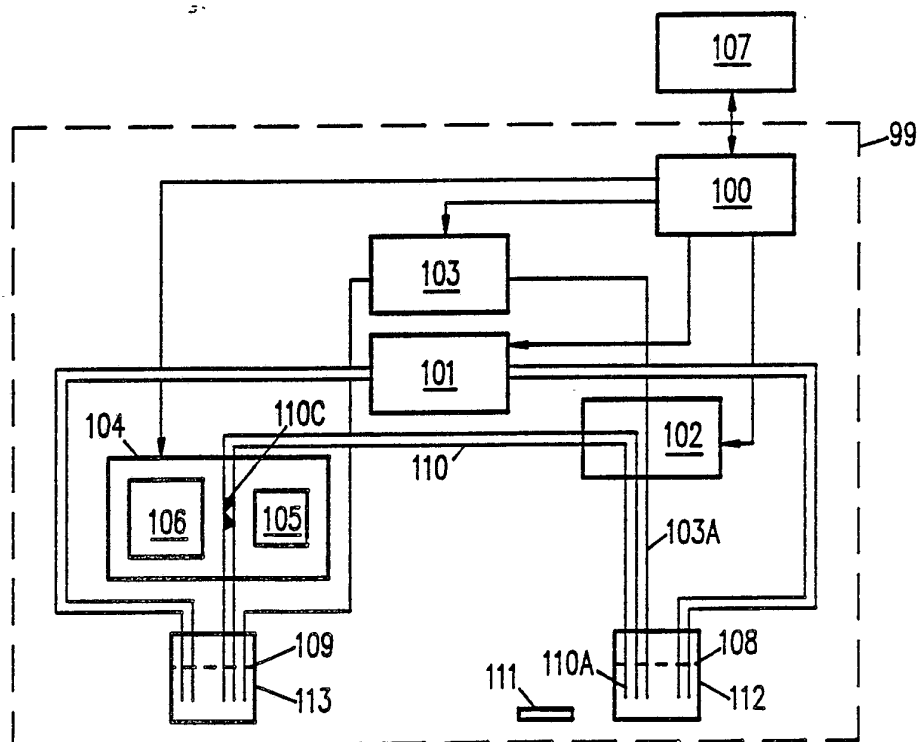


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(54) Title: A DETECTOR FOR FLUORESCENCE AND ABSORPTION SPECTROSCOPY



(57) Abstract

Automated capillary electrophoresis tests can be performed using the capillary electrophoresis instrument (99) of this invention. The capillary electrophoresis instrument (99) has means for automatically purging and replenishing electrolytes (101), for automatically entering a sample into the capillary tube (110) and for automatically performing capillary electrophoresis. A detector (104) for simultaneous detection of absorbance and fluorescence provides means for quantifying the species separated by electrophoresis.

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A DETECTOR FOR FLUORESCENCE AND
ABSORPTION SPECTROSCOPY

Robert G. Brownlee

RELATED APPLICATION

5 This application is related to U.S. Patent Application
Serial No. 07/187,769, entitled "An Auto-Sample System and
Related Apparatus for Capillary Electrophoresis," filed by
Robert G. Brownlee, assigned to the assignee of the present
invention and incorporated herein by reference.

10 BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

15 The invention relates generally to a subsystem for
fluorescence and absorption spectroscopy and more specifi-
cally to an apparatus for simultaneously generating
absorption and fluorescent data for a single sample.

DESCRIPTION OF THE PRIOR ART

20 High performance liquid chromatography (HPLC), gel
electrophoresis and capillary electrophoresis are all used
for biochemistry applications such as separation of DNA
molecules. Electrophoresis is a separation technique
produced by the migration of charged molecules (or
particles) in an electrolyte under the influence of an
electric field. Smaller or more highly charged sample
molecules move faster than larger or lower charged
25 molecules. Hence, each species of the sample molecules is
divided into bands which pass or reach a fixed point at
different times.

30 In gel electrophoresis (GE), the electrolyte is usually
supported by a porous hydrophilic polymer matrix, the gel,
coated on a sheet of glass, sandwiched together with another
glass plate and sealed on each side with a gasket. The

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samples are applied to the top edge of the gel. The bottom edge of the gel sandwich is placed vertically in a reservoir containing a buffered electrolyte. A second reservoir is placed on top of the sandwich and filled with buffered electrolyte. Each reservoir contains an electrode connected to the proper output of a DC power supply. A voltage of up to two thousand volts is applied to the sample. A typical GE run may take six hours. After the run the plate must be stained to visualize the GE bands of interest.

The success of electrophoresis in most applications depends upon the effective utilization of a stabilizing medium such as polymer gels. The gels stabilize the separation medium against convection and flow which would otherwise disrupt separations. A large body of modern electrophoresis technology is devoted to understanding and to controlling the formation of electrophoresis gels.

Gel electrophoresis, as commonly practiced, is generally not considered a true instrumental method of analysis. Instrumental versions of gel electrophoresis analogous to column chromatography are still in the developmental stages in most cases. The presence of the stabilizing gels has prevented the adaptation of electrophoresis to on-line detection quantification or automated operation and consequently gel electrophoresis is still a manual intensive methodology.

Capillary electrophoresis has been developed as an alternative to column chromatography and gel electrophoresis because capillary electrophoresis is up to ten times faster than gel electrophoresis and is more accurate. In capillary electrophoresis, the time, expense and variability of packing a chromatographic column or casting an electrophoresis gel are obviated. Small samples are separated and analyzed in a few minutes. The sharpness of separations is enhanced by the use of narrow-bore tubing, since this minimizes the thermal gradients and the

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consequential convective turbulence and diffusion of sample components. Capillary separations avoid the "eddy migration" problems which are encountered when stabilizing media such as electrophoretic gels or chromatographic packings are used.

A schematic of a prior art capillary electrophoresis system is illustrated in Figure 1. A high voltage power supply 10 provides a high voltage to a first electrode 17 which is mounted in a first reservoir 16 containing an electrolyte. A first end 15 of a capillary tube 14 is also suspended in reservoir 16. A second end 13 of capillary tube 14 is mounted in a second reservoir 12 containing an electrolyte and a second electrode 11 connected to ground through power supply 10. A detector 18 is mounted around capillary tube 14.

Capillary tube 14, used to bridge the gap between two electrolyte reservoirs 12, 16, is typically a fused silica capillary tube 50 microns in diameter and about 50 cm long. Tube 14 is first filled with electrolyte and then approximately five nanoliters of sample solution are introduced at end 15 of capillary tube 14, and an ultraviolet (UV) light is passed through the diameter of the capillary tube by the detector near end 13 of tube 14. When a 20-30 KV potential is applied across capillary tube 14, electrophoresis causes all charged sample molecules to travel along capillary tube 14 at different velocities and to pass through the illuminated section of capillary tube 14 at different times. The sample molecules are detected by a photosensor placed opposite the UV light source in the case of an absorbance detector, or at right angles to the light source in the case of a fluorescence detector. All sample components whether anions, cations, or neutrals are eventually swept through the detector as peaks, sometimes called bands, and the output signals from the detector are analyzed to identify the characteristics of the sample.

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Since the migration time for a species in the sample is dependent upon the length of capillary tube 14, the electrophoretic mobility of the species, and the applied voltage, species having different electrophoretic mobilities will pass through the detector at different times. The separation efficiency of the various species in the sample in terms of the total number of theoretical plates is dependent upon the mobility, the applied voltage and the diffusion coefficient. Hence, high separation efficiencies are best achieved through the use of high voltages. Also, column length plays no role in the separation efficiency but column length has an influence on the migration times and hence the time required for analysis of a sample.

The CE system, as illustrated in Figure 1, potentially provides rapid, high resolution online detection capability that is not attainable with gel electrophoresis equipment. Capillary electrophoresis circumvents the labor intensive manual procedures of experiment preparation, sample manipulation, data generation and interpretation which is inherent in gel or other stabilizing medium electrophoresis techniques. Further, the quantity of sample required to perform capillary electrophoresis is significantly less than that of the other methods.

A variety of methods for introducing the sample into the capillary tube have been used. Displacement techniques such as direct injection, gravity flow or siphoning and suction are commonly used since these techniques are simple and produce separations which accurately reflect the relative concentration of sample constituents. Other techniques for introducing the sample involve the principle of electromigration. In these applications, the samples are introduced into the capillary tube by a short duration electrical current. Both electrophoretic and electro-osmotic forces can contribute to the sample movement in these techniques. Devices using a sample splitter (See M.

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Deml, F. Foret and P. Bocek, "Electric Sample Splitter for Capillary Zone Electrophoresis," J. Chrom. 346 pp. 159-165 (1985)), a micro injector (See R.A. Wallingford and A.G. Ewing, "Characterization of a Micro Injector for Capillary Electrophoresis," Anal. Chem. 59, pp. 678-681 (1987)), and a rotary injector (See T. Tsuda, T. Mizuno, and J. Akiyama, "Rotary-Type Injector for Capillary Zone Electrophoresis," Anal. Chem. 59, pp. 799-800 (1987)) have been reported.

In capillary electrophoresis separations utilizing electroendosmotic flow, the sample components are introduced at the high voltage anode side of the apparatus. This is the opposite of most conventional electrophoretic techniques.

Fused silica is most commonly used for electrophoretic capillaries. Capillary tube inner diameters of 50-100 microns with wall thicknesses of less than 200 microns are used in most applications. Capillary lengths of 10-100 centimeters are most often used. While as described above, the species separation is theoretically independent of capillary length, and a shorter tube would seem advantageous in minimizing band broadening caused by diffusion and sample interactions. Practical considerations of Joule heat dissipation dictate the length of the capillary tube.

The regulated direct current high voltage power supplies have had potentials up to 50 kilovolts. These voltages generate microamp currents through the capillary tube. Again, while the theoretical considerations indicate that faster separations are obtained with higher voltage potentials, there are practical limits imposed by heat dissipation requirements. In addition, excessively high voltages may result in corona discharge through the capillary tube and elsewhere within the instrument.

The small scale of analysis in capillary electrophoresis requires ultra-sensitive detection instrumentation. Hence, no convenient universal detector

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for every conceivable type of sample molecule exists. Multiple detection methodologies have been used. Capillaries have been used for years in "on-column" detection among several areas of the separation sciences, most notably, liquid and gas chromatography. Hence, much of the detection instrumentation for capillary electrophoresis is drawn from these areas. Ultraviolet wave length detectors are commonly used for analysis of amino acids, peptides, proteins, nucleic components, as well as some carbohydrates, drugs and other molecules of biological significance. In one prior art application, capillary electrophoresis zone detection was accomplished with fluorescence detectors and ultraviolet absorption detectors. Both detectors were separately used in an on-column mode (See J.W. Jorgenson and K.D. Lukacs, "Capillary Zone Electrophoresis," Science, Vol. 222, pp. 266-272, October 21, 1983).

Commercial absorption detectors and fluorescence detectors are available which may be modified for use in capillary electrophoresis. In one prior art absorption detector, shown in Figure 2, a low wavelength ultraviolet light 20, typically between 200 nanometers and 280 nanometers, is incident upon a diffraction grating 21. Grating 21 separates the light into different wavelength components and one of these components 24 is passed through the sample measurement region 23 of capillary tube 22. The light 25 emerging from the sample is incident upon a first photodiode 26. Actually, as shown in Fig. 2, the light from grating 24 is divided into two parts, one which passes through the sample and a second which is incident upon a second photodiode 27. The signal from photodiode 27 is used as a reference so that as the relative intensity from light source 20 changes, the signal from first photodiode 26 is corrected for the changes in light source 20.

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This apparatus has two primary limitations. First, only one wavelength at a time is incident upon the sample. To use a second wavelength, grating 21 must be mechanically repositioned so that a different wavelength is incident upon capillary tube 22. The second problem with this absorption detector is designing a holder for capillary tube 22 so that capillary tube 22 is not damaged while performing the measurement, changing samples, or loading the sample into the capillary tube.

In prior art fluorescence detectors, an ultraviolet (UV) light source, having a selected wavelength, illuminates the sample. The wavelength of the ultraviolet light causes certain molecules to fluoresce and emit light at wavelengths different from the wavelength of the incident UV light. A spectrophotometer is oriented at an angle, typically 90°, from the light source so that the emitted visible light (between 450 and 630 nm) is measured against a black background.

Fluorescence detection is inherently very sensitive and biomolecules can be detected using fluorescence via a fluorescence tag for proteins or a "stain" for DNA. The spectrophotometer, used in fluorescence detectors, typically has a grating and a photomultiplier tube. The grating reflects a specific wavelength of the fluorescent light from the sample upon a photomultiplier tube. The photomultiplier tube provides great sensitivity, but using such a tube inherently restricts the detector to a single channel. Rotation of the grating is required for the selection of a different wavelength.

To overcome the limitations of single wavelength operation, a detector has been developed which utilizes a spectrophotometer and a diode array. In this detector, the light from the sample is incident upon a grating. The grating disperses the light from the sample into a spectrum of wavelengths. Instead of having a single photodiode or

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photomultiplier tube to intercept the light from the grating, an array of up to 1000 photodiodes on a single semiconductor chip is used.

5 In this self scanning diode array detector, electronic sensing circuitry measures the charge on a capacitor associated with each diode by quickly scanning the capacitors with a video type signal. Thus, the relative light intensity incident upon each diode is measured. Each diode in the array corresponds to two nanometers in
10 bandwidth and so the measurement provides a complete spectrum. However, the rapid switching from one diode to another introduces electronic noise, which in turn limits the sensitivity of the detector. Thus, this detector generates a spectrum from which information about the
15 chemistry of the sample can be ascertained by looking at the relative absorption at different wavelengths, but the detector does not provide the sensitivity of single channel detectors.

20 The prior art detectors are not easily adapted for use in capillary electrophoresis. Each detector requires a special holder for the capillary tube and the combination of the thin capillary tube and the requirement for changing electrolytes and samples makes an automated apparatus impractical. While as previously described, capillary
25 electrophoresis has significant advantages over gel electrophoresis, the detector limitations inhibit the development of an automated instrument.

A detector which provides the sensitivity of the single channel absorption detector or fluorescence detector and the
30 advantages of a multichannel detector is currently not available. Further, separate detectors are used for fluorescence and absorption measurements. Thus multiple tests are required to obtain fluorescence and absorption data. A system having an integral absorption and
35 fluorescence detector with multiple channel capability would

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significantly enhance the flexibility and utilization of capillary electrophoresis and in fact all HPLC.

While capillary electrophoresis is faster and more accurate than gel electrophoresis, prior art systems still require multiple manual manipulations. For example, manual filling and replacement of the electrolyte is frequently required during electrophoresis tests. Accordingly, capillary electrophoresis measurements, while not as manually intensive as the gel electrophoresis measurements, still require some amount of manual intervention.

SUMMARY OF THE INVENTION

The capillary electrophoresis instrument of this invention includes an automated means of sampling nanoliter samples from a sample tray, an automated means for replacing the running electrolyte and the detector electrolyte used in capillary electrophoresis, an automated means for performing capillary electrophoresis tests and a single detector for simultaneously measuring both absorbance and fluorescence.

The capillary electrophoresis instrument of this invention includes a capillary tube; a programmable high voltage power supply with a first and second electrode; a vacuum tight detector electrolyte bottle containing a electrolyte, a first of the high voltage electrodes and a first end of the capillary tube; up to four running electrolyte bottles; and a sample tray. An autosampler head provides a means for moving a second high voltage electrode and the second end of the capillary tube between the four running electrolyte bottles and the sample tray. A replenishment system containing a pump, valve system, a waste bottle and electrolyte supply bottles is connected to the detector electrolyte bottle and is coupled to the running electrolyte bottles by the autosampler head so that the electrolytes in the detector electrolyte bottle and the running electrolyte bottles can be evacuated and refilled.

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A system controller in the capillary electrophoresis instrument, which receives specified commands from either a personal computer or a teletype, controls the autosampler head, the high voltage supply, the replenishment system and the electrophoresis test sequence. The system controller also controls the detector and the associated electronics.

The detector contains a pulsed light source; a continuous light source; a polychromator containing a grating; and a photodiode array. The capillary tube is positioned at the entrance slit of the polychromator such that light emerging from a sample in the capillary tube is incident upon the grating. Light from the continuous light source, used for absorption measurements, is focused on a section of the capillary tube so that the light passes through the capillary tube and enters the polychromator. Light from the pulsed light source, used for fluorescent measurements, is focused on the same section of the capillary tube at a selected angle from the slit of the polychromator such that light from the pulsed light source does not directly enter the polychromator.

The visible fluorescent light excited by the pulsed light source and the unabsorbed light of the continuous light source both enter the polychromator. The unabsorbed light from the continuous light source is primarily comprised of short wavelengths while the pulsed fluorescent light is primarily comprised of longer visible wavelengths. The grating in the polychromator separates the unabsorbed continuous light and the pulsed fluorescent light into discrete wavelengths. The diode array is oriented so that individual diodes in the array receive the discrete wavelengths provided by the grating. Further, selected diodes in the array are used to measure absorbance, i.e., the shorter wavelengths while other diodes measure fluorescence, i.e., the longer wavelengths. The continuous nature of the unabsorbed light and the pulsed nature of the

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fluorescent light provides a means for effectively eliminating contributions to the fluorescence measurements from the unabsorbed light and conversely any contributions from the fluorescent light to the absorption measurements.

5 To perform a capillary electrophoresis test a series of samples are entered in a microtiter tray and a running electrolyte is placed in the running electrolyte bottles. Also, the external computer is used to supply commands to the system controller which describe the locations of
10 hardware in the capillary electrophoresis instrument, the test conditions, and the sequence of tests to be performed. After the system controller initializes the capillary electrophoresis instrument, the specified tests are performed.

15 Specifically, the system controller first checks to see whether a directive has been supplied to purge and refill the electrolyte in any of the running electrolyte bottles or the detector electrolyte bottle. If such a command has been given, a level sensor is automatically lowered into the
20 appropriate bottle and then the replenishment system is used to evacuate the bottle until the level sensor indicates that the bottle is dry. Upon receipt of the dry signal from the level sensor and a subsequent fill command, the system controller automatically raises the level sensor to a "full"
25 position and proceeds to refill the electrolyte bottle with electrolyte from the running electrolyte storage bottle. When the electrolyte reaches the level of the sensor, the sensor sends a command to the system controller indicating that the bottle is full and the system controller then stops
30 the filling of the bottle and raises the level sensor to a location where it does not impede the capillary electrophoresis tests.

Next, the system controller automatically moves the sample end of the capillary tube and an associated high
35 voltage electrode into the electrolyte in a selected running

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electrolyte bottle. The replenishment system is used to draw a vacuum on the detector electrolyte bottle so that running electrolyte from the running electrolyte bottle is drawn into and through the capillary tube. After the
5 capillary tube is filled, the system controller moves the capillary tube and the associated high voltage electrode into a sample in a selected well in the microtiter plate. Following previously supplied instructions from the external microcomputer or teletype, the system controller draws the
10 sample into the capillary tube using either a vacuum or a high voltage.

After drawing the sample into the end of the capillary tube, the system controller automatically returns the sample end of the capillary tube to the electrolyte bottle and
15 applies a high voltage so as to maintain either a constant selected voltage or a selected current across the capillary. The controller can also change either the voltage or the current with time. The high voltage causes the sample to separate and move through the capillary tube
20 in bands. The bands pass through the fluorescence and absorbance detector which is mounted close to the second end of the capillary tube. As the sample molecules pass through the detector, light from the ultraviolet light source causes the molecules to fluoresce and this fluorescence is passed
25 into the polychromator which disperses it onto a first subset of diodes in a photodiode array. Similarly, some of the short wavelength light from the continuous light source is absorbed by the sample as the sample passes through the detector. The polychromator disperses the unabsorbed light
30 from the continuous light source onto a second subset of diodes in the photodiode array. The analog output signals from both subsets of diodes are processed, digitized, corrected for changes in the output intensity of the light sources and provided to the output terminal of the
35 electrophoresis instrument.

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Hence, the instrument of this invention provides a completely automated means for performing capillary electrophoresis and an unique detector which includes both fluorescence and absorption capability in conjunction with multichannel capability. The multichannel fluorescence and absorption detector approaches the sensitivity of the prior art single channel detectors by reducing the electronic noise limitations of the prior art self scanning diode array detectors by using a discrete amplifier for each diode. The detector sensitivity is further enhanced by the use of broader bandpass detection, i.e., 16 nm per diode instead of 2 nm as in the prior art detector.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram of a prior art capillary electrophoresis apparatus.

Figure 2 illustrates a prior art single channel absorption detector for use in capillary electrophoresis.

Figure 3 illustrates a general block diagram of the capillary electrophoresis instrument of this invention.

Figure 4 illustrates the capillary electrophoresis instrument of this invention.

Figures 5a and 5b are a detailed block diagram of the capillary electrophoresis instrument of this invention.

Figures 6a through 6e are wiring diagrams for the capillary electrophoresis instrument of this invention.

Figure 7 illustrates detector module 104 of this invention.

Figures 8a through 8g are a front view, a top view, a section A-A view, a section B-B view, a left side view, a right side view, and a bottom view, respectively, of capillary tube holder 232.

Figure 9 illustrates the general spatial relationship of the components in the detector of this invention. The figure is not drawn to scale.

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Figure 10 is a simplified schematic representation of the replenishment system of this invention.

Figures 11a and 11b are a schematic of detector preamp circuit 220 of this invention.

5 Figures 12a through 12d are a schematic of the fluorescence signal processing circuit 225 of this invention and a timing diagram representative of each channel in circuit 225.

10 Figures 13a through 13c illustrate the wavelength of the light sources of this invention and the range of absorbance and fluorescence wavelengths.

Figures 14a and 14b illustrate the screw and clamp respectively used to secure capillary tube 110 in capillary tube holder 232 of this invention.

15 DETAILED DESCRIPTION

20 The capillary electrophoresis instrument of this invention includes a detection system which simultaneously measures absorption and fluorescence induced by light from a light source, a replenishment system for automatically replacing the running electrolyte and the detector electrolyte and an autosampler for automatically analyzing up to 96 different samples. A block diagram of the electrophoresis instrument of this invention is shown in Figure 3. A system controller 100, a VME 3U style 10 Mhz 25 68000 microprocessor VME bus master in one embodiment, interfaces with a replenishment system 101, an autosampler 102, a high voltage power supply 103, and a detection system 104 including light source 105 and polychromator 106.

30 Either an external computer 107 or a teletype provides instructions to system controller 100. System controller 100 interprets these instructions and automatically performs capillary electrophoresis using a specified sample. The system controller 100 may also be directed to replace electrolyte 108 in running electrolyte reservoir 112 and

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electrolyte 109 in detector electrolyte reservoir 113 using replenishment system 101.

To perform a capillary electrophoresis test, system controller 100, following commands from external computer 107, moves capillary tube end 110A and electrode 103A to sample 111 using autosampler 102. Sample 111 is entered in capillary tube 110 using high voltage from high voltage power supply 103 or vacuum created by replenishment system 101.

After the sample is introduced into capillary tube 110, system controller 100 causes autosampler 102 to move capillary tube end 110A and associated high voltage electrode 103A into the electrolyte of running electrolyte bottle 112. A programmed high voltage from high voltage supply 103 is then applied across capillary tube 110 so that the sample is moved by electrophoresis through capillary tube 110. Simultaneously, the system controller activates detection system 104.

In one embodiment, light source 105 contains both a xenon flash tube and a deuterium lamp. The deuterium lamp generates a beam of light which passes through a first slit and lens imaging system so that the light is focused precisely on the center of capillary tube 110. A second lens imaging system with or without a slit, focuses the light from the xenon flash tube on the inner diameter of capillary tube 110. As described below, the light sources are oriented about a sample region 110C of capillary tube 110 so that fluorescence and absorption measurements can be obtained simultaneously. Here, sample region 110C means the region of capillary tube 110 where the bands of different species of sample molecules are detected and not the end 110A of capillary tube 110 where the sample is initially introduced. The capillary tube and the capillary tube holder are described more completely below. Detector 104 of this invention provides an increased capability over prior

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art detectors because both fluorescence and absorption measurements can be made without changing the on-column detector.

5 Polychromator 106 contains a grating oriented such that the light from the deuterium lamp and fluorescent light excited by light from the xenon flash lamp are simultaneously incident upon the grating. As described more completely below, the grating is selected such that the fluorescent light is projected onto a first subset of
10 photodiodes and the deuterium light, which is not absorbed by the sample molecules in capillary tube 110, is focused upon a second subset of photodiodes in detector 104. Accordingly, detector 104 simultaneously measures both absorbed light and fluorescent light.

15 Output signals from the photodiodes responding to the deuterium lamp are passed to a signal processor where signals are first processed by an analog system. The output signals from the analog system are passed through an analog to digital converter and then the 12-bit digital output
20 signals from the analog to digital converter are provided to the output lines of the electrophoresis instrument. In another embodiment, the absorbance signals from the diode array are processed directly by an analog to digital converter which generates 16-bit digital output signals.

25 The fluorescent photodiode output signals, which are typically about ten microseconds in duration, are amplified and captured in a sample and hold circuit and then passed through an analog to digital converter. For each flash of the xenon flash lamp, the detector output signal is divided
30 by a flash lamp reference signal to reduce the effect of flash to flash intensity differences. In one embodiment, the flash rate is 100 Hz, ten ratioed values are averaged to further improve the signal to noise ratio and reduce the data transfer rate to 10 Hz. This 10 Hz data is provided to
35 the output terminals of the capillary electrophoresis

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instrument. The capillary electrophoresis instrument separates, for example, very large DNA molecules (chromosomes), virus particles, and cell organelles.

The capillary electrophoresis (CE) instrument 99 of this invention, as illustrated in Fig. 4, is 34 inches wide by 23 inches deep by 18 inches high at its highest point. AC power is provided through the rear panel to an AC power entry module having an integrated RFI line filter, and a fuse. A UL recognized, CSA certified, and VDE approved power entry module, such as that made by Corcom 5EFM4S, is used in the CE instrument. In viewing the instrument from the front, the left side 120 of instrument 99 has a fan outlet. The right side 121 of instrument 99 contains a fan inlet 124 and a RS232 serial communication port 122, which is used to link an IBM PC-AT compatible computer or a teletype to CE instrument 99, and two strip chart recorder output channels with BNC output connectors 123.

The front of instrument 99 has two removable covers. A first front cover 125 encloses autosampler 102 mechanism, electrolyte replenishment system bottles, polychromator 106 and a high voltage power supply 103. All of these components may need frequent access by the operator. This cover when opened activates an interlock which turns off the HV power supply 103 and informs system controller 100 (Fig. 3), a central processing unit (CPU), of CE instrument 99 that the interlock was triggered. The second front cover 126 encloses the electronic circuits, DC power supplies, transformer and other components.

On the front of the CE instrument are three light emitting diodes (LED) 127, 128, 129 and a capillary current monitor 130. The LEDs indicate the RUN, SAMPLE, or ERROR conditions of CE instrument 99. When the ERROR LED 129 is lit, one of the following errors have occurred:

1. A safety interlock has been triggered;

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2. A high level current limit has been exceeded and Joule heating of the electrolyte may result;
3. A low level current limit has been exceeded and air bubbles may be present within the capillary tube; or
4. The communication between the PC-AT and the CE instrument has been lost.

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SAMPLE LED 128 indicates that an autosampler head, described below, is moving the capillary tube input end 110A to either a microtiter well or a running electrolyte (RE) bottle, also described below. High voltage is not applied while SAMPLE is lit. RUN LED 127 indicates that the CE instrument is taking data, high voltage is applied to the capillary tube ends, and a sample is being quantified by the electronics of CE instrument 99.

20
25
30
The autosampler access door is part of a carriage 131 and opens to the right side of the instrument. (See Figure 4). Autosampler carrier 131 slides from the external load position to the internal operate position on a rail, and holds the four running electrolyte bottles (RE1 thru RE4) and either a 96 well microtiter plate 132 or a tray for eight discrete bottles (not shown). After loading the microtiter plate 132 and running electrolyte bottles RE1 through RE4 on carrier 131, carrier 131 is pushed to the left into instrument 99, against a light spring force, until the door closes and the latch is engaged. After the batch of samples has been processed by CE instrument 99, the door latch is automatically released by CPU 100 and the processed sample tray slowly slides out of the instrument on carrier 131.

35
Access to instrument 99 via carrier 131 and its integral door is prohibited during a test since up to 30 KV is present across capillary tube 110. Carrier 131 and door are sensed as being closed by an infrared opto sensor. The carrier is released to open by a solenoid release mechanism.

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Capillary electrophoresis instrument 99 is illustrated in the system block diagram of Figs. 5a and 5b and wiring diagrams Figs. 6a through 6e. In one embodiment, the CE instrument 99 interacts with an IBM PC-AT clone 107 having:

- 1.) 80286 Processor;
- 2.) 10 Mhz Clock (no wait states);
- 3.) 1 Mega-byte of Fast RAM;
- 4.) One 1.2 Megabyte Floppy Disk Drive;
- 5.) One Serial and One Parallel Port;
- 6.) Enhance AT Keyboard;
- 7.) 44 Megabyte Full Height Hard Disk with 28ms access time;
- 8.) Streaming Tape Back-up;
- 9.) EGA Card;
- and 10.) 14" EGA Monitor.

As described more completely below, the external computer 107 or teletype provides set-up conditions to CE instrument 99 for batch processing of samples. External computer 107 could also perform post-processing of the data acquired by CE instrument 99, generate reports results via the EGA monitor and other similar functions. The post-processing functions are dependent upon the specific use of CE instrument 99 and accordingly are not considered further.

The CE instrument 99 batch processes either a single microtiter plate 132 with 96 individual sample wells or individual sample vials. The CE instrument (Fig. 5b) contains up to four different running electrolyte (RE) bottles RE1, RE2, RE3, RE4, and one detector electrolyte (DE) bottle 113. The automatic reagent replenishment system, described more completely below, for both the running electrolyte (RE) and for the detector electrolyte (DE) bottles may be automatically implemented after each sample run.

The autosampler has four main functions:

1. To move end 110A of capillary tube 110 (Fig. 5b) and associated platinum (Pt) electrode 103A between a selected microtiter well and a running electrolyte bottle RE1, RE2, RE3, RE4 and to immerse capillary tube end 110A and electrode 103A in the fluid of the selected microtiter well or

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running electrolyte bottle;

2. To lower a level sensor 202 sequentially into each of the four running electrolyte bottles to measure if sufficient electrolyte is present for a test;
3. To release sample tray and running electrolyte bottles to the operator via the movable sliding sample carrier 131; and
4. To deliver a reagent fill tube 203 over each of the running electrolyte bottles during a reagent replenish cycle.

The autosampler consists of an autosampler head 204 which holds an end 110A of capillary tube 110 and high voltage platinum electrode 103A, a thermistor 202 and associated tube 203 from replenishment system 101.

Thermistor 202 and tube 203 are coupled to autosampler head 204 by a solenoid controlled arm. The autosampler also contains three stepper motors 205, 206, 207, stepper motor control circuits 208, 209, motor drivers 210, 211, 212, an autosampler interface 213 and a position sensor interface 214.

When carrier 131 is inserted and the door is closed, sample end 110A of capillary tube 110 with associated high voltage Pt electrode 103A are moved from the sample well to the running electrolyte bottle and vice versa by the X, Y and Z stepper motor driven head 204, sometimes called the autosampler head.

The X axis is defined as motion parallel to the carrier movement. Viewing the instrument from the front (Fig. 4), the X axis is from left to right. The Y axis is from the front of the instrument to the back and the Z axis is vertical.

