

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) **A1**

(22) Date de dépôt/Filing Date: 2004/07/20

(41) Mise à la disp. pub./Open to Public Insp.: 2006/01/20

(51) Cl.Int.⁷/Int.Cl.⁷ G01N 21/64, G01N 33/58, G01N 37/00

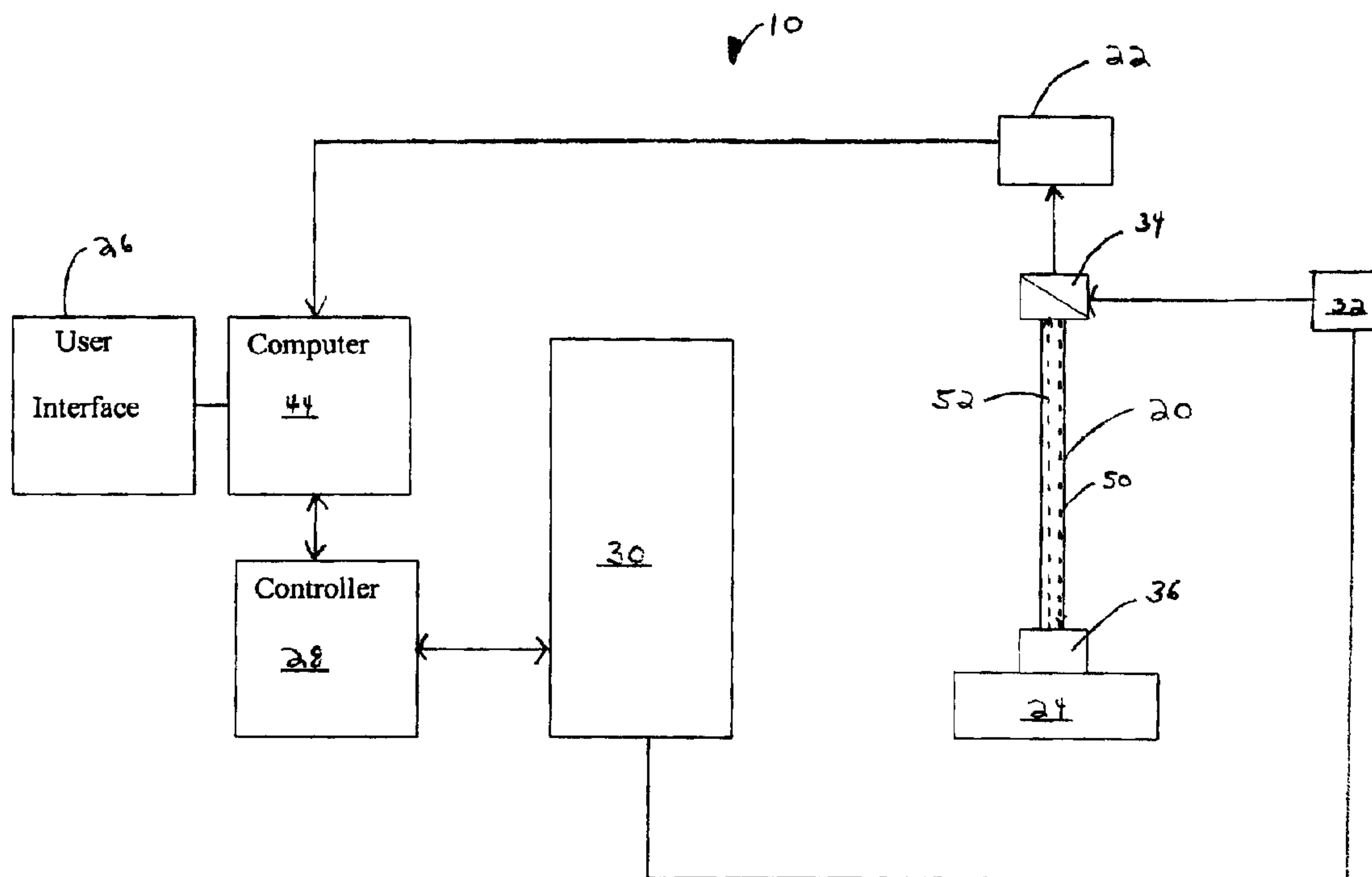
(71) Demandeur/Applicant:
BIOPHYS, INC., CA

(72) Inventeurs/Inventors:
LEA, PETER, CA;
SZABADOS HAYNES, NICOLE, CA;
DYKSTRA, COLIN, CA;
DING, SHI-FA, CA;
SCHAIBLE, UWE DIRK, CA;
VON STYP-REKOWSKI, NORMAN HANS, CA

(74) **Agent:** DIMOCK STRATTON LLP

(54) Titre : SYSTEME ET METHODE DE LECTURE RAPIDE DE MACRO-MATRICES ET DE MICRO-MATRICES

(54) Title: SYSTEM AND METHOD FOR RAPID READING OF MACRO AND MICRO MATRICES



(57) **Abrégé/Abstract:**

An analyte reading system which includes a reader unit for rapidly detecting and evaluating the outcome of an assay to measure the presence of analytes in a sample. Quantitative and qualitative measurements of analyte concentration in a sample may be rapidly obtained using the reader device with algorithms which ascertain the nature of the assay and perform a comparison against a calibration sample. The reader device can scan preset areas of an assay device in order to provide focal points for the reader device and evaluate the volume of the test sample in the assay device. The reading portion of the assay slide has at least one test dot for detecting the presence of the analyte and the signal intensity of the labelled analyte, and processes the detected signal using an algorithm which provides an accurate output measurement indicating the quantity of the analyte in the test sample. The reader device can read the analyte as a random array format, print and read the analyte to be measured in a fixed array format, and print and read the analyte in a hybrid format consisting of both fixed and random arrays.

ABSTRACT

An analyte reading system which includes a reader unit for rapidly detecting and evaluating the outcome of an assay to measure the presence of analytes in a sample. Quantitative and qualitative measurements of analyte concentration in a sample may be rapidly obtained using the reader device with algorithms which ascertain the nature of the assay and perform a comparison against a calibration sample. The reader device can scan preset areas of an assay device in order to provide focal points for the reader device and evaluate the volume of the test sample in the assay device. The reading portion of the assay slide has at least one test dot for detecting the presence of the analyte and the signal intensity of the labelled analyte, and processes the detected signal using an algorithm which provides an accurate output measurement indicating the quantity of the analyte in the test sample. The reader device can read the analyte as a random array format, print and read the analyte to be measured in a fixed array format, and print and read the analyte in a hybrid format consisting of both fixed and random arrays.

SYSTEM AND METHOD FOR RAPID READING OF MACRO AND MICRO MATRICES

Field of the Invention

The present invention relates to a device for the reading and data analysis of an assay
5 device for analysis of analytes.

Background of the Invention

Micro and macro matrices of bacteria and their respective toxic proteinaceous
contaminants account for several million cases of food-related illness and about 9,000
deaths per year in the United States. Contaminated processed food, poultry and meat
10 products etc. are a major cause of these deaths and illnesses. The five most common
pathogens infecting food products and especially poultry and meat products are E. coli
O157:H7, Salmonella species, Listeria species, Listeria monocytogenes and
Campylobacter jejuni.

Similarly, contamination of water supplies also causes illness and death. The United
15 States Environmental Protection Agency has determined that the level of E. coli in a
water supply is a good indicator of health risk. Other common indicators are total
coliforms, fecal coliforms, fecal streptococci and enterococci. Currently, water
samples are analyzed for these microorganisms with membrane filtration or multiple-
tube fermentation techniques. Both types of tests are costly and time consuming and
20 require significant handling. They are not, therefore, suitable for field-testing.

Many disease conditions, such as bacterial and viral infections, many cancers, heart
attacks and strokes, for example, may be detected through the testing of blood and
other body fluids, such as saliva, urine, semen and feces for markers that have been
shown to be associated with particular conditions. Early and rapid diagnosis may be
25 the key to successful treatment. Standard medical tests for quantifying markers, such
as ELISA-type assays, are time consuming and require relatively large volumes of
fluid.

