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(54) Title: NEW APPARATUS AND METHODS FOR DISEASE DETECTION

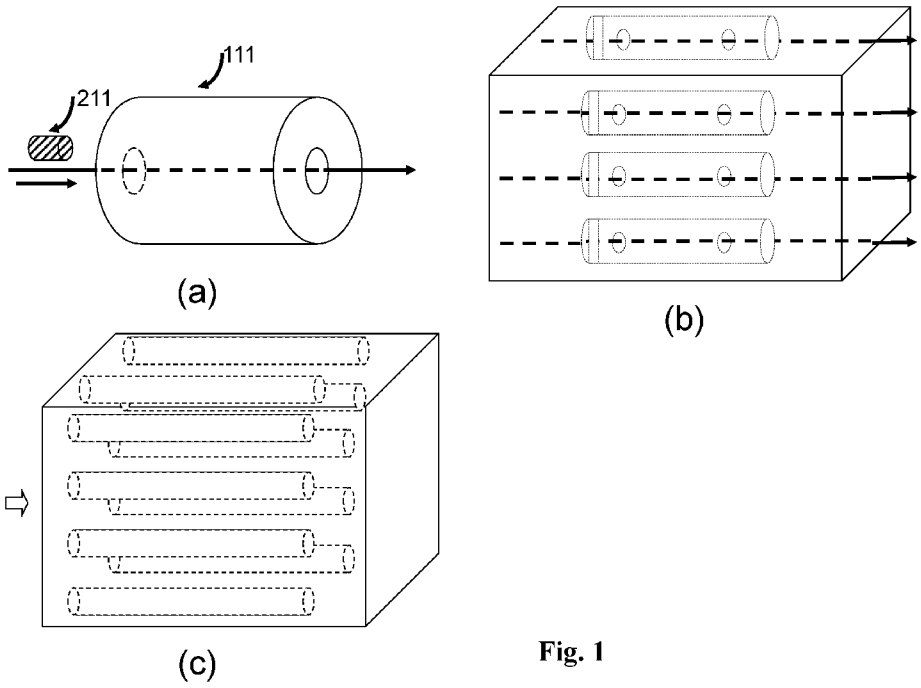

Fig. 1

(57) Abstract: The invention relates to apparatus and methods for apparatus for detecting presence or monitoring profession of a disease in a biological subject, comprising a chamber in which the biological subject passes through, and at least one detection transducer placed partially or completely in the chamber; wherein information related to properties of cells in the biological subject and of cell-surrounding media is detected by the detection transducer and collected for analysis to determine whether the disease is likely to be present with the biological subject or to determine the status of the disease, thereby providing the ability to continuously determine or monitor progression of the disease.

[Continued on next page]

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NEW APPARATUS AND METHODS FOR DISEASE DETECTION

Cross-Reference to Related Applications

[01] This application claims priority to US Application No. 62/661,361, filed April 23, 2018, US Application No. 62/678,846, filed May 31, 2018, US Application No. 62/741,843, filed October 5, 2018, US Application No. 62/776,605, filed December 7, 2018, US Application No. 62/818,909, filed March 15, 2019, and US Application No. 62/830,354, filed April 5, 2019, the contents of all of which are incorporated herein by reference in their entireties.

Background of the Invention

[02] Many diseases are difficult to be detected by a single approach or methodology. In particular, many serious diseases with high morbidity and mortality, including cancer and heart diseases, are difficult to diagnose at an early stage with high sensitively, specificity and efficiency, by using one piece of detection equipment. Current disease diagnosis devices typically detect and rely on a single type of macroscopic data and information such as body temperature, blood pressure, or scanned images of the body. For example, to detect serious diseases such as cancer, each of the diagnosis apparatus commonly used today is based on one imaging technology, such as x-ray, CT scan, or nuclear magnetic resonance (NMR). While used in combination, these diagnosis apparatuses provide various degrees of usefulness in disease diagnosis. However, each of them alone cannot provide accurate, conclusive, efficient, and cost-effective diagnosis of such serious diseases as cancer at an early stage. Further, many of the existing diagnosis apparatus have a large size and are invasive with large footprint, such as x-ray, CT scan, or nuclear magnetic resonance (NMR).

