequency, so that the sample (4) is alternating in and out of the beam;

b) generating a wavelength control signal by rectifying the alternating component of the intensity control signal; and

c) using the wavelength control signal to tune the wavelength of one of the radiations to reduce the alternating

component of the intensity control signal substantially to zero.

3. The method according to Claim 1, where the matrix (4) exhibits pulsatile variations of thickness or composition, and the instantaneous value of the intensity control signal is recorded at a constant phase of the pulsatile cycle.

4. The method according to Claim 1 wherein the analyte is glucose and the matrix is the human body, the extinction coefficient of the human body being determined from a wavelength calibration at a point on the body where, or during a time when, the glucose concentration is low.

5. Measurement apparatus to determine an unknown concentration of an analyte that absorbs electromagnetic radiation and is dissolved or dispersed in a matrix sample (4), comprising:

a) generating means (1,2) for generating a probe beam of the said radiation which contains, alternating in time, two different and substantially monochromatic wavelengths;

wherein the improvement comprises:

b) the wavelengths being adjusted such that, at the two wavelengths, the extinction

caused by the combined effects of absorption and scattering in the matrix (4) is equal, but the absorption produced by the analyte is different at one wavelength;

c) optical means (32) for transmitting this beam into a matrix sample (4);

d) collecting means (34) for collecting the beam after it has traversed the sample, said collecting means comprising a detector (5) sensitive to both said wavelengths and providing in response an alternating-current signal;

e) electronic means to rectify (7) and integrate (8) the alternating current signal from the detector (5) at the wavelength-alternating frequency to produce an intensity control signal;

f) control means (12) for controlling the intensity ratio of the wavelengths using the said intensity control signal so that the alternating-current signal from the detector (5) is maintained substantially at zero;

g) comparison means to compare the said intensity control signal to a reference signal to produce a concentration signal; and

h) conditioning and display means (14) to condition and display the said concentration signal.

6. Apparatus according to Claim 5, comprising in addition:

a) translating means (16,17) for moving a sample (4) in and out of the probe beam at a frequency substantially lower than the wavelength-alternating frequency;

b) means (18,19) for amplifying and rectifying the alternating-current component of the intensity control signal occurring at the frequency of sample insertion to produce a wavelength control signal; and

c) tuning means (13) for adjusting one of the wavelengths using the wavelength control signal.

7. Apparatus according to Claim 5, in which the analyte is glucose to be measured in human or animal body tissue, the two different wavelengths being between about 1 to 2.5 μm.

8. Apparatus according to Claim 5, in which the optical means and the collecting means include one or more optical elements (22,23) that are semi-permanently attached to the sample (4) and can be repeatedly taken out of and returned to

the measurement apparatus, together with the sample, to allow the measurement to be repeated a multiple of times at constant geometrical relationship to the sample (4).