Accordingly, for the prevention of infection of consumers through contaminated food and water and detection of many disease conditions there is a need for the accurate and rapid identification of microorganisms and markers of the health of a patient. The accurate, rapid detection and measurement of microorganisms, such as bacteria,
5 viruses, fungi or other infectious organisms and indicators in food and water, on surfaces where food is prepared, and on other surfaces which should meet sanitary standards is, therefore, a pressing need in industrial, food, biological, medical, veterinary and environmental samples. Further, in routine inspection of industrial products for microbiological contamination there is a need for the early detection of
10 contamination which will lead to more rapid release of safe products, and for the rapid, accurate detection and measurement of micro-organisms which are not pathogenic but have a role in the determination of a product's shelf life.

A variety of assay methodologies have been used for determining the presence of analytes in a sample of interest. Assays for detecting some microorganisms require
15 that the samples be cultured. In this assay, the typical practice is to prepare a culture growth medium (an enrichment culture) that will favour the growth of an organism of interest. A sample such as food, water or a bodily fluid that may contain the organism of interest is introduced into the enrichment culture medium. Typically, the enrichment culture medium is an agar plate where the agar medium is enriched with
20 certain nutrients. Appropriate conditions of temperature, pH and aeration are provided and the medium is then incubated. The culture medium is examined visually after a period of incubation to determine whether there has been any microbial growth. It could take several days to obtain results and requires a technician to read the agar plates by visual inspection which can lead to errors.

25 There are presently many examples of one-step assays and assay devices for detecting analytes in fluids. One common type of assay is the chromatographic assay, wherein a fluid sample is exposed to a chromatographic strip containing reagents. A reaction between a particular analyte and the reagent causes a colour change on the strip, indicating the presence of the analyte. In a pregnancy test device, for example, a urine
30 sample is brought into contact with a test pad comprising a bibulous chromatographic strip containing reagents capable of reacting with and/or binding to human chorionic

gonadotropin ("HCG"). The urine sample moves by capillary flow along the bibulous chromatography strip. The reaction typically generates a colour change, which indicates that HCG is present. While the presence of a quantity of an analyte above a threshold may be determined, the actual amount or concentration of the analyte is
5 unknown. Accordingly, there is a risk that a pathogen may be present below a level sufficient for either the test to detect its presence, or for the individual assessing the test strip to visually observe the colour change of the test strip.

Assays have been developed for providing a quantitative measure of the presence of pathogens or analytes of interest. In such a typical test assay, a fluid sample is mixed
10 with a reagent, such as an antibody, specific to a particular analyte (the substance being tested for), such as an antigen. The reaction of the analyte with the reagent may result in a colour change that may be visually observed, or in chemiluminescent, bioluminescent or fluorescent species that may be observed with a microscope or detected by a photodetecting device, such as a spectrophotometer or photomultiplier
15 tube. The reagent may also be a fluorescent or other such detectable-labelled reagent that binds to the analyte. Radiation that is scattered, reflected, transmitted or absorbed by the fluid sample may also be indicative of the identity and type of analyte in the fluid sample.

In a commonly used assay technique, two types of antibodies are used, both specific to
20 the analyte. One type of antibody is immobilized on a solid support. The other type of antibody is labeled by conjugation with a detectable marker and mixed with the sample. A complex between the first antibody, the substance being tested for and the second antibody is formed, immobilizing the marker. The marker may be an enzyme, or a fluorescent or radioactive marker, which may then be detected. See, for example,
25 U.S. Pat. No. 5,610,077, which is incorporated herein by reference.

In order to quantitatively measure the concentration of an analyte in a sample and to compare test results, it is usually necessary to either use a consistent test volume of the fluid sample each time the assay is performed or to adjust the analyte measurement for the varying volumes.

There is therefore a need for a device which can efficiently, rapidly and accurately read an assay for determining the presence of analytes in a sample and for determining the quantity of respective analytes in the sample. There is a need for an assay reading device that permits a user to assess the results of the assay carried out in an efficient,
5 simple and reliable manner.

Summary of the Invention

The present invention provides an analyte reading system which includes an analyte reader unit for rapidly detecting and evaluating the outcome of an assay to measure the presence of analytes in a sample. Quantitative and qualitative measurements of
10 analyte concentration in a sample may be rapidly obtained using the reader device with preset algorithms which also ascertain the nature of the assay being read, provide controls and can prevent erroneous duplication of measurement of that assay.

According to a method of the present invention, the reader device can detect from a reading area of an assay device control indicators from which the system can calculate
15 or ascertain the nature of the assay or assays conducted in the assay device, the volume of sample and other control conditions such as the response of standard samples to provide a reliable calibration within the assay device for the analyte reading system.

According to another aspect of the present invention, the reader device can scan preset
20 areas of an assay device in order to provide focal points for the reader device and evaluate the volume of the test sample in the assay device. This aspect of the invention permits the reader device to adjust the analyte measurement for varying volumes.

According to one aspect of the invention there is provided analyte reader unit for
25 reading and measuring the outcome of an assay on an assay slide containing a fluorescently labelled analyte, comprising

a positioning stage for holding the assay slide in a desired position,

a light sensor, and

an optical system comprising

5 an excitation light source for illuminating a fluorescently labelled
 analyte, and a dichroic mirror for reflecting excitation light to the
 analyte and light emitted by the fluorescent dye to pass through to the
 light sensor.

According to another aspect of the present invention, there is provided a reading
system for reading and measuring the outcome of an assay on an assay slide
containing a fluorescently labelled analyte, comprising

10 a positioning stage for holding the assay slide in a desired position,
 a light sensor,

an optical system comprising

 an excitation light source for illuminating a fluorescently labelled
 analyte, and
15 a dichroic mirror for reflecting excitation light to the analyte and light
 emitted by the fluorescent dye to pass through to the light sensor, and

a computer for processing the signal detected by the light sensor to generate a
measurement of analyte density on a detected portion of the assay slide.

20 According to yet another aspect of the present invention, there is provided a method of
reading an assay slide containing a fluorescently labelled analyte, comprising the steps
of:

- a. illuminating a portion of the assay slide containing a test sample.
- b. detecting an intensity of light emitted by the test sample in a single image
field, and
- c. generating a measurement of analyte density in the test sample based on said
25 intensity detection.

According to another aspect of the present invention, there is provided, a method of reading an assay slide containing a fluorescently labelled analyte, comprising the steps of:

- a. illuminating a portion of the assay slide containing a test sample of unknown analyte density and a portion of the assay slide containing a calibration sample of known analyte density with an excitation light,
- b. detecting an intensity of light emitted by the test sample and an intensity of light emitted by the calibration sample in a single image field, and
- c. comparing the intensity of light emitted by the test sample to the intensity of light emitted by the calibration sample to generate a measurement of analyte density in the test sample.

Brief Description of the Drawings

In drawings which illustrate by way of example only a preferred embodiment of the invention,

Figure 1 is a schematic view of an analyte reader system of the present invention;

Figure 2 is a schematic view of the analyte reader of the invention;

Figure 3 is an assay device that can be read by the reading system of Figure 1;

Figure 4 is a reading portion of the assay device shown in Figure 3;

Figure 5 is a graph showing a relationship between fluorescent intensity of test dots and known antigen concentration in a sample;

Figure 6 is a graph showing a relationship between fluorescent intensity of calibration dots and the amount of antigen in the calibration dots;

Figure 7 is a graph showing a relationship between the antigen concentration in the sample and the amount of antigen in the calibration dots;

Detailed Description of the Invention

The present invention provides an analyte reading system and method for the rapid reading of macro and micro matrices such as that illustrated in Figure 1. As illustrated in Figure 1, in the preferred embodiment the analyte reading system 10 comprises a
5 analyte reading unit 20, which is preferably a microscope such as that illustrated in Figures 1 and 2, having an imaging device such as a CCD camera 22 which transmits signals to a general purpose computer 44 integrated into the system. The microscope 20 has a stage 24. The stage movement (x and y axes) for assay positioning and focusing (z axis) for image clarity and resolution are controlled by servo motors
10 through a suitable user interface 26, such as a touch-pad or touch-screen control board, which preferably also provides switches for the light sources. In the preferred embodiment the PC is programmed to process the signal returned by the CCD camera 22 to provide accurate assay identification and results, as described in detail below; however the PC could also be programmed to control the functions of the analyte
15 reading unit via suitable user displays and touch-screen activation of functions. The microscope has an optics assembly 62. Optics assemblies known in the art may be used for the purposes of the present invention. The microscope 20 also has a dichromatic mirror 34 and a focus mechanism 36. A laser 32 is connected to the dichromatic mirror 34.