[03] Even the newly emerged technologies such as those deployed in DNA tests usually rely on a single diagnosis technology and cannot provide a comprehensive, reliable, accurate, conclusive, and cost-effective detection for a serious disease. In recent years, there have been some efforts in using nano technologies for various biological applications, with most of the work focused on one type of gene mapping and moderate developments in the field of disease detection. For instance, Pantel et al. discussed the use of a MicroEelectroMechanical Systems (MEMS) sensor for detecting cancer cells in blood and bone marrow *in vitro* (see, e.g., Klaus Pantel et al., *Nature Reviews*, 2008, 8, 329); Kubena et al. disclose in U.S. Patent Number

6,922,118 the deployment of MEMS for detecting biological agents; and Weissman et al. disclose in U.S. Patent Number 6,330,885 utilizing MEMS sensor for detecting accretion of biological matter.

[04] In sum, to date, most of above described technologies have been limited to isolated diagnosis technology for sensing, using systems of relatively simple constructions and large dimensions but often with limited functions, and lack sensitivities and specificities. Further, the existing technologies require multiple times detection by multiple apparatus. This will increase costs and affect achieved degree of sensitivity and specificity as well.

[05] Current cancer screening and prognosis IVD methods typically include bio-markers, circulating tumor cells (CTC), and genomics (such as circuiting tumor-DNA (ct-DNA)). While each of the above-mentioned technology offer a number of advantages, they also have a number of limitations, which include inability to detect cancer early, relatively low sensitivity and specificity, and in some cases, inability to detect certain types of cancer (for example, esophageal cancer and brain tumor). Bio-markers are not effective for early stage cancer detection, but also lack markers for a number of cancer types. In the case of CTC and ct-DNA, signals occur only after solid tumor has been formed, making early stage cancer detection relatively. See, e.g., Jiasong Ji et al., J Clin Oncol 33, 2015; Xuedong Du et al., J Clin Oncol 33, 2015; Geng Xi Jiang et al., J Clin Oncol 33, 2015; Hongmei Tao et al., J Clin Oncol 33, 2015; Chetan Bettegowda et al., Science Translational Medicine, 2014, 6 (224):224; J Phallen et al., Science Translational Medicine, 2017, 9 (403): 2415; BL Khoo et al., Science Advances, 2016, 2 (7):e1600274; I Garcia-Murillas et al., Science Translational Medicine, 2015, 7 (302): 302; C Abbosh et al., Nature, 2017, 545 (7655):446-451; RS Herbst et al., and Nature, 2018, 553 (7689):446.

[06] These drawbacks call for novel solutions that provide reliable and flexible diagnosis apparatus using multiple diverse technologies and bring improved accuracy, sensitivity, specificity, efficiency, non-invasiveness, practicality, conclusive, and speed in early-stage disease detection at reduced costs.

Summary of the Invention

[07] The present invention in general relates to a novel technology for detecting disease, in which a number of different classifications of biological information are collected in a device and processed or analyzed.

[08] It also relates to a novel technology for assessing risk levels of disease and cancer occurrence, and differentiating healthy individuals from possible disease or cancer individuals.

[09] In traditional technology, typically only one level of biological information is collected (one dimensional), while in this novel technology, at least two levels (classifications) of information can be collected (seven dimensional, or seven factor interactions). Compared with traditional technology which typically focuses on one parameter or one level (for example, bio-marker at protein level), signal and information collected in this novel technology can be collected in a number of forms, and non-linearly amplified. There are additional 2-factor and three-factor interactions which can be collected and analyzed, which maybe missing in other technologies, since they typically only measure one type of biological information.