In one embodiment, each axis has three infrared opto interrupt position sensors. The three positions are called HOME, REF and END. Viewing the CE instrument from the front, HOME position is located at the center of running

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electrolyte bottle, RE1. Bottle RE1 is the running electrolyte bottle closest to the front of instrument 99 (Fig. 4). For both the X and Y axis these sensors, i.e., the HOME position sensors, are positioned 0.1 inches before a mechanical stop. For the Z axis the HOME position is in the up, fully retracted, position 0.1 inches before a mechanical stop. The END opto sensor for each axis is at the end of travel opposite to the HOME sensor. The END opto sensor limit is encountered before hitting a mechanical stop. Viewing the CE instrument from the front, END corresponds to autosampler head 204 being positioned to the left side, in the front position and fully down. The third sensor associated with each axis is a reference (REF) sensor. For both the X and Y axis, the REF sensors are located at running electrolyte bottle RE1. For the Z axis, the REF position was chosen as being 0.250 inches down from the Z axis HOME position. In another embodiment, the REF sensors are not used, and each axis has only the HOME and END sensors.

One of the functions of the autosampler is to sense the electrolyte level in the running electrolyte bottles RE1-RE4 using thermistor 202 which is encased in a stainless steel sleeve. Thermistor 202 is heated and in air is at a temperature of approximately 10 degrees above the ambient temperature. The thermistor's resistance, when it is lowered into the cooler room temperature electrolyte, changes rapidly. Conditioning electronics in position sensor interface circuit 214 sense this change and signal 68000 VME CPU 100, the system controller, that the sampled electrolyte bottle contains an electrolyte. Autosampler head 204, which moves end 110A of capillary tube 110 and HV electrode 103A as a pair, also carries level sensing thermistor 202 and Teflon running electrolyte replenish tube 203 as a pair. Replenish tube 203 and thermistor 202 are mounted on head 204 about one inch away from capillary tube

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110 and electrode 103A. Replenish tube 203 and thermistor 202 as a pair can be lowered one half inch beyond the tip of capillary tube 110 by a solenoid driven plunger. When the solenoid is de-energized, replenish tube 203 and thermistor 202 are retracted one half inch above tip 110A of capillary tube 110. When the autosampler is sampling or taking an acquisition run, the solenoid is de-energized and so replenish tube 203 and thermistor 202 are retracted above tip 110A of capillary tube 110. When the autosampler is sensing the presence of liquids, replenish tube 203 and thermistor 202 are extended beyond capillary tube 110 by the plunger. During a running electrolyte replenish cycle, replenish tube 203 and thermistor 202 are inserted by autosampler head 204 into the RE bottle near the top of the bottle to sense when the proper electrolyte level has been reached.

With replenish tube 203 and thermistor 202 suspended on autosampler head 204, the running electrolyte bottle liquid levels must be tested in series. Any of the running electrolyte bottles RE1, RE2, RE3, RE4 (Fig. 5b) can have its level sensed, but only running electrolyte bottles having the same electrolyte can be replenished because in this embodiment, the capillary electrophoresis instrument has only one running electrolyte supply bottle. Cross contamination between electrolytes is not a problem.

Since the running electrolyte bottle, end 110A of capillary tube 110 and Pt electrode 103A can have 30 KV present during a test, a proper spacing of about 2.5 cm must be kept between the capillary/electrode combination and any metal surface. Capillary tube 110 and electrode 103A are held parallel to each other, approximately 0.125 inches apart, by autosampler head 204, for a distance of about 2 cm.

In one embodiment, thermistor 202 has a resistance of 10K ohms at 25° C and a response time including electronic

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circuit delays of two seconds upon entry into the electrolyte. A thermistor such as that manufactured by YSI under part number 44106 is suitable for use in the autosampler.

Two identical stepper motor controllers 208, 209 are used to control the autosampler stepper motors 205, 206, 207. Each controller can independently control two stepper motors. Since the autosampler has three motors, second controller 209 has one spare motor controller.

Stepper motor controller cards 208, 209 are 3U style cards which are controlled by 68000 CPU 100. Stepper motor controllers 208, 209 communicate with 68000 CPU 100 via VME bus connector P1. The stepper motor controller can provide either full or half step outputs to motor driver circuits 210, 211, 212. The signals from autosampler position sensors (HOME, REF, END), described above, are supplied to stepper motor controllers 208, 209 for each axis. Controller card 208 is for the X and Y axis while controller card 209 is used for the Z axis. In one embodiment, cards 208, 209 are programmed for half-stepping with a maximum output rate of 1000 half-steps per second. The stepper motor controllers are programmed to control 4 phase motors and receive signals from three limit switches per autosampler axis where the signals received are TTL level compatible active low. The base address for the X axis and Y axis controller is FF00XX, while the base address for the Z axis controller is FF01XX. A controller such as that manufactured by Matrix Corporation and supplied under Part No. MS-DSC is suitable for use in the CE instrument.

Motor driver circuits 210, 211, 212 function as power drivers for four phase stepper motors 205, 206, 207 respectively. The phase input signals from stepper motor controllers 208, 209 are amplified and sent to a stepper motor 205, 206, 207. Each motor driver circuit generates a unipolar output drive signal. The CE instrument uses three of these boards -- one for each axis of the autosampler. A

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motor driver such as that provided by Matrix Corporation under part number USD with 8 amps per phase can be used.

5 The position sensor interface 214 is mounted on autosampler head 204. The position sensor interface contains no electronics. The position sensor interface simply routes the wiring for the Y and Z axis stepper motors 206, 207 to autosampler interface circuit 213 and provides a physical location for the three position sensors (HOME, REF, END) associated with each axis, as described above. The
10 infrared opto sensors used for position sensing are Opto 22 (OPT-865).

Autosampler interface circuit 213 provides the interface electronics for all of the autosampler position sensors; interface electronics for sensing when running
15 electrolyte level sensor 202 is in the non-extended position; interface electronics for sensing when the sample tray door is closed; a signal to open the sample door; and a common interface path between the X, Y and Z stepper motors 205, 206, 207 and the three motor driver circuits 210, 211,
20 212. While one embodiment of the interface between system controller 100 and stepper motors 205, 206, 207 has been described, this embodiment is illustrative only and is not intended to limit the scope of the invention. In view of the principles of this invention, one skilled in the art can
25 use other circuit configurations to interface controller 100 and the stepper motors. A summary of the characteristics of the autosampler and sensors in the autosampler is given in Table 1.

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TABLE 1Autosampler Total Travel

X - axis = 0.0026 inches/step

Y - axis = 0.0026 inches/step

Z - axis = 0.004 inches/step

Maximum Travel Time

X - axis = 2 inches/sec.

Y - axis = 2 inches/sec.

Z - axis = 0.8 inches/sec.

Maximum Steps/Second Provided by the Controller

X, Y - axis = 1000 half steps/sec.

Z - axis = 1000 half steps/sec.

Positional Accuracy Required

X, Y - axis = 0.040 inches

Z - axis = 0.010 inches

Carrier/Door Release Solenoid

Operating = +12 vdc - normally not powered.

Power Dissipation Continuous Duty = 2.5 watts

Life Expectancy = 2.5×10^6 cycles

Part Number = R8X7DC-12

Manufacturer = Regdon

Positional Sensors

- (1) X, Y, Z axis opto interruptor (infrared) three for each axis representing the HOME position, REF position, and END position
- (2) Door Closed opto interruptor (infrared)
- (3) Level Sensor HOME position opto interruptor (infrared)

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The autosampler of this invention makes capillary electrophoresis a true instrumental method of analysis. Unlike the prior art methods which contained only a single electrolyte and required manually positioning the sample container, the electrodes, and the electrolytes, the autosampler automatically introduces the sample into the capillary tube and can move the capillary tube to any one of four running electrolyte bottles. Further, as described below, the autosampler in conjunction with the replenishment system can change the electrolyte in any one of the four running electrolyte bottles. Hence, manual intervention for capillary electrophoresis testing is no longer required.

A first function of detector module 104 is to excite the sample passing through capillary tube 110 in sample region 110C with UV light from xenon flash lamp 105A and at a 90 degree angle to the incident light from lamp 105A measure the fluorescence emitted by the sample in capillary tube 110. A second function of detector module 104 is to illuminate capillary tube 110 at a 180 degree angle from the entrance slit to polychromator 106 so that the detector module can simultaneously measure absorbance.

The main components of detector module 104, as illustrated in Fig. 5b, are: polychromator 106; xenon flash lamp 105A; xenon flash lamp power supply 215; deuterium lamp 105B; deuterium lamp power supply 216; diode array 217; capillary tube 110; and a capillary tube holder (not shown).

In one embodiment, the polychromator housing is fabricated from a single piece of aluminum which has been stress relieved. A holographic concave grating 218 is mounted inside the aluminum housing and is positioned to disperse the incoming light into its component wavelengths which are in turn focused by grating 218 onto a diode array 217. A grating used in one embodiment was 37 x 37 mm with 560 grooves per mm and a radius of curvature of 97 mm. Such a grating is manufactured by American Holographics, and

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provided under catalog number 456.20.

The geometry of polychromator 106 is designed so that grating 218 spreads the incoming light over the plane of photodiode array 217. For the previously described grating, the centers of the adjacent photodiodes are separated by wavelengths of about 16 nanometers. Photodiode array 217 has 35 identical elements which are sensitive to the wavelengths of interest. However, only 16 photodiodes are currently utilized, five for UV absorbance measurements and ten for fluorescence measurements. Also, one diode is used to generate a reference signal in UV absorbance measurements. The wavelengths of light, the corresponding diode of the array selected, and the dye names, which generate fluorescent light at the specified wavelength, are listed in Table 2. In Table 2, two embodiments are given. In the first embodiment, sixteen channels are used as described above. In the second embodiment, eighteen channels are used and the two additional channels are identified in Table 2.

20

TABLE 2UV Absorption Measurement

	Wavelength	Diode Array	
		Number	
25	214 nm	D1	Absorbance
	230 nm	D2	Absorbance
	246 nm	D3	Absorbance
	262 nm	D4	Absorbance
	278 nm	D5	Absorbance
	422 nm	D15	Absorbance
30			Reference

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Fluorescence Measurement

	Wavelength	Diode Array Number	(Wavelength) Dye
5	454 nm	D16	(450-470) Coumarin, (450) OPA, (460) Hoechst
	470 nm	D18	(475) Fluorescamine
	486 nm	D19	
10	502 nm	D20	--Used only for 18 channel detector--
	518 nm	D21	(520) Fluorescein
	534 nm	D22	(520-550) NBD
	550 nm	D23	(550) Rhodamine, (550) Dansyl
15	566 nm	D24	(560) Eosin, (570-580) Trimethylrhodamine
	582 nm	D25	(580-590) Ethidium Bromide, Lissamine Rhodamine
20	598 nm	D26	
	614 nm	D27	(620) Texas Red
	630 nm	D28	(633) HeNe Laser -- Used only for 18 channel detector--
25			

Diode array 217, which is mechanically mounted with two preamp boards (Figs. 11a and 11b), is adjustable for fine tuning the wavelength calibration. The adjustment allows moving the array through 4 mm of the spectrum. When the alignment is complete, two lock down screws are tightened.

The polychromator housing in addition to housing grating 218 also provides an optically rigid mounting

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surface for xenon flash lamp 105A, the capillary tube holder (not shown), UV reference preamp 219, signal preamp 220, filter 221, deuterium lamp 105B, and glass cover slip 222. A more detailed layout drawing of detection module 104 is shown in Fig. 7.

For a CE fluorescence measurement, high energy xenon flash lamp 105A excites the sample in capillary tube 110 with ultraviolet light. The broadband xenon light pulse is filtered with a 340 nm filter 221. The range of wavelengths from lamp 105A and the wavelengths transmitted by filter 221, described more completely below, are illustrated in Fig. 13a. The range of fluorescent wavelengths excited by the light passed through filter 221 is illustrated in Fig. 13c.

Flash lamp 105A, flash lamp socket, and discharge capacitor are housed in a steel enclosure 223 which in turn is mounted on polychromator 106 assembly. As described more completely below, the lamp arc is precisely aligned with respect to capillary tube 110. Lamp assembly 223 is designed so that a replacement flash lamp needs no alignment upon installation. However, if a small adjustment is necessary, a 3 point screw mechanism gives an additional 0.010 inch adjustment of the arc position. Lamp assembly 223, in one embodiment, contains Hamamatsu xenon flash lamp (L2436), Hamamatsu flash lamp socket (E2438), and a 0.1 uf capacitor with WVCW 3000 volts such as Condenser Products Company (KMOC-3M01ES). Lamp assembly 223 is connected to xenon flash lamp power supply 215 by a shielded cable.

Xenon flash lamp power supply 215 charges the 0.1 uF storage capacitor to 1000 vdc. When a trigger signal is received from 68000 CPU 100, the stored energy is discharged into xenon flash lamp 105A creating a high intensity light pulse. Xenon flash lamp 105A and power supply assembly 215 are capable of producing a light pulse at a maximum rate of 100 Hz.

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For a CE absorption measurement, deuterium lamp 105B generates short wavelength UV light with very little light output above 300 nm (See Fig. 13b). This light is reflected by a mirror 308 onto sample region 110C of capillary tube 110. Deuterium lamp 105B is described in further detail below.

Deuterium lamp power supply 216 provides a high DC voltage for the electrodes of deuterium lamp 105B and a trigger voltage to initiate discharge of deuterium lamp 105B. The anode current is supplied through a floating constant current regulator/preregulator circuit and a voltage feedback loop maintains a constant 12-volt drop across the current regulator/preregulator circuit, irrespective of the load or line conditions. The starting circuit in power supply 216 provides a ramped high voltage pulse to deuterium lamp 105B, and at the moment of ignition of the deuterium lamp, this voltage is instantaneously removed. The lamp discharge is maintained by the current regulator circuit. A deuterium lamp power supply 216 having these characteristics is provided by Mimir Instruments, Model No. 430.

The polychromator spreads the wavelengths of emitted light, shown in Table 2, onto a 35 element array of which only 16 elements or diodes are used in one embodiment. An array manufactured by Hamamatsu under part number S2313-35Q was used in one embodiment. The specifications for this array are given in Table 3.

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TABLE 3

	Number of Elements:	35
	Dark Current:	150 pA @ VR = 10v
	Junction Capacitance:	140 pF @ VR = 10v
5		60 pF @ VR = 0v
	Spectral Range:	190 - 1050 nm
	Peak Wavelength:	720 nm
	Radiant Sensitivity	80 ma/W @ 200 nm
		230 ma/W @ 930 nm
10	Diode Element Size:	4.4 x 0.94 mm
	Effective Area:	4.1 mm ²

The detector module 104 consisting of deuterium lamp 105B, mirror 308, xenon flash lamp 105A, filter 221, glass cover slip 222, polychromator 106 including grating 218 and photodiode array 217, provides a significant enhancement in fluorescence and absorption measurement techniques over prior art detectors. UV absorption and fluorescence measurements are performed by a single device. The high sensitivity of prior art discrete channel detectors is maintained and a multiplicity of spectral information is obtained from photodiode array 217.

The signals from diode array 217 are individually amplified by discrete channels so that the noise introduced by the scanning of prior art photodiode arrays is not present. Further, the detector is maximized for sensitivity rather than spectral resolution by monitoring a few wavelengths with 16 nm resolution rather than 400 wavelengths at 2 nm resolution as with the prior art diode array detector. This is most important for the fluorescence measurement, as the emitted fluorescent light is about 30 nm at full width, half height (FWHH). In this embodiment, the digital signals, derived from the output signals of several diodes, can be added for the best signal to noise

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measurement, without degrading the UV absorbance measurements which need better spectral resolution.

The detector preamp circuit 220 (Figs. 11a and 11b) contains diode array 217, 16 low noise amplifiers, and the diode biasing selector. Sixteen preamps are associated with the six absorbance diodes and the ten fluorescence diodes as defined in Table 2. The diode biasing selector is a signal which switches the common diode cathodes between +12V for fluorescence measurements and ground for absorbance measurements. The diode biasing minimizes the capacitance of the diodes for fast pulse response for fluorescence measurements and minimizes the dark current for absorbance measurements. If both detection modes are required, the bias is applied.

The fluorescence preamp circuit provides an additional stage of gain for the 16 channels. The 10 fluorescence channels are AC coupled (pulsed) while the 6 absorbance channels are DC coupled (continuous).

A fluorescence signal processor circuit 225 (Figs. 12a through 12d) converts the 10 channels of fluorescence pulse data and xenon flash lamp reference data into DC voltages which are subsequently processed by A/D converter 227 into 12 bit digital data. The 11 channels of information are each input to fluorescence signal processor circuit 225 (Fig. 12a through 12d) in the form of a pulse between 5 and 20 microseconds wide and a peak amplitude of approximately 20 mv. These signals are amplified, integrated and sent to a sample and hold amplifier. The gating time and the constants in circuit 225 are selected such that any continuous signal, which may be generated by longer wavelength light from deuterium lamp 105B upon the fluorescence photodiodes, as defined in Table 2, is effectively filtered from the input signals.

Three additional voltage signals are supplied to fluorescence signal processor circuit 225 from system I/O

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controller 228. These three voltage signals are a voltage monitor signal VMON from high voltage power supply 103, a current monitor signal IMON from the capillary holder, which indicates the current through capillary tube 110, and a vacuum monitor signal from replenishment system interface 229.

These three signals are simply routed to A/D converter 227. The six absorbance preamp signals are also not processed. The absorbance preamp signals are sent to absorbance signal processing circuit 226. Fluorescence signal processing circuit 225 is on a 6U style printed circuit card suitable for mounting in a VME chassis.

Absorbance signal processor circuit 226 is also on a 6U style printed card which resides in the VME chassis. As described above, the six absorbance signals from preamp 220 are supplied to circuit 226 via fluorescence signal processor circuit 225. The five channels of data and the reference channel are processed and then routed back to the fluorescence signal processor 225 and subsequently to A/D circuit 227. Analog ratioing of the absorbance channels with a deuterium light source reference signal, generated by the photodiode in photodiode array 217, and an auto zeroing function are performed by absorbance signal processor 226. The analog signal processing is required to obtain the accuracy needed for absorbance measurements because the twelve bits of information from analog to digital converter 227 are not suitable for direct processing of the absorbance data from preamp circuit 220. The absorbance signal processor circuit 226 is essentially a DC circuit because the absorbance input signals are continuous rather than pulsed. Accordingly, the coupling and time constants in this circuit are selected to minimize any signals generated by short wavelength fluorescence interacting with the absorbance photodiodes.

In yet another embodiment, analog to digital converter

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227 is selected to provide 16 bits of digital information, i.e., 16 bit words. This word length provides the required accuracy and so absorbance signal processor 226 is not needed. The absorbance signals are passed directly to analog to digital converter 227. Alternatively, analog to digital converter 227 may be maintained in the capillary electrophoresis instrument and another analog to digital converter which generates 16 bits of digital data used to replace absorbance signal processor 226. In this case, the absorbance signals from fluorescence signal processor 225 are converted by the 16 bit analog to digital converter and the digital data is supplied directly to system controller 100. The path from the present absorbance signal processor circuit 226 through the fluorescent signal processor to the analog to digital converter 227 is not utilized in this embodiment.

In yet another embodiment, very low cost single channel 16 bit CMOS chips (Crystal Simiconductor Corp. part number CS5501) are used to process the signals from the absorbance photodiodes. These components are used in a custom printed circuit board instead of the multiplexed high speed 16 bit analog to digital converter from Matrix, described above.

Xenon flash lamp reference preamp 219 samples the filtered light from xenon flash lamp 105A. The output signal of preamp 219 is sent to fluorescence signal processor circuit 225. This signal is used to initiate the timing sequences and signal processing of fluorescent data. The reference channel is also digitized by A/D converter 227. Since a xenon flash lamp light pulse varies in amplitude from pulse to pulse, the signal processing software in the 68000 CPU 100 ratios all of the fluorescent channels with the reference channel after the digitization of the data.

Capillary tube 110 (Fig. 5b) is made of fused silica and is manufactured by Polymicro, of Arizona. Tube 110 is

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coated with polyamide to allow bending of the tube without shattering. This coating allows capillary tube 110 to flex as the autosampler moves sample end 110A of capillary tube 110 from a sample well to a selected running electrolyte bottle. The portion 110C of capillary tube 110 at detector 104 must be transparent to the UV light pulse and the light from the deuterium lamp. Therefore, the polyamide coating is burned away for a length of about 10 mm at location 110C where the light enters polychromator 106 after first passing through capillary tube 110. The outer protective coating of polyamide must be burned off for a length of about 1 cm with a flame prior to mounting in the detector. An ordinary propane cigarette lighter is used for this purpose.

The location of sample region 110C with respect to end 110B of capillary tube 110 is not critical so long as sample region 110C can be placed in capillary holder 232 and end 110B is near the bottom of detector electrolyte bottle 113. The exposed silica tube is very brittle and so this 10 mm portion of capillary tube 110 is rigidly held in place in capillary tube holder 232, as described below. Capillary tube 110 has an typical inner diameter of about 50 microns, an outer diameter of about 320 microns and a length which ranges from about 30 cm to about 75 cm.

Capillary tube holder 232, as illustrated in Figs. 8a through 8g, holds the capillary tube 110 stationary; supports the two imaging lens holders for light from xenon UV lamp 105A and light from deuterium lamp 105B; and provides a vacuum tight connection to detector electrolyte bottle 113 for stationary end 110B of capillary tube 110. Detector electrolyte bottle 113 also has two teflon tubes which are part of the replenishment system. One teflon tube, which has an outer diameter of 0.125 inches, provides up to 22 inches of vacuum during a sample introduction. The other teflon tube, which has a 0.063 inch outer diameter, is used to evacuate spent electrolyte and to add fresh

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electrolyte, as described below.

As shown in Figs. 8a through 8g capillary tube holder 232 is a multiple-purpose element. First, at the bottom 232A of holder 232 is detector electrolyte bottle 113 and a
5 Viton O-ring 243. The bottle is mechanically held in place against O-ring 243 so as to form a vacuum-tight seal. The two teflon tubes are inserted through holes 240, 241. Platinum electrode 103B is inserted through hole 242 which extends through capillary holder 232. The electrode is
10 passed through hole 242 and then glued into position. Capillary tube 110 is passed through hole 244 which also extends through the body of capillary tube holder 232. Hole 244 is half round at the bottom of hole 245 in which capillary tube 110 can rest. A threaded hole 245 is
15 provided for use in securing capillary tube 110 in capillary tube holder 232. Hole 245 is threaded to accept a screw 390 (Fig. 14a) which has a free-turning disc 391 (Fig. 14b) mounted on the end going into holder 232. The screw is threaded into the sample holder until the disc encounters a
20 captive O-ring, and after the disc contacts the O-ring an additional quarter turn of the screw securely fastens capillary tube 110 in place without damaging tube 110. Ultraviolet light from xenon lamp 105A enters the capillary tube through port 246. An apex 247, shown in section B-B of
25 Fig. 8d, is designed to trap any of the light passing through capillary tube 110 so that the light is not reflected into polychromator 106. The light from deuterium lamp 105B enters through port 248. Both the light from deuterium lamp 105B and the fluorescent light from the
30 sample exit holder 232 to polychromator 106 through port 249. The other features in capillary tube holder 232, as shown in Figs. 8a through 8g, are used to secure capillary tube holder 232 to polychromator 106, xenon lamp 105A and deuterium lamp 105B, as shown in Fig. 7.

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A programmable high voltage (0-30 KV) power supply 103 introduces sample components electrophoretically into capillary tube 110 prior to the electrophoretic separation. As shown in Fig.6b, two platinum wire electrodes 103A, 103B electrically connect high voltage power supply 103 to detector electrolyte bottle 113 and to a selected running electrolyte bottle respectively. Both bottles contain an electrolyte solution and capillary tube 110 connecting the two bottles is filled with electrolyte, as previously described. After autosampler head 204 moves electrode 103A and sample end 110A of capillary tube 110 to one of the sample wells containing sample solution, a voltage, typically 30 KV, is applied across electrode 103A and platinum electrode 103B in detector electrolyte bottle 113. The electrical circuit is now complete and the sample components electrophoretically flow from the sample well into capillary tube 110. The voltage is then turned off and autosampler head 204, under the direction of 68000 CPU 100, moves capillary tube 110A and electrode 103A into the electrolyte of a running electrolyte bottle and a high voltage is again applied. The sample now travels through tube 110 from running electrolyte bottle RE1 (Fig. 5b) past polychromator 106 into detector electrolyte bottle 113, separating as it travels.

The CE instrument has two different HV power supply 103 configurations, a plus 0 to plus 30 KV programmable in 7.32 volt increments, and a minus 0 to minus 30 KV programmable in 7.32 volt increments. Only one polarity unit can be installed at a time, but the units are designed to be easily exchanged by the user.

The specifications for high voltage power supply 103 are given in Table 4.

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TABLE 4Option 1

5 Output Polarity: Plus (0-30KV)
 Manufacturer: Gamma High Voltage
 Research, Inc.
 Part Number: OEM version in custom case
 like #RC10 - 30 P-VM
 Voltage Monitor
10 Output: Plus (0 - 3 vdc) corresponds
 to plus (0-30 KV)
 Maximum Continuous
 Output Current: Plus 330 microamps

Option 2

15 Output Polarity: Minus (0-30 KV)
 Manufacturer: Gamma High Voltage Research,
 Inc.
 Part Number: OEM version in custom case
 like #RC10 - 30 N-VM
 Voltage Monitor
20 Output: Minus (0 - 3 vdc) corresponds
 to minus (0 - 30 KV)
 Maximum Continuous
 Output Current: Minus 330 microamps

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Common specifications for both options 1 and 2:

	Input Voltage:	+28 vdc +/- 10% @ 800 ma
	Line Regulations:	0.01% maximum
	Load Regulations:	0.01% maximum
5	Ripple:	0.05% maximum
	Output Connector:	Alden 8111LSFP
	Input Connector:	9 pin Sub-D

CPU controller 100, sometimes called the system controller, used in the CE instrument is a VME 3U style 10 Mhz 68000 microprocessor VME bus master. Controller 100 controls all electronic processing and control functions and communicates serially (RS232) with PC-AT 107. The functions of controller 100 are described more completely below. The controller's specifications are listed in Table 5.

15

TABLE 5

	Manufacturer:	Matrix Corporation
	Part Number:	MS-CPU00B
	EROM:	256K zero wait state EROM
20	SRAM:	64K zero wait state, dual ported to the VME bus static RAM, with battery back up
	Serial Ports:	Two RS232 serial communication ports. Only one serial port is used by the CE instrument. It is used to communicate to the PC-AT at 9600 baud
25	Timer:	16 bit programmable timer for generating periodic interrupts
30		

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NOVRAM: 256 bit non-volatile RAM
Front Panel: Software abort and reset
switch
run and fail light
5 Size: 3U

The system software was written in Forth and was compiled using Laboratory Microsystems, Inc. Metacompiler for the 68000. The machine code is presented in Appendix A and incorporated herein by reference. To use the machine
10 code a reference address of 4000 H must be used upon loading the program.

The overall system electronics architecture was planned to minimize interconnection wiring. Circuits having a "*" in Figs. 5a and 5b are mounted in a 6U style 7 slot VME card
15 cage. The function of the VME chassis is to link the 68000 CPU VME bus controller to the other VME cards used by the CE instrument. The chassis is configured with seven VME slots, three of which are 3U style cards and four of which are for the 6U style cards. The mother board for the VME chassis
20 has signal terminating resistors designed into the back plane. The chassis is made by ELMA Electronic Inc., part number 11-216-42-615.

A/D converter 227 changes analog voltage signal channels to digital signals which are then read and
25 processed by 68000 CPU 100. The analog signal channels are:

1. 5 Absorbance Channels
2. 10 Fluorescence Channels
3. HV Power Supply Voltage (VMON)
4. HV Power Supply Current (IMON)
- 30 5. Vacuum Sensor Voltage
6. Xenon Flash Lamp Reference Signal
7. Deuterium Lamp Reference Signal

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The specifications for A/D converter 227 are listed in Table 6.

TABLE 6

	Manufacturer:	Matrix Corporation
5	Part Number:	MS-AD12E
	Resolution:	12 bits
	Number of Channels:	32 single ended
	Input Range:	+(0 - 10v)
	Programmable Gains:	1, 10, 100, 500, 1,000
10	Settling Time:	20 microseconds
	Input Impedance:	60 Megaohms.
	Size:	3U
	Base Address:	FF0200

The replenishment system is described more completely below, but briefly, replenishment system interface 229 transfers signals from system controller 100 which turn ON and OFF valves V1 through V5, and transfers signals about the liquid levels in waste bottle 251, running electrolyte supply bottle 252, and detector electrolyte supply bottle 253 to controller 100. Replenishment system interface 229 also measures the vacuum present in waste bottle 251 (up to 22 inches of Hg).

The system I/O controller is a 6U VME card which resides in the VME chassis. Its basic function is to provide an I/O interface between 68000 CPU 100 and autosampler interface circuit 213, fluorescence signal processor circuit 225, replenish system interface 229 and front panel 230 displays.

System I/O controller 228 receives signals on the status of the interlocks and the position of the running electrolyte level sensor position as to being extended or not. Circuit 228 also passes to the fluorescence signal

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processor 225 high voltage and current readings as well as the vacuum reading from waste bottle 251 and various control signals. In addition, circuit 228 passes signals which control and read the status of replenishment system interface 229; which control the biasing voltage to photodiode array 217; which fire xenon flash lamp 105A; and which turn on and off deuterium lamp 105B. System I/O controller circuit 228 also passes control voltages 0 to 10V to power supply 103 and two channels of D/A data to the strip chart recorder outputs.

As described previously, the CE instrument can use various high voltage power supplies. In addition to those described, a power supply which rapidly switches from a positive high voltage through zero to a negative high voltage can be used in the CE instrument. The system I/O controller circuit 228 includes a high voltage rectifier and a current rectifier for processing the VMON signal and the IMON signal respectively from a rapidly switched power supply.

The CE instrument contains two power supplies. The first power supply is a linear power supply which provides +28 vdc at 1.0 amps to HV power module 103. The +28 volt supply is manufactured by Power One Incorporated, part number HB28-1-A.

The second DC power supply is a switching power supply which provides:

+5 vdc @ 12 amps for the digital logic;

+/- 12 vdc @ 1.5 amps for the analog electronics; and

+12 vdc @ 5.0 amps for the motors and relays.

The manufacturer is SQV, part number SQV100-1222-4.

A detector capable of simultaneous measurements of fluorescence and absorption of a sample in a thin capillary having an inner diameter of about 50 microns and an outer diameter of about 320 microns as in the capillary

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electrophoresis instrument presents many problems. The light from the light sources must be focused such that light is incident only upon the sample in capillary tube 110. If ambient light passes around capillary tube 110 and enters polychromator 106 or reenters capillary tube 110, the ambient light inhibits accurate measurement of the absorption spectrum and the fluorescence spectrum. The small size of the capillary makes simultaneous measurements using separate polychromators for absorption and fluorescence impractical so that both spectra must be measured by a single polychromator.

The detector 104 of this invention uses a photodiode array 217 and a single polychromator 106, as previously described, for both fluorescence and absorption measurements. Since in absorption measurements, wavelengths less than 300 nanometers are primarily of interest and in fluorescence measurements wavelengths greater than 400 nanometers are of interest (Fig. 13c), the various wavelengths provided by polychromator 106 to photodiode array 217 are sufficient for both measurements.

There is some overlap of excitation light from the flash lamp assembly onto the diodes of less than 300 nm and some visible light from the deuterium lamp assembly falling on the diodes above 400 nm. However, the circuitry and measurement methods of this invention eliminate most of the potential problems introduced by this overlap.

The first step in minimizing the effect of the overlap in the selection of the light sources. Xenon flash lamp 105A in the capillary electrophoresis instrument provides light having wavelengths from less than 200 nanometers to greater than 650 nanometers (Fig. 13a). Since xenon flash lamp 105A is used for the fluorescence measurements, the entire bandwidth of light generated is not required, but rather a specific wavelength which causes a wide range of samples to fluoresce is needed. Accordingly, the excitation

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light from flash lamp 105A is filtered by a 1/2" diameter filter having a broad band pass centered about a 340 nanometer wavelength. (Fig. 13a.) Accordingly, visible light, which would interfere with the fluorescence measurement, is rejected. Filters having such a characteristic include a Hoya number U340 filter or Schott number UG11 filter. Filter 221 was coated, in one embodiment, with a single layer anti-reflection coating on each side which was optimized for minimum reflectance at 340 nanometers. Xenon flash lamp 105A provides a pulse of light about 10 microseconds in duration.