20 The system 10 has a controller 28 that is connected to the computer 44. The controller 28 is also connected to an options assembly 30. The options assembly 30 received signals from the controller 28. The options assembly controls the laser 32 that is adapted to apply energy to the dichromatic mirror 34 that forms part of the microscope 20.

25 In operating the system, a user places an assay device that is to be read onto the stage 24. The system then applies an initialization and calibration routine. The assay device preferably has an identification dot that is detected by the system and provides instructions regarding what assay is to be read and accordingly which routines and calculations need to be carried out. In reading an assay device, the laser 32 applies
30 light energy to the dichromatic mirror 34. Light beam 50 is reflected from the

dichromatic mirror 34 onto a sample on the stage 24. A return light beam is reflected off the sample to the CCD camera 22. The signal from the CCD camera 22 is relayed to the computer 44 where pre-programmed routines are performed on the image to make the required calculations. The results of the calculations performed are relayed
5 the user interface 26.

In one embodiment of the invention the analyte reading system is designed to detect microorganism antigens marked or coated with an indicator such as a fluorescent labelled antibody. In this embodiment the analyte reading system can be used to determine the concentration in a given sample of the microorganism antigen. The
10 antigen concentration, which can be used as a measure of the microorganism concentration from a sample, such as a food sample, can then be compared with an acceptable analyte concentration limit and a pass/fail response reported to the user.

In this embodiment of the invention the analyte reader unit is adapted to read and detect specifically labelled analytes in an assay slide or assay chip in which the analyte
15 sample is placed. One fluorescent dye suitable for labelling bacteria for use in the designed assay chip is Alexafluor™ 647nm dye. It is the assay chips which are presented to the analyte reader for scanning. One skilled in the art will appreciate that alternatives to fluorescent labelling can also be used. Whatever labelling system is used, the light source (which may include electromagnetic radiation ranging from
20 ultraviolet to infrared) for imaging and the detector must be matched, and may be collectively referred to as the imaging system.

In one embodiment of the invention the analyte reader unit, illustrated in Figures 1 and 2, consists of seven parts: an optical system, a positioning stage, a stage controller board, an embedded computer, a monochrome CCD camera, a touch screen LCD
25 display, and a power supply board. In this embodiment the entire unit is housed in a case or containment means.

In a preferred embodiment the optical system consists of five parts: a light source such as a laser light source, a light emitting diode (LED) ring light source, a filter cube, a microscope objective lens, and an optical tube with focussing. In this embodiment the
30 laser light source preferably has a peak spectral emission at 635 nm. The laser spectral

emission at 635 nm then passes through an excitation filter of the filter cube. This excitation filter is used to control the bandwidth and wavelength of light that will reach the assay chip assay chip in the analyte reader unit. In this embodiment the excitation filter allows only the 635nm emission line from the laser light source to be
5 passed to the filter cube's dichroic mirror, which then reflects this light down the axis of the optical tube towards the microscope objective lens. The laser light is focused on the assay chip assay chip by the microscope objective lens and causes the labelling marker, in this embodiment the AlexafluorTM 647nm fluorescent dye attached to the antibody bound (directly or indirectly) to the analyte to fluoresce and emit light with a
10 peak intensity at 668nm.

In this embodiment a WSTech UH5-15G-635 15mW 635nm laser diode module is used to provide illumination of the assay chip for producing fluorescence of the dye. This is a class IIIb laser. However, a person skilled in the art will appreciate that different light sources, including different laser light sources, will be suitable as an
15 excitation light source. The desirable laser will be dictated by the peak emission wavelength and the excitation wavelength for the labelling marker. As an alternative to laser illumination it is possible to use other excitation sources, for example, an LED or mercury vapour lamp, provided the desired excitation energy is transmitted by that light source in sufficient intensity to produce a detectable fluorescence in the sample.

20 In this embodiment a custom moulded filter cube is used to hold the excitation filter, dichroic mirror, and emission filter in the most suitable position to allow for illumination of the assay chip from above through the microscope objective lens. The filter cube also preferably interlocks with the excitation light source via an adjustable flange. In this embodiment of the filter cube, a ChromaTM Technology Corporation
25 Z635/20x 635nm (12.5mm) narrow bandpass interference filter, is used as an excitation filter. This filter has a full width-half maximum bandwidth of 20 nm. In combination with this excitation filter a ChromaTM Technology Corporation Z635RDC 635nm (20mmx30mm) dichroic mirror is used to reflect the laser light down the axis of the optical tube towards the microscope objective. This dichroic
30 mirror allows lower frequency light such as the light emitted from the fluorescent dye to pass straight through the dichroic mirror toward the image detector, in this

embodiment a CCD camera comprising a CMOS image sensor. This embodiment of the filter cube a ChromaTM Technology Corporation HQ685/50m 685nm (25mm DIA) bandpass filter is used as an emission filter. This filter has a full width-half maximum bandwidth of 50nm. This filter prevents any reflected laser light that passes through the dichroic mirror from reaching the CMOS image sensor. The sensor device is held in a fixed position relative to the filter cube. In this embodiment, a camera board is mounted to the top of the filter cube so that the image sensor is held in a fixed position relative to the filter cube.

The fluorescent emitted light is then focused by the microscope objective lens as it passes back up the optical tube to the dichroic mirror. The fluorescent light passes through the dichroic mirror and then through the emission filter of the filter cube. The emission filter removes any reflected laser light in the image and allows only the fluorescent emitted light to pass to the image sensor device.

In a preferred embodiment of the invention, the assay chip containing the labelled test sample also has focus spots. To ensure accuracy in this embodiment of the invention, the analyte detector device ideally will focus the optical system by reference to the focus spots carried on the assay chip. When the analyte detector device is focussing by imaging the focus spots on the assay chip in this embodiment the laser light source used to provide the excitation of the labelled sample is prevented from illuminating the assay chip. This may be achieved in a variety of ways such as switching off the laser or blocking the light from the laser light source from entering the filter cube. The bright field illumination of the assay chip for imaging of the focus spots in this embodiment is provided by side illumination of the assay chip from the LED ring light source. In one embodiment the bright field side illumination of the assay chip is provided by four LumexTM SSL-LX5093SRC/E 3500mcd 660nm high brightness LEDs which are used in an LED ring around the microscope objective.

A suitable microscope objective lens for this embodiment of the invention is an Edmund Industrial OpticsTM R43-906 4x plan achromatic commercial grade standard microscope objective lens with a working distance of 13.9mm, which is used to focus

an image of the bacteria on the CCD image sensor. This objective lens is designed to produce an image at 150mm from the top edge of the objective lens.

In this preferred embodiment of the device of the invention, a light-impervious metal optical tube is used to house the optics of the optical reading unit. The purpose of this optical tube is to prevent interference with the detected signal, the excitation light and emitted light by peripheral or external light sources. This optical tube is grooved and the entire assembly is anodized to reduce the reflection of light and prevent reflection of light from the optical assembly directly onto the image sensor. The optical tube provides a conduit for the light from the excitation source and the emitted light from the labelled analyte between the microscope objective lens and the filter cube. In this preferred embodiment the microscope objective lens is attached to the lower end of the optical tube and the filter cube is attached to the upper end of the optical tube. One way in which the filter cube and microscope objective lens can be attached to the optical tube is using threaded attachment.

In the preferred embodiment of the invention a Point Grey Research Dragonfly IEEE-1394 monochrome CCD camera is used to capture images of fluorescing analytes. This camera contains an ICX204AL 1/3" black and white, 1024x768 pixel, CCD image chip with a pixel size is 4.65um x 4.65um. The camera in this embodiment is powered from the IEEE-1394 bus and has an interface protocol which is compliant with the IEEE IIDC DCAM V1.3 specification.

Thus, the analyte reading system of the invention can be used to carry out a preferred embodiment of the method of the invention, which comprises illuminating a portion of the assay slide containing a test sample of unknown analyte density and a portion of the assay slide containing a calibration sample of known analyte density with the excitation light; detecting an intensity of light emitted by the test sample and an intensity of light emitted by the calibration sample in a single image field; and comparing the intensity of light emitted by the test sample to the intensity of light emitted by the calibration sample to generate a measurement of analyte density in the test sample.

The optical tube is also provided with a focussing means, in this embodiment using a stepper motor focussing assembly. In an embodiment of the optical tube a Hayden Switch and InstrumentTM 26463-12-003 26mm 12V captive unipolar linear actuator stepper motor is used to move the lower end of the optical tube along the Z axis. The
 5 Z-axis is perpendicular to the plane defined by the assay chip in position on the positioning stage. Thus movement in this Z-axis provides focussing of the microscope objective lens on the assay chip.

