US 20050239192A1

(19) **United States**

(12) **Patent Application Publication**

(10) **Pub. No.: US 2005/0239192 A1**

(43) **Pub. Date: Oct. 27, 2005**

Nasarabadi et al.

(54) **HYBRID AUTOMATED CONTINUOUS NUCLEIC ACID AND PROTEIN ANALYZER USING REAL-TIME PCR AND LIQUID BEAD ARRAYS**

(75) Inventors: **Shanavaz L. Nasarabadi**, Livermore, CA (US); **Richard G. Langlois**, Livermore, CA (US); **Billy W. Colston JR.**, San Ramon, CA (US); **Evan W. Skowronski**, Odenton, MD (US); **Fred P. Milanovich**, Lafayette, CA (US)

Correspondence Address:
Eddie E. Scott
Assistant Laboratory Counsel
Lawrence Livermore National Laboratory
P.O. Box 808, L-703
Livermore, CA 94551 (US)

(73) Assignee: **The Regents of the University of California**

(21) Appl. No.: **11/159,008**

(22) Filed: **Jun. 21, 2005**

Related U.S. Application Data

(63) Continuation-in-part of application No. 10/643,797, filed on Aug. 19, 2003.

(60) Provisional application No. 60/406,159, filed on Aug. 26, 2002.

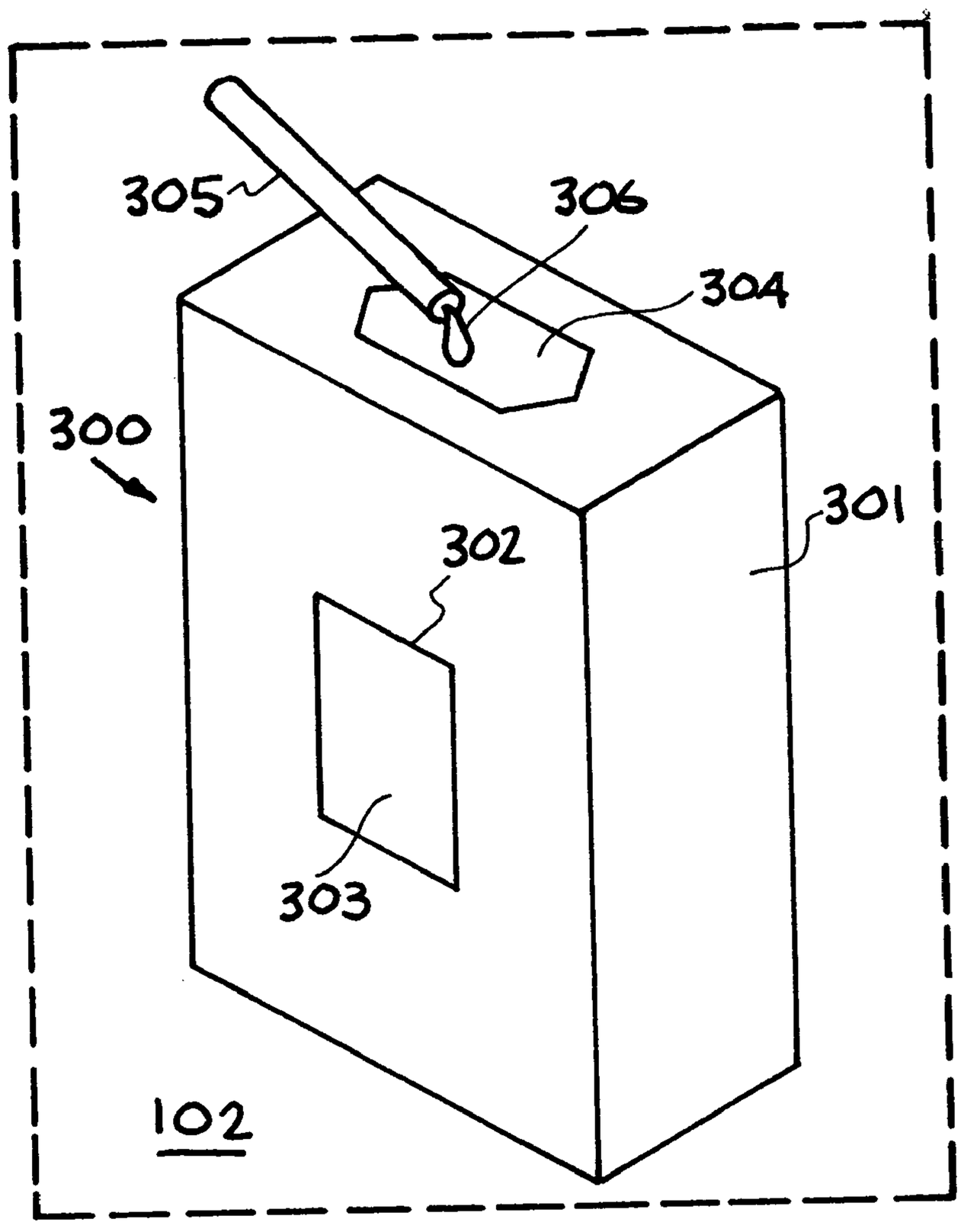
Publication Classification

(51) **Int. Cl.**[7] **C12M 1/34**; C12M 3/00

(52) **U.S. Cl.** **435/287.1**

(57) **ABSTRACT**

A nucleic acid assay system for analyzing a sample using a reagent. A sample and reagent delivery unit is operatively connected to a thermal cycler for delivering the sample and the reagent to the thermal cycler. A hybridization chamber is operatively connected to the thermal cycler. A flow cytometer is operatively connected to the hybridization chamber.



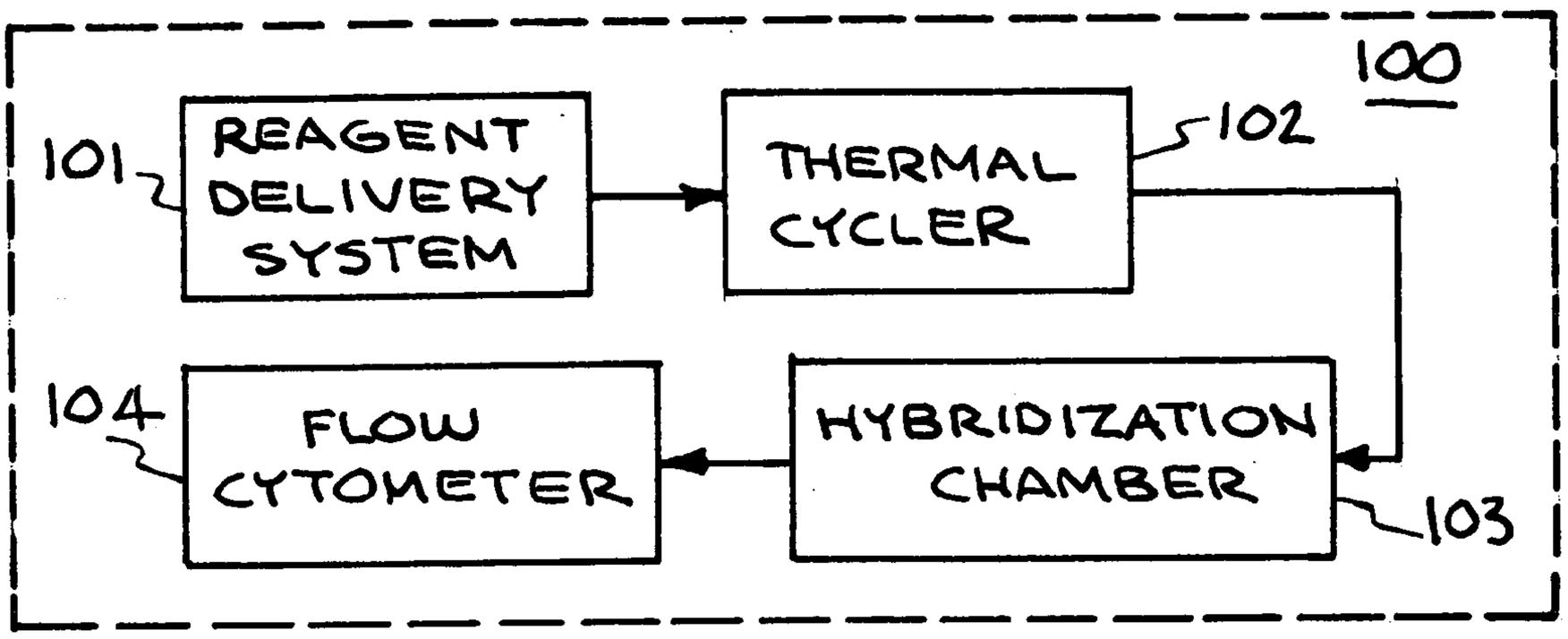

100
101
REAGENT DELIVERY SYSTEM
THERMAL CYCLER
102
104
FLOW CYTOMETER
HYBRIDIZATION CHAMBER
103

FIG. 1

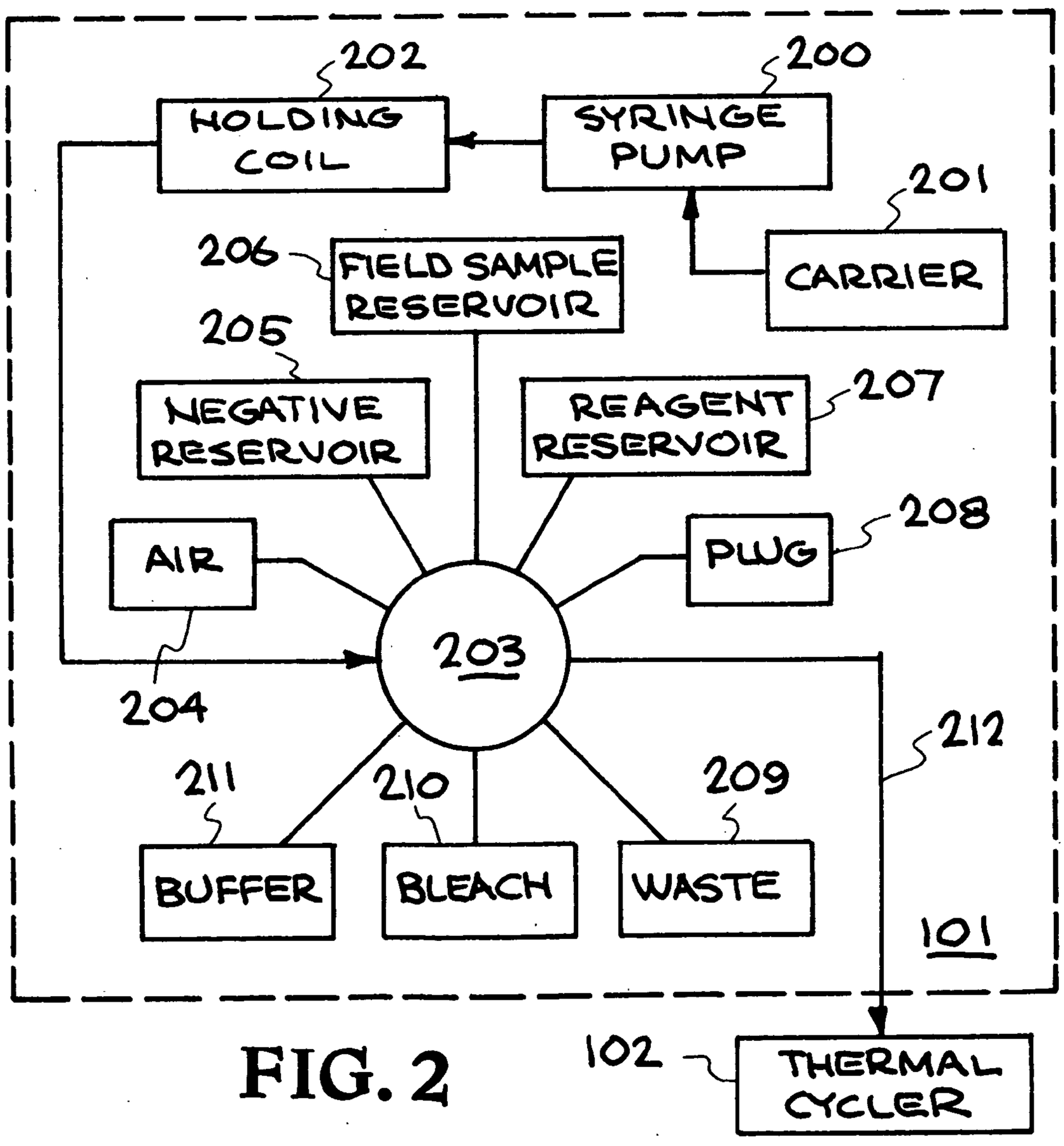

202
200
HOLDING COIL
SYRINGE PUMP
201
206
FIELD SAMPLE RESERVOIR
CARRIER
205
NEGATIVE RESERVOIR
REAGENT RESERVOIR
207
AIR
PLUG
208
203
204
212
211
210
209
BUFFER
BLEACH
WASTE
101
102
THERMAL CYCLER