Deuterium lamp 105B provides light having wavelengths of 200 nanometers and longer, but as the wavelength increases the light intensity decreases very rapidly (Fig. 13b). Thus, light from deuterium lamp 105B has only a small percentage of wavelengths greater than 300 nanometers, e.g., most of the light intensity from deuterium lamp 105B is less than 300 nanometers.

The difference between a continuous light source and a pulsed light source provides the means for eliminating problems caused by the overlap of the absorption spectrum and the fluorescence spectrum. The absorption spectrum represents a basically continuous output signal from the diode array while the fluorescence spectrum has an alternating current aspect caused by the pulsed light. Thus, the circuitry used to measure the absorption signals is effectively DC coupled so as to eliminate contributions from the fluorescent signals, i.e., the time constants and coupling reject high frequency signals, such as the fluorescence signals. Conversely, the circuitry used to measure the fluorescent signals are effectively AC coupled to eliminate contributions from the absorption spectrum, i.e., the time constants, coupling, and gating are such that continuous signals, such as the absorbance signals, are eliminated.

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Finally, the longer wavelengths of light from deuterium lamp assembly 105B can excite some fluorescence. However, the magnitude of this fluorescence is sufficiently small that it is insignificant in comparison to the fluorescence excited by light from xenon lamp 105A. The use of two light sources in the CE instrument is illustrative only and is not intended to limit the scope of the invention. Using the principles of this invention, one skilled in the art could use a single light source with appropriate optical systems for both fluorescence and absorbance measurements. Further, the detector output signal processing systems, described previously, could be modified to accommodate detector output signals generated by a single light source.

To provide further accuracy in the measurements, as described previously, a xenon reference detector 219 and a reference diode in diode array 217 for deuterium lamp 105B are included in the capillary electrophoresis instrument. The reference signals provide means for correcting for amplitude variations of the source light. The reference diode for deuterium lamp 105B measures a long wavelength component from lamp 105B which is not absorbed. Since this wavelength is within the fluorescent spectrum, the coupling described above is used to separate the signal portion from deuterium lamp 105B from the signal portion from the fluorescent spectrum.

As described previously, the light from xenon flash lamp 105A must be precisely positioned so that the light is directly incident upon only the inner diameter of capillary tube 110. Further, since xenon flash lamp 105A has a finite lifetime, the apparatus holding xenon flash lamp 105A is designed to maintain proper alignment of the lamp's arc after replacement of flash lamp 105A. The arc 300 (Fig. 9, which is not drawn to scale) is aligned with a long axis parallel to a vertical plane 400 through sample region 110C. The housing of lamp 105A has fine adjustments which

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are used to provide a maximum fluorescence signal with a sample in capillary tube 110. The alignment of the cylindrical axis of lamp housing 105A with respect to optical axis 402 which is perpendicular to plane 400 passing through capillary tube 110 is not critical. Lamp 105A is attached to a mounting base 301 with a spring action strap 302 across the middle of lamp 105A. The end 303 of the lamp assembly has a pivot adjustment, which is implemented by a screw actuating a pad (not shown) protruding through mounting base 301. By adjusting the screw, arc 300 is aligned in plane 401 which is normal to optical axis 402 passing through arc 300. A pin (not shown) protruding through base 301 maintains the height of the arc on the optical axis of the instrument.

Immediately in front of xenon flash lamp 105A is a metal strip 304 having a slit which functions like a pinhole. The width of the slit is approximately fifteen times greater than the inner diameter of capillary tube 110 and the height of the slit is also approximately fifteen times greater than the height of sample region 110C in capillary tube 110.

Light from slit 304 encounters 340 nanometer filter 221, described previously, and after passing through filter 221, the filtered light is incident upon reference window 222. Reference window 222 is a microscope cover slip glass set at a 45 degree angle to optical axis 402 to reflect a sample of the ultraviolet light to reference detector 219. Reference window 222 is 0.1 mm thick and must be large enough to provide a clear aperture of 0.25 inches minimum diameter in a plane (not shown) perpendicular to optical axis 402. In one embodiment, reference window 222 has a magnesium fluoride antireflection coating on each side to reduce the total light reflected to reference detector 219 to about 3% of the irradiance from filter 221.

Light from reference window 222 encounters a first

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source imaging lens 305. Lens 305 and slit 304 are an imaging system. For reference slit 304, which is approximately fifteen times larger than the inner diameter of capillary tube 110, source imaging lens 305 has a 9.5 mm focal length, a 0.25 inch diameter and is a plano-convex ultraviolet grade fused silica lens sold by Oriel under Catalog number 41115. For ultraviolet light, the focal length of lens 305 is actually 9.1 mm and the paraxial back focus is 7.7 mm. The back surface of lens 305, i.e., the surface closest to window 222, is located 0.314 inches in front of the center of capillary bore 244 of capillary sample holder 232 (Fig. 8a). This distance is established by a ray trace and the distance is shorter than the paraxial image position by an amount controlled by spherical aberration. After sample region 110C is encountered, holder 232 (Fig. 8d) has a conical hole 247 countersunk on the lens axis beyond capillary tube 110 to act as a trap for the excitation light.

The size of slit 304, described above, and source imaging lens 305 are an illustration of one embodiment only and are not intended to limit the scope of the invention. Other slit sizes and source imaging lenses can be used. The only requirement is that light incident upon sample region 110C must be limited to prevent polychromator 106 from receiving light from surrounding surfaces whose light reflecting properties are uncontrolled. To further limit the reflected light, the light passes through a black, light tight assembly.

The filtered UV light incident upon sample region 110C in capillary tube 110 causes the sample to fluoresce and the fluorescent light enters polychromator 106 at a 90 angle to the incident UV light.

Light from deuterium lamp 105B, used for absorption measurements, is on optical axis 403 (Fig. 9) passing directly through capillary tube 110 and into polychromator

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106. Deuterium lamp 105B is a continuous extended light source and so a slit mask 307 is mounted in front of the source to provide an initial imaging of the light. Light from slit 307 encounters a mirror 308 which in turn reflects the light onto a lens 306. Lens 306 projects the light from slit 307 onto the bore of capillary tube 110 so as to provide maximum sensitivity. The incident light is focused so that light entering polychromator 106 passes through a path in sample region 110C, preferably a maximum path length. Hence, the width of slit 307 is limited so that the incident light falls on a region near the center of the diameter of capillary tube 110. Lateral adjustment of the slit position is made so as to obtain a maximum reading from polychromator 106 for a sample in capillary tube 110. If the slit is fixed, mirror 308 can be adjusted to align the slit image with the capillary tube.

Two different mirrors can be used in the CE instrument. In a first embodiment (not shown), a dichroic beam splitter filter is utilized as mirror 308. This mirror reflects wavelengths from about 190 to 350 nanometers and transmits wavelengths of about 400 to 700 nanometers. The range between 350 nanometers and 400 nanometers is a transition range. Hence, in this embodiment, mirror 308 is used as folding mirror for the ultraviolet light and as a window for the visible light. Hence, mirror 308 reflects the wavelength range from 190 to 350 nanometers onto lens 306 and passes the longer wavelengths from deuterium lamp 105B through mirror 308. In this embodiment, a reference detector is mounted so as to receive the longer wavelengths passed through mirror 308. The reference channel generates the signal used to adjust the absorbance signals for variations in intensity of lamp 105B.

In a second embodiment, illustrated in Fig. 9, a reflector mirror is used as a steering mirror 308 and all of the light from deuterium lamp 105B is reflected onto lens

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306. In this embodiment, a sixth diode detector in diode array 217 is used as a reference channel for measurement of variations in the amplitude of light from deuterium lamp 105B, as previously described.

5 The other elements of the detector are identical to those described previously for the fluorescence detector. However, the wavelength calibration adjustment is made by moving the detector assembly to get a selected pair of detector array elements to produce a specified ratio of
10 signals for a given spectra light output from the calibration source.

 In polychromator 106, the fluorescent light and the unabsorbed light from deuterium lamp 105B are incident upon a flat field concave holographic grating 218 (Fig. 5b)
15 having a size of 37 x 37 mm with 560 grooves per millimeter. The grating can be obtained from American Holographic under Catalog number 456.20. This grating has an overall diffraction efficiency of about 30%. The grating is optimized to have an efficiency of about 33% at a
20 wavelength of around 350 to 360 nanometers and the efficiency of the grating is something less than 30% at the long wavelength end of the grating around 600 nanometers.

 The precise mounting of holographic grating 218 in polychromator 106 and the size of polychromator 106 are
25 dictated by the manufacturer's specifications. For the grating described above, the polychromator housing wall has a thin edged aperture with a diameter such that an F/2.5 cone is formed from the slit position along the optical axis of the grating. The grating slit position is occupied by
30 capillary tube 110. As described above, the region about capillary tube 110 is covered and baffled so that stray reflected light is prevented from falling on the internal walls of polychromator 106. However, should any stray light reach the internal walls of polychromator 106, the walls are
35 relieved or milled out so as to keep them some distance from

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the cones of light from sample region 110C. In one embodiment, additional stray light rejection is provided by a baffle in the cavity of polychromator 106.

5 The grating is ultimately positioned so that the optical axis from the light source to the grating slit, and that from the grating slit to the vertex of the grating all lie in the same horizontal plane. As shown in Fig. 7, the polychromator housing wall is removed, and grating 218 is inserted and placed against four mounting brackets 275A,
10 275B which cover the corners of grating 218. The corners of grating 218 are not used and so this method of mounting grating 218 does not affect the efficiency of polychromator 106. Grating 218 is held in place by a fixture applied to the back side of grating 218. Photodiode array 217 is
15 mounted on the polychromator housing so that the 214 nanometer wavelength light from grating 218 falls nominally at the middle of the first detector element. The circuit board containing photodiode array 217 is enclosed in a shielding box. The board is mounted so that the board can
20 be adjusted ± 2 mm in a horizontal direction along the plane of photodiode array 217. This adjustment provides means for wavelength calibration of the detector signals, as previously described.

The electrolyte replenishment subsystem of this
25 invention is shown in Fig. 10. The electrolyte replenishment subsystem includes two 100 ml supply bottles 252, 253 which contain the running electrolyte and the detector electrolyte. Running electrolyte supply bottle 252 is connected to running electrolyte bottle RE1 by a 1/8 inch
30 tube through valve V4 and detector electrolyte bottle 113 is connected by a 1/8 inch tube through valve V5 to supply bottle 253. A 500 ml waste bottle 251 is similarly connected by 1/8 inch tubing to running electrolyte bottle RE1 through valve V4 and to detector electrolyte bottle 113
35 through valve V5. Each of the bottles in replenishment

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system 410 has a thermistor level sensor, as previously described. A pump 254 capable of providing either a 22 inch Hg vacuum or pressure is coupled to running electrolyte supply bottle 252 and to detector electrolyte supply bottle 253 by a valve V1 and to waste bottle 251 by a valve V2. Waste bottle 251 also has a sensor which is connected to a vacuum sensor gauge. Valve V1 is a pressure vent valve while valve V2 is a vacuum vent valve. Valve V3 is a timed injection valve while valves V4 and V5 are refill or purge valves for the running electrolyte bottle RE1 and the detector electrolyte bottle 113. Fig. 10 illustrates only a single running electrolyte bottle RE1, but, as previously described, autosampler head 204 moves the 1/8 inch tube and level sensor to any of the four running electrolyte bottles. Accordingly, in Fig. 10, running electrolyte bottle RE1 (Fig. 5b) has been selected.

To empty and fill running electrolyte bottle RE1 the external data communication device 107 (Fig. 5a) provides system controller 100 in the capillary electrophoresis instrument with the command "PREPURGE" or "POSTPURGE" directed to a running electrolyte bottle. These commands are described more completely in Appendix B which is incorporated herein by reference. When system controller 100 receives either of these commands, controller 100 opens valves V1, V3 and V5 and closes valves V2 and V4. Next, the level sensor is lowered to the bottom of running electrolyte bottle RE1 and pump 254 is turned on to full vacuum. The pump continues to run until the level sensor in running electrolyte bottle RE1 senses that bottle RE1 is dry.

After bottle RE1 is dry, if controller 100 receives a "FILL" command (See Appendix B) for the running electrolyte bottle, the other level sensors are checked to make sure that replenishment system 410 is in a configuration suitable for proceeding with the refill operation. If the sensors show that system 410 is in a configuration for proceeding,

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i.e., an adequate liquid level is present in the bottles, the level sensors are raised to the "full" position. Conversely, if an error is detected in the check of the other level sensors, such as running electrolyte supply bottle 252 is empty, then the empty and refill cycle is aborted.

After the level sensors are raised to the "full" position, valves V2, V3 and V4 are opened and valves V5 and V1 are closed by system controller 100 and then pump 254 is turned on so that a positive pressure is created in running electrolyte supply bottle 252 and consequently the running electrolyte is transferred to running electrolyte bottle RE1. When the level sensor detects the electrolyte, pump 254 is turned off and system controller 100 proceeds with the next instruction.

Purging and filling of detector electrolyte bottle 113 is similar to the purging and filling of running electrolyte bottle RE1. However, in this process the commands are directed to the detector electrolyte bottle (See Appendix B). In this process, system controller 100 purges detector electrolyte bottle 113 by opening valves V1, V3 and V4 and closing valves V2 and V5. System controller 100 turns pump 254 on so that pump 254 creates a vacuum. Pump 254 runs until detector electrolyte bottle 113 is evacuated of electrolyte. After the evacuation, if a "FILL" command is received, system controller 100 continues to refill bottle 113 by opening valves V2, V3 and V5 and closing valves V4 and V1. Pump 254 is then turned on so that detector electrolyte is pumped from detector electrolyte supply bottle 253 to detector electrolyte bottle 113.

While the replenishment system of this invention has been described using only one running electrolyte bottle, one detector electrolyte bottle, one waste bottle and two supply bottles, this illustration is not intended to limit the scope of this invention. In view of this description,

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one skilled in the art could use additional valves, waste bottles, running electrolyte or detector electrolyte bottles as well as multiple supply bottles.

The replenishment system has significant advantages over the prior art. This system eliminates manual intervention in capillary electrophoresis tests. Also, the ability to frequently and easily change the electrolyte is significant because the possibility of a degraded electrolyte distorting test results is eliminated. Accordingly, this invention not only makes capillary electrophoresis easier, but also makes such testing more reliable and reproducible.

Replenishment subsystem 410 is also used for a timed injection cycle which draws a sample from the microtiter tray into capillary tube 110. In this operation, system controller 100 (Fig. 5a) first positions autosampler head 204 (Fig. 5b), using the X and Y stepper motors 205, 206, over the sample location in the microtiter tray and then uses Z stepper motor 207 to lower capillary tube 110 to reach the bottom of the sample. Next valves V1, V3, V4 and V5 are opened and valve V2 is closed. Then, in response to the command from external computer 107 (See Appendix B), system controller 100 turns on vacuum pump 254 and runs pump 254 until a specified vacuum level is reached. System controller 100 maintains the vacuum by toggling valve V2. System controller 100 then closes valve V3 for a specified time. The closing of valve V3 causes the sample to be drawn into capillary tube 110. After the specified time valve V3 is reopened and subsequently capillary tube 110 and the high voltage electrode 103A are moved to the running electrolyte bottle selected by commands to system controller 100.

System controller 100, as described above, receives commands from external device 107 and then controls the hardware within the capillary electrophoresis instrument so as to perform the desired tests. Controller 100 controls

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the three stepper motor controllers so as to position the capillary tube in a desired location, the level sensors, and the replenishment system. Controller 100 provides gain control to multichannel analog digital converter 227, which
5 converts the analog high voltage power supply voltage and current signals, the pressure/vacuum signals and related parameters, as well as controlling the multichannel digital to analog converter which generates signals which in turn supply analog output signals for the strip chart recorder
10 terminals, described previously. Finally, system controller 100 controls the serial communications port to the external PC 107, the front panel LED status indicators, the xenon flash lamp trigger control, and the system integrity interlocks.

15 The software program in system controller 100 has three basic modes which are the power on initialize mode, the command mode and the test mode. In the power on initialize mode, system controller 100 initializes program parameters, initializes hardware in the capillary electrophoresis
20 instrument, sets ASCII communication to "terminal" mode and moves autosampler head 204 to the HOME positions. The communication error LED on the front of the capillary electrophoresis machine is also turned on and a question mark signal sent to external PC 107. After the question
25 mark is sent, the microprocessor waits for a status request from PC 107 and after receiving the status request the communication error LED is turned off and system controller 100 enters the command mode. In the command mode, system controller 100 responds to all commands from PC 107 and
30 after receiving the command sends the signal "OK" back to PC 107. These commands include status, configuration data, test parameters, operational commands and data commands. If the command received is "STARTTEST", system controller 100 transmits the "OK" response and then transfers to the test
35 mode. As described previously, the machine language Forth

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program for the system controller is given in Appendix A. In Appendix B, the commands to the system controller from the external PC or teletype are defined, and incorporated herein by reference.

5 In the test mode, the system controller 100 acquires and transmits data to PC 107 per the test parameters which were specified during the command mode. When the test is completed the phrase test complete is sent followed by a 0 for the data and "OK". If an escape character is received
10 during the test or when the test is complete, system controller 100 moves the autosampler head 204 to the HOME position and returns to the command mode.

 After the power on cycle described previously, and before the test command is given, external PC 107 is used to
15 configure the EC instrument. When the power is initially applied, no assumptions are made as to hardware coordinate locations. Thus, PC 107 must provide this information sometime prior to transmitting the test parameters for the first test. Once the system is configured, the definitions
20 are retained until changed by PC 107 or until the capillary electrophoresis instrument is turned off. The parameters that must be defined include the locations for the running electrolyte bottles and the test samples, the level sensors for each of the running electrolyte bottles, the running
25 electrolyte supply bottle, the detector electrolyte supply bottle and the waste bottle. The purge controls, described previously, to purge the waste bottle, to purge a selected running electrolyte bottle and the running electrolyte
 bottle to be refilled using selected supply bottles. The
30 specific commands are given in Appendix B.

 After the initial setup the test parameters must next be specified. The test parameters instruct system controller 100 as to which running electrolyte bottle to use, whether the running electrolyte is to be purged and
35 refilled, what aspiration method to use, what the high

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voltage test conditions are, and other parameters required for the test. Specifically, for each test, the running electrolyte and test sample must be specified and the aspiration method for drawing the test sample into capillary tube 110, i.e., either a vacuum or an applied voltage, must be specified.

After the initialization of the parameters which describe the test, the capillary tube run time power must be defined. There are two modes for controlling the power across the capillary tube during a test. The first mode is a voltage profile mode where a voltage vs. time profile is specified, e.g., the voltage may be changed in a ramp like fashion, and the second mode is a current mode wherein the current values, that must be maintained during the test, are specified. These modes are mutually exclusive. In the voltage profile mode, the applied voltage across the capillary tube is programmed to change as a function of time. The maximum number of profiles settings is limited to 50.

The final parameters that which must be specified are channel numbers of the output data vs. associated detectors. System controller 100 can group the output signals from the sixteen individual diode detectors into any desired combination to form detector groups. A single arithmetic value is reported during the test for each detector group. The grouping consists of adding together individual detector signals. Since fluorescence emission wavelengths are typically 30 nm FWHH, the signals from two or three diodes can be summed to obtain a better signal to noise ratio. A group can consist from 1-16 detectors and any combination of the 16 detectors can be used to define the group. A maximum of four groups is allowed.

After the parameters for initializing the position of the autosampler head and the parameters for the test are entered, the test command is given and system controller 100

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automatically performs the following sequence of operations. After receiving the test command, the status of the sample door is first checked and if the sample door is open an error command is transmitted to PC 107 and system controller 100 returns to the command mode. If the sample door is closed, then the standard response of "OK" is transmitted to PC 107, and the level sensor moved over the running electrolyte bottle specified for the test and lowered into the running electrolyte container. If a prepurge or fill command is given these commands are completed at this time. In the prepurge directive the plumbing is set as required, the vacuum pump is initialized and the vacuum is drawn for a period of time as described previously. After the selected running electrolyte bottle is evacuated, the pump is turned off and the replenishment system is set to exhaust the atmosphere.

The fill directive causes system controller 100 to raise the level sensor to the level defined in the fill directive, set the plumbing to the defined running electrolyte supply bottle and to start the pump for pressure. The pump continues to operate until the level sensor detects liquid and turns off the pump as previously described. After either the refill or purge command, if the level sensor does not detect a liquid level, an error flag is set. If a liquid level is detected, the level sensor is moved to the storage position. Since there is liquid in the running electrolyte bottle, the test is ready to commence and the autosampler head is moved to the running electrolyte bottle that will be used for the test and the capillary tube is lowered into the running electrolyte bottle. The plumbing is then set to draw a vacuum on the running electrolyte bottle and the vacuum pump is turned on. After a selected period of time, as specified in the test parameters, the pump is turned off and the exhaust is set to atmosphere. This operation fills the capillary tube with

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running electrolyte for the test. Next, autosampler head 204 moves the end of capillary tube 110A to the specified sample and the sample is drawn into the capillary either using the vacuum or the high voltage, as previously described. After the sample is drawn into the capillary tube, the end of the capillary tube is moved to the running electrolyte bottle and lowered into the bottle.

The test is commenced using either the specified voltage or current profile data. After the data is acquired, the high voltage is turned off and an end of data is transferred to PC 107 and the capillary tube is retracted from the running electrolyte. At this time, if a postpurge operation is specified, the running electrolyte is purged as previously described. The capillary tube is moved to the home position and the test is completed. The system controller completes all tests using a specified running electrolyte bottle before proceeding with tests using another running electrolyte bottle.

It will, of course, be understood that modifications of the present inventive automated capillary electrophoresis instrument including the absorbance and fluorescence detector will be apparent to others skilled in the art. For example, the detector can be employed with either column chromatography or tube gel electrophoresis. Consequently, the scope of the present invention should not be limited by the particular embodiments described above, but should be defined only by the claims set forth below and equivalents thereof.

APPENDIX A

[illegible]

S1234660780000824F52464C466A301E81563C1C3E3568004EF5780083584F52466346805B
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S9030000FC

APPENDIX B

Defining running electrolytes and test samples requires the following command

Nx Ny Nz DEFxyz name<Cr>

5 where:

N is the numeric value of the subscripted coordinate to be associated with "name".
-15000 <= N <= 15000

10 name is an alpha-numeric string of 20 characters or less, containing at least 1 alpha.

EXAMPLE: 500 100 20 DEFxyz RE1<Cr>
Defines RE1 to be at the indicated
X=500, Y=100, Z=20 position.

NOTE:

15 The System Controller places no significance on the alpha-numeric string characters in "name". In the above example "RE1" is most likely a running electrolyte bottle. However, the character significance of the name is known to the System PC not the System Controller. To the System
20 Controller, the string "RE1" is shorthand for the assigned coordinates.

The format for defining the liquid level sensor vs. "name" relationship is:

name N =GUAGE#<CR>

25 where:

name is an alpha-numeric string, as defined above.
N is the level sensor number (1-4).

EXAMPLE: RE3 2 - GUAGE#<Cr>
Instructs the System Controller to use the #2
30 level sensor anytime an operation on RE3 requires level information.

To inform the System Controller which of the two plumbing systems in the replenishment system is associated with which running electrolyte bottle, the following format is used.

5 name N =PURGE#<Cr>

where:

name is an alpha-numeric string previously defined
N is the plumbing identifier (1 or 2)

EXAMPLE: RE3 1 =PURGE#<Cr>

10 Instructs the Sample Handler to use plumbing
 #1 when emptying RE3.

To define which running electrolyte supply bottle is plumbed to which running electrolyte bottle, the following format is used.

15 name N =RESERVOIR#<CrLf>

where:

name is an alpha-numeric string previously defined
N is the plumbing identifier (1 or 2)

EXAMPLE: RE4 1 =RESERVOIR#<CrLf>

20 Instructs the System Controller to use the
 plumbing associated with the supply bottle #1
 when filling RE4.

After establishing names for the Running Electrolyte (RE) and the Test Sample (TS) coordinate definitions, the names assigned can be used to define the RE & TS for the next test.

The format for defining the Running Electrolyte (RE) is:

name =RE

Defines "name" as the Running Electrolyte

The format for defining the Test Sample (TS) is:

name =TS

5

Defines "name" as the Test Sample

EXAMPLE:

RE4 =RE<Cr>

Specifies that the Running Electrolyte be
drawn from the location labeled RE4

A1 =TS<Cr>

10

Specifies that the Test Sample be drawn from
the sample location labeled A1

The format for establishing the aspiration method to
draw the sample into the capillary tube is:

Nv Nt type =ASPERATE<Cr>

15 where:

type is one of two character strings, "VOLTS" or
"VACUUM".

20 Nv is the value of the aspiration force in units
of mmHg (torr) for vacuum or volts for
electromotive.

Nt is the time to apply the force in units of
seconds.

EXAMPLE:

30000 20 VOLTS =ASPERATE<Cr>

25 Defines the aspiration to consist of applying
30kv across the capillary for 20 seconds.

100 60 VACUUM - ASPERATE<Cr>

Defines the aspiration to consist of applying a 100 torr vacuum to the capillary tube for 60 seconds.

Pretest Purge is a directive to empty a RE container at the beginning of a test, and would be generally be followed by a FILL directive. To establish this directive in the next test protocol, the following format is used:

Nv Nt name PREPURGE<Cr>

where:

10 Nv is the vacuum level to use for the purge.
 Nt is the time in seconds to apply the vacuum.
 name is an alpha-numeric string defined above for
 the running electrolyte bottle and the
 associated plumbing.

15 The PREPURGE directive is not retained for subsequent tests. Consequently, all tests requiring a pretest purge must be so directed before the test is started.

EXAMPLE: 200 60 RE1 PREPURGE<Cr>

20 Directs that RE1 be emptied before starting the next test, using a 200 torr vacuum for 60 seconds.

Posttest purge follows the same format and operation as the Pretest purge using the directive POSTPURGE.

Nv Nt name POSTPURGE<Cr>

25 The posttest purge is performed at the end of the next test. After the purge is completed, the posttest directive is cleared as in the case of the PREPURGE.

The format for the FILL directive is:

name Nz FILL<Cr>

where:

name is the alpha-numeric string defined above.

5 Nz is the vertical position to which the level sensor is to be lowered for gauging the fill level.

There are two modes for controlling the power across the capillary during a test:

- 10 1. VPROFILE, which specifies a voltage vs. time power control
2. ICONSTANT, which specifies a constant current value to be maintained during the test.

These modes are mutually exclusive. Transmitting a VPROFILE directive clears any previous ICONSTANT directive and any associated settings. The same is true when an ICONSTANT directive is transmitted: i.e., any previous VPROFILE directive and associated settings are cleared.

During the course of a test the applied voltage across the capillary tube can be programmed by the operator to change as a function of time. These voltage vs. time profiles are transmitted to the System Controller by the PC prior to starting a test. The profile information is transferred as voltage level, delta time information. That is, the voltage data transferred to the System Controller is the new voltage setting and the time data is the period of time over which the System Controller is to change from the currently running value to the new setting. The profile information is transmitted as a block of data using a directive followed by the data. A "-1" in the data stream marks the end of the profile settings. Anytime voltage profile settings are transmitted, the entire profile must be transmitted; one specific setting in the profile cannot be separately edited. The format for transferring the voltage

profile is:

```

5          VPROFILE<Cr>
            v1 t1<Cr>
            v2 t2<Cr>
            ...
            vn tn<Cr>
            -1<Cr>

```

where:

```

10      vn   is the voltage level in volts
          0 <= vn <= 30000 volts
      tn   is the time interval in seconds within which
          to make the change to vn
          0 <= tn <= 30000 seconds
      -1   is the end of data mark

```

15 The maximum number of profile settings is limited to 50.

EXAMPLE:

```

          VPROFILE<Cr>
          2000 0<Cr>   -directs an initial step to 2000
                        volts
          10000 30<Cr> -directs a 30 second up ramp
20                        from 2000 to 10000 volts
          10000 60<Cr> -directs a hold at 10000 volts
                        for 60 seconds
          8000 0<Cr>   -directs a step to 8000 volts
          2000 30<Cr>   -directs a 30 second down ramp
25                        to 2000 volts
          -1<Cr>       -directs that the profile data
                        is complete.

```

The format of the constant current directive is:

Ni Nt ICONSTANT

where:

Ni is the value of the constant current in
microamps
50 \leq Ni \leq 500 microamps
5 Nt is the time interval of the test in second
0 \leq Nt \leq 15000 seconds

The format for defining a group of detector signals is:
n1 n2 n3 ... Nd Ng =GROUP

where:

10 n is a detector number (1-16)
Nd is the number of detector data signals in the
group
Ng is the group # (1-4)

15 EXAMPLE: 10 1 2 =GROUP
Defines group #2 to be 1 detector signal,
detector 10.

8 9 10 10 11 12 6 3 =GROUP
Defines group #3 to be 6 detector signals,
wherein detector 10 is used twice.

CLAIMS

I claim:

1. A fluorescence and absorption detector comprising:
a light source for providing light incident upon
5 and interacting with a sample, with emerging light from
such a sample being characteristic of said sample;
means, receiving said emerging light, for
separating light into a number of different
wavelengths; and
10 means, receiving each of said different
wavelengths from said means for separating light, for
providing an output signal corresponding to each of
said different wavelengths.
2. A detector as in Claim 1 wherein said light source
15 comprises a first light source and a second light source,
said first light source having a wavelength of light causing
a sample to fluoresce and said second light source having
wavelengths absorbed by such a sample, wherein said first
light source providing light incident upon a first region of
20 such a sample and said second light source providing light
incident upon a second region of such a sample.
3. A detector as in Claim 2 wherein said first light
source comprises a xenon flash lamp.
4. A detector as in Claim 2 wherein said second light
25 source comprises a deuterium lamp.
5. A detector as in Claim 2 further comprising a
filter being placed between said first light source and a
sample so that light incident upon such a sample from said
first light source consists essentially of said wavelength

which causes such a sample to fluoresce.

6. A detector as in Claim 5 wherein said filter comprises approximately about a 340 nanometer wavelength filter.

5 7. A detector as in Claim 5 further comprising a first imaging system placed between said first light source and a sample wherein light from said first light source is projected upon only a first selected portion of said first region of such a sample.

10 8. A detector as in Claim 7 wherein said first imaging system comprises a slit mask and a lens.

15 9. A detector as in Claim 2 further comprising a second imaging system placed between said second light source and a sample wherein light from said second light source is projected upon only a selected portion of said second region of such a sample.

10. A detector as in Claim 9 wherein said second imaging system comprises a slit mask and a lens.

20 11. A detector as in Claim 2 wherein said means for separating light receives emerging light from a sample at a first selected angle from said first region.

12. A detector as in Claim 11 wherein said means for separating light receives emerging light from a sample at a second selected angle from said second region.

25 13. A detector as in Claim 12 wherein said first selected angle is 90°.

14. A detector as in Claim 12 wherein said first selected angle is 180°.

15. A detector as in Claim 2 wherein said means for separating light comprises a grating.

5 16. A detector as in Claim 15 wherein said means for providing an output signal comprises a photodiode array.

17. A detector as in Claim 16 further comprising a multiplicity of amplifiers operatively connected to said diode array, each diode in said photodiode array receiving
10 light from said means from separating light having one of said multiplicity of amplifiers.