[010] This novel technology can be used for cancer screening, assisting in diagnosis, prognosis, and follow-up tests with improved sensitivity and specificity, ability to detect cancer early, ability to detect major diseases, pre-cancer diseases and over 20 types of cancer, cost effective, and no side effects.

[011] The novel technology offers several advantages that cannot be achieved by the traditional technology: (1) ability to detect over 20 cancer types in one test, including some cancer types which cannot be detected by other in vitro tests (e.g., esophageal cancer, cerebral cancer), covering over 80% of all cancer incidences; (2) capability of early stage cancer detection; (3) high sensitivity and specificity (75%～ 90% on over 20 types of cancer); (4) no side effects; (5) high speed, fully automated operations without human intervention; (6) statistical difference between cancer group and non-cancer disease group including inflammation – significantly lower false positives (higher specificity); (7) easy process, no difference between fasting blood testing and non-fasting blood testing, and (8) highly cost effective.

[012] Accordingly, one aspect of this invention relates to an apparatus for detecting presence or monitoring progression of a disease in a biological subject, comprising a chamber in which the biological subject passes through, and at least one detection transducer placed partially or completely in the chamber; wherein information related to properties of cells in the biological subject and of cell-surrounding media is detected by the detection transducer and collected for analysis to determine whether the disease is likely to be present with the biological subject or to determine the status of the disease, thereby providing the ability to continuously determine or monitor progression of the disease.

[013] The information can be collected over the course of months to years to monitor the change in the said information. The information can be utilized to track and screen diseases comprising cardiovascular diseases, diabetes, liver diseases, lung diseases, and cancer. The information can also be utilized to track evolution from healthy stage to disease stage, to pre-caner state, to early cancer stage, and to late cancer stage. The evolution can be continuous and monitored continuously. The information and its evolution also can be utilized to screen and diagnosis disease status and stage.

[014] In some embodiments, the properties of the cells and cell-surrounding media comprise cell signaling, cell surface properties, or cell-to-cell interaction properties; and the detected information is collected for analysis to as to whether the disease is likely to be present with or within the biological subject. For example, the cell surface properties can include cell surface tension, cell surface area, cell surface charge, cell surface hydrophobicity, cell surface potential, cell surface protein types and compositions, cell surface bio-chemical components, cell surface signaling properties, cell surface mutations, or cell surface biological components; the cell to cell interaction properties can include cell to cell affinity, cell to cell repulsion, mechanical force, electrical force, gravitational force, chemical bonding, bio-chemical interactions, geometrical matching, bio-chemical matching, chemical matching, physical matching, biological matching, or cell to cell signaling properties; the cell to cell signaling properties can include signaling method, signaling strength, cell surrounding media its properties to which signal is transmitted, or signaling frequency; and the cell signaling can include cell signal type, cell signal strength, cell signal frequency, cell interactions with cell media to which cell signal is transmitted, or cell interactions with other biological entities to which signal is transmitted..

[015] In some embodiments, the cell surrounding media can include blood, proteins, red blood cells, while blood cells, T cells, other cells, gene mutations, DNA, RNA, or other biological entities.

[016] In some embodiments, the cell surrounding media properties include a thermal, optical, acoustical, biological, chemical, physical-chemical, electro-mechanical, electro-chemical, electro-chemical-mechanical, bio-physical, bio-chemical, bio-mechanical, bio-electrical, bio-physical-chemical, bio-electro-physical, bio-electro-mechanical, bio-electro-chemical, bio-chemical-mechanical, bio-electro-physical-chemical, bio-electro-physical-mechanical, bio-electro-chemical-mechanical, physical, an electric, magnetic, electro-magnetic, or mechanical