A metal frame is used to keep the filter cube, optical tube, image board, and positioning stage in fixed positions relative to each other. The positioning stage is
 10 used to move the assay chip in the X-Y plane relative to the microscope objective lens. The Y-axis is along the short dimension of the plane of the assay chip which is perpendicular to the longitudinal axis of the optical tube. The assay chip is inserted onto the positioning stage along the Y-axis of the assay chip. The X-axis is along the long axis of the plane of the assay chip which is perpendicular to the longitudinal axis
 15 of the optical tube. The positioning stage can be moved in the X-Y axis using two motors, for example two Hayden Switch & InstrumentTM motors. In one embodiment a 26mm 12V captive unipolar linear actuator stepper motor is used to drive the stage in the X-axis over a 12.7mm total displacement distance. Similarly, a 26mm 12V non-captive unipolar linear actuator stepper motor is used to drive the stage in the Y-
 20 axis over a 38.1mm total displacement distance. These examples of motors have a step size of 0.005" (or approximately 12.7 μ m).

The reference (or home) position for the positioning stage is found by moving the positioning stage to a preset position (usually to the limit of its range of movement in the X and Y-axes). At the reference position an electrical contact is established with
 25 two detector switches mounted on the positioning stage. One type of detector switch suitable for this application is PanasonicTM Type ESE11HS1. Optionally, the positioning stage can be controllably moved to the locations of several reference marks or points on the assay chip for accurate optical calibration.

The positioning stage controller board (or stage controller board) controls movement
 30 of the positioning stage and controls the illumination of the assay chip and positioning

stage by switching on or off the excitation light source (e.g. laser light source), and by switching on or off the side illumination light source (e.g. the ring LED). Control of the stage controller board is achieved by commands sent via an interface, e.g. an RS232 interface. Status messages are also sent back over the same interface.

5 The stage controller board in a preferred embodiment of the invention comprises several components and circuits: a microcontroller; an RS232 level converter; stepper motor drivers; a excitation light source driver or drivers; a side illumination light-source (or LED) driver; connectors and jumpers. In the preferred embodiment a MicrochipTM PIC16F876A-I/SP operating at 12MHz is used to control the positioning
10 stage. The microcontroller in this embodiment of the invention has the following features: 8Kx14 Flash program memory; 368x8 Data memory; 256x8 EEPROM (Electrically Erasable Programmable Read-Only) memory; 22 general purpose I/O lines (Input/Output); watchdog timer; power on reset and brown out detector; USART (Universal Synchronous Asynchronous Receiver Transmitter); timers; and in-circuit
15 serial programming

In the microcontroller of the above embodiment an ECSTM Inc. ECS-120-32-1 12MHz 32pF parallel resonant crystal with an effective series resistance of 30ohms, a 1mW drive level, and a fundamental mode of vibration provides the microcontroller's internal oscillator circuit. The 22 general purpose I/O lines are as shown in Table 1
20 below. If the microprocessor does not reset properly, if an infinite loop occurs, if the program counter gets lost, or if the timer interrupt ceases to be activated then the microcontroller is reset to a known state. This is achieved using an internal watchdog timer enabled with a prescaler of 1:128 to produce a watchdog timeout period between 0.9s and 4.2s. If the watchdog timer is not cleared within this time period, a reset is
25 generated. The watchdog timer is strobed once at the top of the main loop only if the timer interrupt has indicated that it is still running. The microcontroller's built in reset circuit is used to reset the microcontroller at powerup. In addition, an internal brownout detector will keep the microcontroller reset if the voltage drops below about 4.0V and will reset the microcontroller once the voltage rises back within the pre-
30 determined acceptable range.

In the microcontroller, the onboard USART is used to communicate with the embedded computer. In this embodiment, the USART is set to run in full duplex asynchronous mode using N-8-1 format (no parity, 8 data bits, 1 stop bit). The baud rate is set to 57600bps. The microcontroller also contains 3 timers, though in this
5 embodiment only one of the timers is used. That timer is used to provide a 2.5ms timer period to run the stepper motors. Three of the port B pins are configured to allow for in circuit programming of the microcontroller. Both high and low voltage programming modes are supported.

10

TABLE 1 – I/O Line Use

I/O Line	Type	Function
RA0	O	X-axis, phase 0, driver 0
RA1	O	X-axis, phase 0, driver 1
RA2	O	X-axis, phase 1, driver 0
RA3	O	X-axis, phase 1, driver 1
RA4	O	Unused
RA5	O	Z-axis, phase 0, driver 0
RB0	O	Unused
RB1	O	Laser driver
RB2	O	LED driver
RB3	I	PGM
RB4	O	Z-axis, phase 0, driver 1
RB5	I	Z-axis home switch (focus)
RB6	I	X-axis home switch (stage left-right)/PGC
RB7	I	Y-axis home switch (stage front-back)/PGD
RC0	O	Y-axis, phase 0, driver 0
RC1	O	Y-axis, phase 0, driver 1
RC2	O	Y-axis, phase 1, driver 0

I/O Line	Type	Function
RC3	O	Y-axis, phase 1, driver 1
RC4	O	Z-axis, phase 1, driver 0
RC5	O	Z-axis, phase 1, driver 1
RC6	O	UART Tx line
RC7	I	UART Rx line

Also in this preferred embodiment, the RS232 level translation for the USART serial interface is provided by a Texas InstrumentsTM MAX232N 5V only, 2 transmitter/2 receiver, RS232 driver. Two Texas InstrumentsTM ULN2003AN darlington transistor arrays are used to drive the three unipolar stepper motors. The stepper motors are powered from +12VDC. The common connection on the devices provides a freewheeling path when the outputs to the stepper motors are turned off. Diodes Inc.TM SD101A schottky diodes are used as ground clamp diodes to prevent the driver outputs from going below ground potential. A Fairchild RFD14N05L N-channel logic level power MOSFET is used to energize the laser light source used as the excitation light source for illumination of the assay chip. The laser is powered from +5VDC. The switching operation of the laser is controlled by application of a logic high signal from the general purpose I/O pins to the gate of the MOSFET. A Fairchild RFD14N05L N-channel logic level power MOSFET is also used to energize the LED light source used for the side illumination of the assay chip. The LED is powered from +5VDC. The switching operation of the LED is also controlled by application of a logic high signal from the general purpose I/O pins to the gate of the MOSFET.

Several connectors are also required to make the electrical connections between components of the stage controller board:

1. A Molex 53109-0410 5.08mm right angle disk drive power connector is used to provide power to the stage controller board. It mates with a standard PC disk drive power cable connector. The pinout of the power connector is as shown below in Table 2.

5

Table 2 – Pinout of Power Connector

Pin	Function
1	+12VDC
2	GND
3	GND
4	+5VDC

10

2. An AMP 747844-6 female right angle DB9 connector is used to connect the stage controller board to a computer serial port using a standard 9 pin serial cable. This connector is configured as a DCE with null cable crossovers for the control lines. The pinout of the 9-Pin Serial Connector is shown below in Table 3:

Table 3 – Pinout of 9 Pin Serial Connector

Pin	Function
1	DCD, connected to 4 and 6
2	RD to PC
3	TD from PC
4	DTR, connected to 1 and 6

Pin	Function
5	GND
6	DSR, connected to 1 and 4
7	RTS, connected to 8
8	CTS, connected to 7
9	RI, connected to GND

3. An AMP 499913-1 5x2 pin 0.1" right angle header with short latches provides an alternate connection to a computer serial port. This connector allows a straight through flat ribbon cable to connect directly to the COM2 port on VIA EPLA M10000/ME6000 motherboards. The pinout of the dual in-line 10 pin serial port connector is shown below in Table 4:

Table 4 – Pinout of Dual in-Line 10 Pin Serial Port Connector

Pin	Function
1	DCD, connected to 4 and 6
2	RD to PC
3	TD from PC
4	DTR, connected to 1 and 6
5	GND
6	DSR, connected to 1 and 4
7	RTS, connected to 8

Pin	Function
8	CTS, connected to 7
9	RI, connected to GND
10	N/C

4. A JST S8B-EH 8 pin 2.5mm side entry shrouded header is used to connect the positioning stage X-axis stepper motor and its associated limit switch to the stage controller board. The mating connector is a JST EHR-8 female
- 5 receptacle housing with JST SHE-001T-P0.6 crimp pins. The pinout of the X-axis connector is as shown below in Table 5.