18. A detector as in Claim 17 wherein said first light source is a pulsed light source.

19. A detector as in Claim 18 wherein said second
15 light source is a continuous light source.

20. A detector as in Claim 19 wherein a first subset of photodiodes of said photodiode array provide pulsed output signals in response to wavelengths from said grating corresponding to said fluorescence, and a second subset of
20 photodiodes of said photodiode array provide continuous output signals in response to wavelengths from said grating corresponding to unabsorbed light from said second light source.

21. A detector as in Claim 20 wherein amplifiers for
25 said first subset of photodiodes pass said pulsed signals and reject continuous signals, and amplifiers for said second subset of photodiodes pass said continuous signals and reject pulsed signals.

AMENDED CLAIMS

[received by the International Bureau
on 31 August 1989 (31.08.89);
original claims 1,3-7,9,11-20 replaced, claim
2 cancelled, new claim 22 added (5 pages)]

1. A fluorescence and absorption detector comprising:

light source means for simultaneously providing a first light beam and a second light beam wherein said first and second light beams are simultaneously incident upon and interact with a sample and emerging light from such a sample is characteristic of said sample;

means, receiving said emerging light, for separating light into a number of different wavelengths wherein said emerging light is separated into different wavelengths; and

means, receiving each of said different wavelengths from said means for separating light, for providing an output signal corresponding to each of said different wavelengths;

wherein said first light beam includes a wavelength of light causing such a sample to fluoresce and said second light beam includes a wavelength absorbed by such a sample;

said emerging light comprises unabsorbed light from said second light beam and fluorescent radiation; and

a first subset of said output signals represent said fluorescent radiation from such a sample and a second subset of said output signals represent said unabsorbed light from such a sample.

3. A detector as in Claim 22 wherein said light source means for providing said first light beam comprises a xenon flash lamp.

4. A detector as in Claim 22 wherein said light source means for providing said second light beam comprises a deuterium lamp.

5. A detector as in Claim 22 further comprising a filter placed in the path of said first light beam so that light incident upon such a sample from said filter consists essentially of said wavelength which causes such a sample to fluoresce.

6. A detector as in Claim 5 wherein said filter comprises approximately a 340 nanometer wavelength filter.

7. A detector as in Claim 5 further comprising a first imaging system placed in the path of said first light beam wherein said first light beam is projected upon only a selected portion of said first region of such a sample.

8. A detector as in Claim 7 wherein said first imaging system comprises a slit mask and a lens.

9. A detector as in Claim 22 further comprising a second imaging system placed in the path of said second light beam wherein said second light beam is projected upon only a selected portion of said second region of such a sample.

10. A detector as in Claim 9 wherein said second imaging system comprises a slit mask and a lens.

11. A detector as in Claim 22 wherein said means for separating light receives emerging light from a sample at a first selected angle from said first light beam.

12. A detector as in Claim 11 wherein said means for separating light receives emerging light from a sample at a second selected angle from said second light beam.

13. A detector as in Claim 12 wherein said first selected angle is about 90°.

14. A detector as in Claim 13 wherein said first selected angle is about 180° .

15. A detector as in Claim 22 wherein said means for separating light comprises a grating.

16. A detector as in Claim 15 wherein said output signal providing means comprises a photodiode array wherein said photodiode array receives light from said separating means.

17. A detector as in Claim 16 further comprising a multiplicity of amplifiers, operatively connected to said diode array, each diode in said photodiode array receiving light from said separating means being operatively connected to one of said multiplicity of amplifiers.

18. A detector as in Claim 17 wherein said first light beam is a pulsed light beam.

19. A detector as in Claim 18 wherein said second light beam is a continuous light beam.

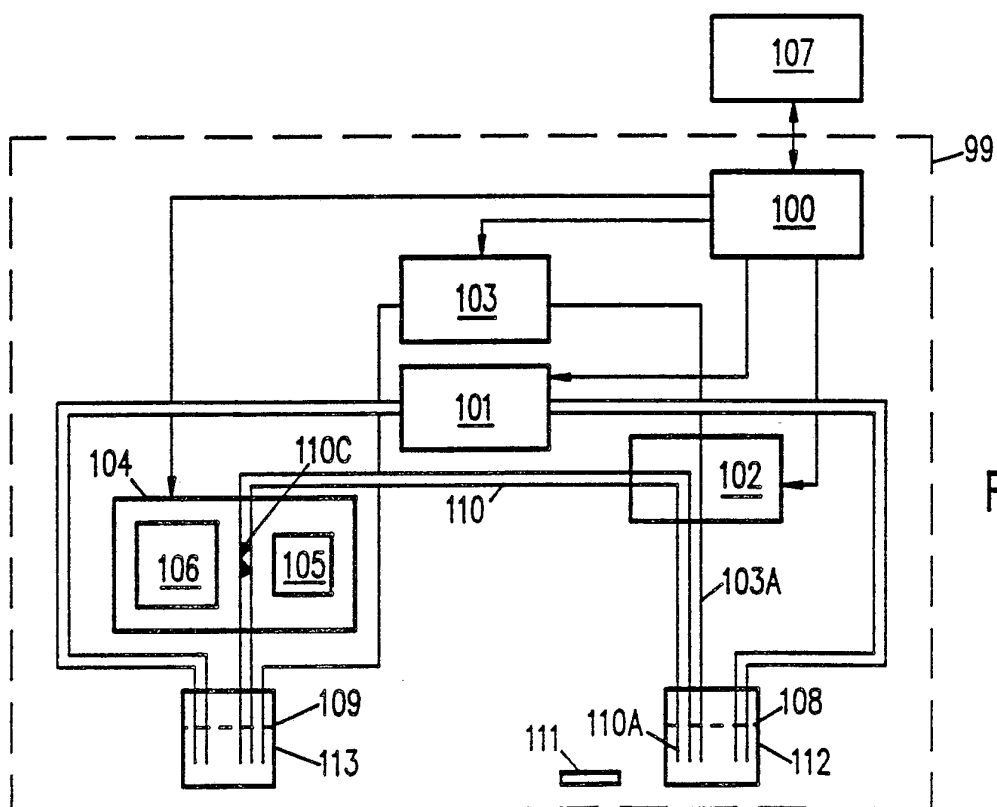
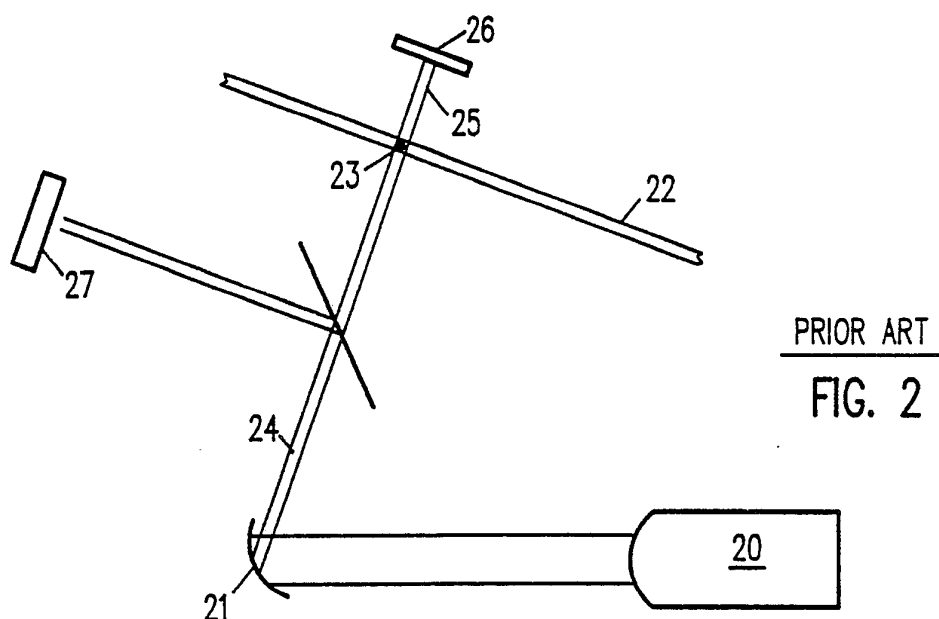
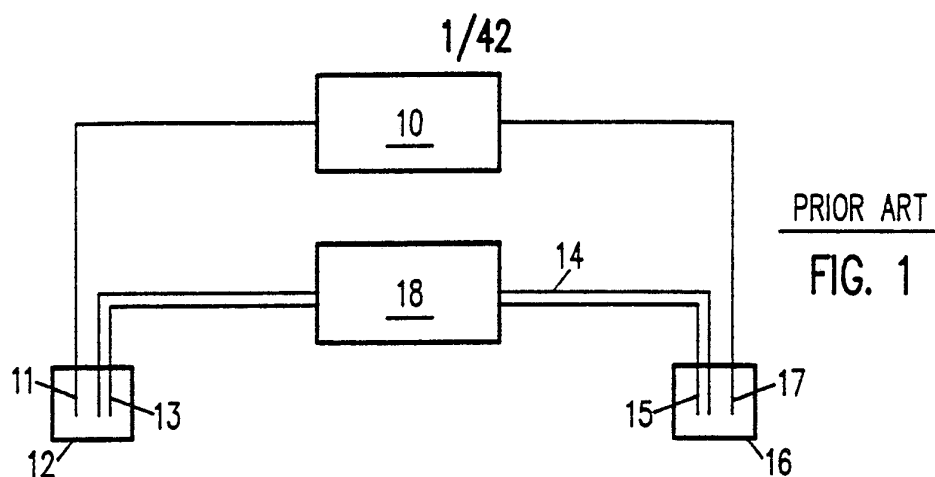
20. A detector as in Claim 19 wherein a first subset of photodiodes of said photodiode array provide pulsed output signals in response to wavelengths from said grating corresponding to said fluorescence, and a second subset of photodiodes of said photodiode array provide continuous output signals in response to wavelengths from said grating corresponding to unabsorbed light from said second light beam.

21. A detector as in Claim 20 wherein amplifiers for said first subset of photodiodes pass said pulsed signals and reject continuous signals, and amplifiers for said second subset of photodiodes pass said continuous signals and reject pulsed signals.

22. A detector as in Claim 1 wherein said first light beam is incident upon a first region of such a sample and said second light beam is incident upon a second region of such a sample so that said beams are incident upon such a sample from different directions.

STATEMENT UNDER ARTICLE 19

Since each of the amendments and the new claim is supported by the Specification and drawings, the amendments have no affect upon the Specification and the drawings. The amendments to the Claims more clearly distinguish Applicant's invention over the cited references.



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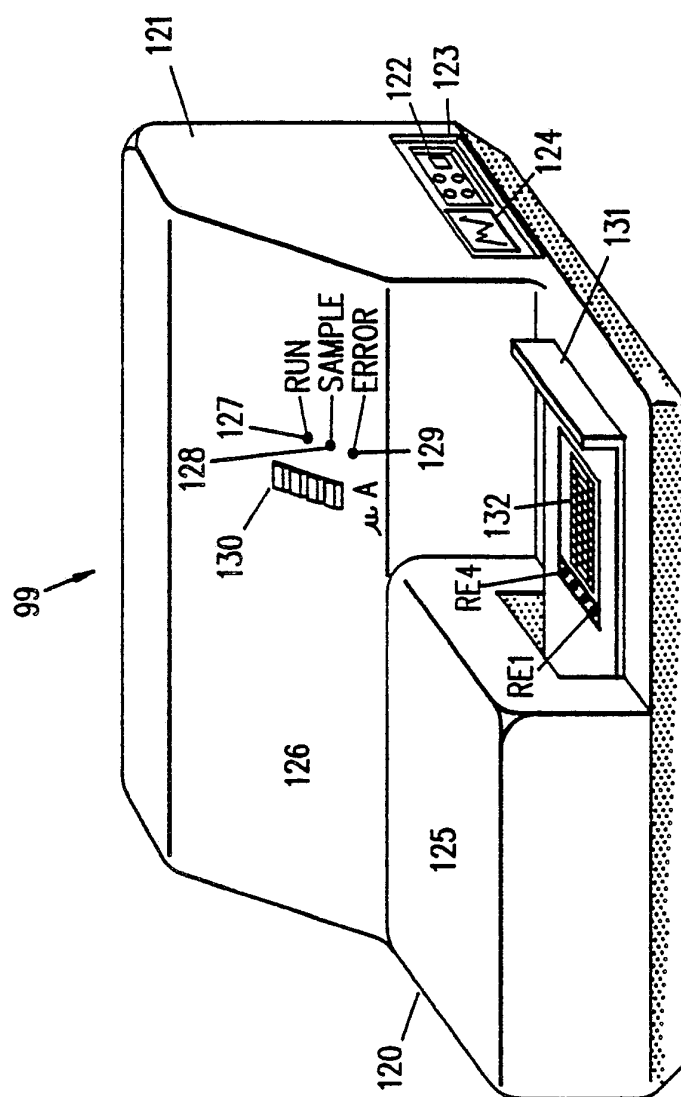


FIG. 4

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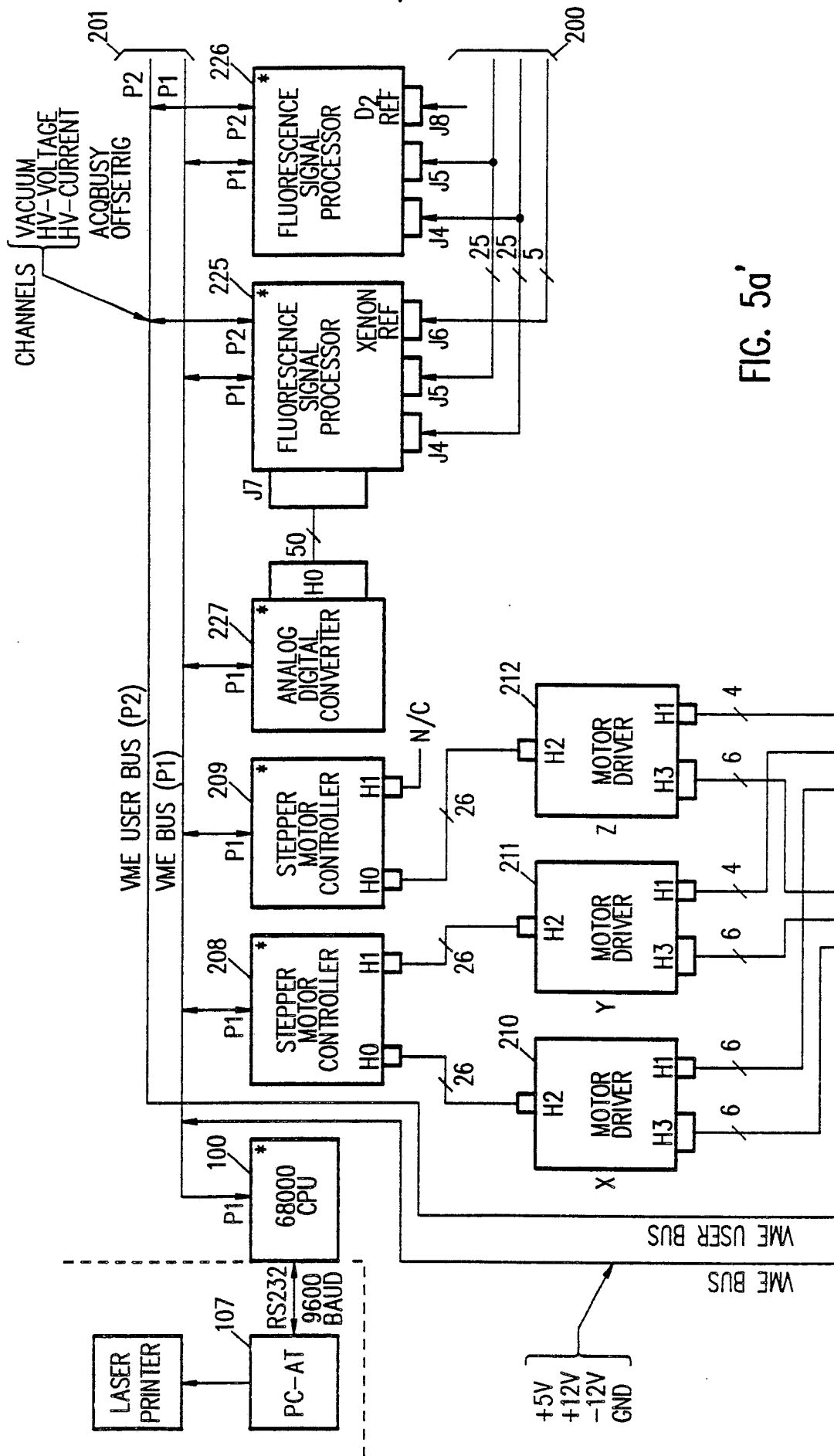


FIG. 5a'

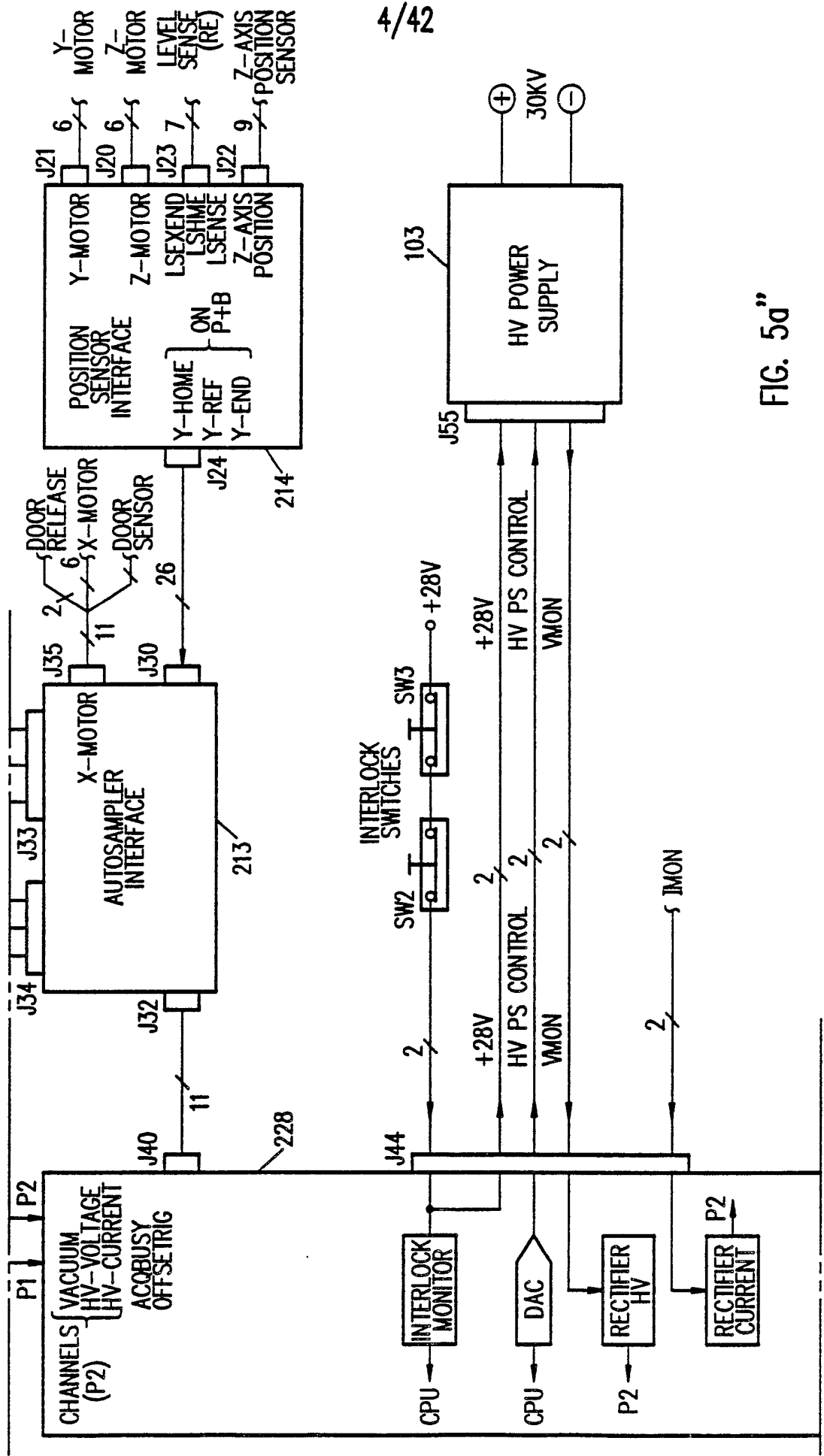


FIG. 5a

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FIG. 5b'
FIG. 5b''

KEY TO FIG. 5b

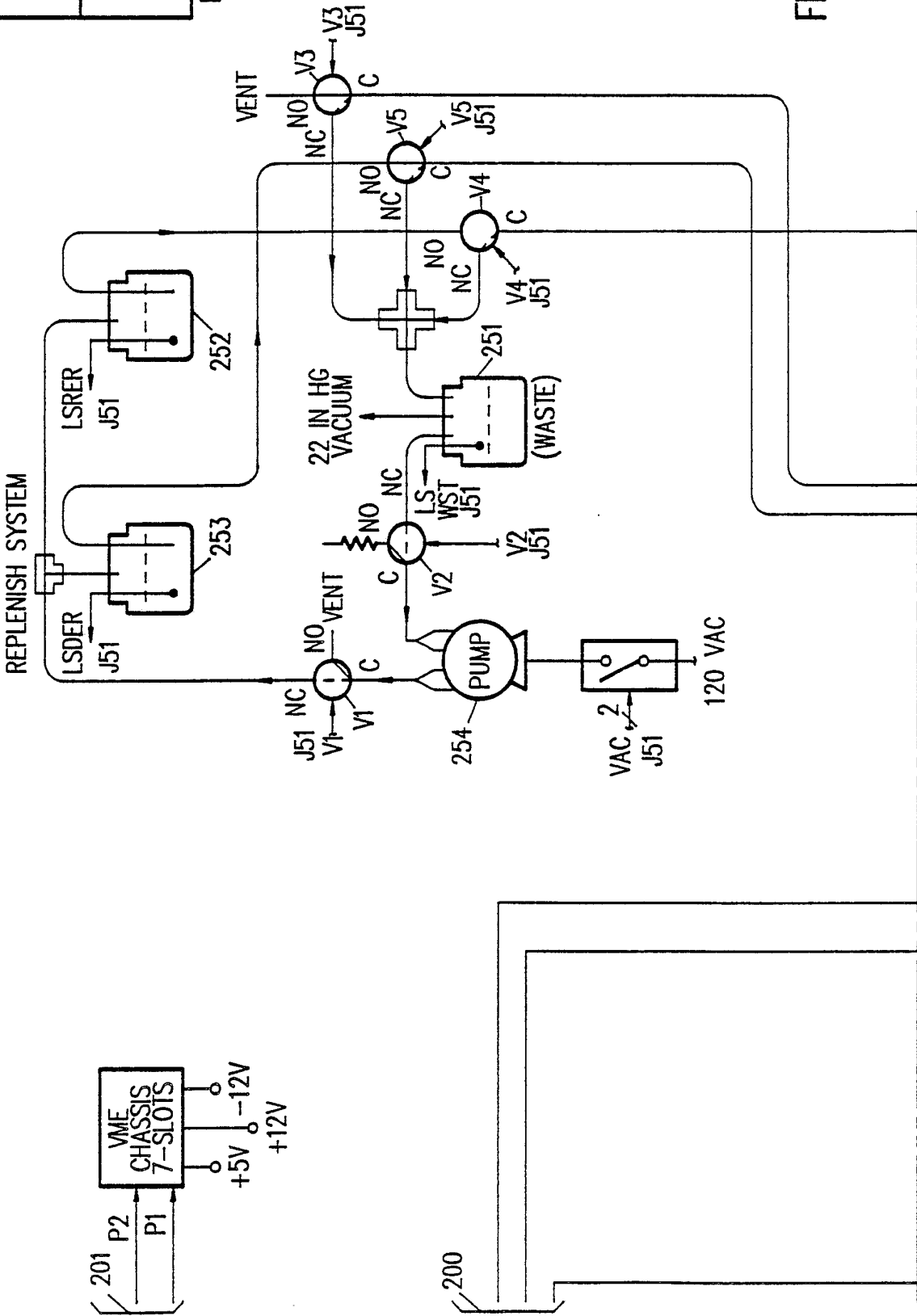


FIG. 5b'

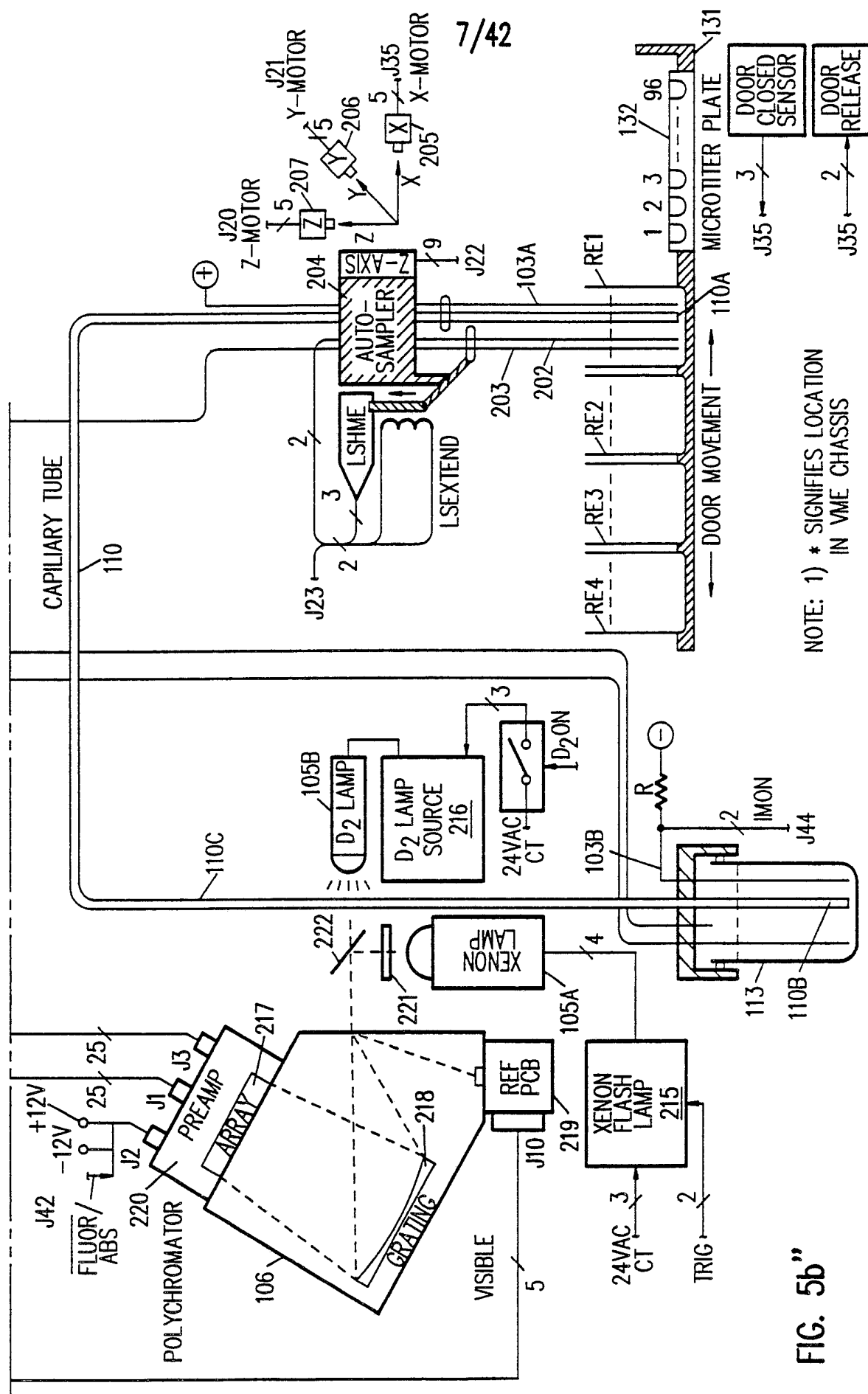
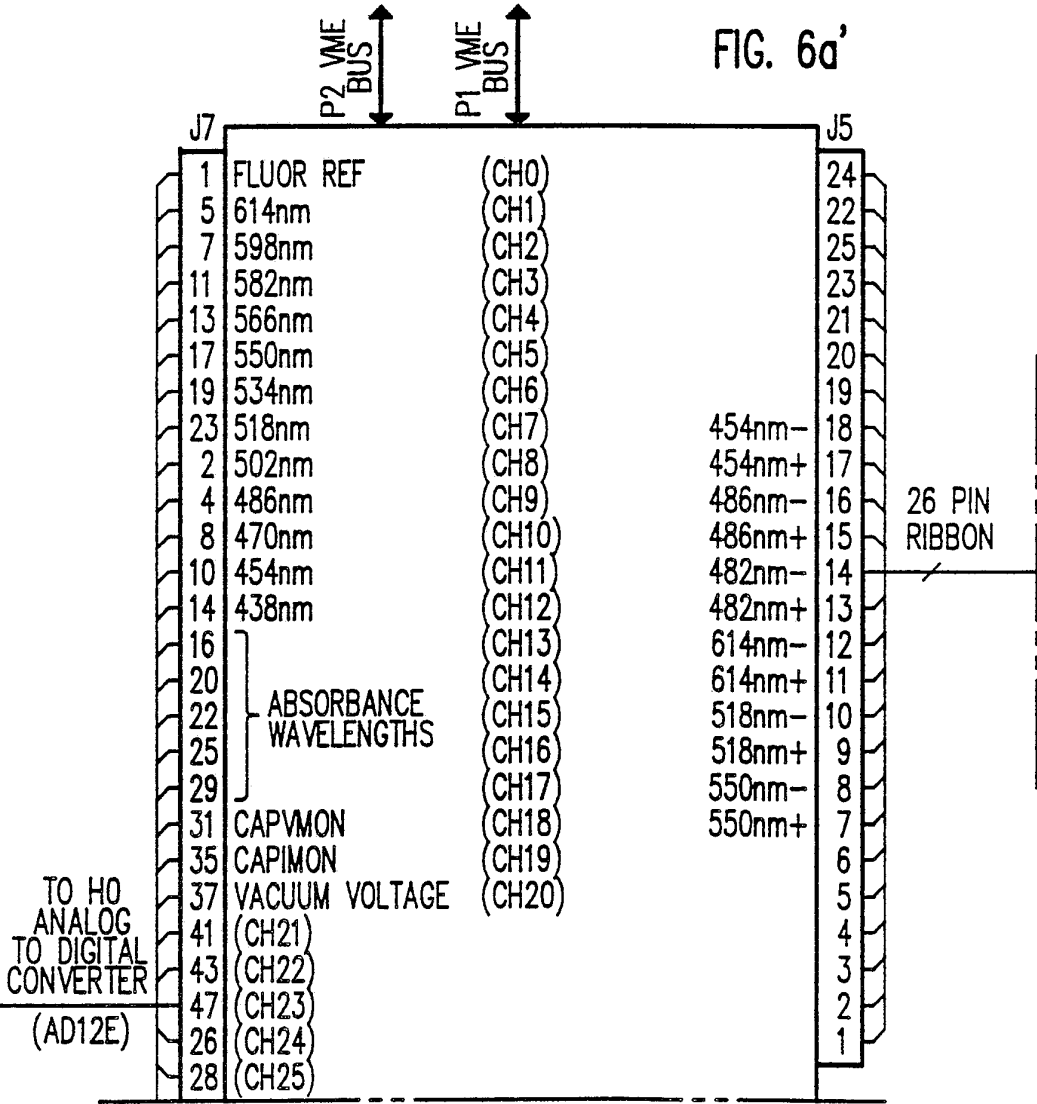


FIG. 5b"