FIG. 2

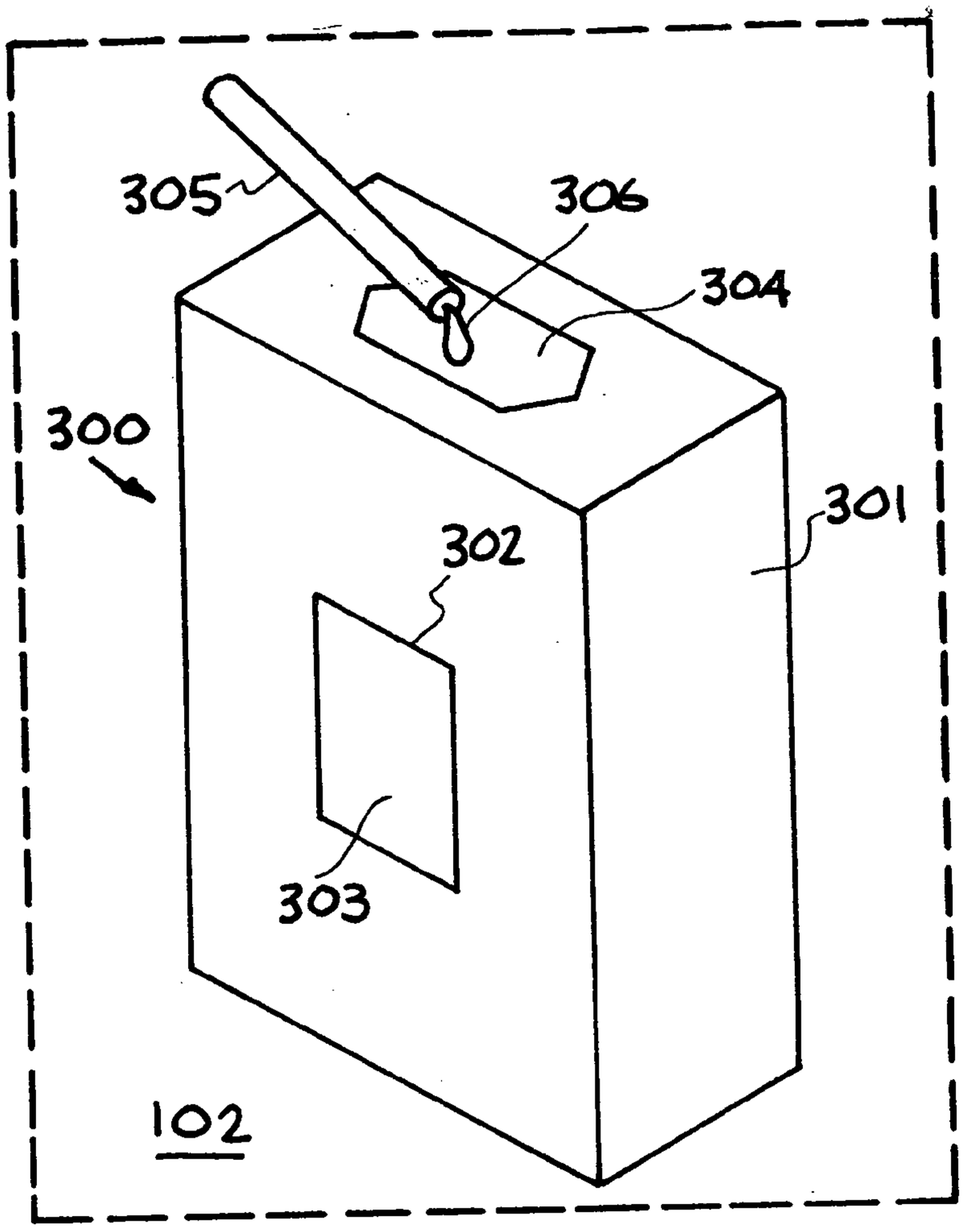

305
306
304
300
301
302
303
102

FIG.3

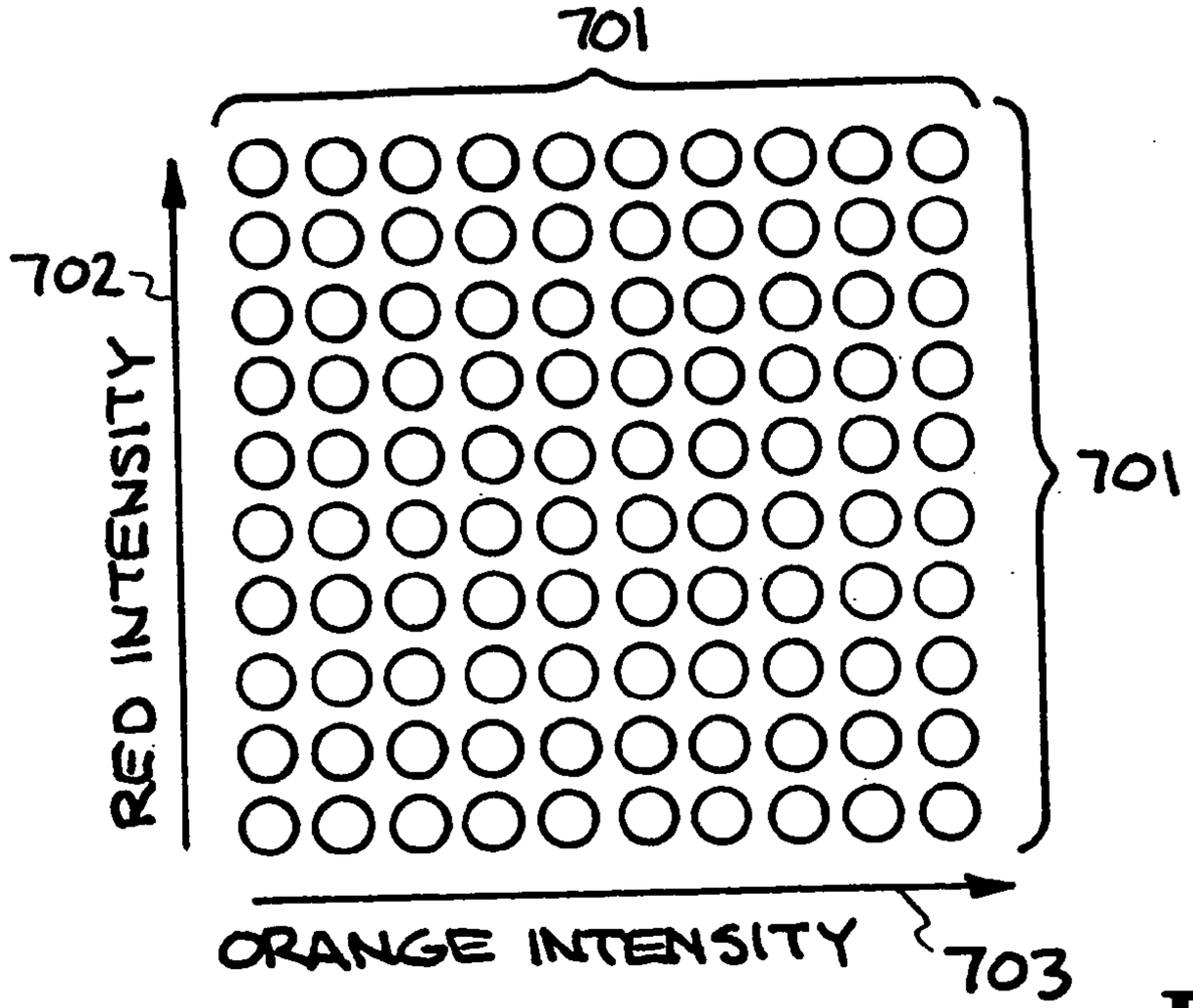

701
702
RED INTENSITY
701
ORANGE INTENSITY
703

FIG.7

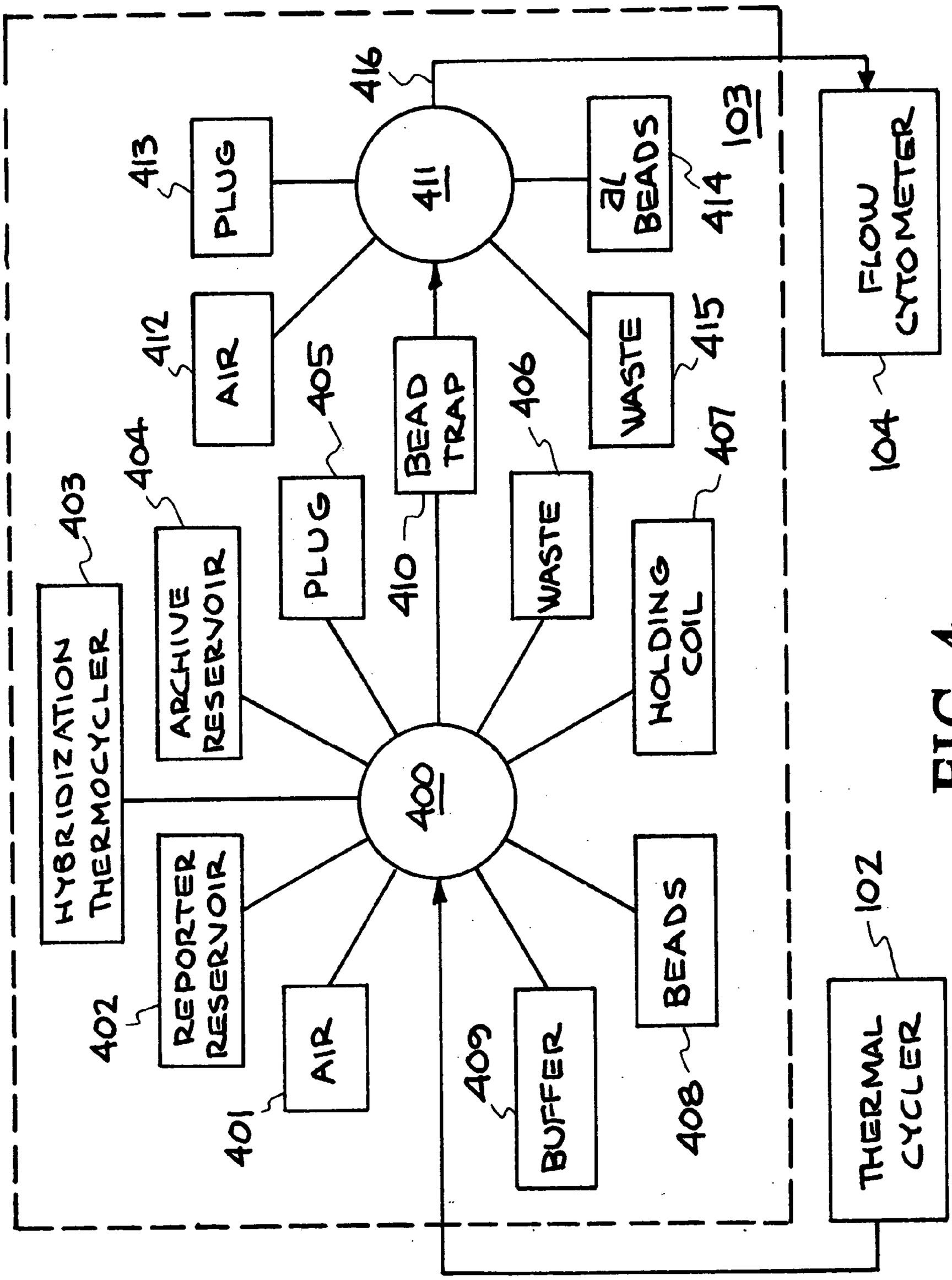

400
401
AIR
402
REPORTER RESERVOIR
403
HYBRIDIZATION THERMOCYCLER
404
ARCHIVE RESERVOIR
405
PLUG
406
WASTE
407
HOLDING COIL
408
BEADS
409
BUFFER
410
BEAD TRAP
411
412
AIR
413
PLUG
414
AL BEADS
415
WASTE
416
103
FLOW CYTOMETER
104
THERMAL CYCLER
102