Table 5 – Pinout of X-axis Connector

Pin	Function
1	X-axis phase 0, driver 0 (Black)
2	X-axis phase 0, power (White)
3	X-axis phase 0, driver 1 (Red)
4	X-axis phase 1, driver 0 (Blue)
5	X-axis phase 1, power (White)
6	X-axis phase 1, driver 1 (Green)
7	X-axis microswitch input
8	X-axis microswitch GND

5. A JST S8B-EH 8 pin 2.5mm side entry shrouded header is used to connect the positioning stage Y-axis stepper motor and its associated limit switch to the

stage controller board. The mating connector is a JST EHR-8 female receptacle housing with JST SHE-001T-P0.6 crimp pins. The pinout of the Y-axis connector is as shown below in Table 6.

Table 6 – Pinout of Y-axis Connector

Pin	Function
1	Y-axis phase 0, driver 0 (Black)
2	Y-axis phase 0, power (White)
3	Y-axis phase 0, driver 1 (Red)
4	Y-axis phase 1, driver 0 (Blue)
5	Y-axis phase 1, power (White)
6	Y-axis phase 1, driver 1 (Green)
7	Y-axis microswitch input
8	Y-axis microswitch GND

5

6. A JST S8B-EH 8 pin 2.5mm side entry shrouded header is used to connect the focusing Z-axis stepper motor and its associated limit switch to the stage controller board. The mating connector is a JST EHR-8 female receptacle housing with JST SHE-001T-P0.6 crimp pins. The pinout of the Z-axis connector is as shown below in Table 7.

10

Table 7 – Pinout of Z-axis Connector

Pin	Function
1	Z-axis phase 0, driver 0 (Black)
2	Z-axis phase 0, power (White)
3	Z-axis phase 0, driver 1 (Red)
4	Z-axis phase 1, driver 0 (Blue)
5	Z-axis phase 1, power (White)
6	Z-axis phase 1, driver 1 (Green)
7	Z-axis microswitch input
8	Z-axis microswitch GND

7. A JST S2B-EH 2 pin 2.5mm side entry shrouded header is used to connect the laser to the stage controller board. The mating connector is a JST EHR-2 female receptacle housing with JST SHE-001T-P0.6 crimp pins. The pinout of the laser connector is as shown below in Table 8.

Table 8 – Pinout of Laser Connector

Pin	Function
1	+5VDC to laser
2	Switched GND

8. A JST S2B-EH 2 pin 2.5mm side entry shrouded header is used to connect the LED to the board. The mating connector is a JST EHR-2 female receptacle

housing with JST SHE-001T-P0.6 crimp pins. The pinout of the LED connector is as shown below in Table 9.

Table 9 – Pinout of LED Connector

Pin	Function
1	Power to LED (current limited)
2	Switched GND

- 5 9. A JST S6B-EH 6 pin 2.5mm side entry shrouded header is used to allow in-circuit serial programming of the microcontroller. The mating connector is a JST EHR-6 female receptacle housing with JST SHE-001T-P0.6 crimp pins. The pinout of the in-circuit serial programming connector is as shown below in Table 10.

10 **Table 10 – Pinout of IN-circuit Serial Programming Connector**

Pin	Function
1	VPP
2	VDD
3	VSS
4	RB7
5	RB6
6	RB3

ICSP programming configuration jumpers are used to isolate the microcontroller from the rest of the system and connect the programming pins to the ICSP connector. A Molex 10-88-1081 0.100" 4x2 pin header is used to select programming or normal mode. The valid jumper configurations are shown below in Table 11.

5

Table 11 – Valid Jumper Configurations

Configuration	Jumpers Installed
Programming mode	1-2, 5-6
Normal mode	3-4, 7-8

The Stage Controller Board software consists of a main loop that processes commands sent to it through the RS232 interface. After reset, the microcontroller hardware is configured, the data structures are initialized, and then the main loop is entered. The main loop is run until power is removed from the system. The commands sent to the Stage Controller Board through the RS232 interface consist of single functions such as moving an axis to a new position, getting the current position of an axis, moving an axis to the home position, and getting the current status of the stage controller system. In response to these commands, the Stage Controller Board performs the desired action and sends back a confirmation or status information.

The main loop performs several functions including for example, re-initializing the configurable hardware or strobing the watchdog timer. In the preferred embodiment the main loop executes commands from the RS232 interface in a state machine format. This prevents commands that take longer to execute from allowing the watchdog timer to time out.

In addition to the main loop there are various interrupt routines that provide realtime control of the stepper motors and manage communications over the RS232 port. For example a timer interrupt routine can be set to trigger when the timer comparator postscaler overflows. This interrupt routine is used to generate the timing for the

stepper motor outputs. In this example the timer interrupt routine, when triggered, would set the timer watchdog timer flag; check and store status of home position sensors; if any stepper motors have finished moving then clear their movement commands; if any stepper motors are being calibrated then update calibration status
 5 based on movement commands and home position sensor status; update stepper motor outputs based on their movement command; and update stepper motor position registers.

A preferred embodiment of the invention includes an embedded computer which is responsible for the GUI interface and all of the processing done in the system. The
 10 internal computer connects to all of the peripheral components of the system and coordinates their functioning. In one preferred embodiment a Via Technologies Inc. EPIA M10000 mini-ITX PC mainboard is used as the embedded computer. In addition, in this preferred embodiment there will also be a hard drive (e.g. a Seagate ST92011A, 20GB, 5400 rpm Notebook hard drive), a RS232 dual serial port card (e.g.
 15 an Axxon Computer Corporation MAP/950 PRO Dual 16950 RS232 PCI serial card plugged into the PCI slot to provide a third and fourth RS232 serial port for the system); and DDR SDRAM memory (e.g Infineon DDR 256MB, 266MHz (PC2100) 184 Pin Memory) which provides all the main memory required by the CPU. The computer may alternatively be a suitably programmed personal computer (PC), or a
 20 specialized computer with an ASIC cpu designed specifically for the analyte reading system.

The analyte reader system also requires a power supply. In the preferred embodiment of the analyte reader system described above an Ituner Networks Corp. PW70-A DC-DC converter is used to generate the voltages required by the system from a single
 25 12VDC source. The DC-DC converter produces ATX compatible voltages. The maximum combined output power of this embodiment of the analyte reader system is 100W. Alternatively an Ituner Networks Corp. AC-DC 12V, 5A switching power adapter is used to produce a 12VDC input to the DC-DC converter from a 110V/220V AC outlet. This provides the analyte reader system with a maximum of approximately
 30 60W of power. Finally, the unit can also be powered from a 12VDC power adapter or

vehicle 12VDC auxiliary outlet. In this way, the 12VDC is fed into the power supply board to provide the voltages needed by the analyte reader system.

In one embodiment of the device of the invention a housing unit is used for the reader system. A modified Digiconcepts Digital 917J Beige ATX 300W Midi Tower Case
5 can be used to house the reader system. A custom made bezel is then used to mount the LCD screen and touchpad in the three 5.25" drive bay slots in the front of the case.

A touchscreen LCD such as an Apollo Display Technologies Ki-1A-063 5.7" 320x240 QVGA colour LCD touchscreen can be used to provide a user interface to the system. In this example embodiment the touchscreen LCD is powered from +5V and +12V
10 from the power supply and interfaces to the embedded PC through 2 RS232 serial ports. During operation the assay chip containing the dye stained bacteria is placed into the reader and a start command is given via the touchscreen. The reader then scans the slide and counts the bacteria. Finally the result is displayed on the display and the assay chip can then be ejected and removed by the user. Examples of other
15 commands that can be sent to the RS 232 interface in a preferred embodiment include: a "cancel" command which flushes the serial communications buffers and cancels any commands in progress; a "read status" command which reads the Stage Controller Board status; a "clear error" command which clears the error status bit; a "read axis position" command which reads the current position of the specified axis; a "calibrate
20 axis" command which calibrates the specified axis by moving it to the home position; a "move axis absolute" command which moves the specified axis to an absolute position relative to the calibrated home position; a "move axis relative" command which moves the specified axis to a position relative to its current position; a "set laser mode" command which sets the laser mode to on or off; and a "set LED mode"
25 command which sets the LED mode to on or off.