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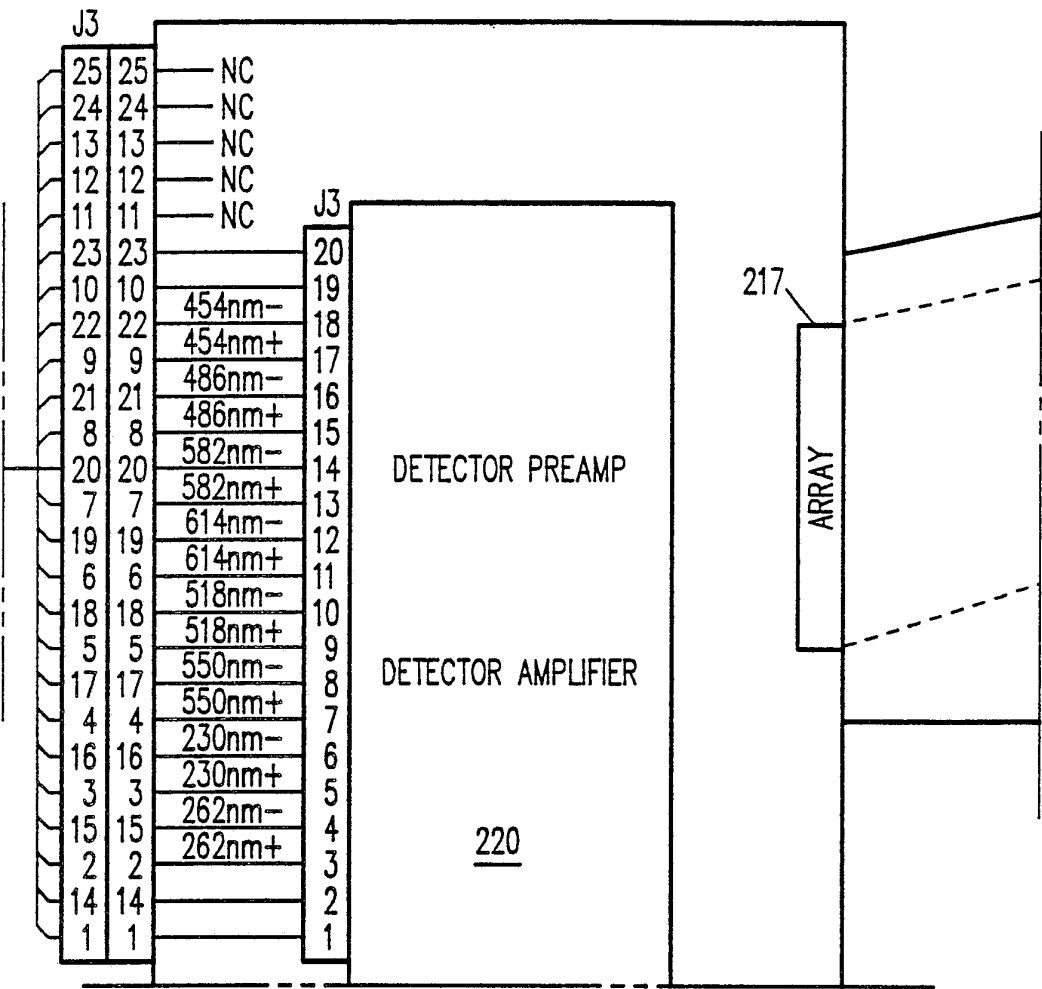
FIG. 6a'	FIG. 6a''	FIG. 6a'''
FIG. 6a''''	FIG. 6a'''''	FIG. 6a''''''

KEY TO FIG. 6a



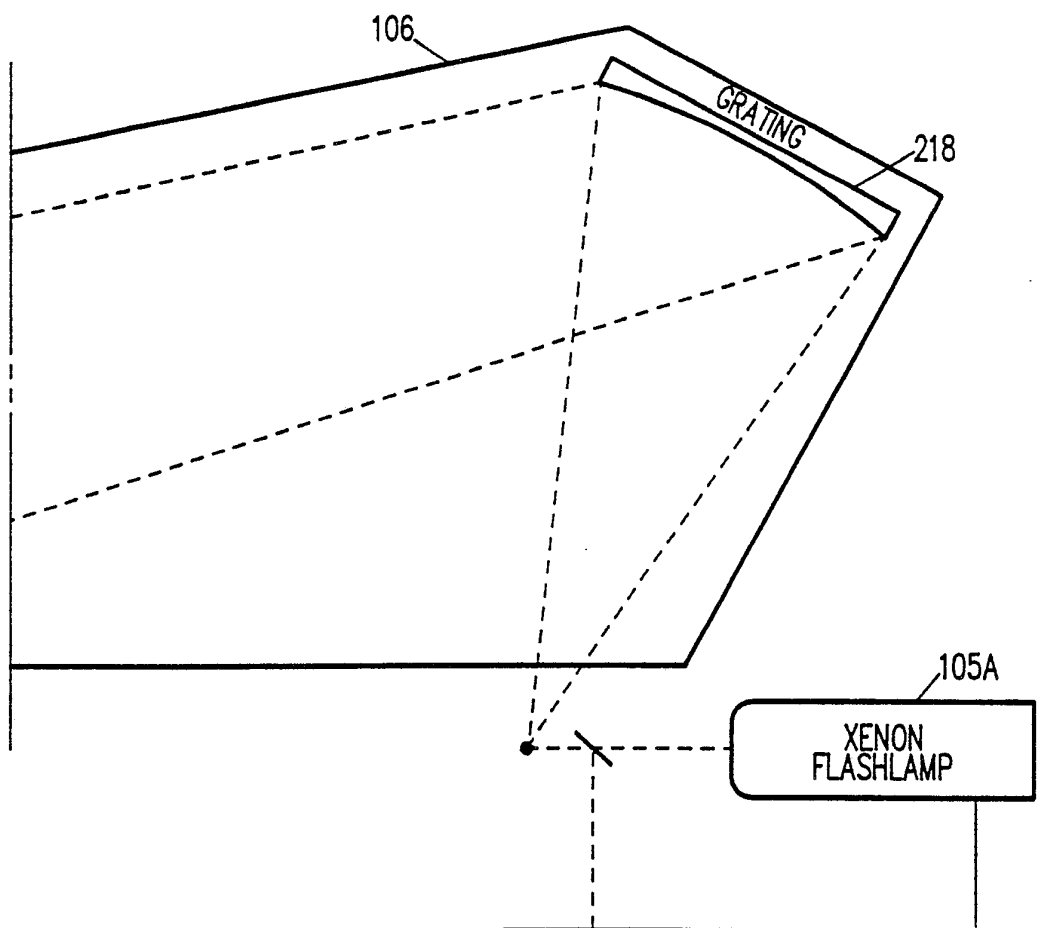
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FIG. 6a''



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FIG. 6a'''



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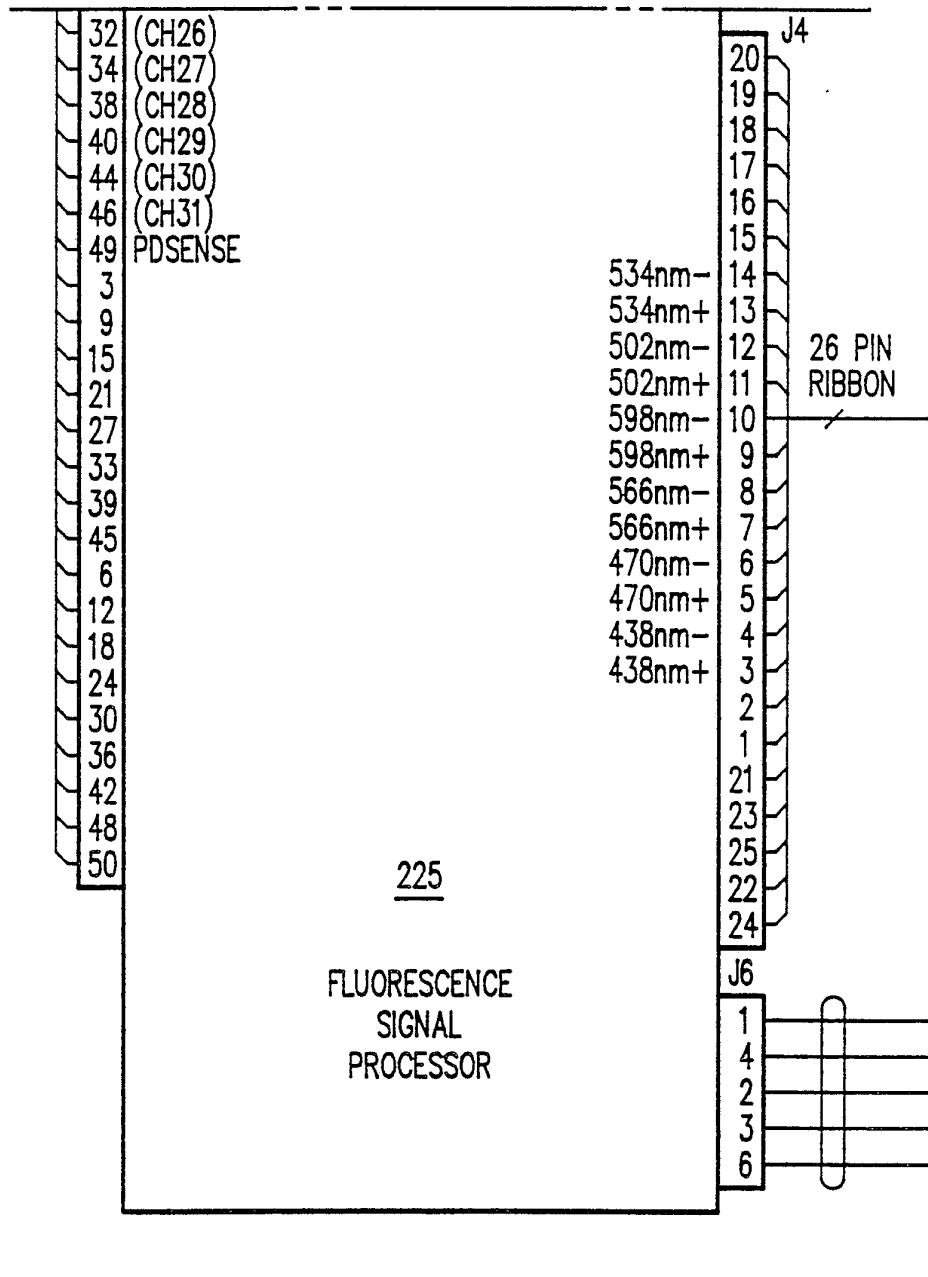


FIG. 6a'''

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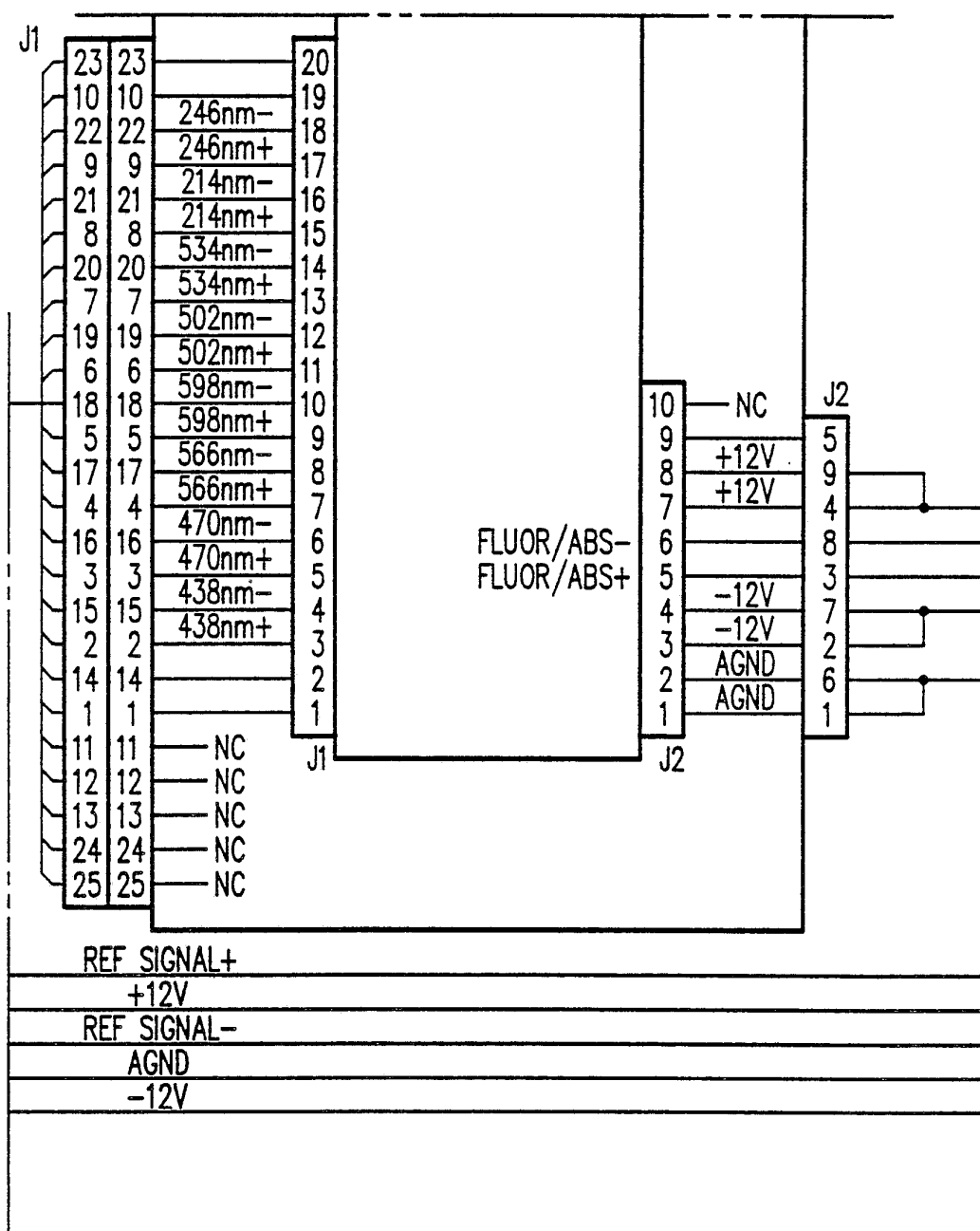


FIG. 6a''''

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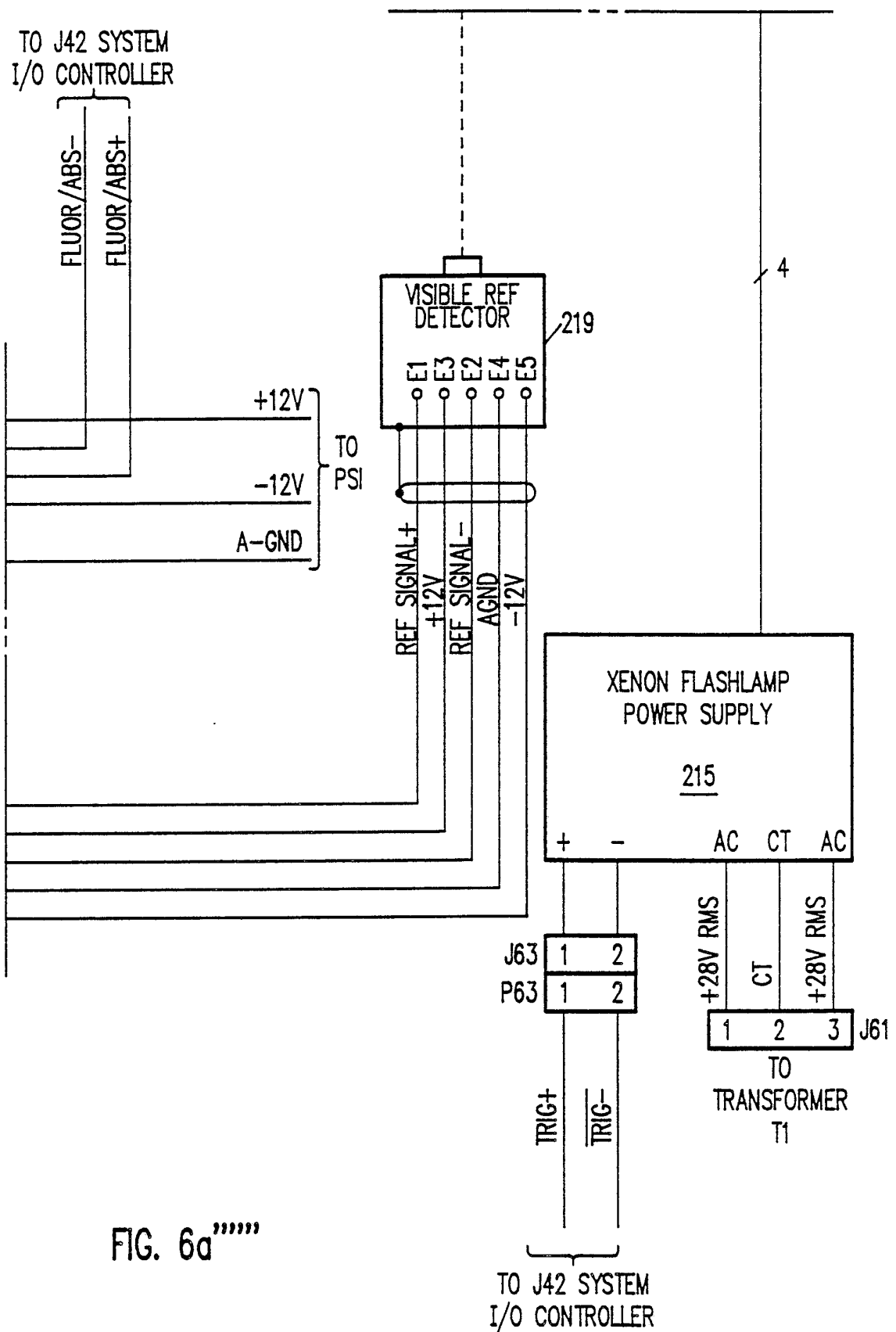


FIG. 6a''''''

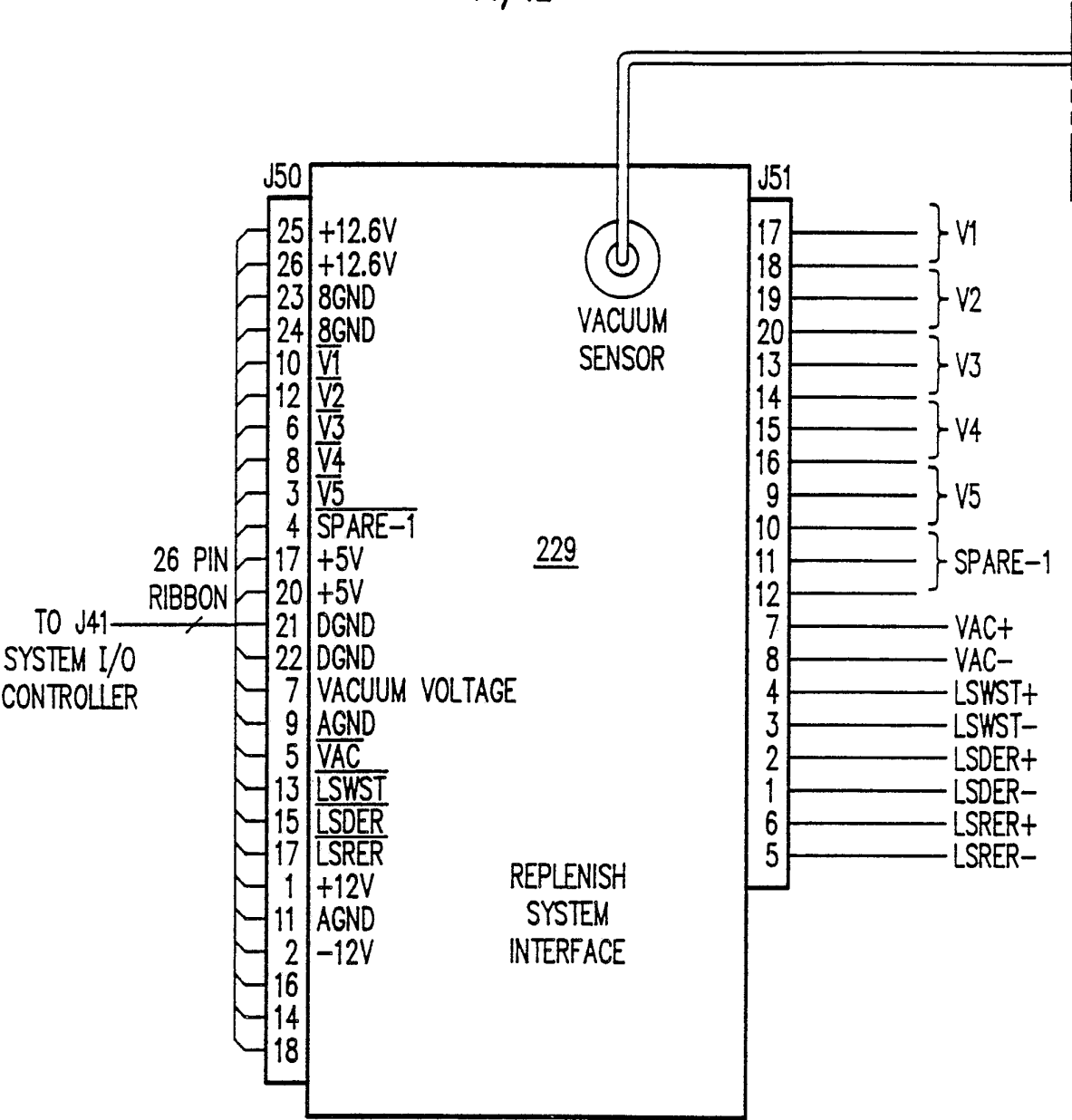
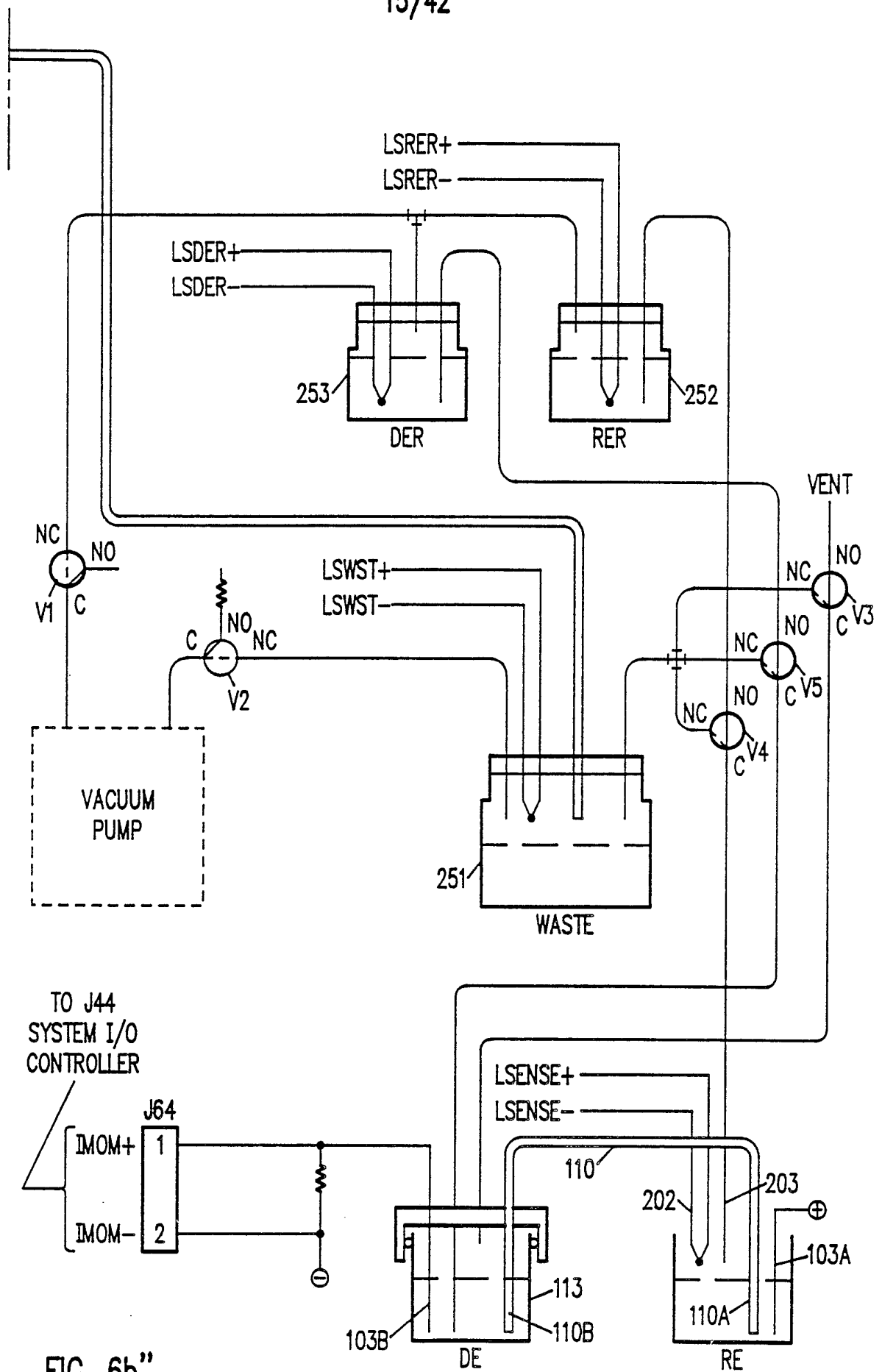


FIG. 6b'

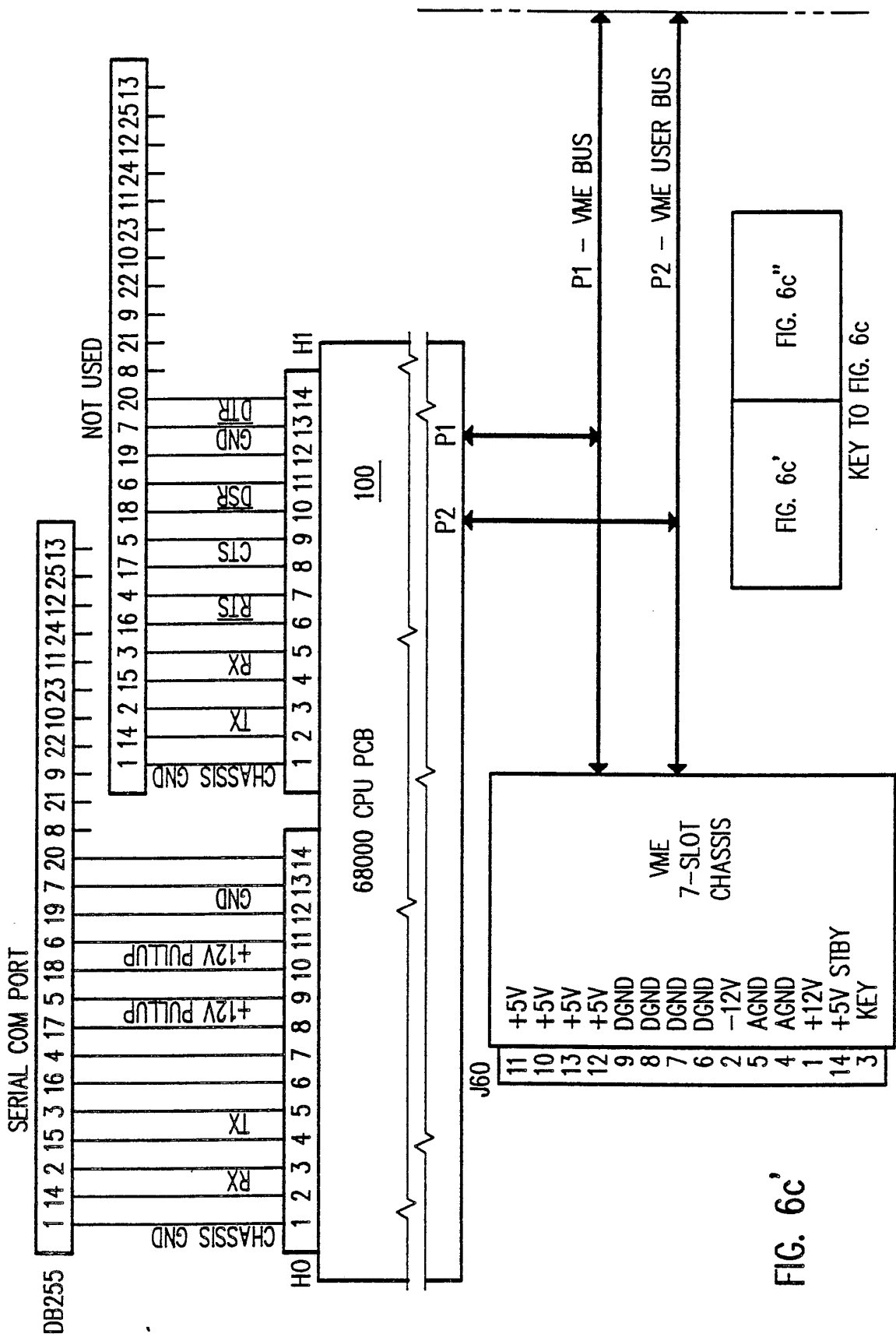
FIG. 6b'	FIG. 6b''
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KEY TO FIG. 6b

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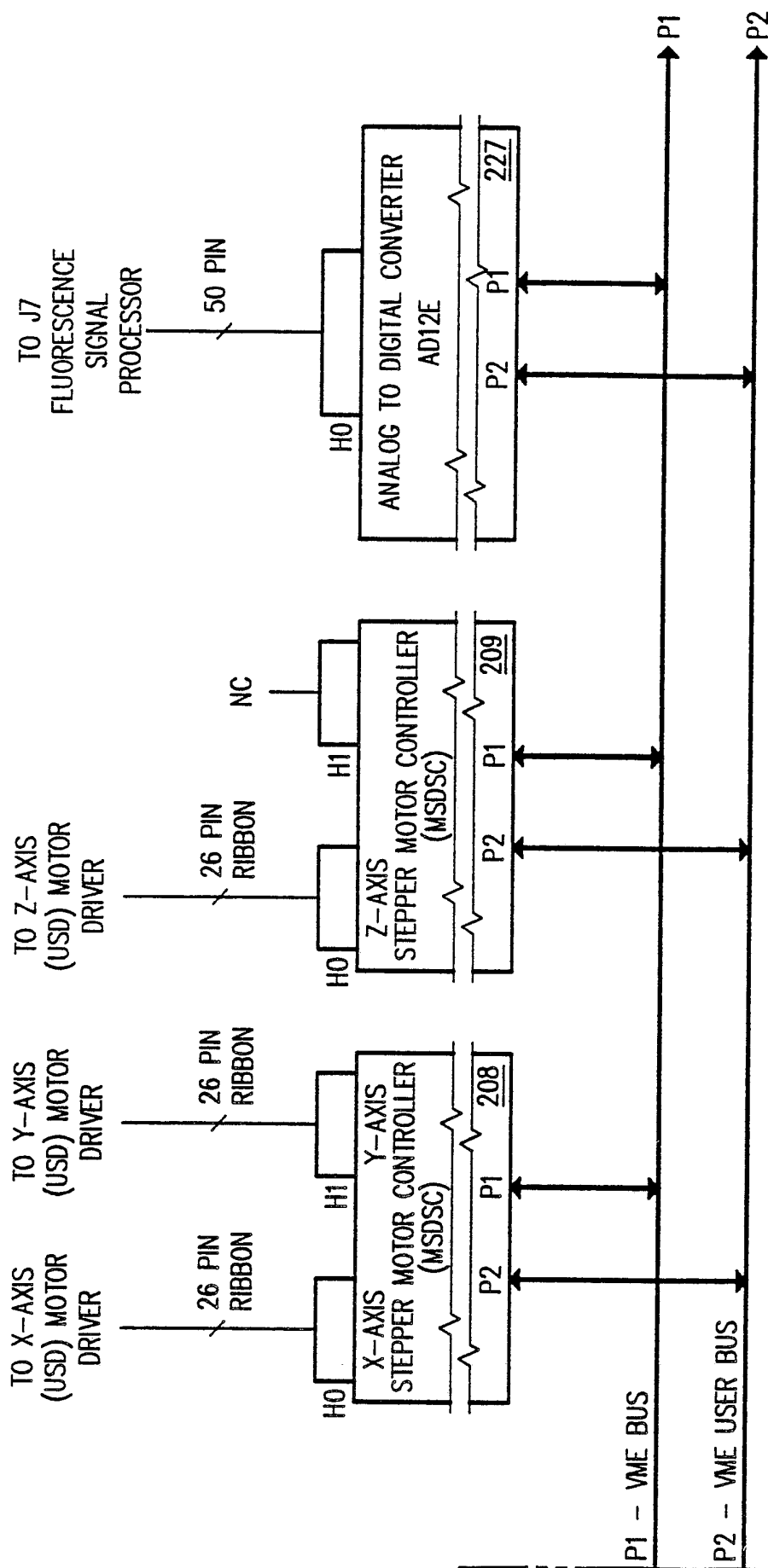