FIG. 4

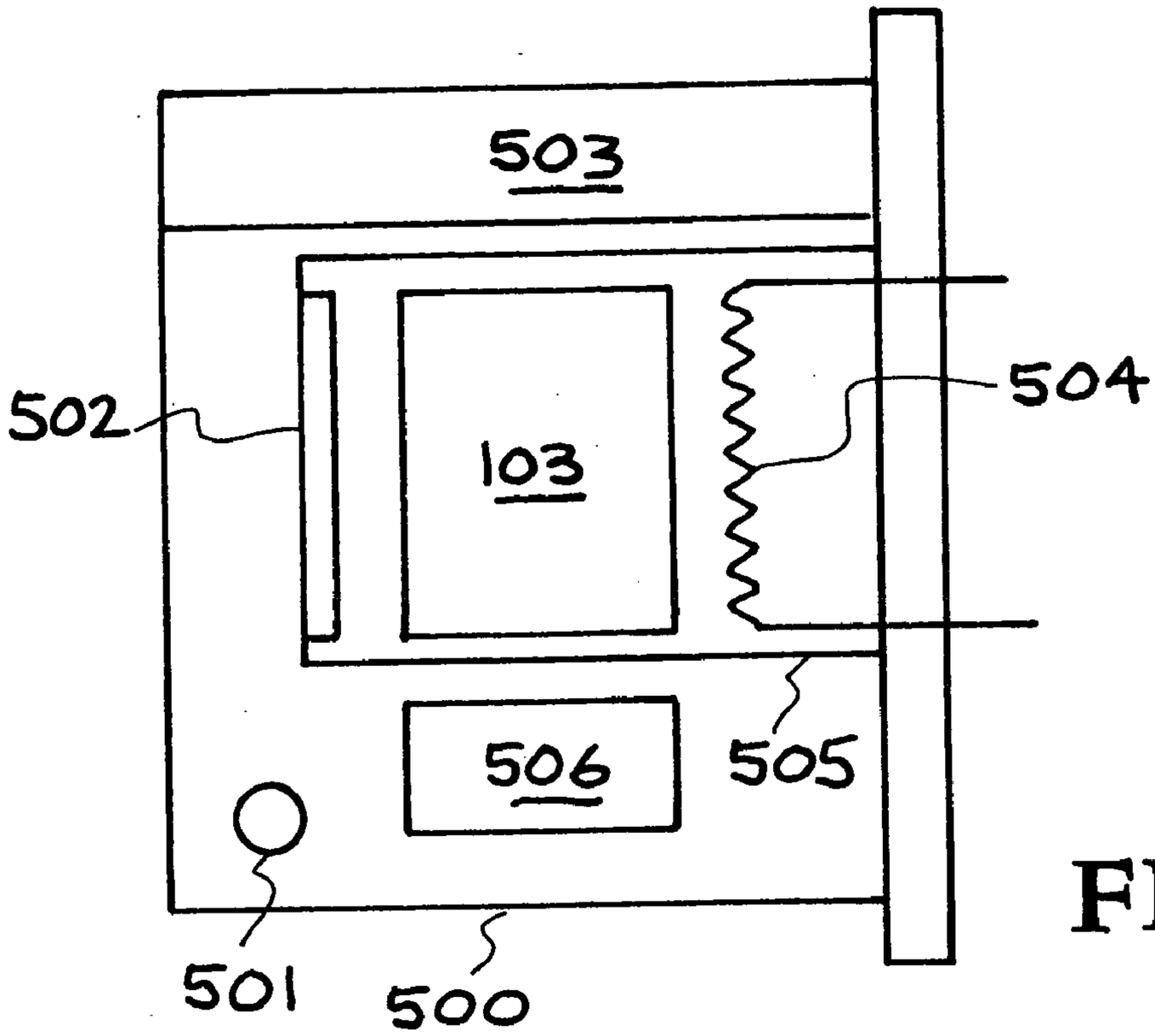

503
502
103
504
506
505
501
500

FIG. 5

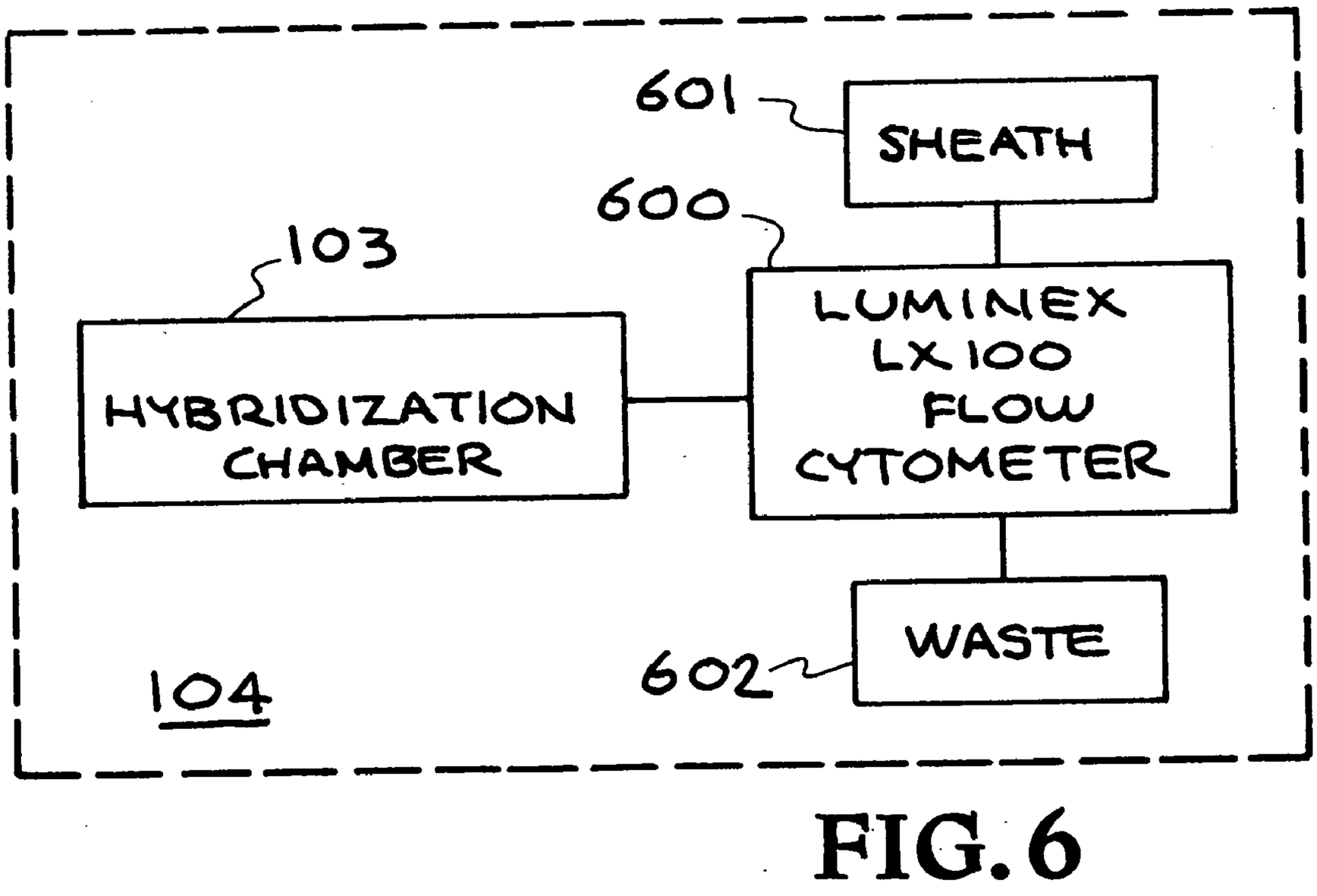

601
SHEATH
600
103
LUMINEX
LX100
FLOW
CYTOMETER
HYBRIDIZATION
CHAMBER
WASTE
602
104

FIG. 6

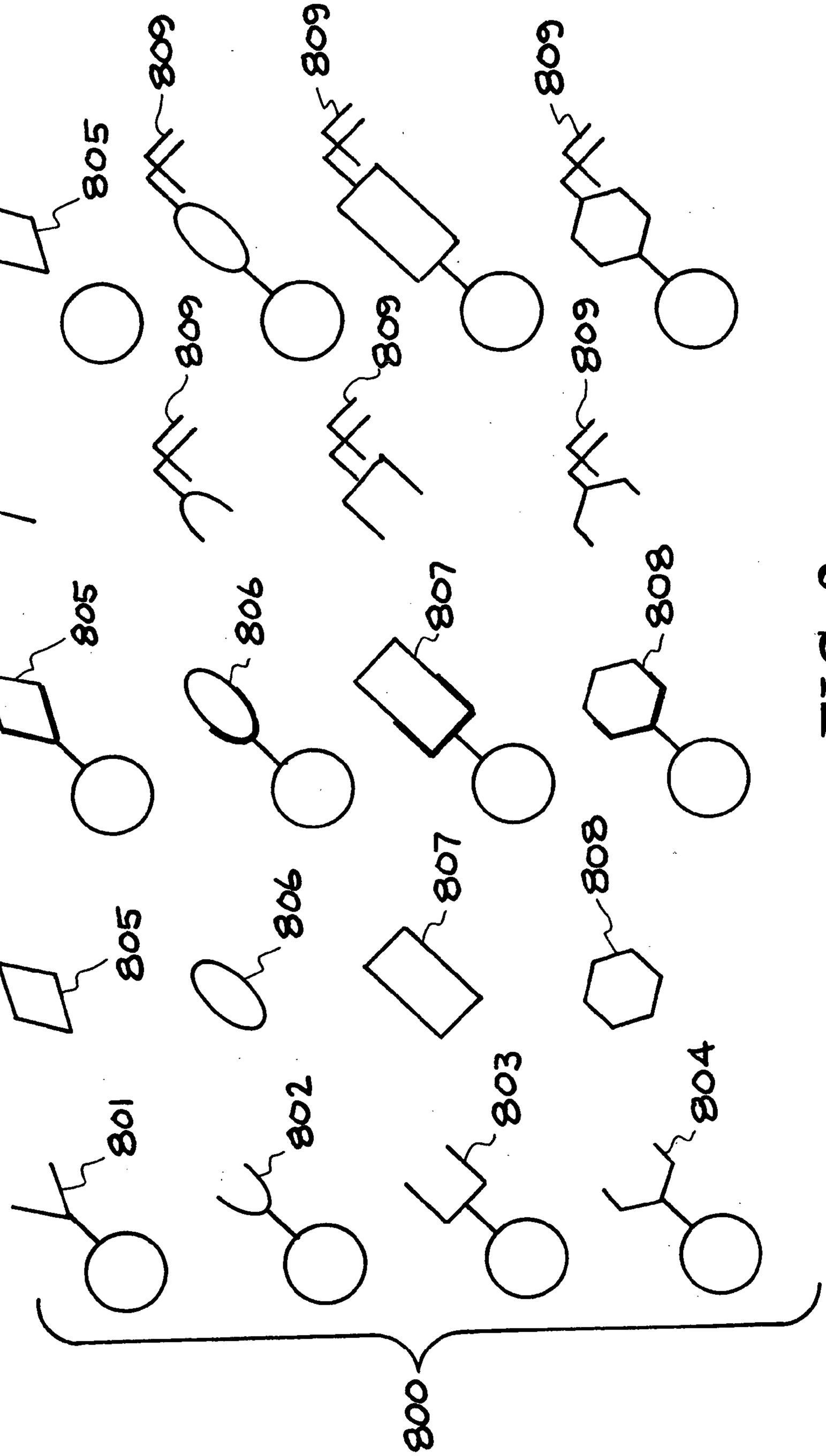


FIG. 8

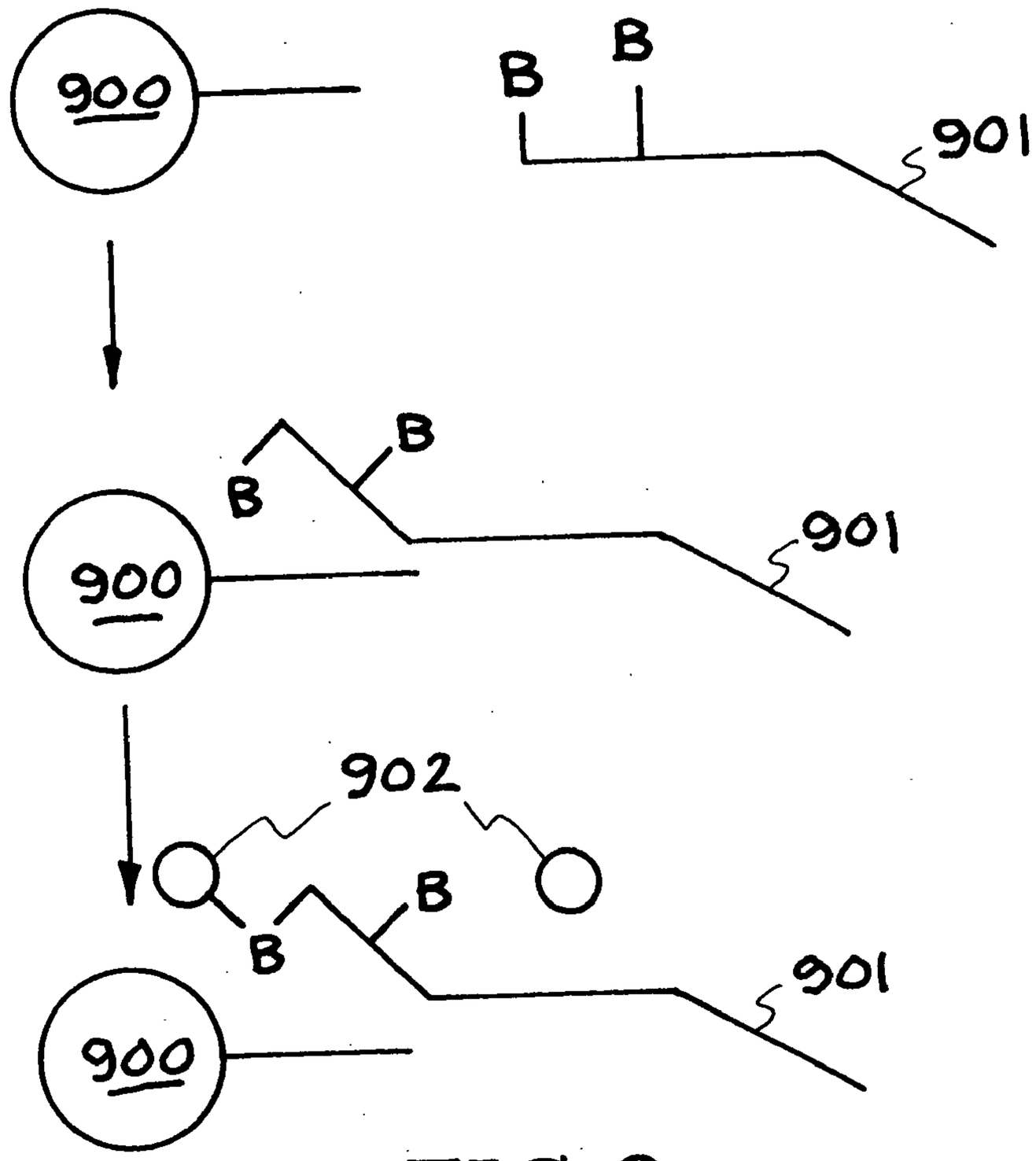


FIG.9

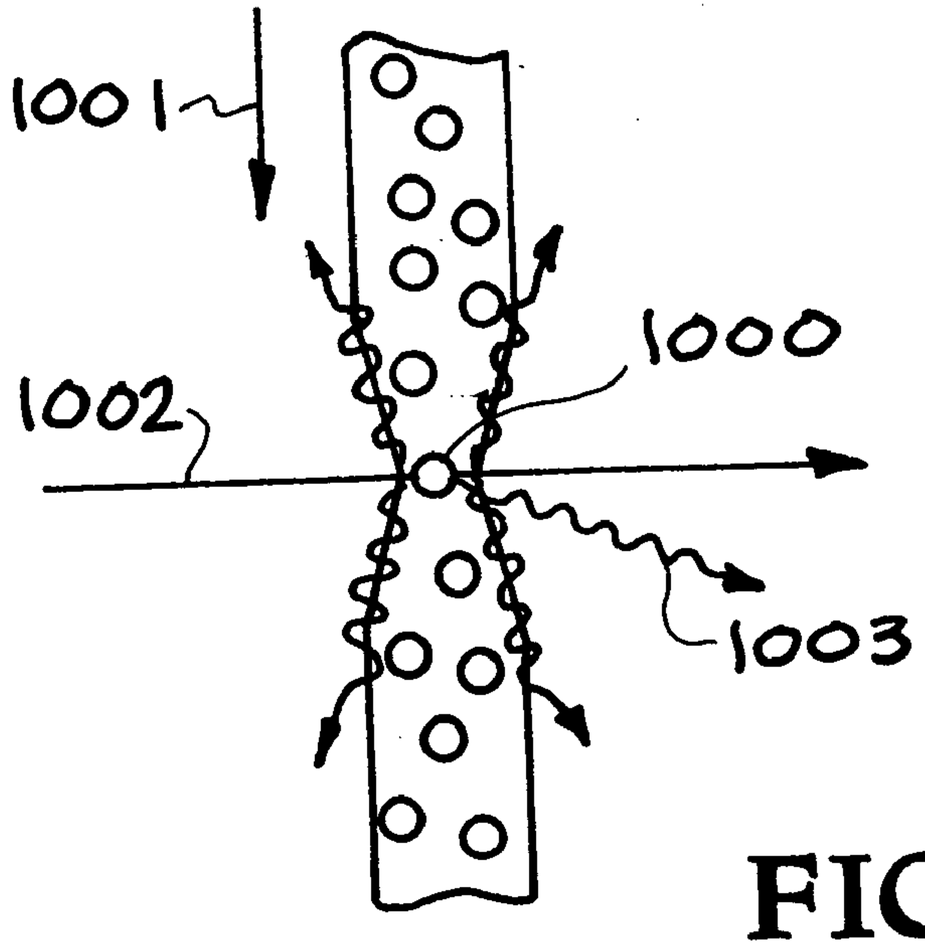


FIG.10

HYBRID AUTOMATED CONTINUOUS NUCLEIC ACID AND PROTEIN ANALYZER USING REAL-TIME PCR AND LIQUID BEAD ARRAYS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a Continuation-in-Part of co-pending U.S. patent application Ser. No. 10/643,797 filed Aug. 19, 2003 and titled, "System for Autonomous Monitoring of Bioagents." U.S. patent application Ser. No. 10/643,797 filed Aug. 19, 2003 claims the benefit of U.S. Provisional Patent Application No. 60/406,159 filed Aug. 26, 2002. U.S. patent application Ser. No. 10/643,797 filed Aug. 19, 2003 and U.S. Provisional Patent Application No. 60/406,159 filed Aug. 26, 2002 are incorporated herein by this reference.

[0002] The United States Government has rights in this invention pursuant to Contract No. W-7405-ENG-48 between the United States Department of Energy and the University of California for the operation of Lawrence Livermore National Laboratory.

BACKGROUND

[0003] 1. Field of Endeavor

[0004] The present invention relates to an assay system and more particularly to a nucleic acid assay system.

[0005] 2. State of Technology

[0006] U.S. Pat. No. 4,022,575 for an automatic chemical analyzer to Elo H. Hansen and Jaromir Ruzicka issued May 10, 1977 provide the following background information, "The ever increasing demand for numbers of analyses in clinical, agricultural, pharmaceutical and other types of analytical control has lead to the development of a large number of various instruments for automated analysis. The development in this field is further being stimulated by the additional advantages gained by automation: increased precision, decreased cost per assay and good reliability of the automated equipment."