The system of the present invention is typically employed to read the results of assays for the presence of microbes although the reader can also be used to read the results of assays for non-biological assays. One such as assay device that is useful for biological assays is shown in Figure 3. The assay device 70, has a substantially planar surface 72
30 having a sample loading portion for receiving a fluid sample and reading portion 80 as

shown in Figure 4. The assay device preferably has microspheres 74 at the junction between the sample loading and reading portions and act as a dynamic filter. The reading portion has printed thereon at least one and preferably at least two test dots 86. More preferably, a plurality of test dots 86 for detecting the presence of the analyte are printed on the reading area 80. The test dots include a reagent that specifically bind to the analyte for which the assay is directed. The reagent is preferably a bound antibody specific for the analyte. The bound antibodies are preferably spaced apart to make each bound antibody available for binding to the test antigen free of steric hindrance from adjacent antigen complexes. Preferably, a non-reactive protein separates the bound antibodies in the test dots.

The reading area 16 has calibration dots 84 printed thereon. The calibration dots include a pre-determined amount of said analyte for reacting with unreacted reagent from the vessel that is bound to a detectable marker. The calibration dots allow the intensity of the label to be correlated to the amount of the antigen present. The intensity of label in the test dots can then be used to derive the quantity of antigen present.

The results of the assay device 70 can be read and calculated by the reader system of the present invention. To determine the concentration of analyte in a sample, the concentrations of two characteristic assay reagents are predetermined. A relationship between a fluorescent intensity of the fixed test dots in a series of samples with known antigen concentrations is determined. An example of a relationship between fluorescent intensity of test dots and known antigen concentration in a sample is shown in the form a graph as shown in Figure 5. Next, a relationship between fluorescent intensity of the calibration dots and the amount of antigen in the calibration dots, determined by using excess detection antibody, as shown in Figure 6. From Figure 5 and Figure 6, an association between the antigen in the sample and the antigen dot concentration is determined as shown in Figure 7. This calibration curve serves as a batch-specific standard curve for the determination of the antigen concentration in the samples. The calibration curve is calculated by the reader system of the present invention based on the light intensities of the calibration dots containing known amounts of analyte.

In the instance of a sample of unknown antigen concentration, the sample is premixed with an excess of detecting antibody. This solution is applied to an assay device such as the assay device shown in Figure 3. The fluorescent intensity of the test dots is normalized against the calibration curve for that particular analyte to provide a
5 normalized test dot value. This normalized test dot value is then read off the calibration curve shown in Figure 7 for that analyte to give the concentration of analyte in the sample.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the embodiments of the invention
10 described specifically above. Such equivalents are intended to be encompassed in the scope of the following claims.

I CLAIM:

1. An analyte reader unit for reading and measuring the outcome of an assay on an assay slide containing a fluorescently labelled analyte, comprising
 - a positioning stage for holding the assay slide in a desired position,
 - a light sensor, and
 - an optical system comprising
 - an excitation light source for illuminating a fluorescently labelled analyte, and
 - a dichroic mirror for reflecting excitation light to the analyte and light emitted by the fluorescent dye to pass through to the light sensor.
2. An assay device according to claim 1 wherein the reader unit further comprises a computer operatively connected to the light sensor for receiving a signal from the light sensor and performing calculations based on said signal.
3. An assay device according to claim 1 wherein the excitation light source is a laser.
4. An assay device according to claim 1 wherein the light sensor is an imaging device.
5. An assay device according to claim 1 further comprising a side illumination means for focussing the optical system on the assay slide.
6. An assay device according to claim 1 further comprising a stage controller board for controlling relative location of the positioning stage in three dimensions relative to the optical system.
7. An assay device according to claim 1 further comprising a user interface for communicating to the user the signal detected by the signal recording means and for input by the user of control commands.

8. A reading system for reading and measuring the outcome of an assay on an assay slide containing a fluorescently labelled analyte, comprising
- a positioning stage for holding the assay slide in a desired position,
 - a light sensor,
 - an optical system comprising
 - an excitation light source for illuminating a fluorescently labelled analyte, and
 - a dichroic mirror for reflecting excitation light to the analyte and light emitted by the fluorescent dye to pass through to the light sensor, and
 - a computer for processing the signal detected by the light sensor to generate a measurement of analyte density on a detected portion of the assay slide.
9. An assay device according to claim 8 wherein the excitation light source is a laser.
10. An assay device according to claim 8 wherein the light sensor is an imaging device.
11. An assay device according to claim 8 further comprising a side illumination means for focussing the optical system on the assay slide.
12. An assay device according to claim 8 further comprising a stage controller board for controlling relative location of the positioning stage in three dimensions relative to the optical system.
13. An assay device according to claim 8 further comprising a user interface for communicating to the user the signal detected by the signal recording means and for input by the user of control commands.
14. A method of reading an assay slide containing a fluorescently labelled analyte, comprising the steps of:

- a. illuminating a portion of the assay slide containing a test sample.
- b. detecting an intensity of light emitted by the test sample in a single image field, and
- c. generating a measurement of analyte density in the test sample based on said intensity detection.

15. A method of reading an assay slide containing a fluorescently labelled analyte, comprising the steps of:

- a. illuminating a portion of the assay slide containing a test sample of unknown analyte density and a portion of the assay slide containing a calibration sample of known analyte density with an excitation light,
- b. detecting an intensity of light emitted by the test sample and an intensity of light emitted by the calibration sample in a single image field, and
- c. comparing the intensity of light emitted by the test sample to the intensity of light emitted by the calibration sample to generate a measurement of analyte density in the test sample.

Application number/numéro de demande: 2 475 191

Figures: 2, 3, 4

Pages: _____

Drawings

Unscannable items
received with this application
(Request original documents in File Prep. Section on the 10th Floor)

Documents reçus avec cette demande ne pouvant être balayés
(Commander les documents originaux dans la section de préparation des dossiers au
10ième étage)