FIG. 6c"

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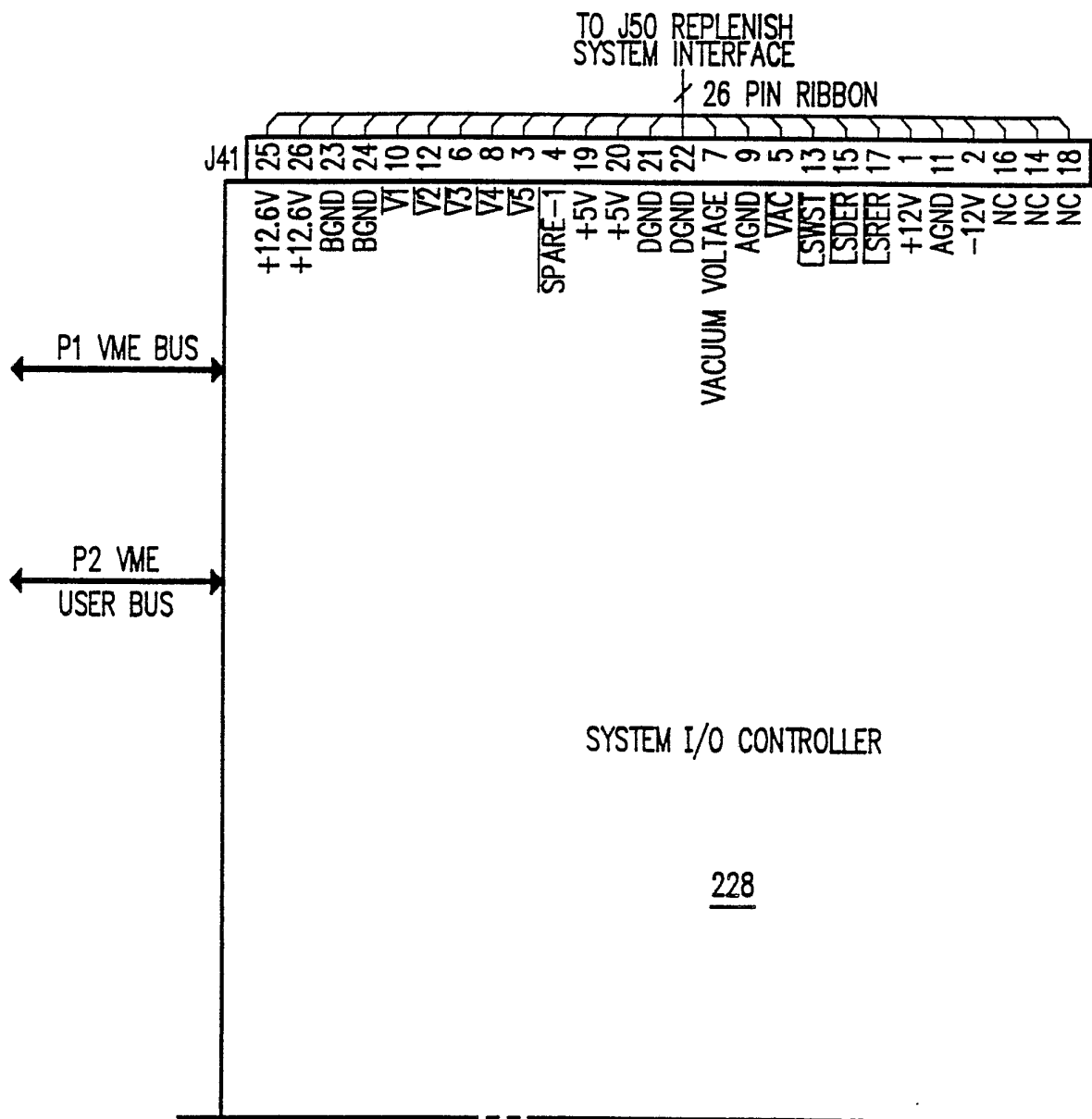


FIG. 6d'

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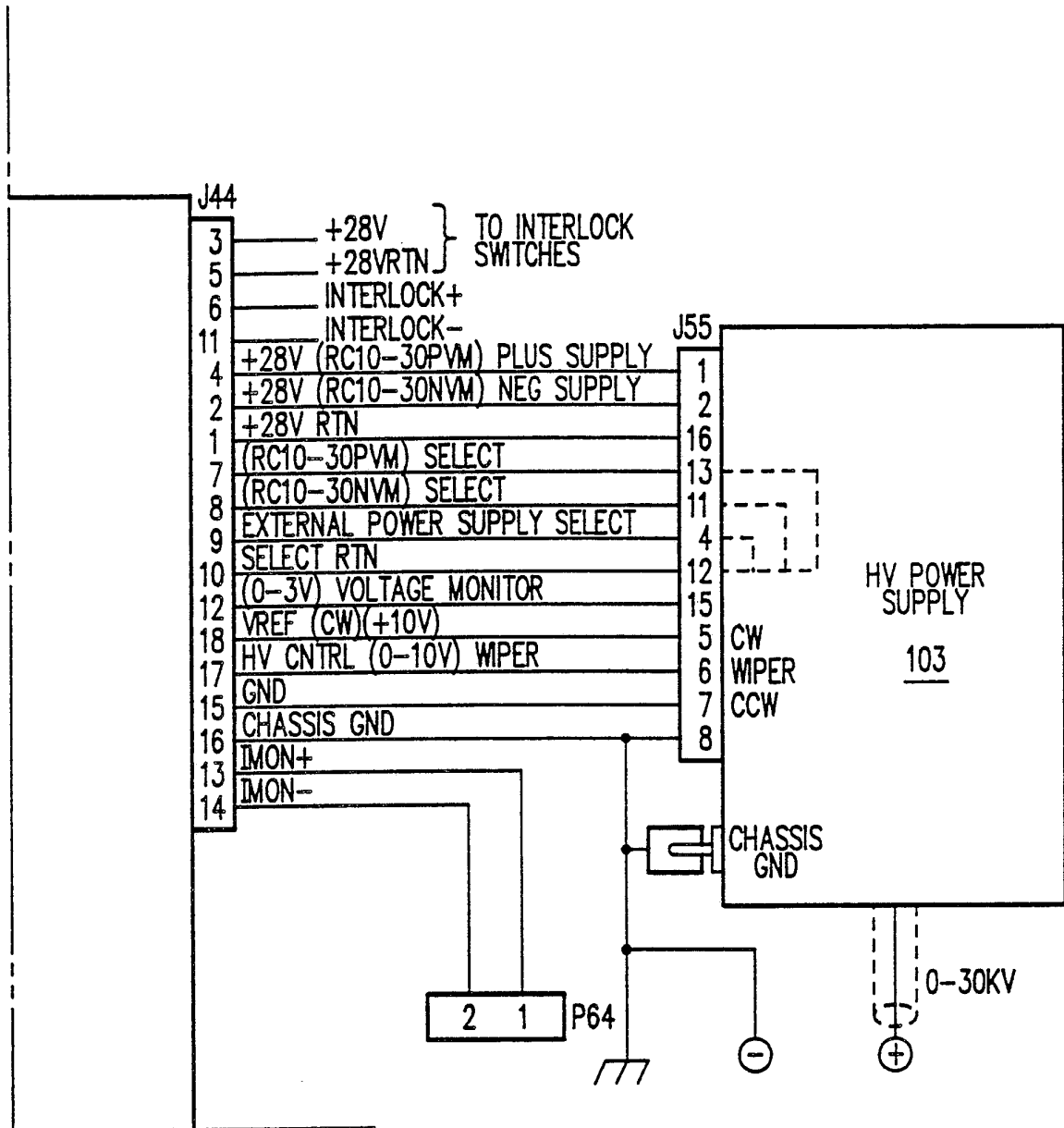


FIG. 6d''

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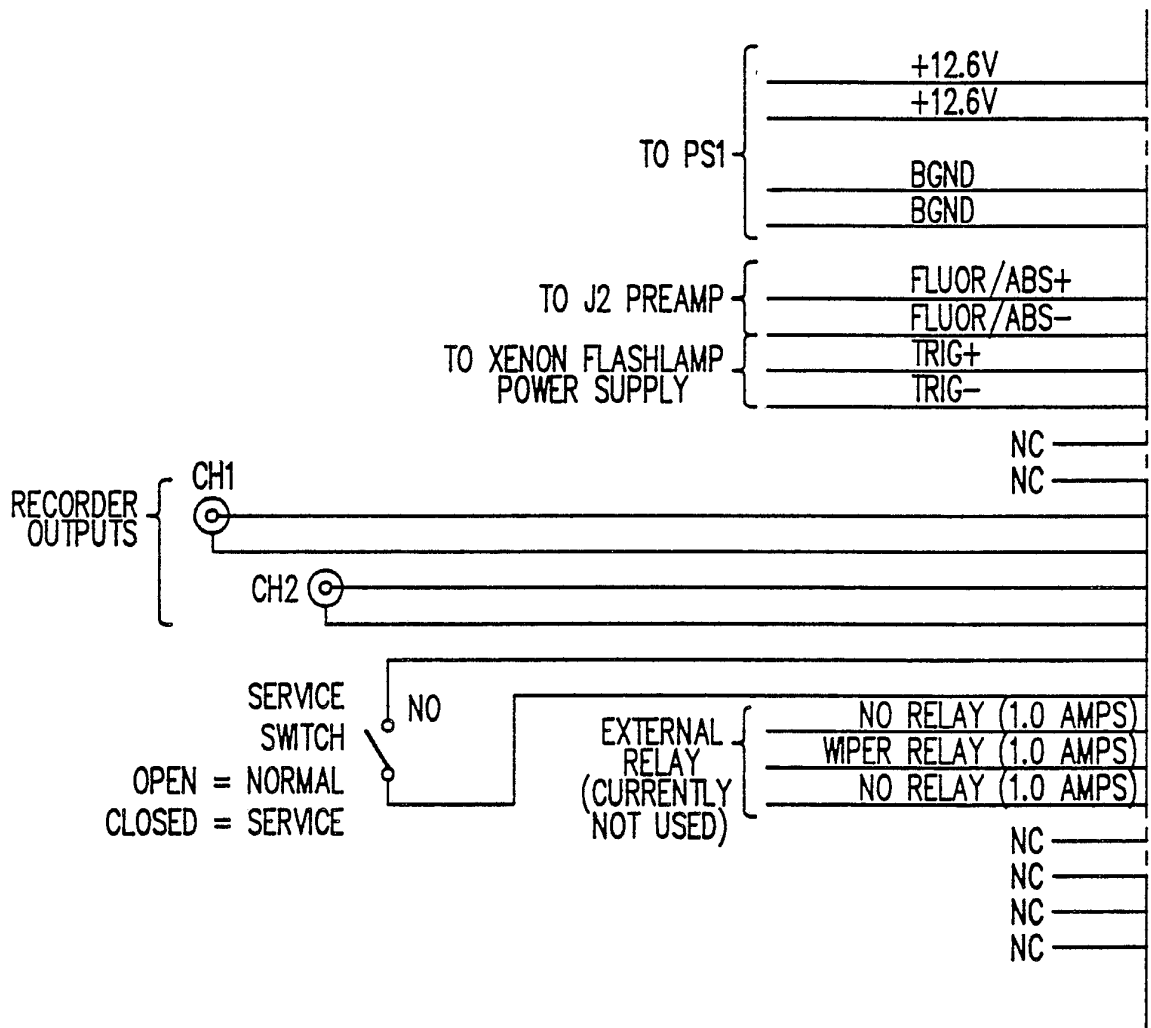


FIG. 6d'''

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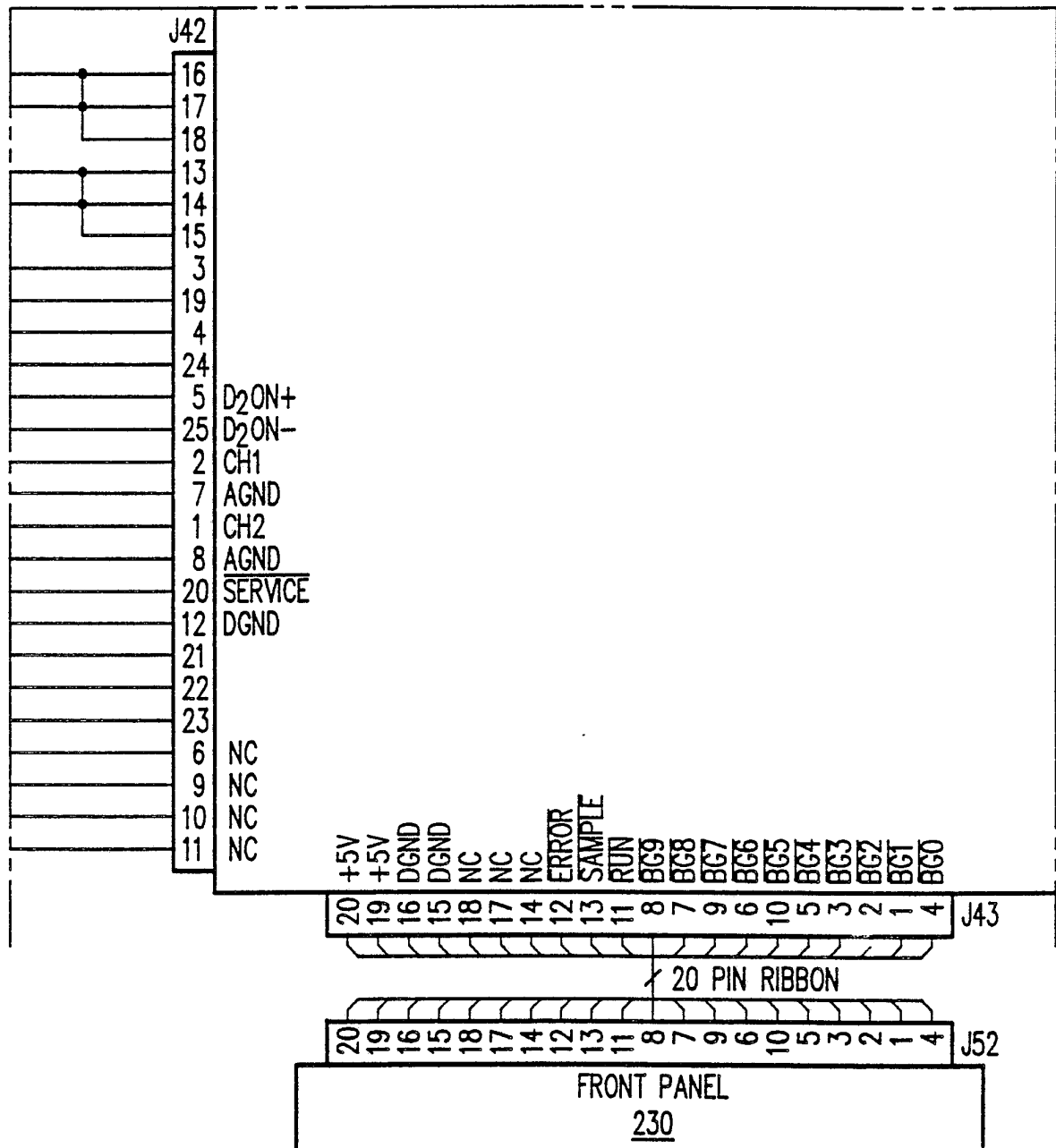


FIG. 6d'''

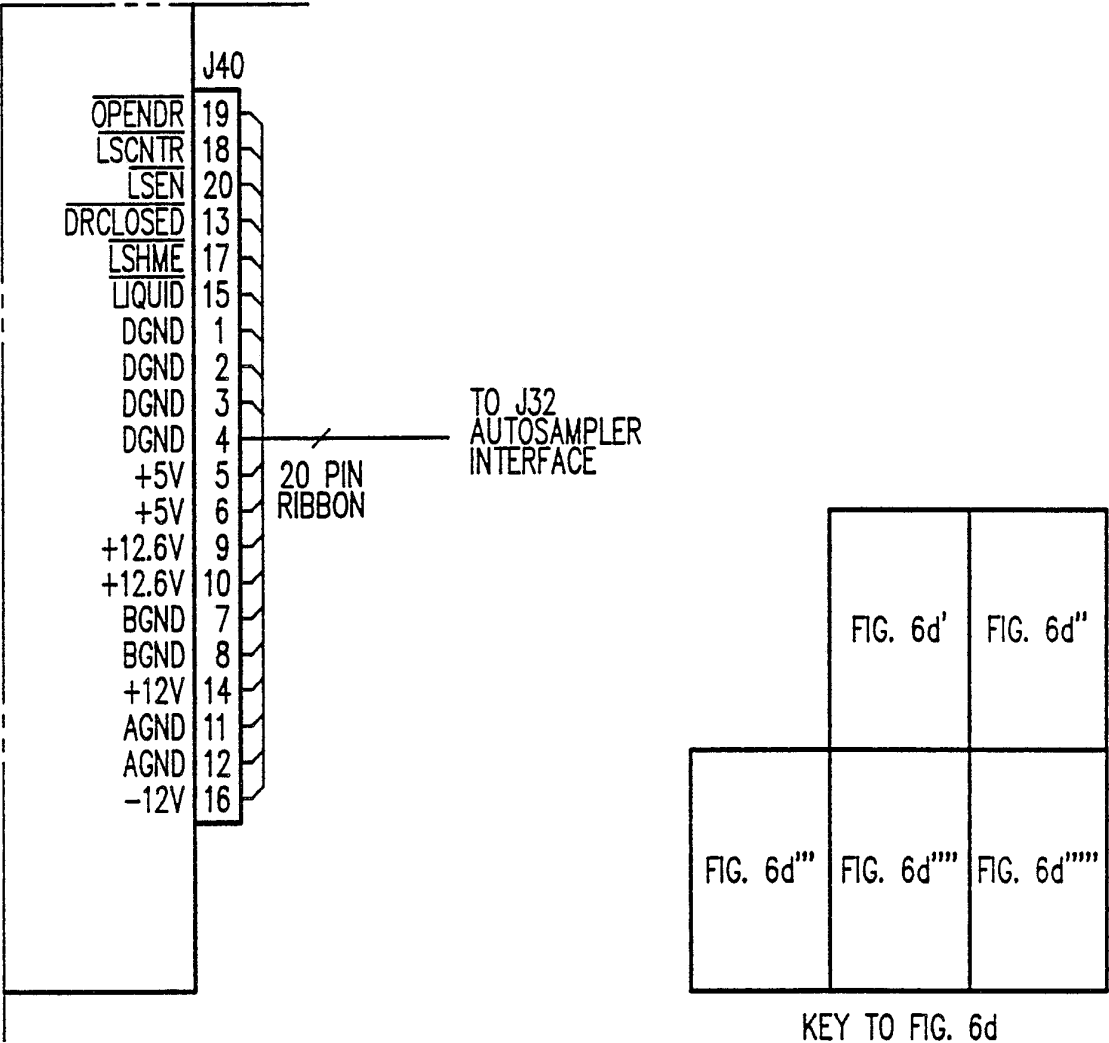


FIG. 6d'''

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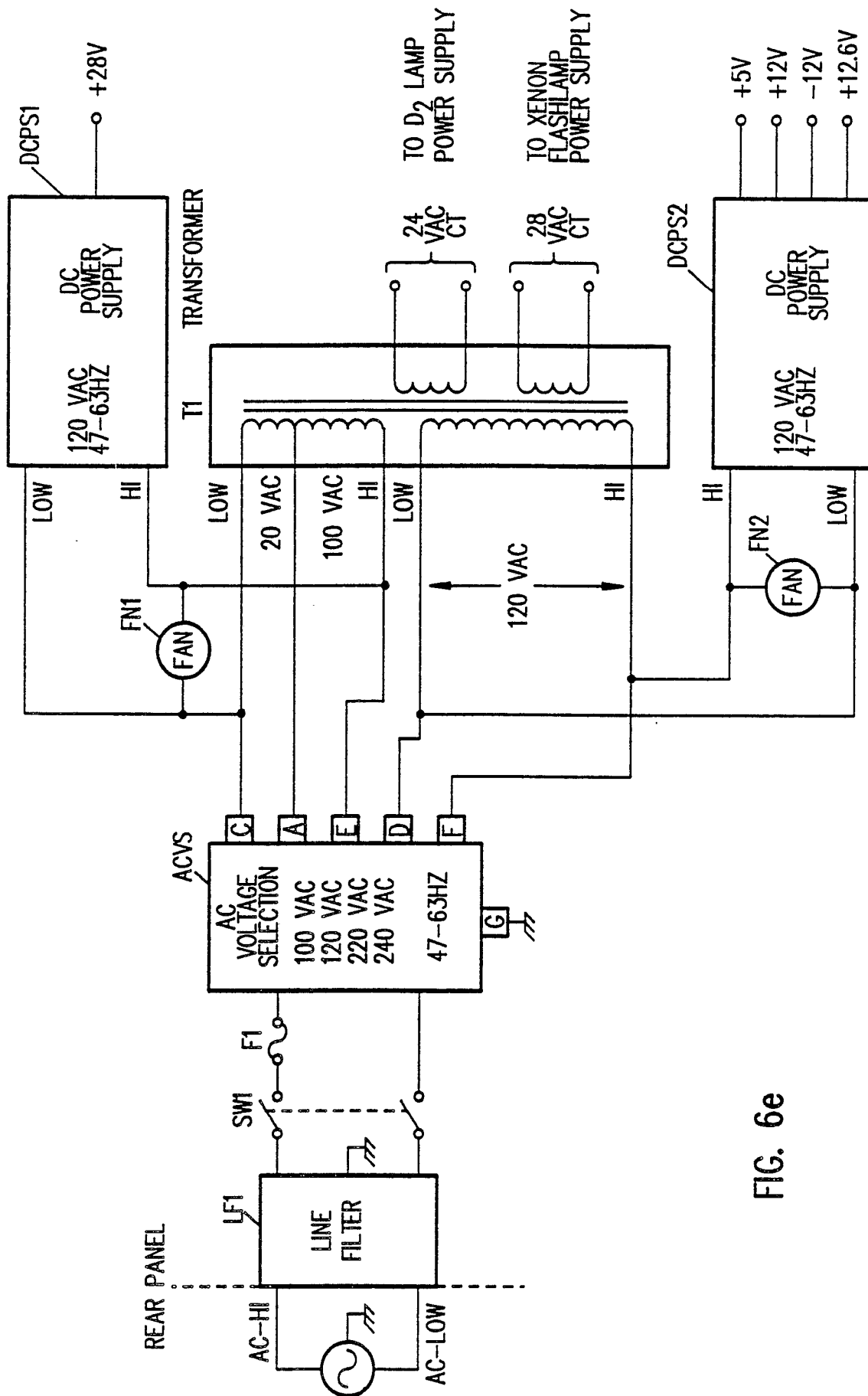


FIG. 6e

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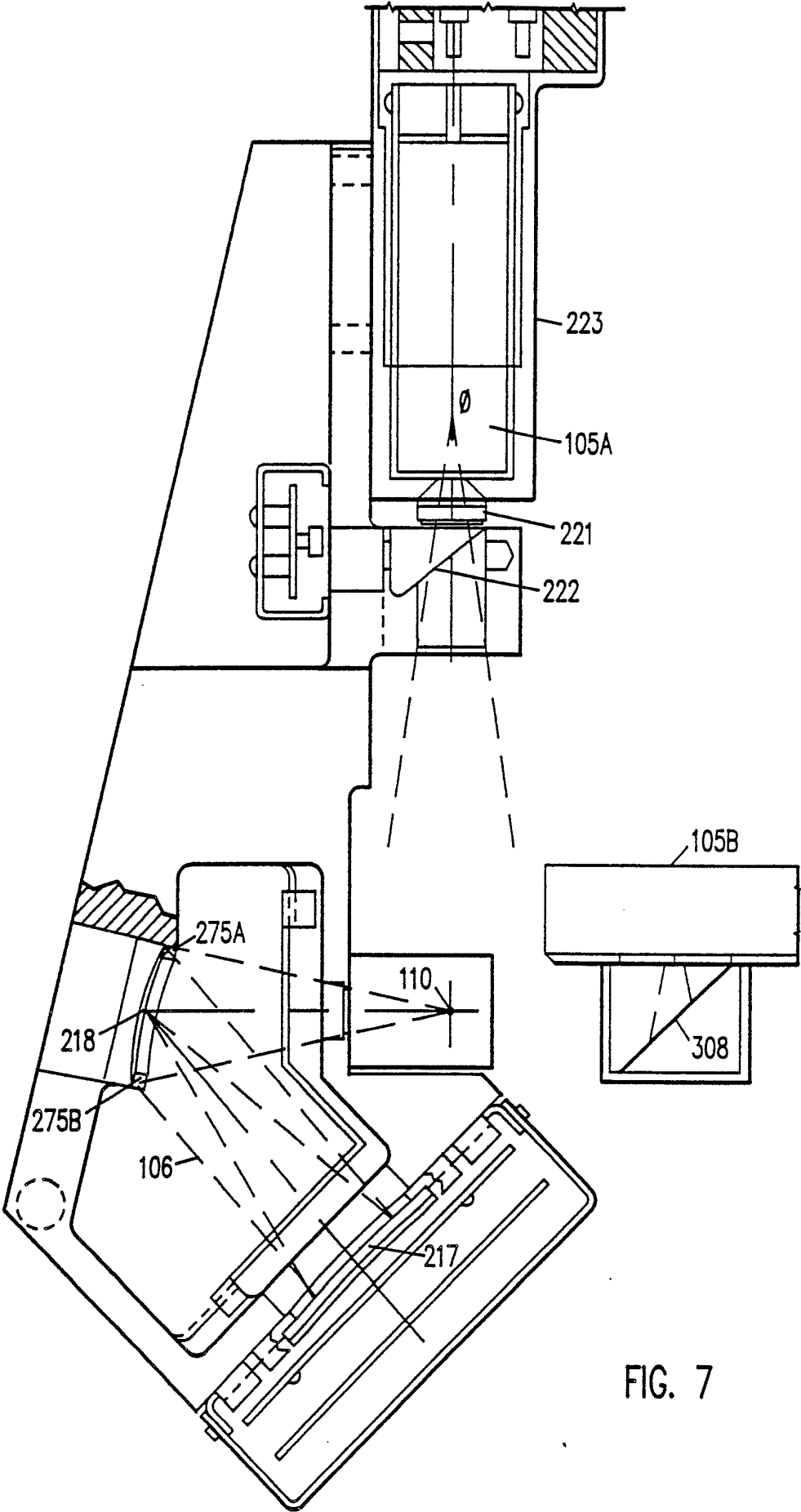


FIG. 7

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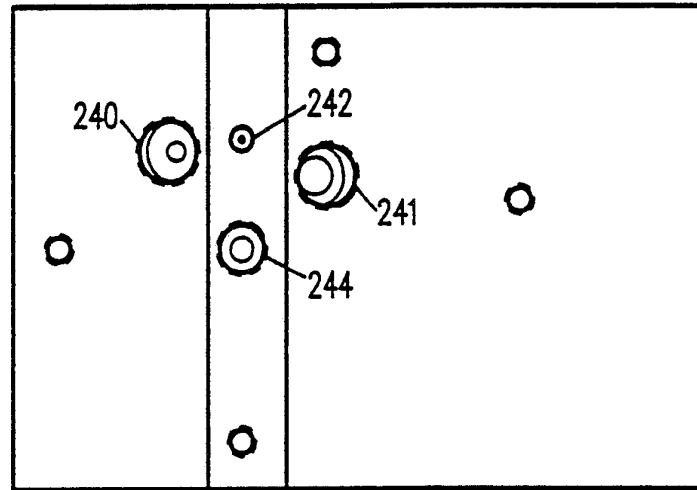


FIG. 8b

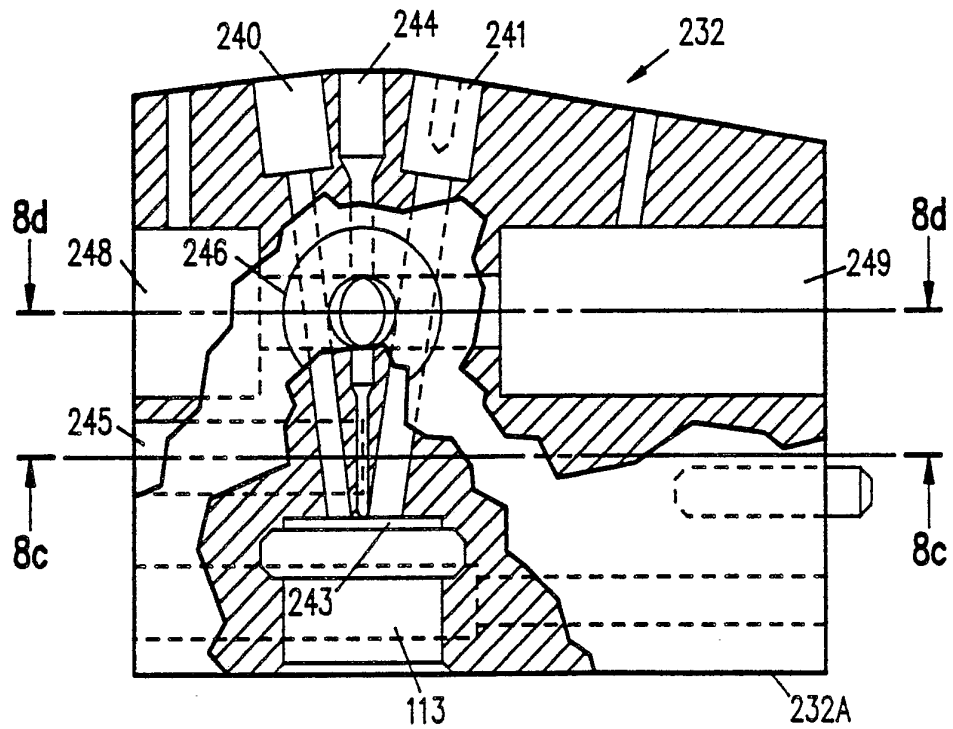


FIG. 8a

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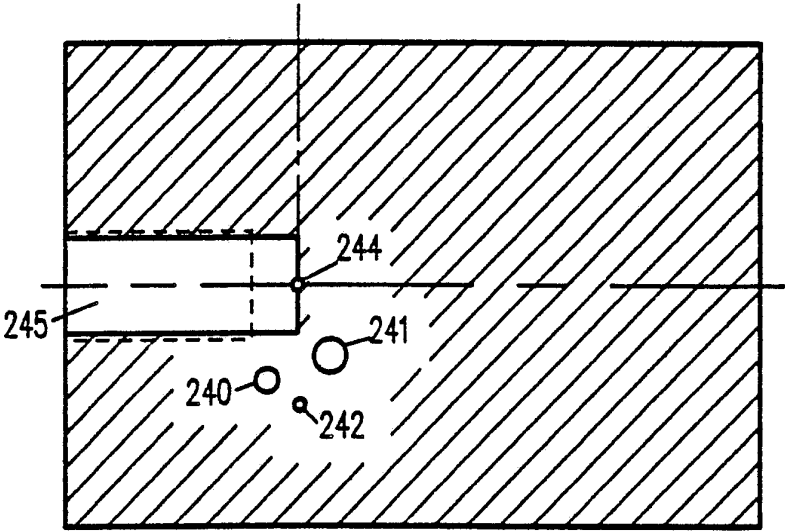