[0007] U.S. Pat. No. 5,589,136 for silicon-based sleeve devices for chemical reactions, assigned to the Regents of the University of California, inventors: M. Allen Northrup, Raymond P. Mariella, Jr., Anthony V. Carrano, and Joseph W. Balch, patented Dec. 31, 1996 provides the following background information: "Current instruments for performing chemical synthesis through thermal control and cycling are generally very large (table-top) and inefficient, and often they work by heating and cooling of a large thermal mass (e.g., an aluminum block). In recent years efforts have been directed to miniaturization of these instruments by designing and constructing reaction chambers out of silicon and silicon-based materials (e.g., silicon, nitride, polycrystalline silicon) that have integrated heaters and cooling via convection through the silicon. Microfabrication technologies are now well known and include sputtering, electrodeposition, low-pressure vapor deposition, photolithography, and etching. Microfabricated devices are usually formed on crystalline substrates, such as silicon and gallium arsenide, but may be formed on non-crystalline materials, such as glass or certain polymers. The shapes of crystalline devices can be precisely controlled since etched surfaces are generally crystal planes, and crystalline materials may be bonded by processes such as fusion at elevated temperatures, anodic bonding, or field-assisted methods. Monolithic microfabrication technology now enables the production of electrical, mechanical, electromechanical, optical, chemical and thermal devices, including pumps, valves, heaters, mixers, and detectors for microliter to nanoliter quantities of gases, liquids, and solids. Also, optical waveguide probes and ultrasonic flexural-wave sensors can now be produced on a microscale. The integration of these microfabricated devices into a single system allows for the batch production of microscale reactor-based analytical instruments. Such integrated microinstruments may be applied to biochemical, inorganic, or organic chemical reactions to perform biomedical and environmental diagnostics, as well as biotechnological processing and defection. The operation of such integrated microinstruments is easily automated, and since the analysis can be performed in situ, contamination is very low. Because of the inherently small sizes of such devices, the heating and cooling can be extremely rapid. These devices have very low power requirement and can be powered by batteries or by electromagnetic, capacitive, inductive or optical coupling. The small volumes and high surface-area to volume ratios of microfabricated reaction instruments provide a high level of control of the parameters of a reaction. Heaters may produce temperature cycling or ramping; while sonochemical and sonophysical changes in conformational structures may be produced by ultrasound transducers; and polymerizations may be generated by incident optical radiation. Synthesis reactions, and especially synthesis chain reactions such as the polymerase chain reaction (PCR), are particularly well-suited for microfabrication reaction instruments. PCR can selectively amplify a single molecule of DNA (or RNA) of an organism by a factor of 10.sup.6 to 10.sup.9. This well-established procedure requires the repetition of heating (denaturing) and cooling (annealing) cycles in the presence of an original DNA target molecule, specific DNA primers, deoxynucleotide triphosphates, and DNA polymerase enzymes and cofactors. Each cycle produces a doubling of the target DNA sequence, leading to an exponential accumulation of the target sequence. The PCR procedure involves: 1) processing of the sample to release target DNA molecules into a crude extract; 2) addition of an aqueous solution containing enzymes, buffers deoxyribonucleotide triphosphates (dNTPS), and aligonucleotide primers; 3) thermal cycling of the reaction mixture between two or three temperatures (e.g., 90. degree.-96.degree., 72.degree., and 37.degree.-55.degree. C.); and 4) detection of amplified DNA. Intermediate steps, such as purification of the reaction products and the incorporation of surface-bending primers, for example, may be incorporated in the PCR procedure. A problem with standard PCR laboratory techniques is that the PCR reactions may be contaminated or inhibited by the introduction of a single contaminant molecule of extraneous DNA, such as those from previous experiments, or other contaminants, during transfers of reagents from one vessel to another. Also, PCR reaction volumes used in standard laboratory techniques are typically on the order of 50 microliters. A thermal cycle typically consists of four stages: heating a sample to a first temperature, maintaining the sample at the first temperature, cooling the sample to a second lower temperature, and maintaining the temperature at that lower temperature. Typically, each of these four stages of a thermal cycle requires about one minute, and thus to complete forty cycles,

for example, is about three hours. Thus, due to the large volume typically used in standard laboratory procedures, the time involved, as well as the contamination possibilities during transfers of reagents from one vessel to another, there is clearly a need for microinstruments capable of carrying out the PCR procedure."

[0008] In an article titled, "U.S. Is Deploying a Monitor System for Germ Attacks," by Judith Miller in The New York Times on Jan. 22, 2003, it was reported, "To help protect against the threat of bioterrorism, the Bush administration on Wednesday will start deploying a national system of environmental monitors that is intended to tell within 24 hours whether anthrax, smallpox and other deadly germs have been released into the air, senior administration officials said today. The system uses advanced data analysis that officials said had been quietly adapted since the September 11 attacks and tested over the past nine months. It will adapt many of the Environmental Protection Agency's 3,000 air quality monitoring stations throughout the country to register unusual quantities of a wide range of pathogens that cause diseases that incapacitate and kill . . . . The new environmental surveillance system uses monitoring technology and methods developed in part by the Department of Energy's national laboratories. Samples of DNA are analyzed using polymerase chain reaction techniques, which examine the genetic signatures of the organisms in a sample, and make rapid and accurate evaluations of that organism . . . . Officials who helped develop the system said that tests performed at Dugway Proving Ground in Utah and national laboratories showed that the system would almost certainly detect the deliberate release of several of the most dangerous pathogens. 'Obviously, the larger the release, the greater the probability that the agent will be detected,' an official said. 'But given the coverage provided by the E.P.A. system, even a small release, depending on which way the wind was blowing and other meteorological conditions, is likely to be picked up.'"

[0009] In an article titled, "Biodetectors Evolving, Monitoring U.S. Cities," by Sally Cole in the May 2003 issue of Homeland Security Solutions, it was reported, "The anthrax letter attacks of 2001, and subsequent deaths of five people, brought home the reality of bioterrorism to Americans and provided a wake-up call for the U.S. government about the need for a method to detect and mitigate the impact of any such future attacks. Long before the anthrax letter attacks, scientists at two of the U.S. Department of Energy's national laboratories, Lawrence Livermore National Laboratory (LLNL) and Los Alamos National Laboratory (LANL), were busy pioneering a "biodetector" akin to a smoke detector to rapidly detect the criminal use of biological agents. This technology is now expected to play a large role in the U.S. government's recently unveiled homeland security counter-terrorism initiative, Bio-Watch, which is designed to detect airborne bioterrorist attacks on major U.S. cities within hours. Announced back in January, Bio-Watch is a multi-faceted, multi-agency program that involves the U.S. Department of Energy, the Environmental Protection Agency (EPA), and the U.S. Department of Health and Human Services' Centers for Disease Control and Prevention (CDC). Many of the EPA's 3,000 air-quality monitoring stations throughout the country are being adapted with biodetectors to register unusual quantities of a wide range of pathogens that cause diseases that incapacitate and kill, according to the EPA. The nationwide network of environmental monitors and biodetectors, which reportedly will eventually monitor more than 120 U.S. cities, is expected to detect and report a biological attack within 24 hours. Citing security reasons, the EPA declined to disclose further details about the program at this time . . . . The Autonomous Pathogen Detection System (APDS) is a file-cabinet-sized machine that sucks in air, runs tests, and reports the results itself. APDS integrates a flow cytometer and real-time PCR detector with sample collection, sample preparation, and fluidics to provide a compact, autonomously operating instrument capable of simultaneously detecting multiple pathogens and/or toxins. 'The system is designed for fixed locations,' says Langlois, 'where it continuously monitors air samples and automatically reports the presence of specific biological agents. APDS is targeted for domestic applications in which the public is at high risk of exposure to covert releases of bioagents—subway systems, transportation terminals, large office complexes, and convention centers . . . . APDS provides the ability to measure up to 100 different agents and controls in a single sample,' Langlois says. 'It's being used in public buildings right now.' The latest evolution of the biodetector, APDS-II, uses bead-capture immunoassays and a compact flow cytometer for the simultaneous identification of multiple biological simulants. Laboratory tests have demonstrated the fully autonomous operation of APDS-II for as long as 24 hours."

SUMMARY

[0010] Features and advantages of the present invention will become apparent from the following description. Applicants are providing this description, which includes drawings and examples of specific embodiments, to give a broad representation of the invention. Various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this description and by practice of the invention. The scope of the invention is not intended to be limited to the particular forms disclosed and the invention covers all modifications, equivalents, and alternatives falling within the spirit and scope of the invention as defined by the claims.

[0011] The present invention provides a hybrid nucleic acid assay system for analyzing a sample using a reagent comprising a thermal cycler, a sample and reagent delivery unit operatively connected to the thermal cycler for delivering the sample and the reagent to the thermal cycler, a hybridization chamber operatively connected to the thermal cycler, and a flow cytometer operatively connected to the hybridization chamber. The present invention also provides a real time nucleic acid assay method for analyzing a sample using a reagent. The method comprises the steps of providing a thermal cycler, providing a hybridization unit, providing a flow cytometer, transporting the sample and the reagent to said thermal cycler for amplification, and analyzing the sample with said flow cytometer utilize beads with each bead having a unique spectral address.

[0012] The hybrid nucleic acid analyzer system has many uses. For example, the hybrid nucleic acid analyzer system has use for clinical analysis of blood bank samples in a continuous 24/7 analysis of pathogens. The hybrid nucleic acid analyzer system has use in diagnostic labs in hospitals for toxin, protein nucleic acid and ménage analysis in clinical samples such as blood, saliva, urine, fecal matter, etc. The hybrid nucleic acid analyzer system has uses as fly

away lab or integrated into a continuous monitoring of environmental samples for detection of Biothreat agents. The hybrid nucleic acid analyzer system also has use in automated processing, amplification and detection of biological molecules in forensic samples. The hybrid nucleic acid analyzer system can also be used a point detector for automated clinical testing, analysis and archiving in event of an outbreak. The hybrid nucleic acid analyzer system can also be used to detect proteins and toxins both in the clinic as well as from the environment.

[**0013**] The invention is susceptible to modifications and alternative forms. Specific embodiments are shown by way of example. It is to be understood that the invention is not limited to the particular forms disclosed. The invention covers all modifications, equivalents, and alternatives falling within the spirit and scope of the invention as defined by the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[**0014**] The accompanying drawings, which are incorporated into and constitute a part of the specification, illustrate specific embodiments of the invention and, together with the general description of the invention given above, and the detailed description of the specific embodiments, serve to explain the principles of the invention.

[**0015**] **FIG. 1** is a fluidic diagram of illustrates one embodiment of a hybrid nucleic acid analyzer constructed in accordance with the present invention.

[**0016**] **FIG. 2** shows additional details of the reagent delivery system of the hybrid nucleic acid analyzer system illustrated in **FIG. 1**.

[**0017**] **FIG. 3** shows additional details of the thermal cycler of the hybrid nucleic acid analyzer system illustrated in **FIG. 1**.

[**0018**] **FIG. 4** shows additional details of the hybridization chamber of the hybrid nucleic acid analyzer system illustrated in **FIG. 1**.

[**0019**] **FIG. 5** shows schematics of the hybridization chamber illustrated in **FIG. 4**.

[**0020**] **FIG. 6** shows additional details of the flow cytometer of the hybrid nucleic acid analyzer system illustrated in **FIG. 1**.

[**0021**] **FIG. 7** shows the beads used in the hybridization chamber and the flow cytometer illustrated in **FIG. 1**.

[**0022**] **FIG. 8** illustrates how the beads are used in the hybridization chamber and the flow cytometer illustrated in **FIG. 1**.

[**0023**] **FIG. 9** provides additional information illustrating how the beads are used in the hybridization chamber and in the flow cytometer illustrated in **FIG. 1**.