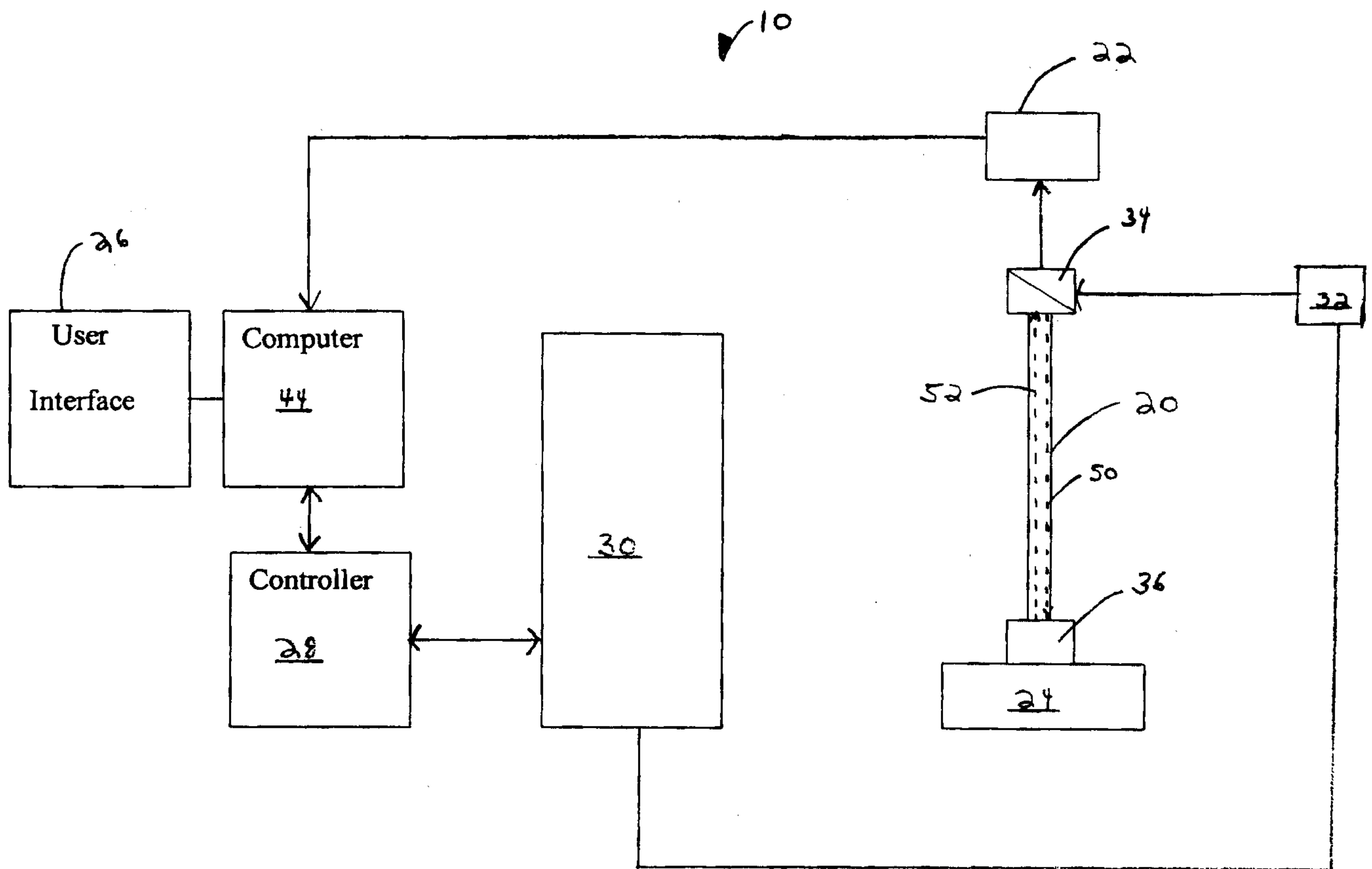
Figure 1

Figure 3

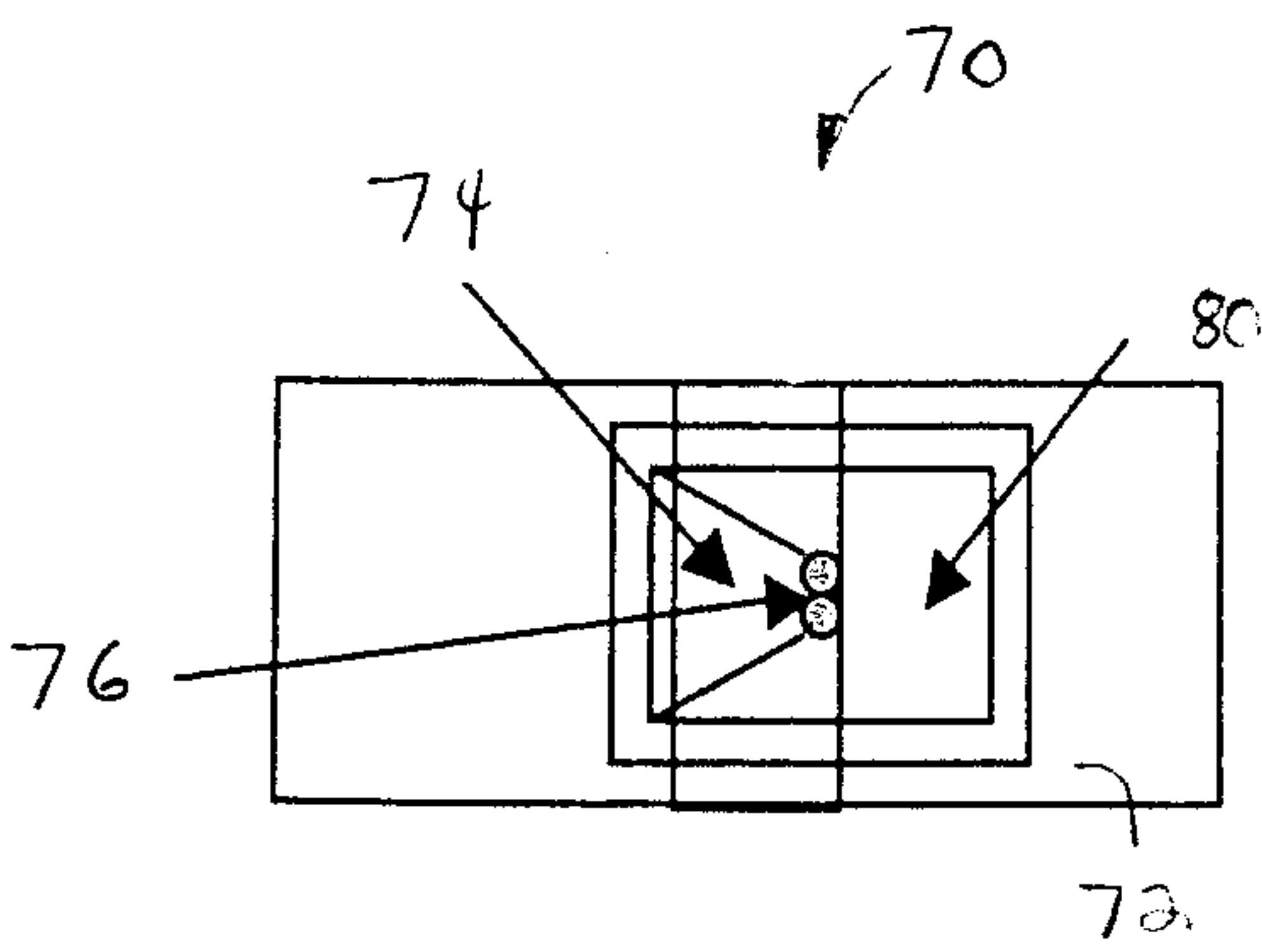


Figure 4

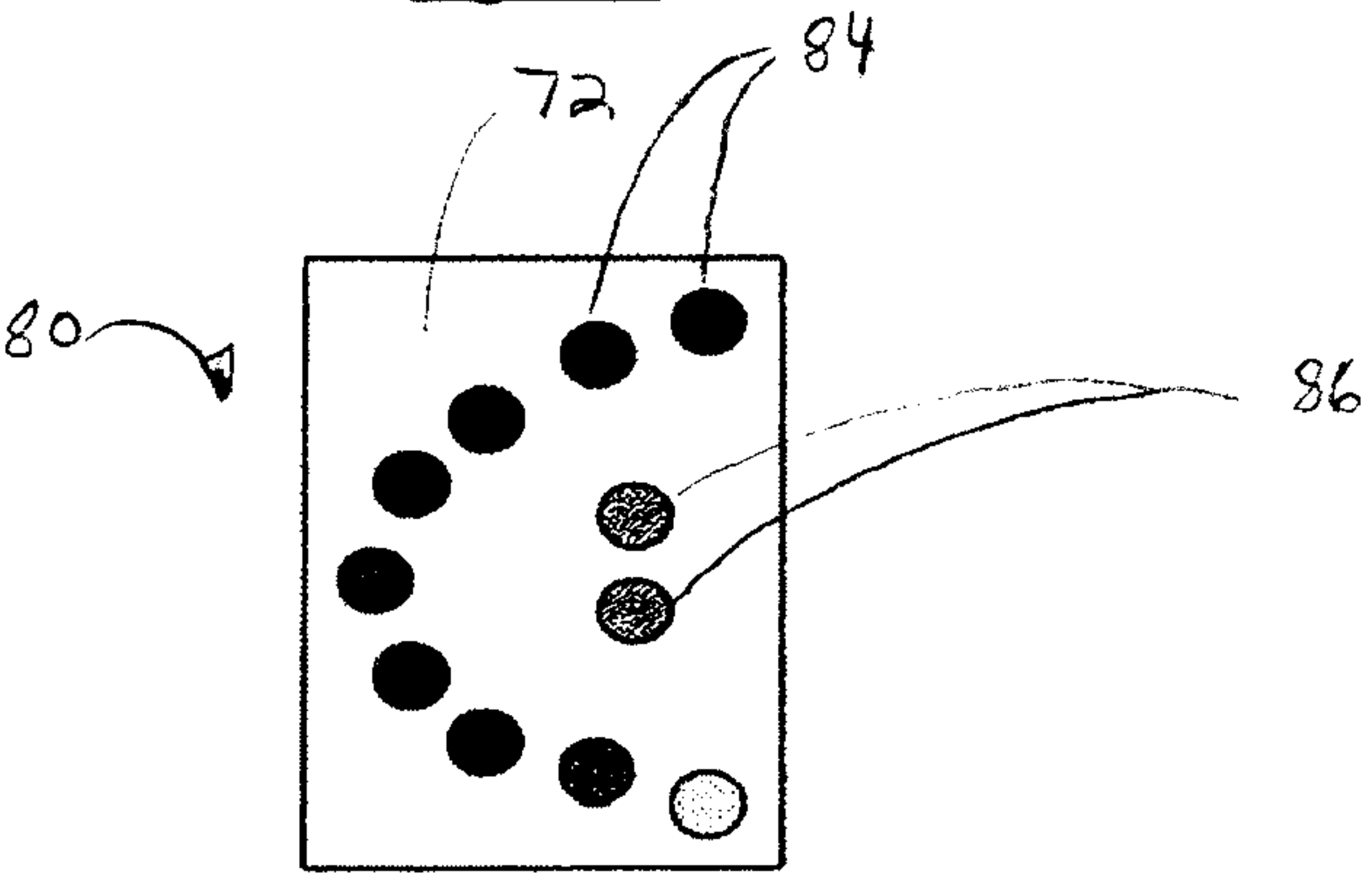