FIG. 8c

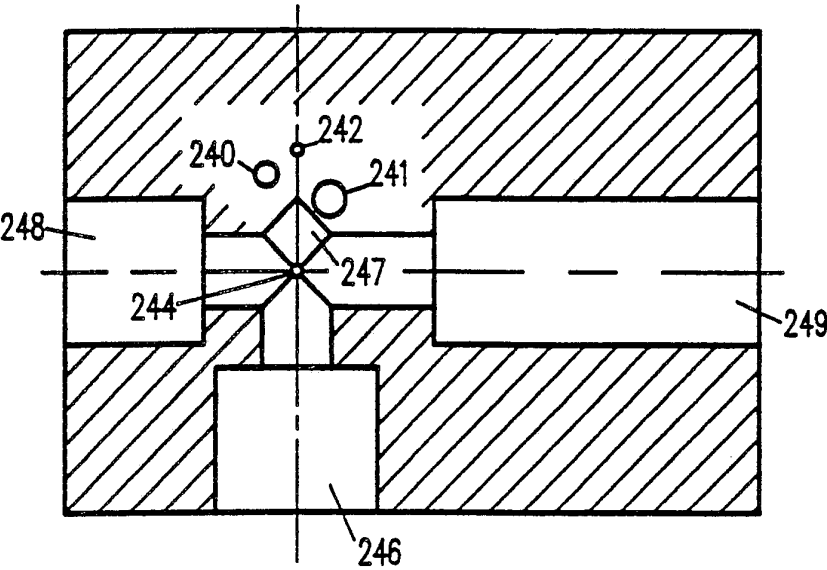


FIG. 8d

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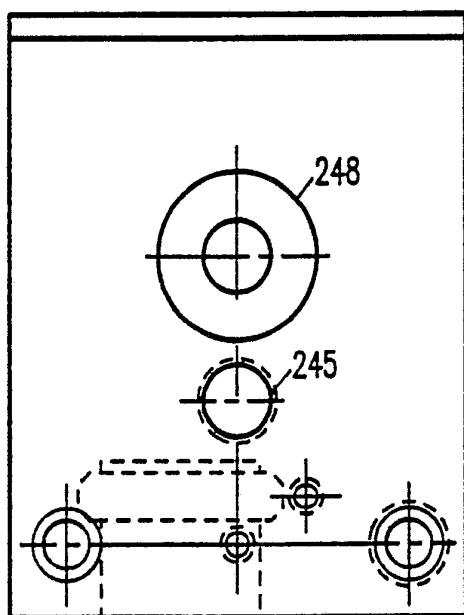


FIG. 8e

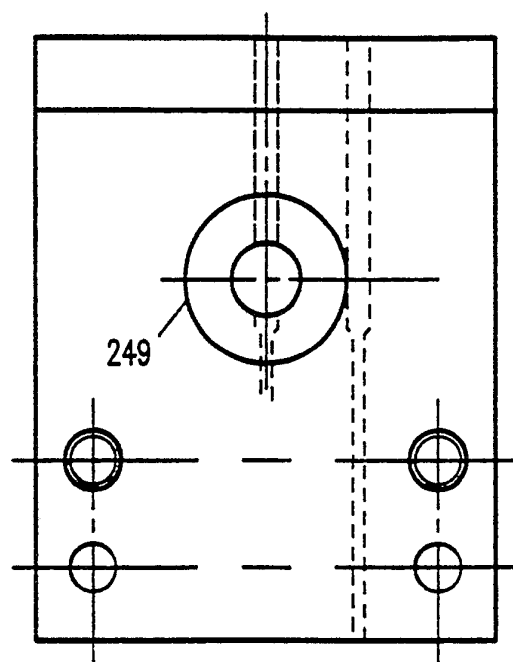


FIG. 8f

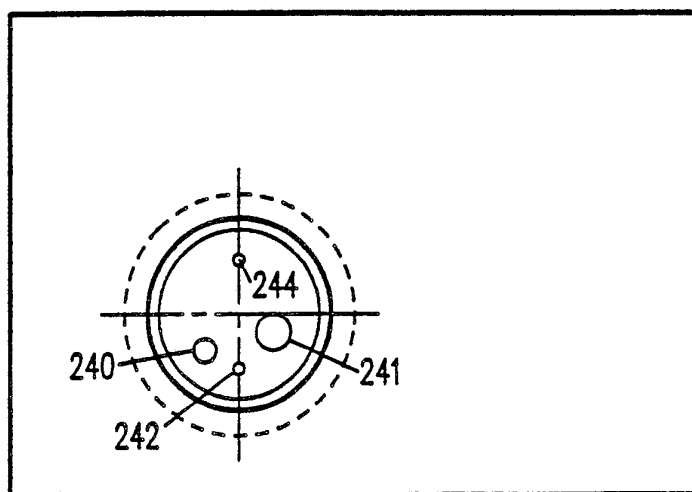


FIG. 8g

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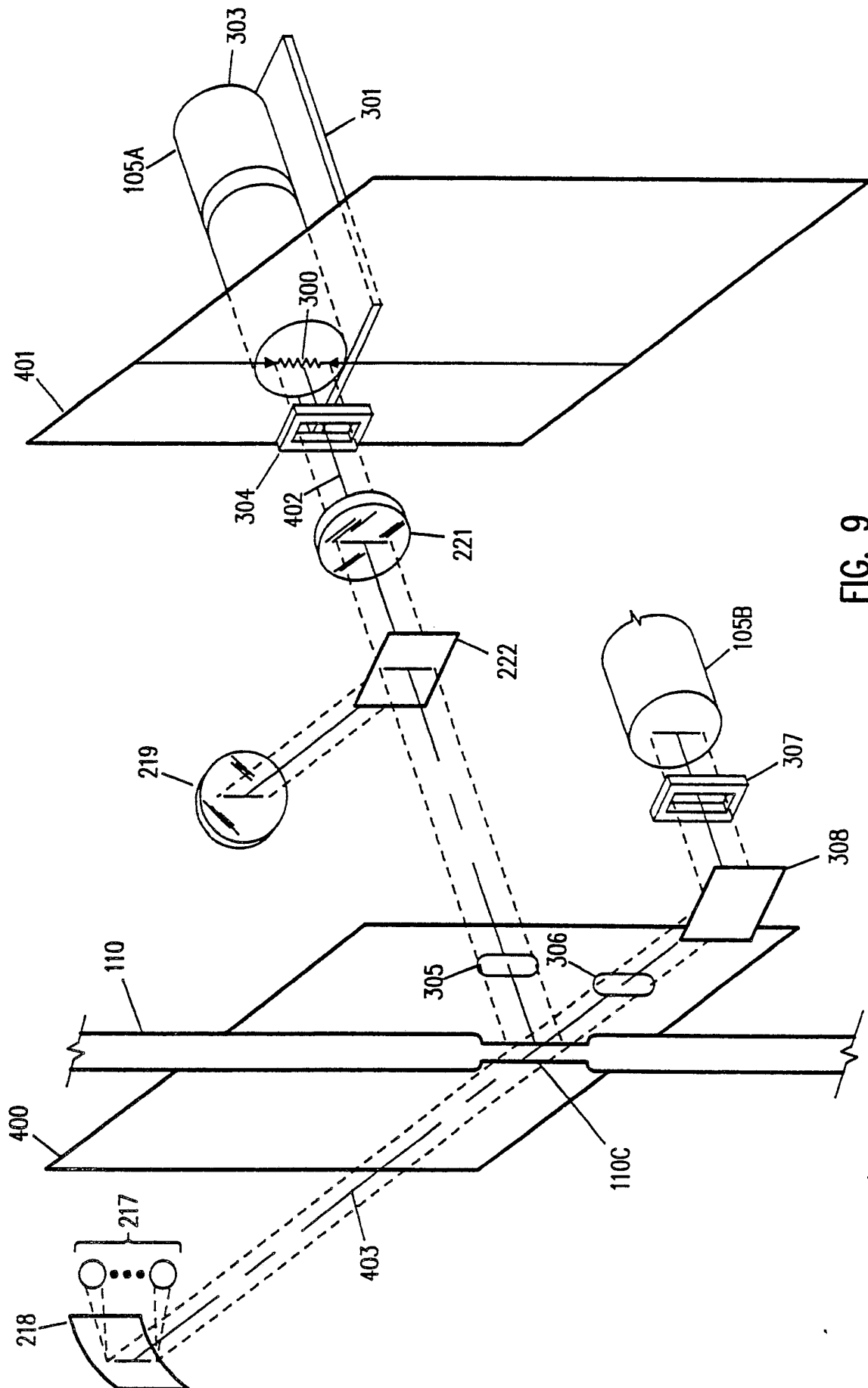


FIG. 9

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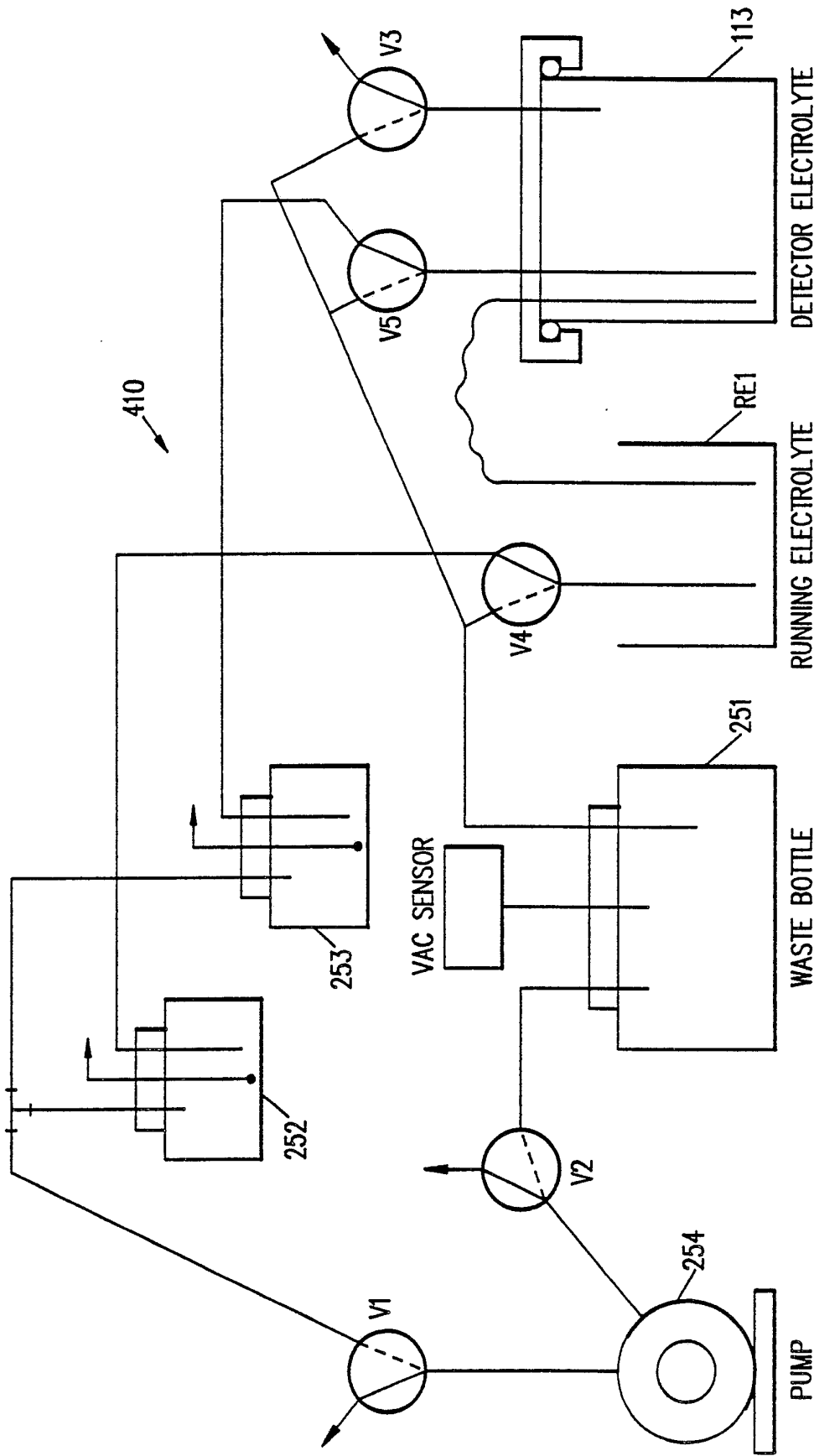
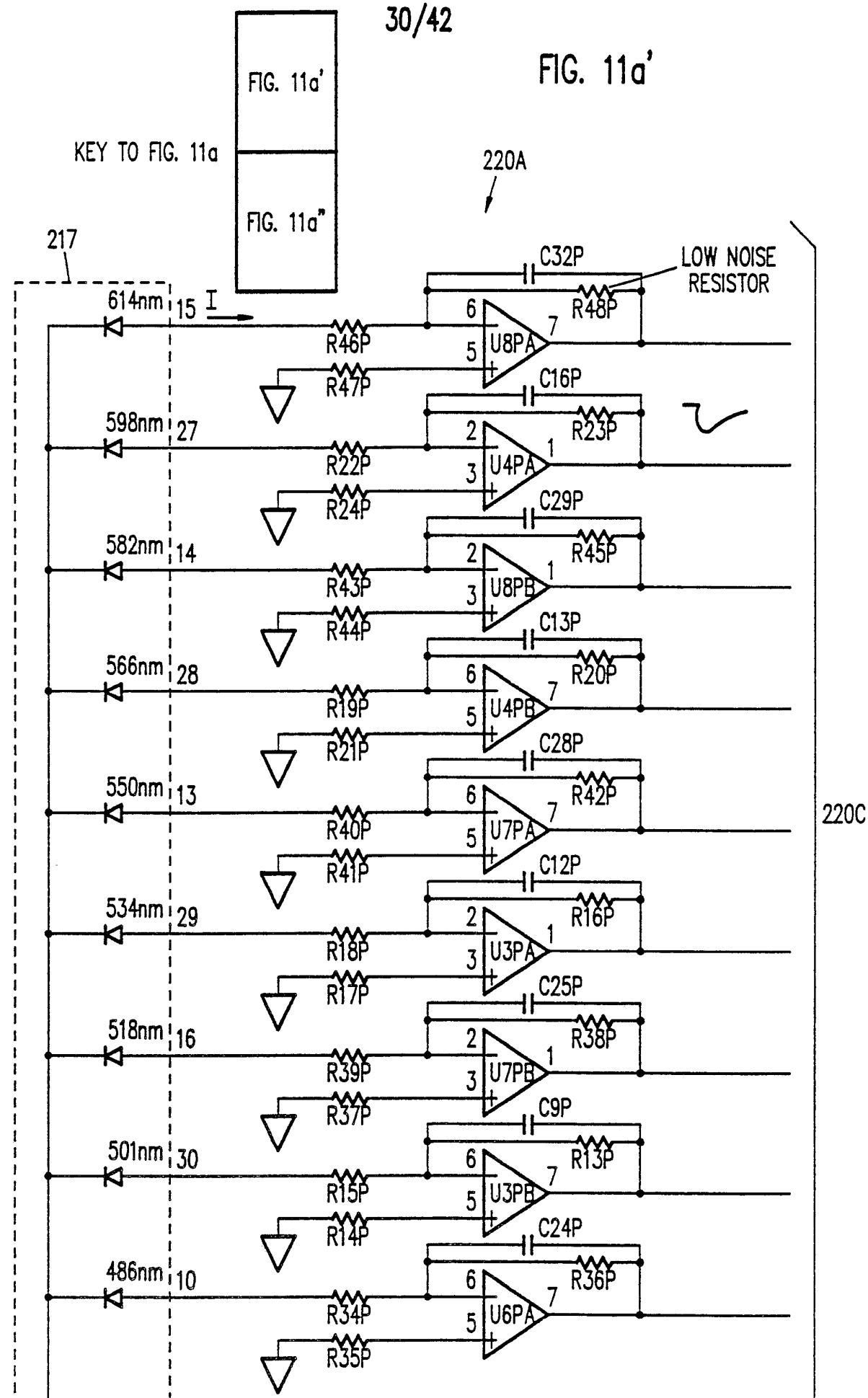


FIG. 10

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FIG. 11a'



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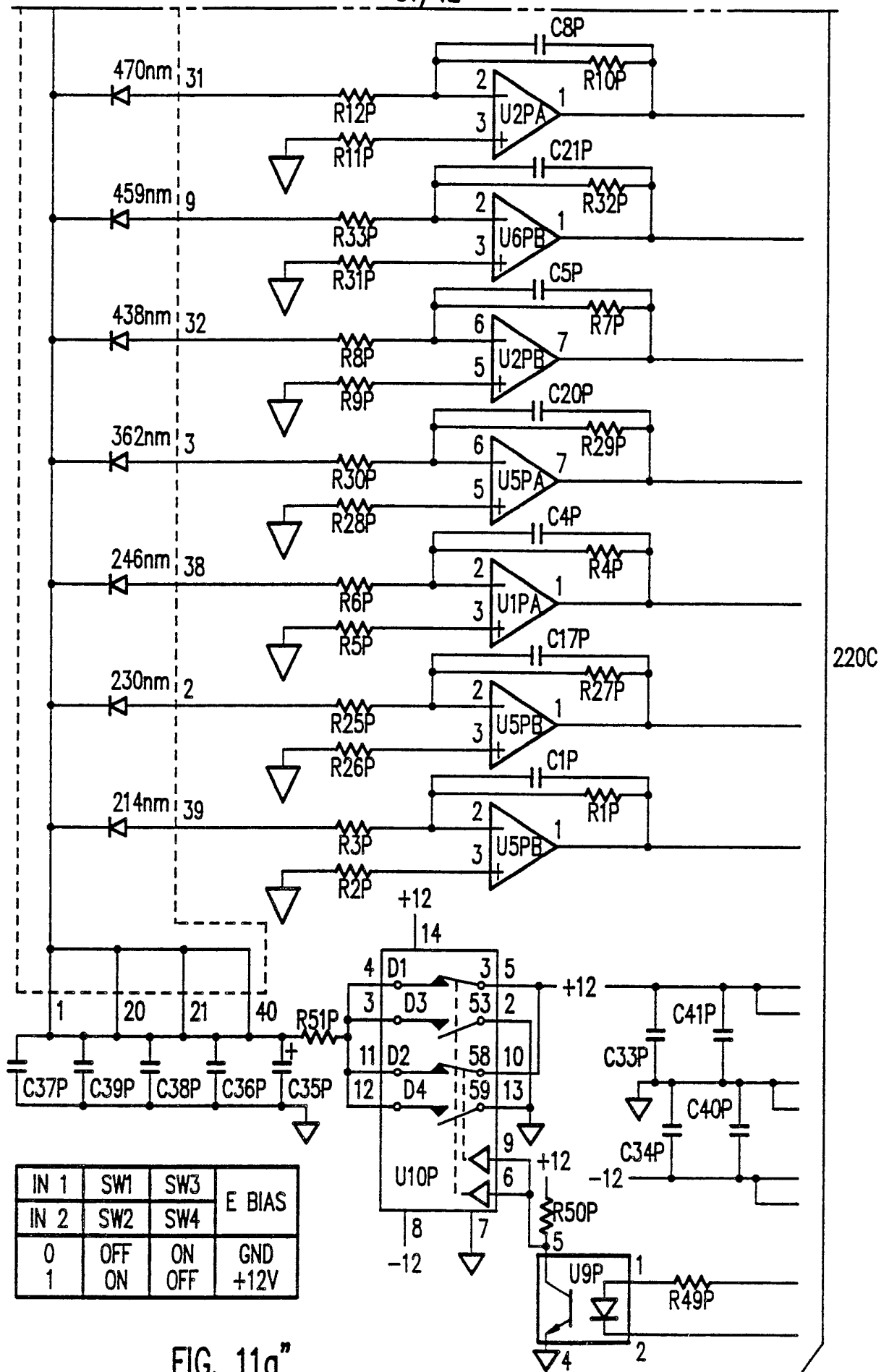
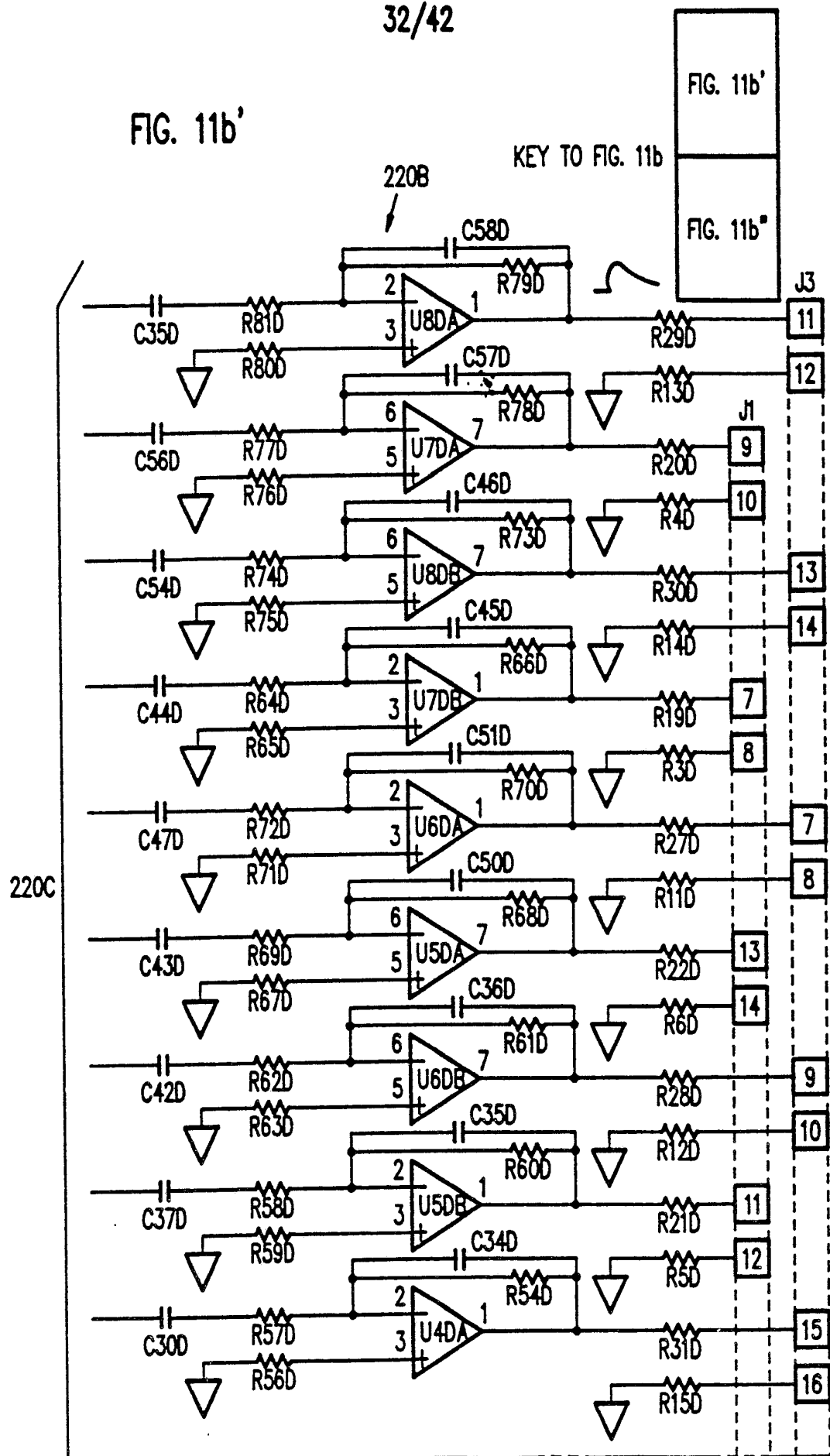


FIG. 11a''

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FIG. 11b'



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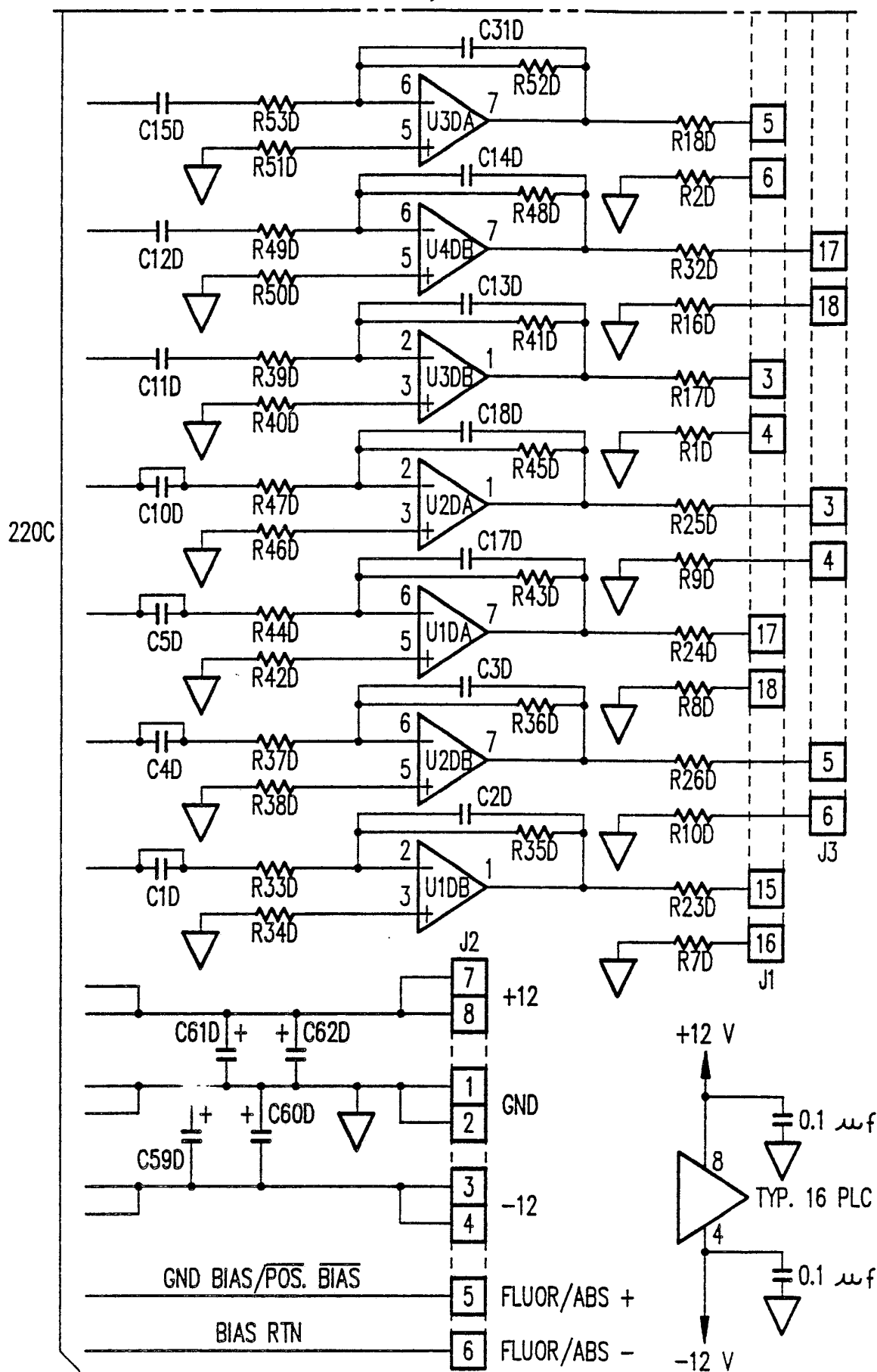


FIG. 11b"

SUBSTITUTE SHEET

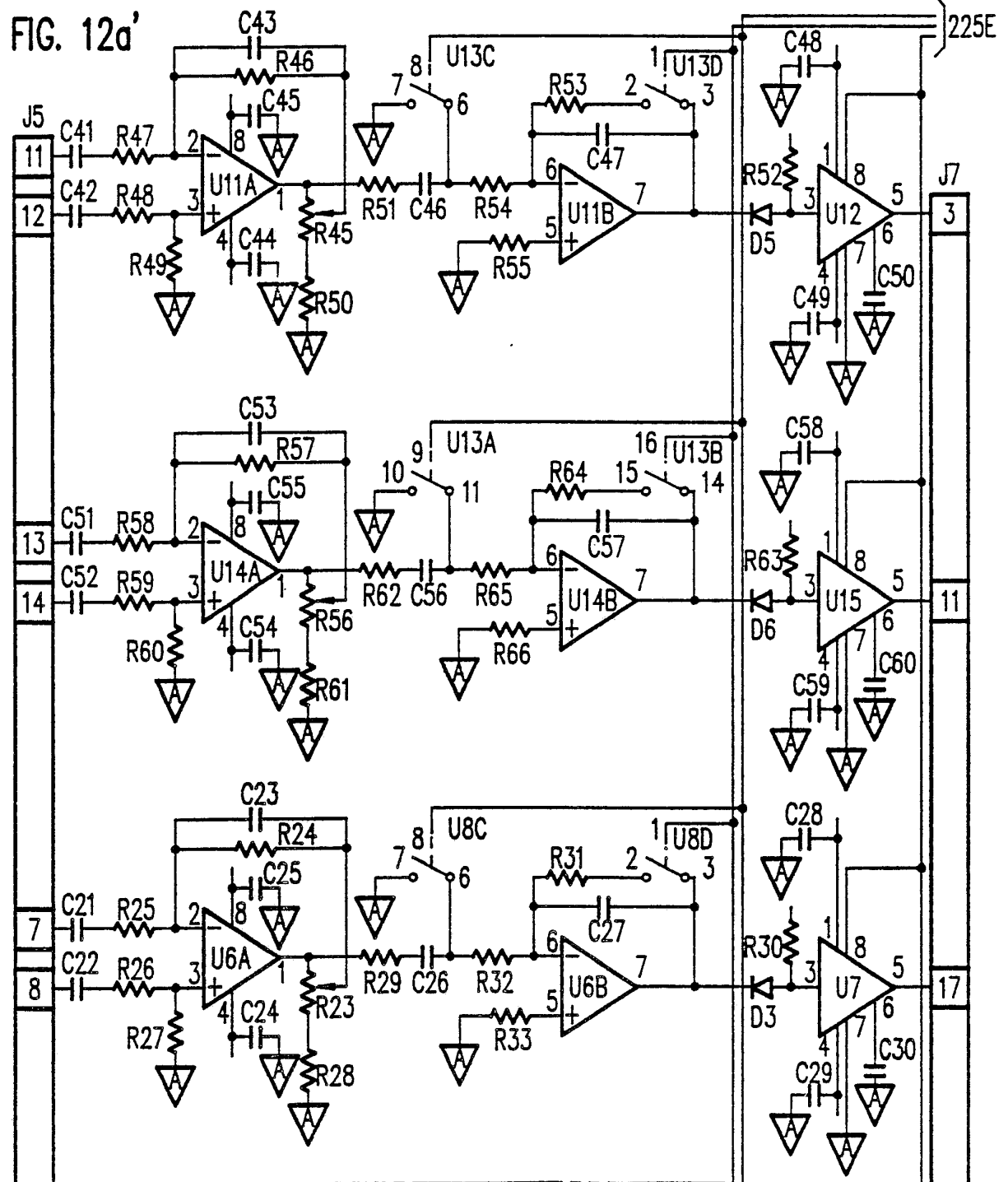
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FIG. 12a'