[**0024**] **FIG. 10** illustrates how the beads are analyzed in the flow cytometer shown in **FIG. 6**.

DETAILED DESCRIPTION OF THE INVENTION

[**0025**] Referring to the drawings, to the following detailed description, and to incorporated materials, detailed information about the invention is provided including the description of specific embodiments. The detailed description serves to explain the principles of the invention. The invention is susceptible to modifications and alternative forms. The invention is not limited to the particular forms disclosed. The invention covers all modifications, equivalents, and alternatives falling within the spirit and scope of the invention as defined by the claims.

[**0026**] Nucleic acid amplification and detection is a widely used technique for conducting biological research. Utilization is applied to an increasing range of applications from diagnostics in bench-top research to the clinical arena, to genomic screening for drug discovery, to toxicology screening for contamination, to identification in a high throughput or conventional analysis capacity. Conventional sample preparation and analysis techniques for performing nucleic acid assays are time-consuming, require trained technicians, and lack precise repeatability. New technical developments are needed to improve the performance of nucleic acid amplification and detection.

[**0027**] Early attempts to automate analytical science turned to robotics, but the high cost of instrumentation and excessive complexity demanded large budgets both in terms of hardware and research effort. With the rapid growth in genomics, and proteomics, and high throughput screening techniques, robotics has enjoyed resurgence. The requirement for large hardware budgets and research resources has not changed.

[**0028**] Referring now to **FIG. 1**, a fluidic diagram of illustrates one embodiment of a hybrid nucleic acid analyzer constructed in accordance with the present invention. This embodiment of the hybrid nucleic acid analyzer is designated generally by the reference numeral **100**. Nucleic acid and protein analyses are usually done on separate analysis platforms. Nucleic acid detection is conventionally done by either Real-time Taqman PCR detection or an end point electrophoresis. Enzyme Linked Immuno assay (ELISA) is the method of choice for protein/antigen analysis.

[**0029**] One embodiment of the hybrid nucleic acid analyzer **100** provides a system for detecting and analyzing the samples: one based on PCR and the other based on flow cytometry. The flow cytometry subsystem uses antibodies to identify pathogens. For the flow-cytometry subsystem, small "capture" beads that are 5 micrometers in diameter are coated with antibodies specific to the target pathogens. The beads are color coded according to which antibodies they hold. Once the pathogens attach to their respective antibodies, more antibodies—those labeled with a fluorescent dye—are added to the mix. A labeled antibody will stick to its respective pathogen, creating a sort of bead sandwich-antibody, pathogen, and labeled antibody. The beads flow one by one through a flow cytometer, which illuminates each bead in turn with a laser beam. Any bead with labeled antibodies will fluoresce. The system can then identify which agents are present, depending on the color of the capture bead.

[**0030**] The hybrid nucleic acid analyzer system **100** comprises a reagent delivery system **101**, a thermal cycler **102**, a hybridization chamber **103**, and a flow cytometer **104**. The reagent delivery system **101** delivers PCR reagents to the thermal cycler **102** autonomously. On completion of cycling in the thermal cycler **102**, the reaction 5 μl is moved to the hybridization chamber **103**. The 5 μl is mixed with 1e6

Luminex beads/ml in the hybridization chamber **103** for hybridization. The hybridized beads are then moved to the flow cytometer **104** for analyses.

[**0031**] The hybrid nucleic acid analyzer system **100** utilizes nucleic acid amplification and detection and sample preparation and analysis techniques and information described in currently co-pending U.S. patent application Ser. Nos. 10/189,319 and 10/643,797, both of which are owed by the Regents of the University of California, the assignee of this application. U.S. patent application Ser. No. 10/189,319 for an "Automated Nucleic Acid Assay System" was filed Jul. 2, 2002 by Billy W. Colston, Jr., Steve B. Brown, Shanavaz L. Nasarabadi, Phillip Belgrader, Fred Milanovich, Graham Marshall, Don Olson, and Duane Wolcott and was published as U.S. patent application No. 2003/0032172 on Feb. 13, 2003. U.S. patent application Ser. No. 10/643,797 for a "System for Autonomous Monitoring of Bioagents" was filed Aug. 19, 2003 by Richard G. Langlois, Fred Milanovich, Billy W. Colston, Jr., Steve B. Brown, Don A. Masquelier, Ray P. Mariella, and Kodomundi Venkateswaran and was published as U.S. patent application No. 2004/0038385 on Feb. 26, 2004. The disclosures of U.S. patent application Ser. Nos. 10/189,319 and 10/643,797 are incorporated herein by this reference.

[**0032**] A LabView interface software system controls the fluidic handling and the operation of the thermal cycler **102**. The LabView interface software system software is integrated into a form compatible with the Graphical User Interface (GUI) used to control and monitor the flow cytometer **104**.

[**0033**] Referring now to **FIG. 2**, additional details of the reagent delivery system **101** of the hybrid nucleic acid analyzer system **100** are shown. The reagent delivery system **101** includes a syringe pump **200** that delivers a carrier **201** to a holding coil **202**. The carrier is available to a zone fluidics system. The zone fluidics system provides sequential injection analysis (SIA).

[**0034**] Zone fluidics defines a general-purpose fluidics tool, allowing the precise manipulation of gases, liquids and solids to accomplish very complex analytical manipulations with relatively simple hardware. Zone fluidics is the precisely controlled physical, chemical, and fluid-dynamic manipulation of zones of miscible and immiscible fluids in narrow bore conduits to accomplish sample conditioning and chemical analysis. A zone is a volume region within a flow conduit containing at least one unique characteristic.

[**0035**] A unit operation in zone fluidics comprises of a set of fluid handling steps intended to contribute to the transformation of the sample into a detectable species or prepare it for manipulation in subsequent unit operations. Examples of unit operations include sample filtering, dilution, enrichment, medium exchange, headspace sampling, solvent extraction, matrix elimination, de-bubbling, amplifying, hybridizing, and reacting. In current analytical practice many of these steps are handled manually or in isolated pieces of equipment. Integration is scant at best, and there is a high degree of analyst involvement. In zone fluidics, sample and reagent zones are subjected to these unit operations in a sequential manner being transported from one unit operation to the next under fluidic control.

[**0036**] Zone fluidics provides an alternative approach whereby unit operations are performed in narrow bore conduits and the transportation medium, instead of being mechanical as in robotics, is fluidic. At the heart of a zone fluidics manifold is a multi-position selection valve. Fluids are propelled and manipulated in the manifold by means of a bi-directional flow pump. A holding coil between the pump and valve is used to stack zones and mix adjacent zones through dispersion and diffusion as is practiced in sequential injection analysis (SIA).

[**0037**] The ports of the multi-position valve are coupled to various reservoirs, reactors, manifold devices, and detectors as indicated. Narrow bore conduits comprise the flow channels and provide fluid contact between manifold devices and components. The term fluid refers to liquids, gases, aerosols, and suspensions. Samples in zone fluidics are not limited to liquids. Rather, gases, and suspensions containing solids or cells are also included. Where solid samples are used, particles are limited to a size that ensures no blockages.

[**0038**] In most cases, reagents are prepared and then coupled to the zone fluidics manifold. The metering capability of the pump and mixing unit operations allow for reagents and standards to be prepared in situ. Reagents can therefore be presented to the zone fluidics manifold in an appropriately designed cartridge as ready-made, reagent concentrates, lyophilized, or crystalline form. Standards can be plumbed to the multi-position valve as discrete reservoirs providing the required range of concentrations. As for reagents though, standards can also be prepared in situ or diluted to cover a larger dynamic range.

[**0039**] In the reagent delivery system **101**, a syringe pump **200** delivers a carrier **201** to a holding coil **202**. The carrier is available to a zone fluidics system. The zone fluidics system provides in sequential injection analysis (SIA). The ports of a multi-position valve **203** of the zone fluidics sequential injection analysis system are coupled to air reservoir **204**, negative reservoir **205**, field sample reservoir **206**, reagent reservoir **207**, plug **208**, waste **209**, bleach reservoir **210**, and bleach reservoir **211** as indicated. The zone fluidics sequential injection analysis system has an outlet **212** that delivers PCR reagents to the thermal cycler **102**.

[**0040**] Referring now to **FIG. 3**, details of the thermal cycler **102** of the hybrid nucleic acid analyzer system **100** of the present invention are shown. Currently available Polymerase Chain Reaction (PCR) thermal cycling units are large cumbersome and non-portable. Some examples of commercially available semi-portable instruments include the iCycler manufactured by Bio-Rad, the Light cycler from Idaho Technologies and the Smart Cycler from Cepheid Inc. Real-time PCR works by including in a reaction mix sequence specific oligonucleotides (primer) that can be extended at its 3' end and a third non-extendable oligonucleotide (probe) that has two fluorescence molecules attached to its 5' and 3' end respectively. Thus the probe is quenched due to the Fluorescence Resonance Energy Transfer (FRET) between the two fluorescent molecules. FRET is dependent on the sixth power of the intermolecular separation of the two fluorophores. In the absence of primer extension, there is no fluorescence signal detected by the fluorimeter. The enzyme DNA polymerase has 5'-3' exo-nuclease activity as well as 5'-3' polymerase activity. During primer extension, the fluorophore is cleaved from the 5' end of the probe and since the fluorophore is no longer quenched, a signal is

detected by the fluorimeter. These instruments are designed for measuring the fluorescence released from sequence specific probes in case of a positive identification. At present the multiplexing of nucleic acid signatures is limited by the number of fluorophores that can be used in the commercial instruments, due to spectral overlap of most of these fluorophores.

[**0041**] The thermal cycler **102** of the present invention can be a unit such as that described in U.S. Pat. No. 5,589,136 issued Dec. 31, 1996 to M. Allen Northrup, Raymond P. Mariella, Jr., Anthony V. Carrano, and Joseph W. Balch and assigned to the Regents of the University of California or in U.S. Pat. No. 6,586,233 issued Jul. 1, 2003 to William J. Benett. James B. Richards, and Fred P. Milanovich and assigned to the Regents of the University of California. The disclosures of U.S. Pat. No. 5,589,136 issued Dec. 31, 1996 and U.S. Pat. No. 6,586,233 issued Jul. 1, 2003 are incorporated herein by this reference. As show in **FIG. 3**, a chamber unit **300** is fabricated of circuit board material. The system can be constructed of materials such as circuit board fiberglass, silicon, ceramics, metal, or glass. Advantages of using circuit board fiberglass is the fact that it is not as thermally conductive as the other materials and the heating is more efficiently applied to the sample rather than being conducted to surrounding materials. Circuit board material is readily available and the technology of producing and working with circuit board material is highly developed. Circuit board material provides lower cost techniques for fabrication. Printed circuit board technology incorporates photolithography, metal etching, numerically controlled machining, and layering technologies to produce the desired device.

[**0042**] As shown in **FIG. 3**, the thermal cycler **102** is generally indicated at **300**. The thermal cycler **102** includes a silicon-based sleeve as a chemical reaction chamber, generally indicated at **301**, constructed of two bonded silicon parts, and which utilizes doped polysilicon for heating and bulk silicon for convective cooling, as described in greater detail hereinafter. The sleeve **301** includes a slot or opening **304** into which reaction fluid, indicated at **306**, from a conduit **305** is inserted into the reaction chamber. The conduit **305** is constructed of plastic, for example, or other material which is inert with respect to the reaction mixture, thereby alleviating any potential material incompatibility issues. The sleeve is also provided with an opening **302** in which is located an optical window **303**, made, for example, of silicon nitride, silicon dioxide, or polymers. The silicon sleeve reaction chamber **301** includes doped polysilicon for heating and bulk silicon for convective cooling, and combines a critical ratio of silicon and silicon nitride to the volume of material to be heated (e.g., liquid) in order to provide uniform heating, yet low power requirements.