Figure 5

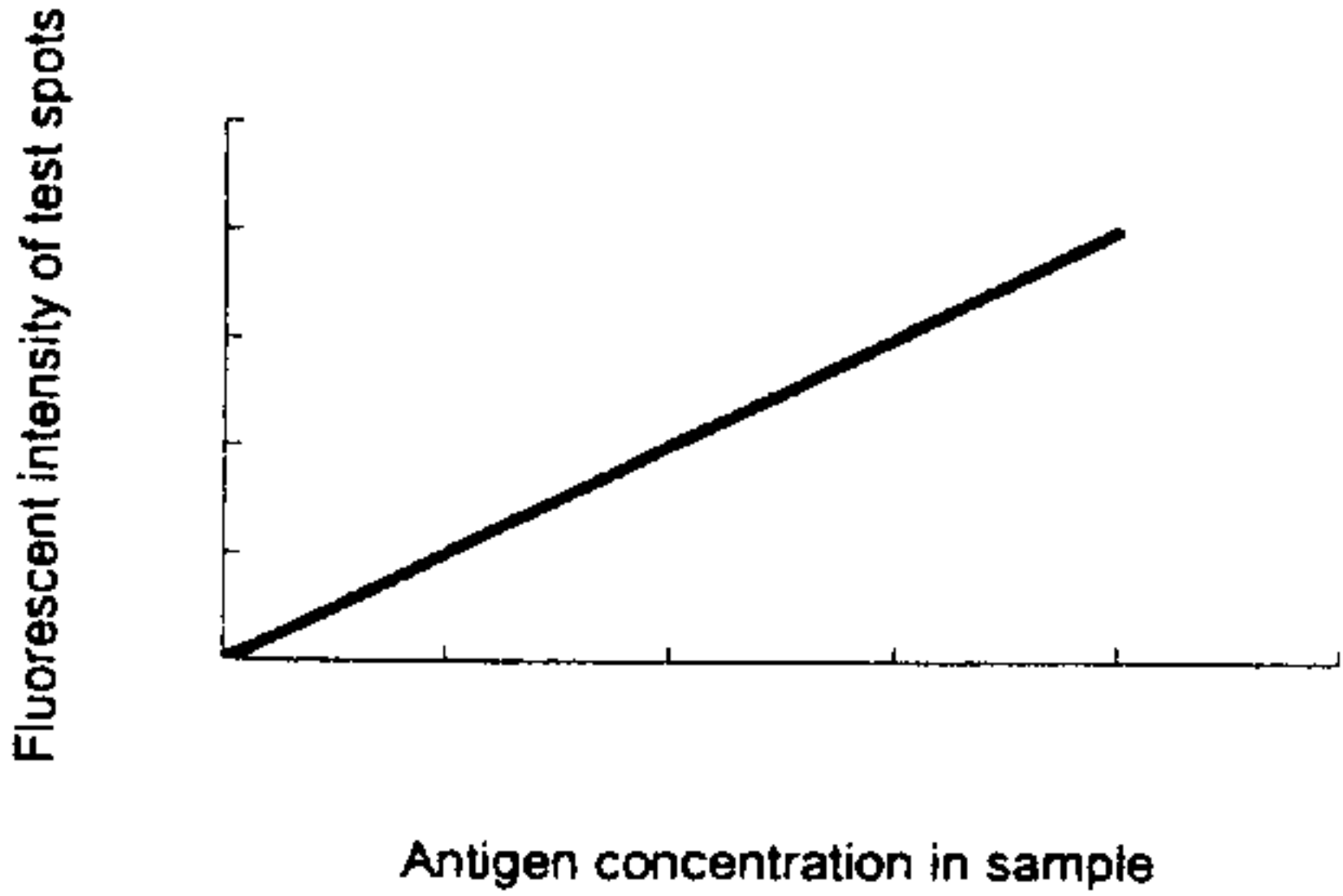


Figure 6

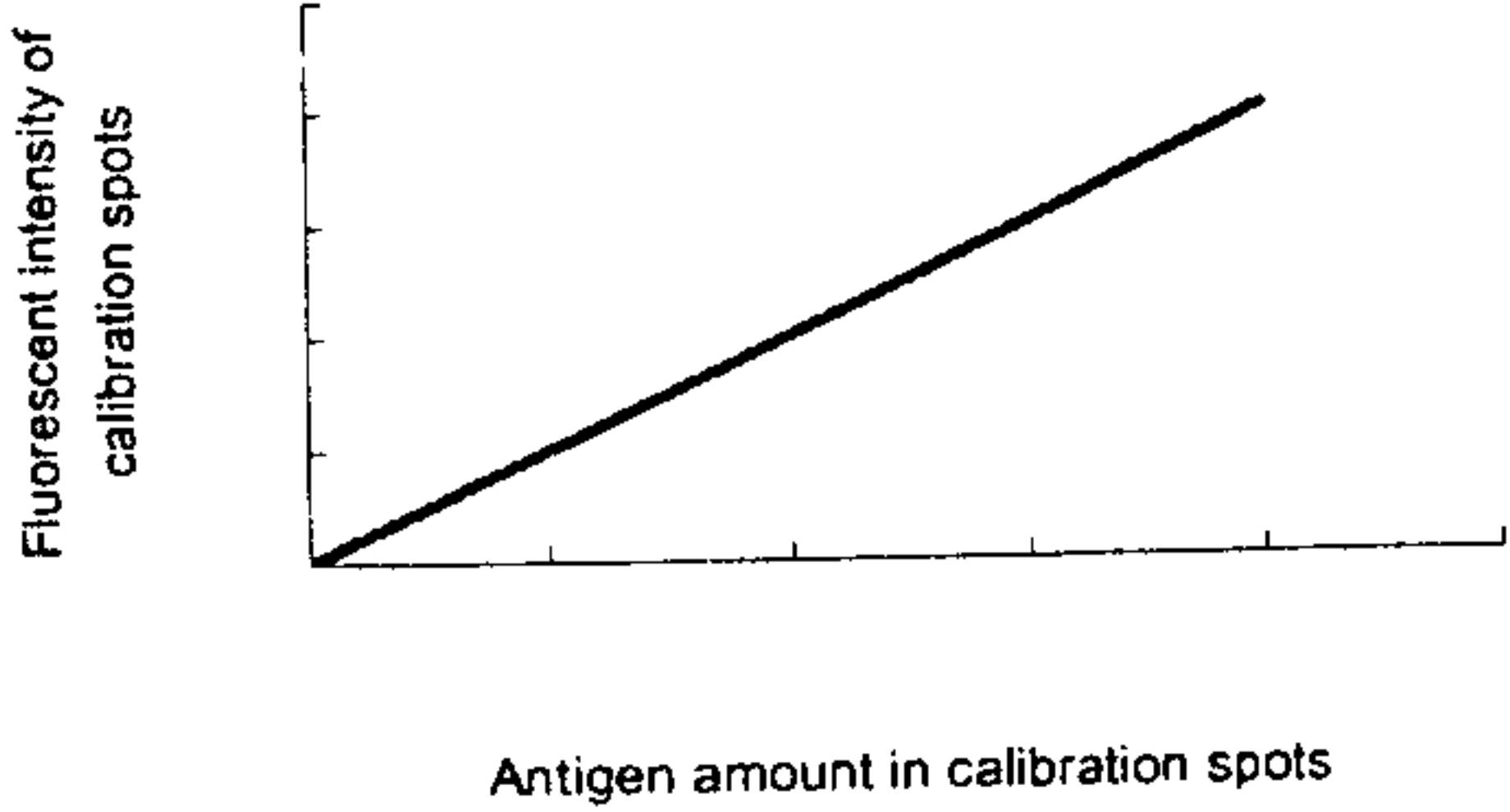


Figure 7