2080691

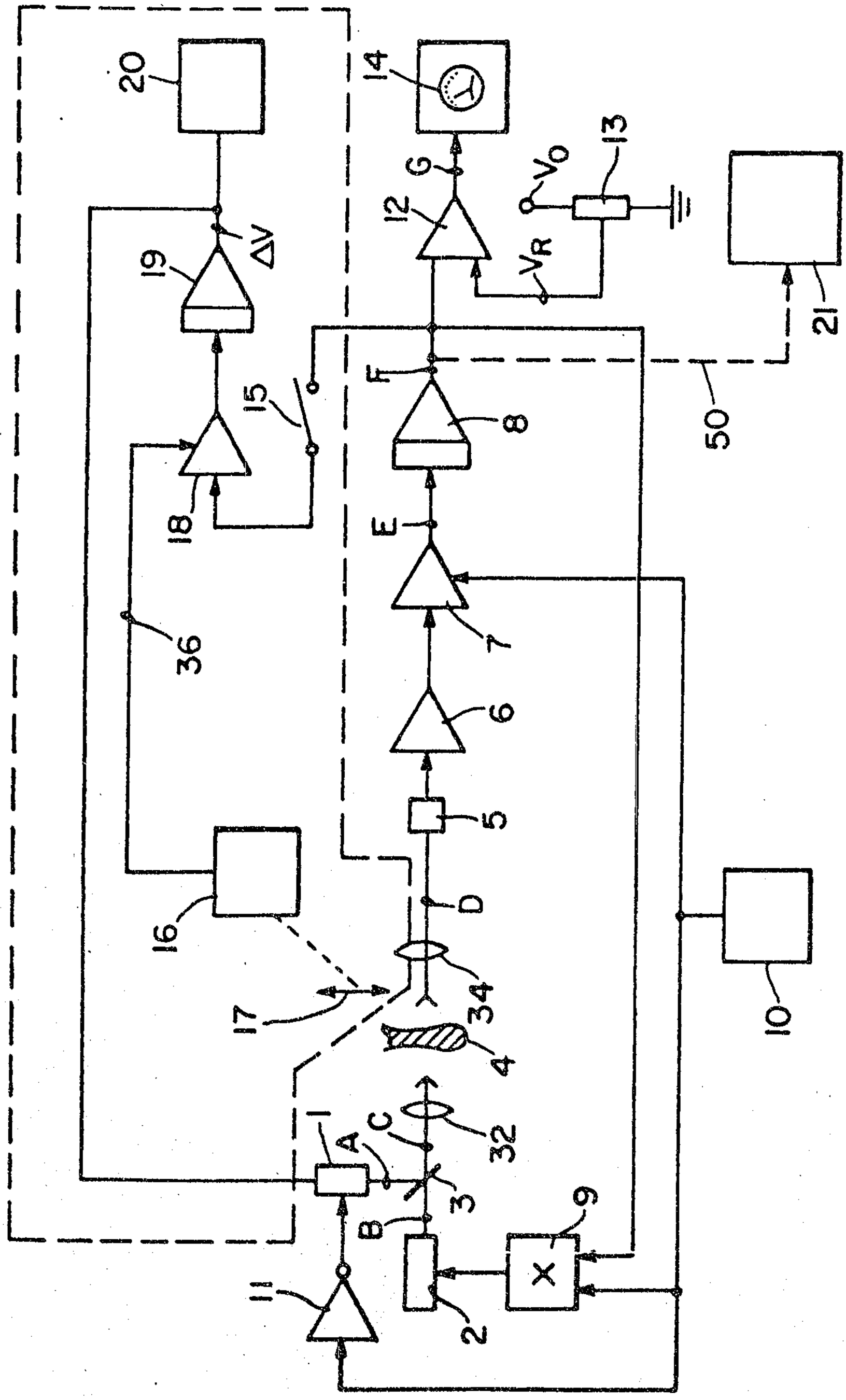


Fig. 1

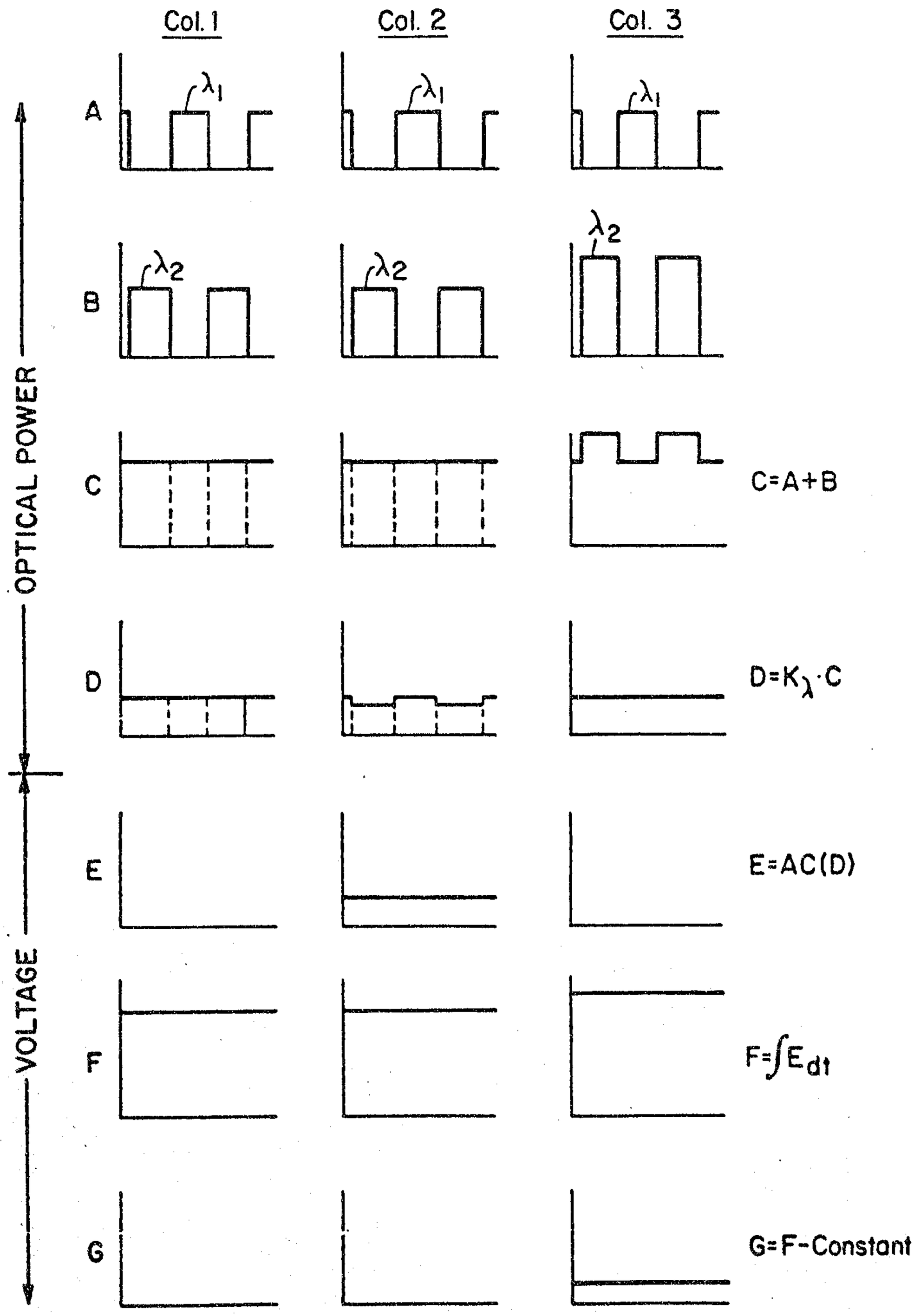

Col. 1
Col. 2
Col. 3
A
B
C
D
E
F
G
λ_1
λ_2
C=A+B
D=K_λ·C
E=AC(D)
F=$\int E_{dt}$
G=F-Constant
OPTICAL POWER
VOLTAGE
TIME