[**0043**] The thermal cycler **102** can be used to rapidly and repetitively provide controlled thermal cycles to the reaction mixture. The thermal conductivity properties of the silicon or similar semiconducting substrate, help speed up the thermal rise and fall times, and allow low power operation. While silicon is unique in its thermal properties, i.e., high thermal conductivity, a combination of silicon, silicon nitride, silicon dioxide, polymers and other materials would provide a combination of thermal conductivity and insulation that would allow thermal uniformity and low power operation.

[**0044**] The Sample and the nucleic acid reaction mix are introduced into the thermal cycler **102** by the Sequential Injection Analysis fluid handling system illustrated in **FIG. 2**. As the sample is continuously driven by convection through the channels it passes through sections of channel that are temperature controlled to be at the upper and lower temperatures required for the PCR reaction. This continuous flow through the PCR temperature zones effectively thermally cycles the sample.

[**0045**] Referring now to **FIG. 4**, additional details of the hybridization chamber **103** of the hybrid nucleic acid analyzer system **100** are shown. In the hybridization chamber **103** the amplified mix is hybridized to luminex beads in-line before being sent to the Flow Cytometer **104** for analysis. A multi-position valve **400** in the hybridization chamber **103** is coupled to air reservoir **401**, reporter reservoir **402**, hybridization thermocycler **403**, archive reservoir **404**, plug **405**, waste **406**, holding coil **407**, beads **408**, and bleach reservoir **409** as indicated. The hybridization chamber **103** uses a simple copper coil around the reaction chamber to heat the bead and PCR mixture to the denaturation temperature. After denaturation, the chamber is brought to its hybridization temperature of 55-60° C. with a fan placed at the bottom of the unit. The multi-position valve **400** delivers the beads and sample to the bead trap **410** and to a multi-position valve **411**. The multi-position valve **411** is coupled to air reservoir **412**, plug **413**, Cal beads **414**, and waste **415** as indicated. The hybridization chamber **103** has an outlet **416** that delivers the hybridized sample to the flow cytometer **104**.

[**0046**] Referring now to **FIG. 5**, schematics of the hybridization chamber **103** of the hybrid nucleic acid Analyzer are shown. A copper tube support **500** provides a support for the hybridization chamber **103** and its associated equipment. As shown in **FIG. 5**, the hybridization chamber **103** includes a temperature control sensor **501**, heatshrink insulation **502**, a muffin fan **503**, a foil heater **504**, tubing **505**, and a fan **506**. The heater **504** heats the chamber to the denaturation temperature of 95° C. followed by a cooling to the required hybridization temperature with the aid of the fans **503** and **506**. The PID temperature control sensor **501** precisely monitors the denaturation and hybridization temperatures within a couple of degrees. The hybridization chamber **103** provides a single step movement of hybridization reagents into the chamber.

[**0047**] In the hybridization chamber **103** the amplified mix is hybridized to luminex beads in-line before being sent to the Flow Cytometer **104** for analysis. The hybridization chamber **103** heats the bead and PCR mixture to the denaturation chamber. After denaturation, the chamber is brought to its hybridization temperature of 55-60° C. with the fans **503** and **506**. The hybridization chamber **103** has an outlet that delivers the hybridized sample to the flow cytometer **104**.

[**0048**] Referring now to **FIG. 6**, additional details of the flow cytometer **104** of the hybrid nucleic acid analyzer system **100** are shown. The flow cytometer **104** comprises a Luminex LX100 Flow Cytometer instrument **600** with a sheath source **601** and a waste reservoir **602**. The hybridized bead array is from the hybridization camber **103** is introduced into the Luminex Flow Cytometer instrument **600** where the beads are interrogated by two lasers, a red laser for the internal discriminator and a green laser for the external

discriminator dyes respectively. Additional details of the flow cytometer **600** and its operation are show in **FIGS. 7, 8, 9**, and **10**.

[**0049**] The protein (toxin or antigen) assay on the liquid bead arrays is a typical sandwich assay. The antibody specific to the antigen or a toxin is attached to the surface of Carboxylated polystyrene beads described above. The antigen is then hybridized to the bead sets followed by a secondary antibody to which is attached the secondary discriminator phycoerythrin.

[**0050**] In order to multiplex more than four signatures, Applicants have designed a Luminex Bead based Array analyzer. With the liquid arrays it is possible to multiplex over 100 different organisms. The discrimination of the polystyrene Luminex bead array is dependent on the precise ratio of two internal discriminator dyes, a red and an infrared dye. The signal intensity on the surface of the bead is dependent on the concentration of the analyte in solution, in our case the amplified DNA of a suspect agent or an antigen or a toxin, whichever the case may be.

[**0051**] Referring now to **FIG. 7**, the beads used in the hybridization chamber **103** and the flow cytometer **104** are illustrated. A 100-plex Luminex liquid array **700** is generated by intercalating varying ratios of red and orange infrared dyes into polystyrene latex microspheres or beads **701**. The process of producing varying ratios of red and orange infrared dyes in the beads **701** is accomplished by increasing the amount of red dye as illustrated by the arrow **702** and increasing the amount of orange dye as illustrated by the arrow **703**. This gives each optically encoded bead **700** a unique spectral address.

[**0052**] Referring now to **FIG. 8**, additional information is provided illustrating how the beads are used in the hybridization chamber **103** and the flow cytometer **104**. The beads designated by the reference numeral **800** are coated with capture antibodies specific for target antigens. Each bead has an attachment site specific for a bioagent. The upper bead has an attachment site **801** for anthrax. The next bead has an attachment site **802** for plague. The next bead has an attachment site **803** for small pox. The next bead has an attachment site **804** for botox. The attachment site **801** for anthrax attaches to the anthrax bioagent **805**. The attachment site **802** for plague attaches to the plague bioagent **806**. The attachment site **803** for small pox attaches to the small pox bioagent **807**. The attachment site **804** for botox attaches to the botox bioagent **808**. After incubation with the antigens, secondary or detector antibodies are added, followed by addition of the fluorescent reporter, phycoerythrin **809** to complete the "antigen sandwich."

[**0053**] Referring now to **FIG. 9**, additional information is provided illustrating how the beads are used in the hybridization chamber **103** and in the flow cytometer **104**. The beads are designated by the reference numeral **900**. **FIG. 9** illustrates bead hybridization with amplified PCR product. The PCR product **901** is added to labeled luminex bead mix and denatured. This is followed by hybridization and addition of SA-PE **902**. The hybridized beads are then detected in the Luminex flow cytometer **104**.

[**0054**] Referring now to **FIG. 10**, an illustration shows how the beads are analyzed in the flow cytometer. The beads are designated by the reference numeral **1000**. The direction of flow is shown by the arrow **1001**. The beads **1000** are interrogated one at a time. As illustrated, one bead **1000** is shown being interrogated. A red laser classifies the bead **1000**, identifying the bead type. Subsequently a green laser **1002** quantifies the assay on the bead surface—only those beads with a complete sandwich will produce a fluoresce **1003** in the green, and the signal is a function of antigen concentration.

[**0055**] The structural details of various embodiments of a hybrid nucleic acid analyzer constructed in accordance with the present invention having been illustrated in **FIGS. 1-10** and described above, the operation of the hybrid nucleic acid analyzer will now be considered. The hybrid nucleic acid analyzer provides an integrated nucleic acid and protein/toxin detection system capable of in-line analysis of a complex sample within an hour or less. The rate limiting step is the rapidity with which the nucleic acid is amplified, the hybridization being instantaneous. The hybrid nucleic acid analyzer has the capability of performing continuous nucleic acid and immunoassays in a multiplex format. The hybrid nucleic acid analyzer is a field deployable instrument for detection of pathogens and toxins in environmental or clinical samples. The hybrid nucleic acid analyzer takes advantage of the multiplexing capability of the Luminex Bead arrays complexed with multiplexed nucleic acid and protein capability developed at the Lawrence Livermore National Laboratory.

[**0056**] The hybrid nucleic acid analyzer has an integrated PCR chamber **102**, DNA Hybridization chamber **103**, and Luminex LX100 flow cytometer **104** controlled by a LabView interface software for the fluidic handling and the operation of the PCR chamber. The software is integrated into a form compatible with the Graphical User Interface (GUI) used to control and monitor the Luminex LX100 flow cytometer. Control and data analysis software routines have been written for controlling the Luminex LX100 flow cytometry. Provisions have been made for the addition of a sample preparation and concentration unit as well as a bead sequestering unit in order to facilitate deep multiplexing of the agents. A sample preparation and concentration strategy involves the use of Silicon pillar chips capable of handling volumes of up to 100 ml or more of the sample, releasing the DNA from the cells through lysis and concentrating it in a small volume for analysis, thus increasing the detection limit many folds.

[**0057**] The fluidics in the instrument is self-contained in order to minimize contamination of the surroundings and the operator. This minimizes contamination of reagents and samples, a feature not available in commercial units. The sample and the nucleic acid reaction mix are introduced into the thermal cycler **102** by Sequential Injection Analysis fluid handling system **101**.

[**0058**] The hybridization chamber **103** uses a simple copper coil around the reaction chamber to heat the bead and PCR mixture to the denaturation chamber. After denaturation, the chamber is brought to its hybridization temperature of 55-60° C. with a fan placed at the bottom of the unit. A thermocouple inserted in the housing of the hybridization chamber as well as on the silicon sleeve of the thermal cycling chamber is used to control the temperatures via feed back from a PID controller. In this instrument the reagents are pre-loaded onto the system so that there is minimal user interface.

[0059] Once the sample is introduced into the instrument **100**, the detection is autonomously done following the sequence of events input by the researcher. Decontamination of the fluidics system is carried out autonomously after each amplification step. The system including the PCR chamber **102**, the hybridization chamber **103**, the tubing carrying the sample to the PCR chamber and all the tubing and fitting downstream from there on are rinsed with 5% household bleach which we have found sufficient to effectively remove all traces of nucleic acids or PCR product from the housing. After every PCR run, a negative control for the agent/agents is amplified in order to determine the efficacy of the decontamination process.

[0060] Manual labor is the major factor for the high cost of sample testing. The software has the capability of stacking a series of fluidic protocols for autonomous analysis. Thus the instrument **100** can be loaded with the reagents and the samples at the beginning of the day and the results can be accessed from a remote location. This cuts the cost of labor as compared to the conventional way of doing analysis. Thus with this instrument it is possible to perform continuous analysis of samples from a known set of reagents with minimal intervention in effect significantly reducing the cost of the assay.

[0061] The hybrid nucleic acid analyzer **100** provides autonomous use of both the thermal cycler **102** and the flow cytometer **104** such that protein analysis can be performed independent of the nucleic acid detection. For detection of antigens or toxins, the sample is introduced directly to appropriately labeled beads followed by hybridization to the secondary antibody and analysis of the assay in the flow cytometer **104**. The hybrid nucleic acid analyzer **100** can be repeatedly decontaminated in between runs with a solution of 5% household bleach.