KEY TO FIG. 12a

FIG. 12a''

225A



SUBSTITUTE SHEET

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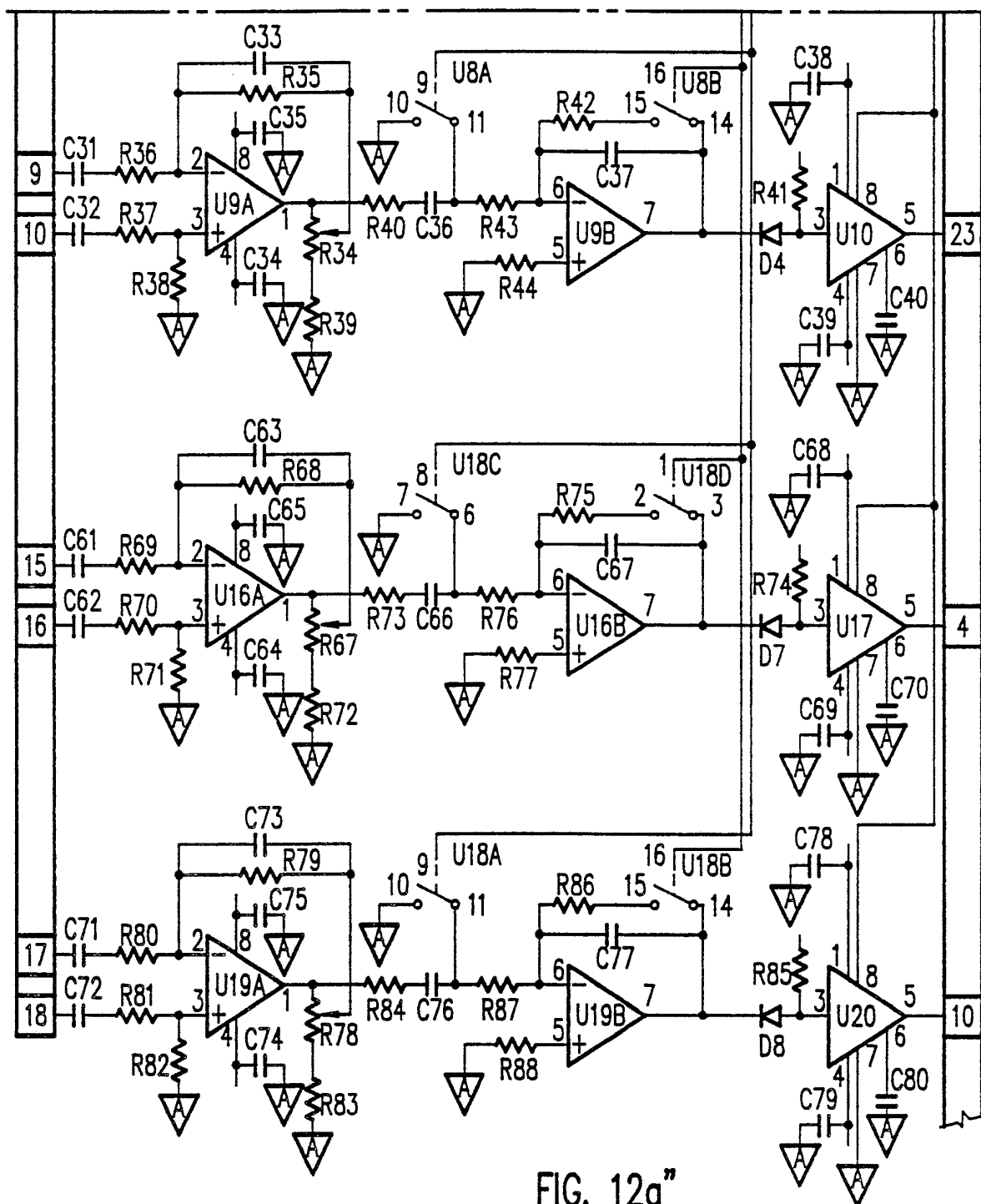


FIG. 12a"

SUBSTITUTE SHEET

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FIG. 12b'

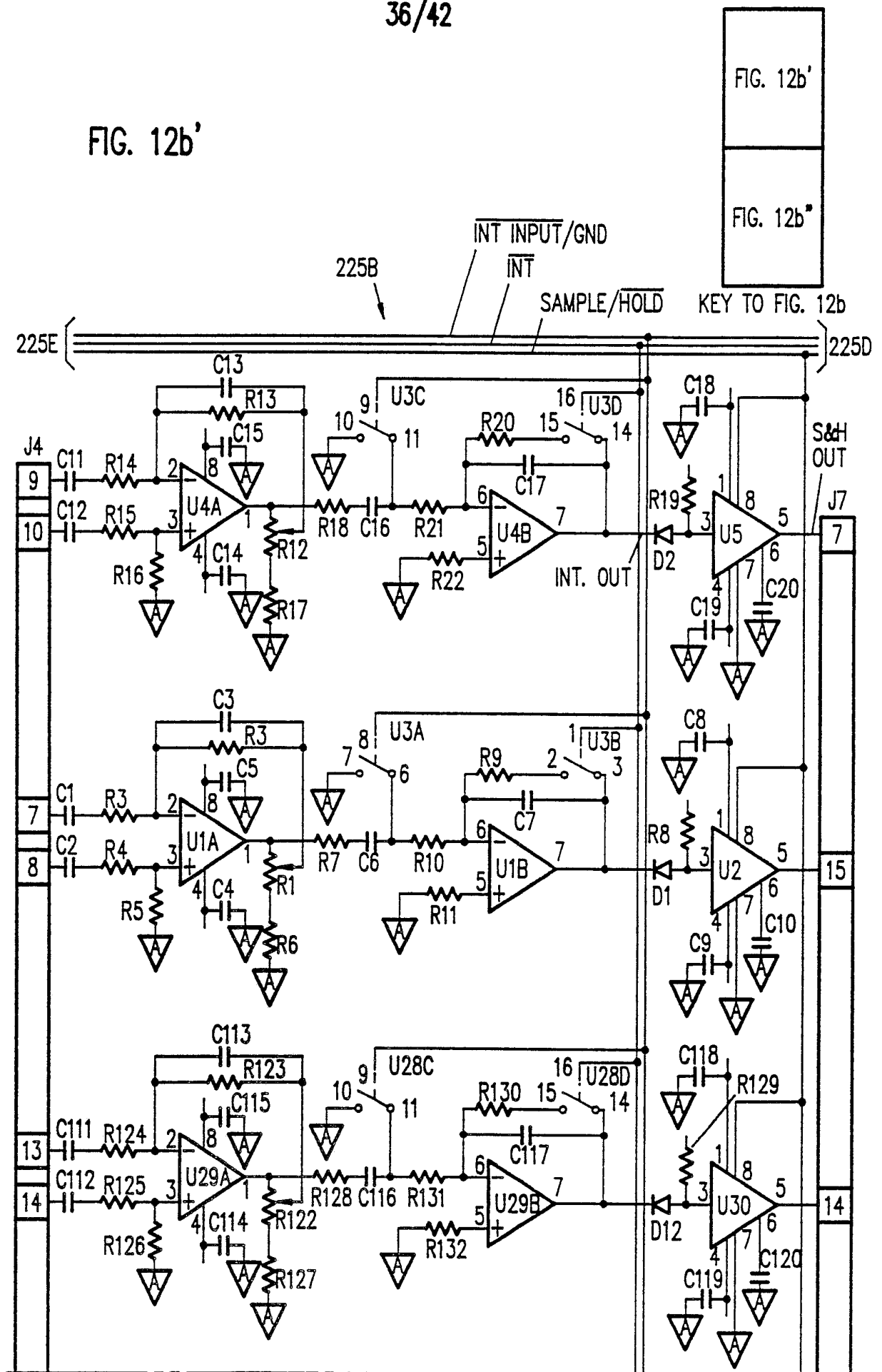


FIG. 12b'

FIG. 12b^u

KEY TO FIG. 12b

S&H
OUT

INT. OUT

INT INPUT/GND

~~XXXXXXXXXX~~
INT

SAMPLE/HOLD

225B

225E | 3

225D

J4

9

10

7

11

13

14

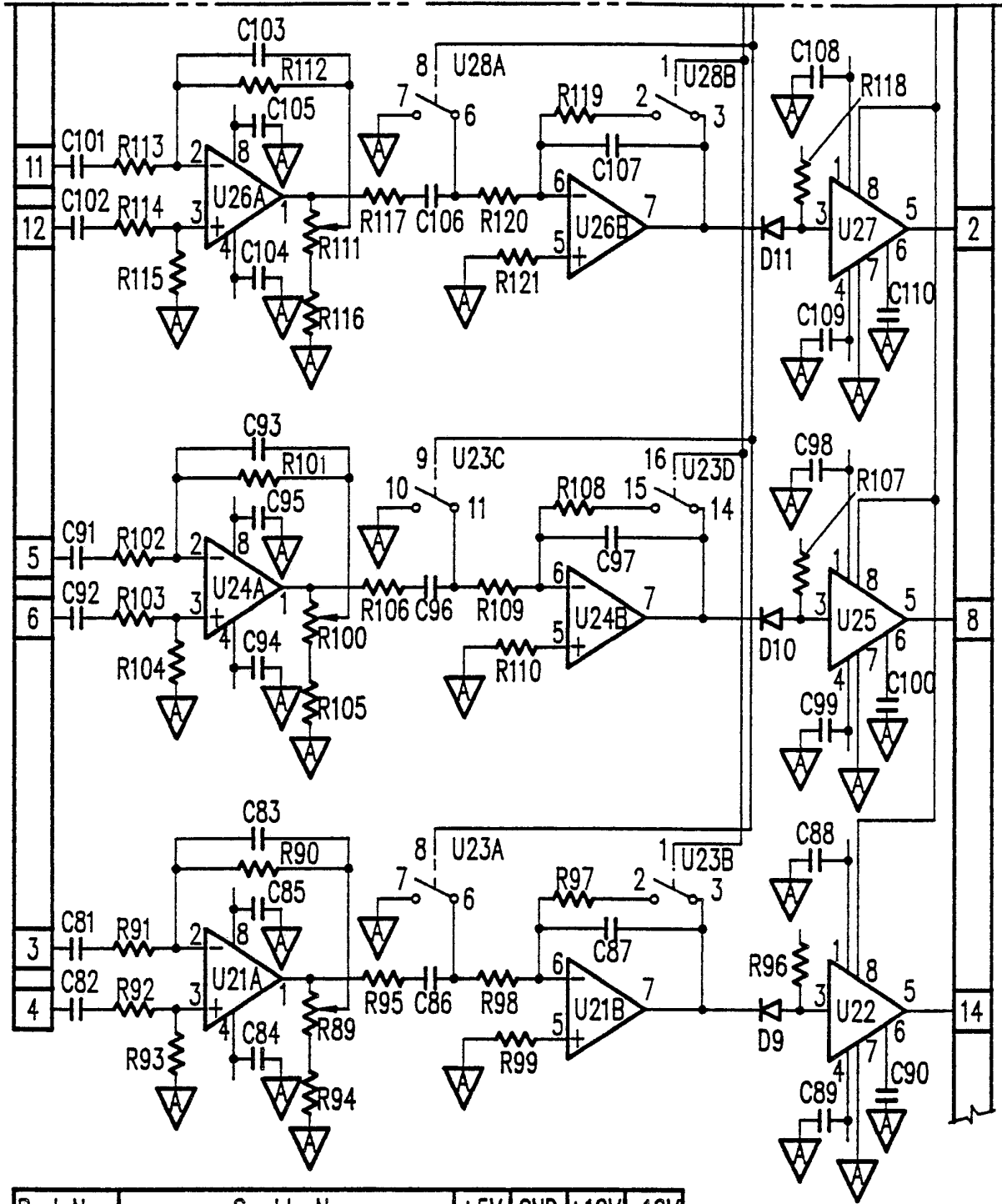
7

[illegible]

15

4

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Part No.	Symble No.	+5V	GND	+12V	-12V
OP215	U1 U4 U6 U9 U11 U14 U16 U19 U21 U24 U26 U29 U31	-	-	8	4
LF398	U2 U5 U7 U10 U12 U15 U17 U20 U22 U25 U27 U30 U32	-	4	1	4
DG202	U3 U8 U13 U18 U23 U28 U33	-	5	13	4
LM311	U503	-	1	8	4
746502	U502	14	7	-	-
746574	U500	14	7	-	-
7465123	U501	16	8	-	-

FIG. 12b"

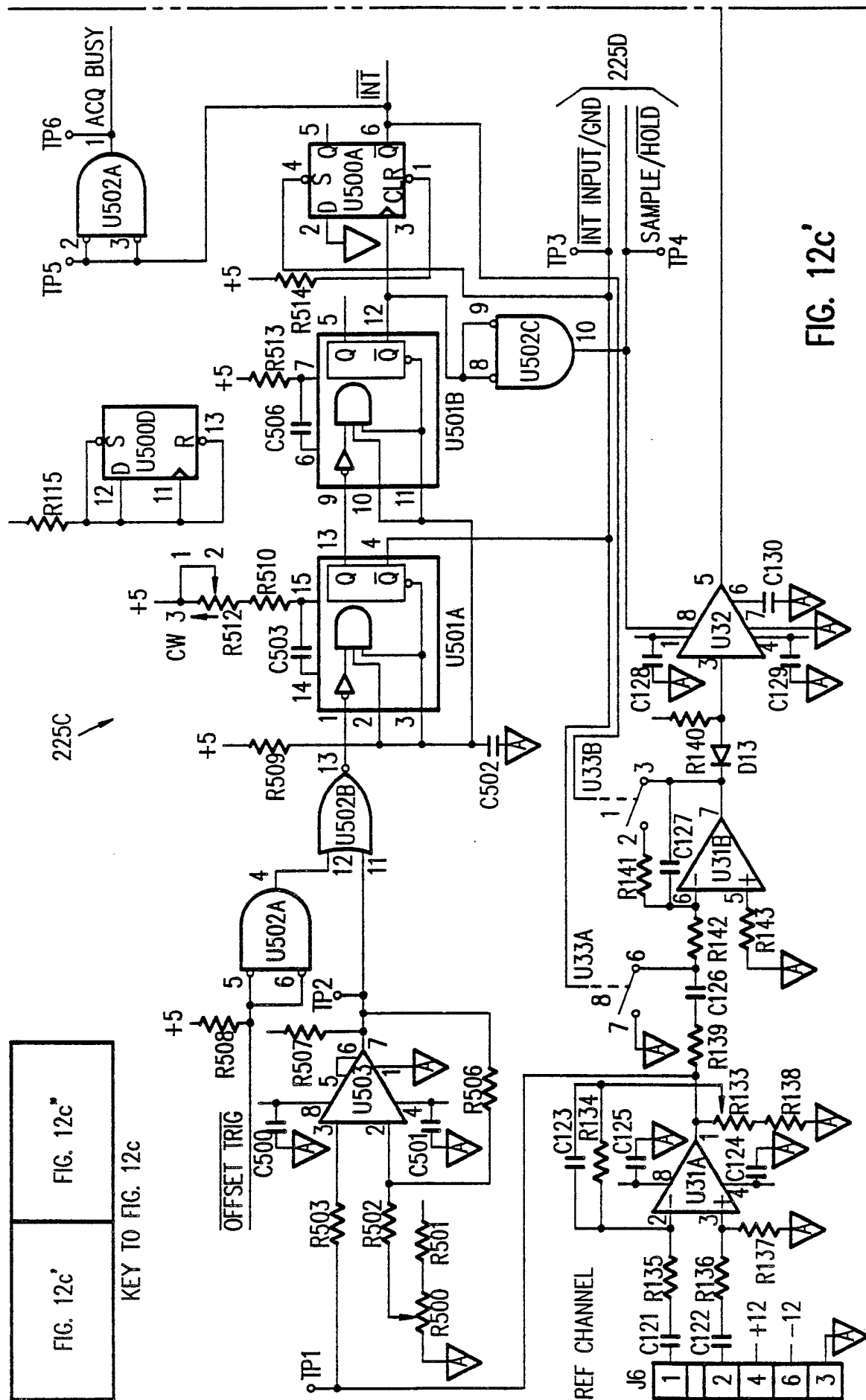
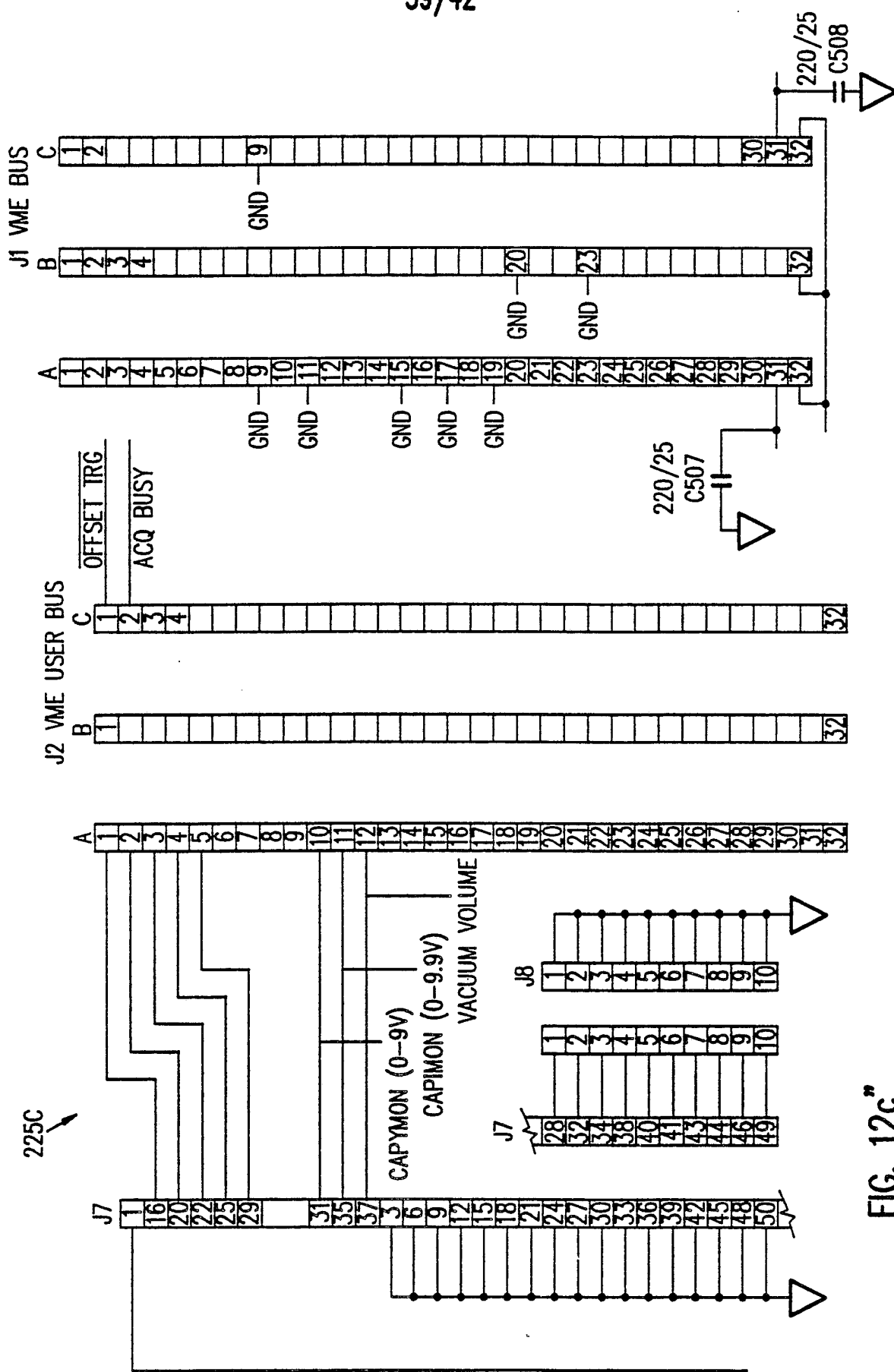


FIG. 12c'

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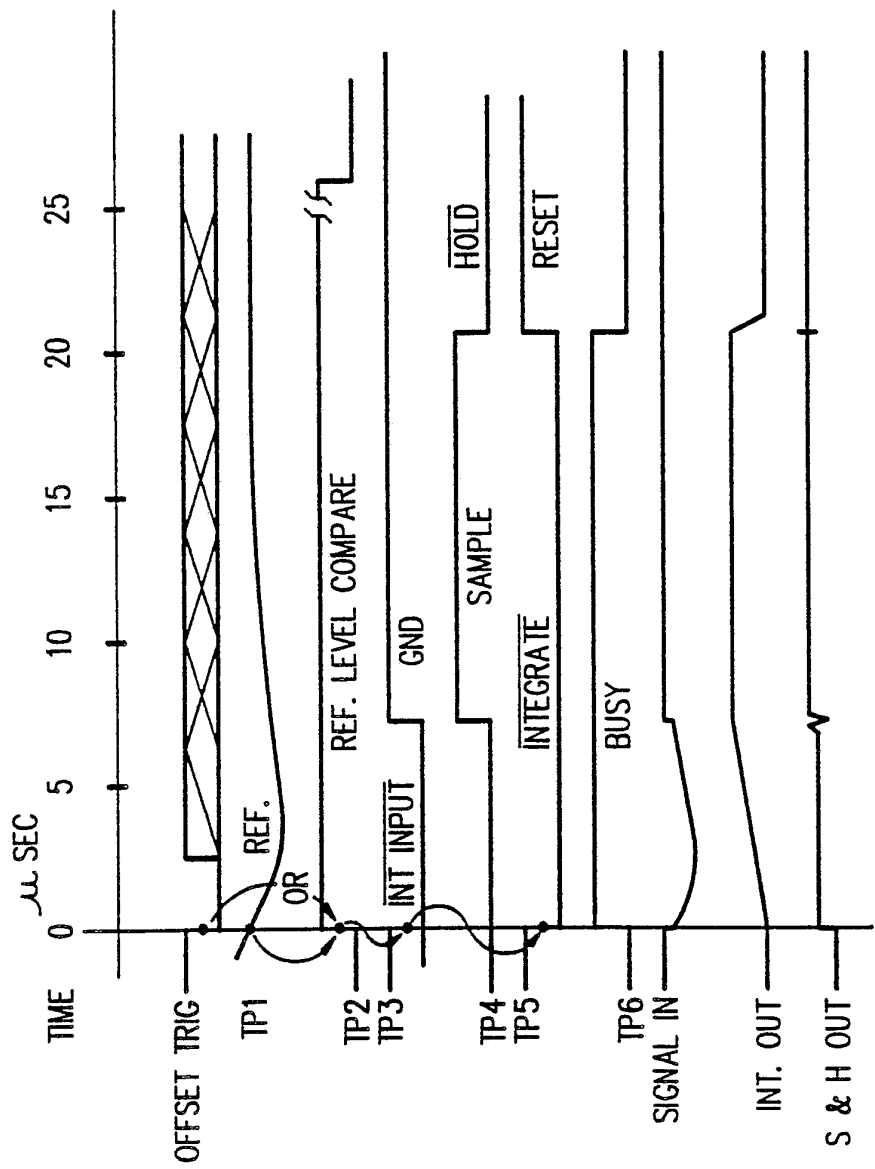


FIG. 12d

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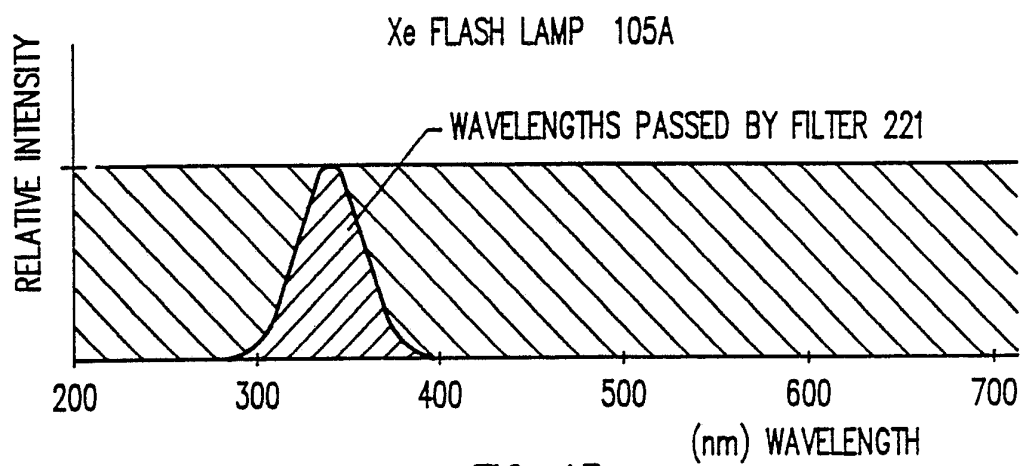


FIG. 13a

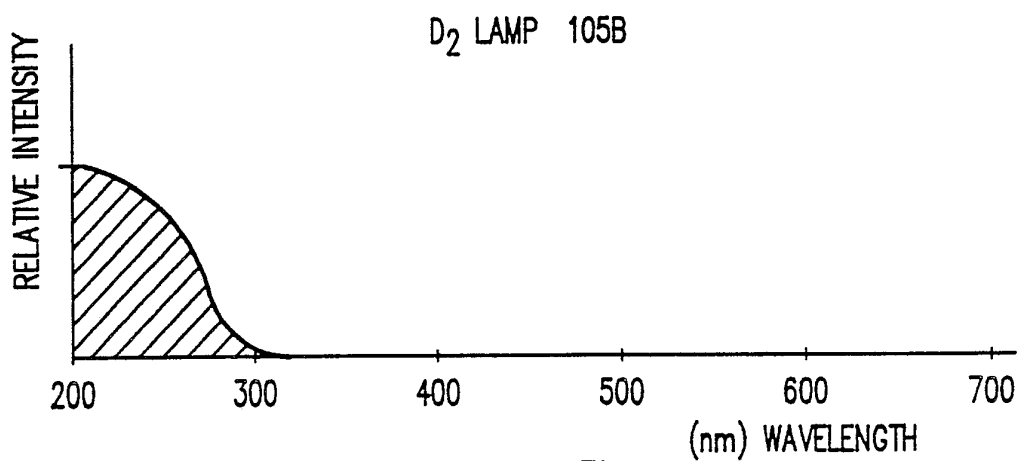


FIG. 13b

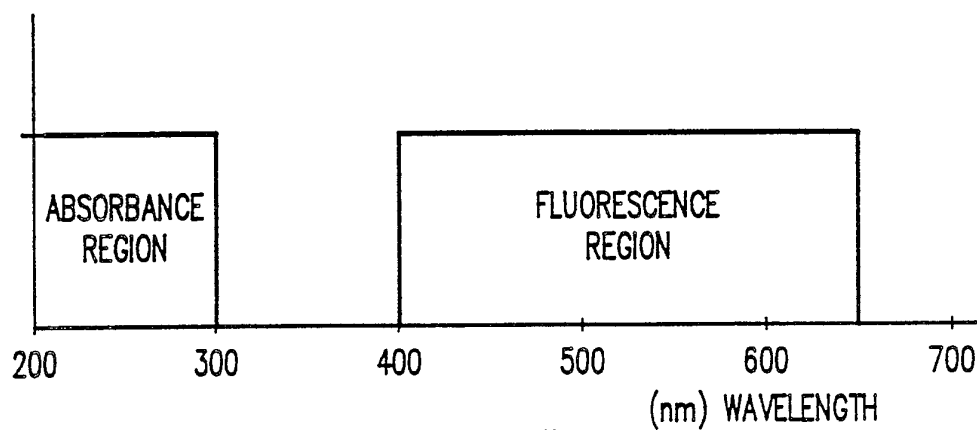


FIG. 13c

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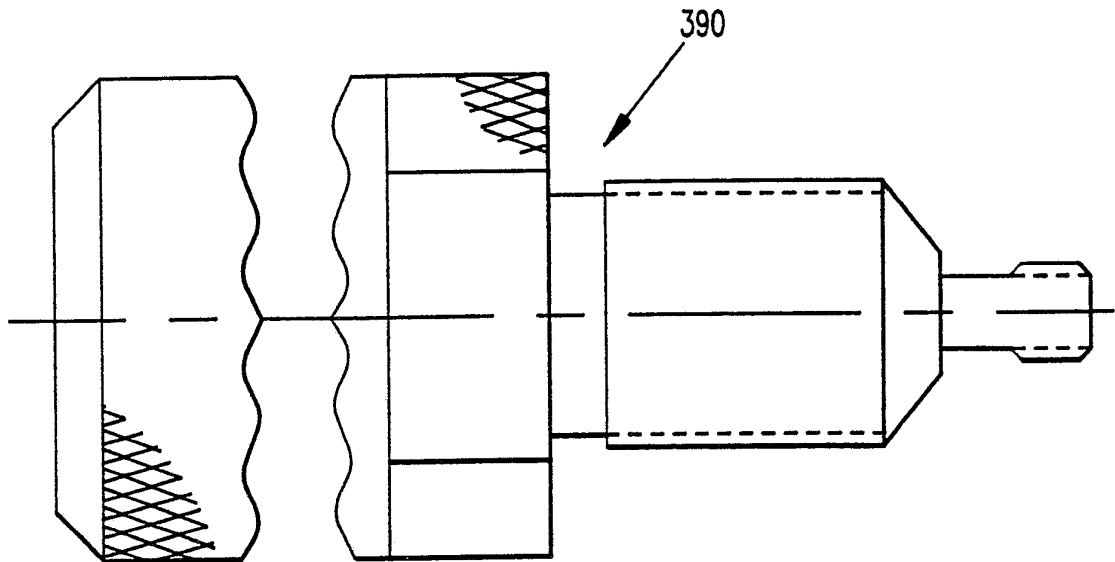


FIG. 14a

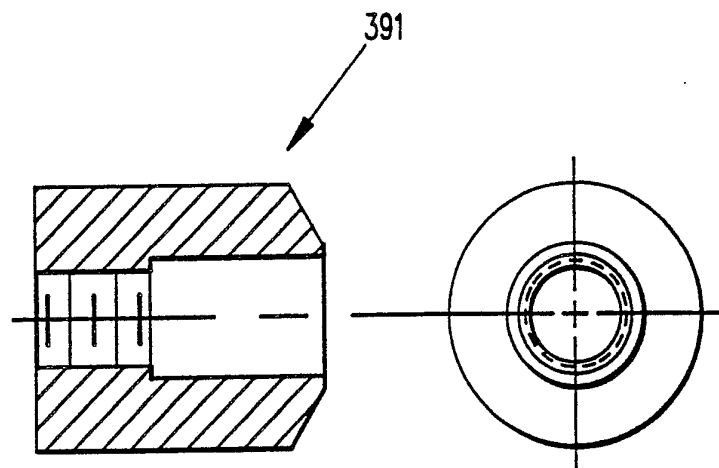


FIG. 14b

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 89/01780

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) ⁷ to both National Classification and IPC IPC ⁴ G01N 21/59; G01N 21/54; G01N 30/74; G01N 30/78 U.S. Cl. 356/72,73		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
U.S.	356/72,73	
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
X	US,A, 4,730,922 (BACH ET AL) 15 March 1988 (15.03.88) note figures 1 and 11.	1-3,5-20
Y	US,A, 4,145,139 (NAKAMURA ET AL) 20 March 1979 (20.03.79) see column 3 at lines 3-7.	4
Y	US,A, 4,699,510 (ALGUARD) 13 October 1987 (13.10.87) see abstract.	21
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"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)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"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</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"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.</p> <p>"&" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report
29 May 1989 (29.05.89)		<div style="font-size: 1.5em; font-weight: bold;">7 JUL 1989</div>
International Searching Authority		Signature of Authorized Officer
ISA/US		Vincent P. McGraw