Fig. 2

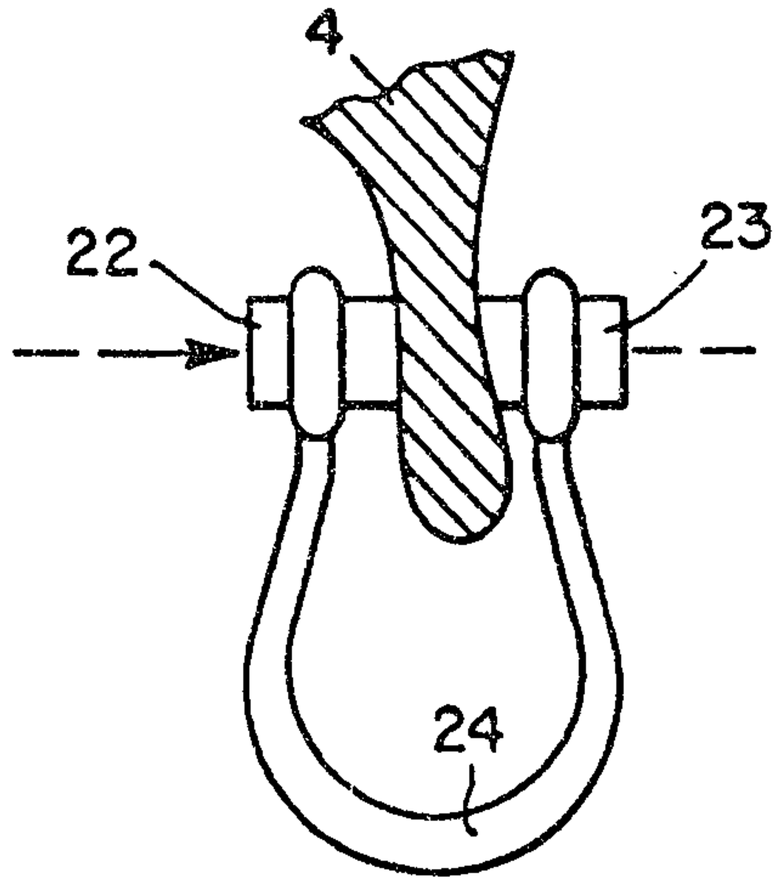
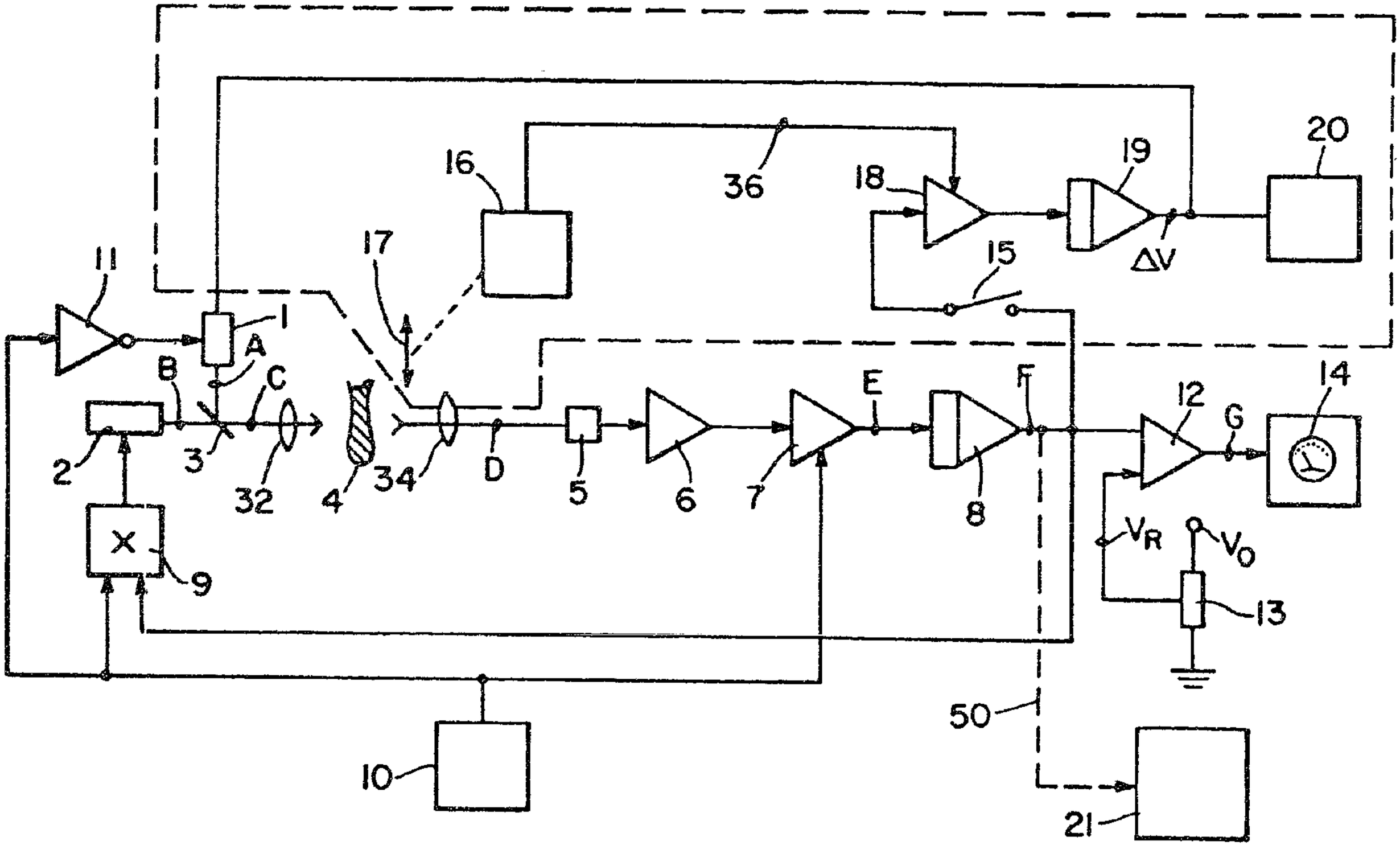


Fig. 3


16
36
18
19
20
17
11
15
ΔV
1
A
B
C
E
F
12
14
G
2
3
32
4
34
D
5
6
7
8
X
9
V_R
V_O
13
10
50
21