[0062] The nucleic acid detection is done by hybridization of the amplified PCR product with the probes attached to the surface of the bead sets via NHS ester linkage chemistry. The PCR product is labeled with Biotin molecules and the hybridization of the product to the beads is followed by streptavidin phycoerythrin addition to the hybridized reaction mix.

[0063] The hybrid nucleic acid analyzer system **100** provides a closed integrated rapid Real-time PCR and multiplex flow analysis instrument for identification of multiplex pathogen and toxin within an hour with minimal exposure to the technician. The hybrid nucleic acid analyzer system **100** combines Real-time flow through PCR with an inline flow cytometer to detect both nucleic acids as well as proteins. Sequential injection analysis (SIA) fluidic system is used to deliver the sample and reagent for in-line mixing, analysis and archiving of samples.

[0064] The unused PCR reaction mix is moved to the waste stream. The hybrid system is decontaminated and made ready for another round of amplification by rinsing with a 5% solution of Household Bleach followed by water rinse. A negative reaction with water substituted for sample is run between sample amplifications to ensure that the system is free of carry over PCR product.

[0065] The hybrid nucleic acid analyzer **100** has many uses. For example, the system **100** has use for clinical analysis of blood bank samples in a continuous 24/7 analysis of pathogens. The system **100** has use in diagnostic labs. The system **100** has use as a fly away lab or integrated into continuous monitoring of environmental samples for detection of Biothreat agents. The system **100** also has use in automated processing, amplification and detection of biological molecules in forensic samples. The system **100** can also be used for automated clinical testing, analysis and archiving in event of an outbreak. The system **100** can also be used to detect proteins and toxins both in the clinic as well as from the environment.

[0066] While the invention may be susceptible to various modifications and alternative forms, specific embodiments have been shown by way of example in the drawings and have been described in detail herein. However, it should be understood that the invention is not intended to be limited to the particular forms disclosed. Rather, the invention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the invention as defined by the following appended claims.

The invention claimed is:

1. A nucleic acid assay apparatus for analyzing a sample using a reagent, comprising:

a thermal cycler,

a sample and reagent delivery unit operatively connected to said thermal cycler for delivering the sample and the reagent to said thermal cycler,

a hybridization chamber operatively connected to said thermal cycler, and

a flow cytometer operatively connected to said hybridization chamber.

2. The nucleic acid assay apparatus of claim 1 wherein said hybridization chamber and said flow cytometer utilize beads with each bead having a unique spectral address.

3. The nucleic acid assay apparatus of claim 1 wherein said hybridization chamber and said flow cytometer utilize polystyrene latex microspheres beads with each bead having a unique spectral address.

4. The nucleic acid assay apparatus of claim 1 wherein said hybridization chamber and said flow cytometer utilize beads with varying ratios of red and orange infrared dyes giving each bead a unique spectral address.

5. The nucleic acid assay apparatus of claim 1 wherein said hybridization chamber and said flow cytometer utilize a 100-plex array of beads with varying ratios of red and orange infrared dyes giving each bead a unique spectral address.

6. The nucleic acid assay apparatus of claim 1 wherein said hybridization chamber and said flow cytometer utilize beads with each bead having a capture antibody specific for a target antigen.

7. The nucleic acid assay apparatus of claim 1 wherein said hybridization chamber and said flow cytometer utilize beads with at least one bead having an anthrax capture antibody specific for a target anthrax antigen.

8. The nucleic acid assay apparatus of claim 1 wherein said hybridization chamber and said flow cytometer utilize beads with at least one bead having a plague capture antibody specific for a target plague antigen.

9. The nucleic acid assay apparatus of claim 1 wherein said hybridization chamber and said flow cytometer utilize

beads with at least one bead having a small pox capture antibody specific for a target small pox antigen.

10. The nucleic acid assay apparatus of claim 1 wherein said hybridization chamber and said flow cytometer utilize beads with at least one bead having a botox capture antibody specific for a target botox antigen.

11. The nucleic acid assay apparatus of claim 1 wherein said hybridization chamber and said flow cytometer utilize beads with each bead having a capture antibody specific for a target antigen and a fluorescent reporter.

12. The nucleic acid assay apparatus of claim 1 wherein said flow cytometer utilizes at least one laser.

13. The nucleic acid assay apparatus of claim 1 wherein said flow cytometer utilizes a red laser.

14. The nucleic acid assay apparatus of claim 1 wherein said flow cytometer utilizes a green laser.

15. The nucleic acid assay apparatus of claim 1 wherein said flow cytometer utilizes a red laser and a green laser.

16. The nucleic acid assay apparatus of claim 1 wherein said hybridization chamber and said flow cytometer utilize beads with each bead having a fluorescent reporter and said flow cytometer utilizes at least one laser for bead interrogation by fluoresce of said fluorescent reporter.

17. The nucleic acid assay apparatus of claim 1 wherein said hybridization chamber and said flow cytometer utilize beads with each bead having a capture antibody specific for a target antigen and a fluorescent reporter and said flow cytometer utilizes at least one laser for bead interrogation.

18. The nucleic acid assay apparatus of claim 1 wherein said hybridization chamber and said flow cytometer utilize beads with each bead having a capture antibody specific for a target antigen and a fluorescent reporter and said flow cytometer utilizes a green laser for bead interrogation by fluoresce of said fluorescent reporter.

19. The nucleic acid assay apparatus of claim 1 wherein said hybridization chamber includes a heater.

20. The nucleic acid assay apparatus of claim 1 wherein said hybridization chamber includes a fan.

21. The nucleic acid assay apparatus of claim 1 wherein said hybridization chamber includes a heater and a fan.

22. The nucleic acid assay apparatus of claim 1 wherein said hybridization chamber includes a heater, a fan, and a temperature control sensor.

23. A nucleic acid assay apparatus for analyzing a sample using a reagent, comprising:

thermal cycler means,

sample and reagent delivery means operatively connected to said thermal cycler means for delivering the sample and the reagent to said thermal cycler means,

hybridization means operatively connected to said thermal cycler means, and

flow cytometer means operatively connected to said hybridization means.

24. The nucleic acid assay apparatus of claim 23 wherein said hybridization means and said flow cytometer means utilize beads with each bead having a unique spectral address.

25. The nucleic acid assay apparatus of claim 23 wherein said hybridization means and said flow cytometer means utilize polystyrene latex microspheres beads with each bead having a unique spectral address.

26. The nucleic acid assay apparatus of claim 23 wherein said hybridization means and said flow cytometer means utilize beads with varying ratios of red and orange infrared dyes giving each bead a unique spectral address.

27. The nucleic acid assay apparatus of claim 23 wherein said hybridization means and said flow cytometer means utilize a 100-plex array of beads with varying ratios of red and orange infrared dyes giving each bead a unique spectral address.

28. The nucleic acid assay apparatus of claim 23 wherein said hybridization means and said flow cytometer means utilize beads with each bead having a capture antibody specific for a target antigen.

29. The nucleic acid assay apparatus of claim 23 wherein said hybridization means and said flow cytometer means utilize beads with at least one bead having an anthrax capture antibody specific for a target anthrax antigen.

30. The nucleic acid assay apparatus of claim 23 wherein said hybridization means and said flow cytometer means utilize beads with at least one bead having a plague capture antibody specific for a target plague antigen.

31. The nucleic acid assay apparatus of claim 23 wherein said hybridization means and said flow cytometer means utilize beads with at least one bead having a small pox capture antibody specific for a target small pox antigen.

32. The nucleic acid assay apparatus of claim 23 wherein said hybridization means and said flow cytometer means utilize beads with at least one bead having a botox capture antibody specific for a target botox antigen.

33. The nucleic acid assay apparatus of claim 23 wherein said hybridization means and said flow cytometer means utilize beads with each bead having a capture antibody specific for a target antigen and a fluorescent reporter.

34. The nucleic acid assay apparatus of claim 23 wherein said flow cytometer means utilizes at least one laser.

35. The nucleic acid assay apparatus of claim 23 wherein said flow cytometer means utilizes a red laser.

36. The nucleic acid assay apparatus of claim 23 wherein said flow cytometer means utilizes a green laser.

37. The nucleic acid assay apparatus of claim 23 wherein said flow cytometer means utilizes a red laser and a green laser.

38. The nucleic acid assay apparatus of claim 23 wherein said hybridization means and said flow cytometer means utilize beads with each bead having a fluorescent reporter and said flow cytometer means utilizes at least one laser for bead interrogation by fluoresce of said fluorescent reporter.

39. The nucleic acid assay apparatus of claim 23 wherein said hybridization means and said flow cytometer means utilize beads with each bead having a capture antibody specific for a target antigen and a fluorescent reporter and said flow cytometer means utilizes at least one laser for bead interrogation.

40. The nucleic acid assay apparatus of claim 23 wherein said hybridization means and said flow cytometer means utilize beads with each bead having a capture antibody specific for a target antigen and a fluorescent reporter and said flow cytometer means utilizes a green laser for bead interrogation by fluoresce of said fluorescent reporter.

41. The nucleic acid assay apparatus of claim 23 wherein said hybridization means includes a heater.

42. The nucleic acid assay apparatus of claim 23 wherein said hybridization means includes a fan.

43. The nucleic acid assay apparatus of claim 23 wherein said hybridization means includes a heater and a fan.

44. The nucleic acid assay apparatus of claim 23 wherein said hybridization means includes a heater, a fan, and a temperature control sensor.

45. A nucleic acid assay method for analyzing a sample using a reagent, comprising the steps of:

providing a thermal cycler,

providing a hybridization unit,

providing a flow cytometer,

transporting the sample and the reagent to said thermal cycler for amplification, and

analyzing the sample with said flow cytometer utilize beads with each bead having a unique spectral address.

46. The nucleic acid assay method of claim 45 wherein said step of analyzing the sample with said flow cytometer comprises utilizing polystyrene latex microspheres beads with each bead having a unique spectral address.

47. The nucleic acid assay method of claim 45 wherein said step of analyzing the sample with said flow cytometer comprises utilizing beads with varying ratios of red and orange infrared dyes giving each bead a unique spectral address.

48. The nucleic acid assay method of claim 45 wherein said step of analyzing the sample with said flow cytometer comprises utilizing a 100-plex array of beads with varying ratios of red and orange infrared dyes giving each bead a unique spectral address.

49. The nucleic acid assay method of claim 45 wherein said step of analyzing the sample with said flow cytometer comprises utilizing beads with each bead having a capture antibody specific for a target antigen.

50. The nucleic acid assay method of claim 45 wherein said step of analyzing the sample with said flow cytometer comprises utilizing beads with at least one bead having an anthrax capture antibody specific for a target anthrax antigen.

51. The nucleic acid assay method of claim 45 wherein said step of analyzing the sample with said flow cytometer comprises utilizing beads with at least one bead having a plague capture antibody specific for a target plague antigen.

52. The nucleic acid assay method of claim 45 wherein said step of analyzing the sample with said flow cytometer comprises utilizing beads with at least one bead having a small pox capture antibody specific for a target small pox antigen.

53. The nucleic acid assay method of claim 45 wherein said step of analyzing the sample with said flow cytometer comprises utilizing beads with at least one bead having a botox capture antibody specific for a target botox antigen.

54. The nucleic acid assay method of claim 45 wherein said step of analyzing the sample with said flow cytometer comprises utilizing beads with each bead having a capture antibody specific for a target antigen and a fluorescent reporter.

* * * * *