property. For example, the thermal property is temperature or vibrational frequency; the optical property is optical absorption, optical transmission, optical reflection, optical-electrical property, brightness, or fluorescent emission; the radiation property is radiation emission, signal triggered by radioactive material, or information probed by radioactive material; the chemical property is pH value, chemical reaction, bio-chemical reaction, bio-electro-chemical reaction, reaction speed, reaction energy, speed of reaction, oxygen concentration, oxygen consumption rate, ionic strength, catalytic behavior, chemical additives to trigger enhanced signal response, bio-chemical additives to trigger enhanced signal response, biological additives to trigger enhanced signal response, chemicals to enhance detection sensitivity, bio-chemicals to enhance detection sensitivity, biological additives to enhance detection sensitivity, or bonding strength; the physical property is density, shape, volume, or surface area; the electrical property is surface charge, surface potential, resting potential, electrical current, electrical field distribution, surface charge distribution, cell electronic properties, cell surface electronic properties, dynamic changes in electronic properties, dynamic changes in cell electronic properties, dynamic changes in cell surface electronic properties, dynamic changes in surface electronic properties, electronic properties of cell membranes, dynamic changes in electronic properties of membrane surface, dynamic changes in electronic properties of cell membranes, electrical dipole, electrical quadruple, oscillation in electrical signal, electrical current, capacitance, three-dimensional electrical or charge cloud distribution, electrical properties at telomere of DNA and chromosome, DNA surface charge, DNA surrounding media electrical properties, quantum mechanical effects, capacitance, or impedance; the biological property comprises protein, cell, genomics, cellular properties (which comprise chemical, physical, bio-chemical, bio-physical, and biological aspects of surrounding liquid, gas and solid of the said cell), surface shape, surface area, surface charge, surface biological property, surface chemical property, pH, electrolyte, ionic strength, resistivity, cell concentration, or biological, electrical, physical or chemical property of solution; the acoustic property is frequency, speed of acoustic waves, acoustic frequency and intensity spectrum distribution, acoustic intensity, acoustical absorption, or acoustical resonance; the mechanical property is internal pressure, hardness, flow rate, viscosity, fluid mechanical properties, shear strength, elongation strength, fracture stress, adhesion, mechanical resonance frequency, elasticity, plasticity, or compressibility.

[017] In some embodiments, the apparatus comprises a micro-electro-mechanical device, a semiconductor device, a micro-fluidic device, bio-chemistry machine, an immunology machine, a voltage meter, or a sequencing machine.

[018] In some other embodiments, the collected information is in the physical, bio-physical, bio-chemical, biological, or chemical form. For example, the physical form of the collected information comprises mechanical, electrical, thermal, thermodynamic, optical, and acoustical properties of the cells or cell surrounding media.

[019] In still some other embodiments, the information is collected after a probe signal is applied to the cells or cell-surrounding media and a response signal is received. The probe signal, for example, can include a physical, bio-physical, bio-chemical, biological, or chemical signal; and the physical signal can include a mechanical, electrical, thermal, thermodynamic, optical, or acoustical signal.

[020] In some embodiments, the disease is a cancer, an inflammatory disease, diabetes, a lung disease, a heart disease, a liver disease, a gastric disease, a biliary disease, or a cardiovascular disease. For example, the cancer can include breast cancer, lung cancer, esophageal cancer, intestine cancer, cancer related to blood, liver cancer, stomach cancer, cervical cancer, ovarian cancer, rectum cancer, colon cancer, nasopharyngeal cancer, cardiac carcinoma, uterine cancer, oophoroma, pancreatic cancer, prostate cancer, brain tumor, or circulating tumor cells; the inflammatory disease comprises acne vulgaris, asthma, autoimmune diseases, autoinflammatory diseases, celiac disease, chronic prostatitis, diverticulitis, glomerulonephritis, hidradenitis suppurativa, hypersensitivities, inflammatory bowel diseases, interstitial cystitis, otitis, pelvic inflammatory disease, reperfusion injury, rheumatic fever, rheumatoid arthritis, sarcoidosis, transplant rejection, or tasculitis; the lung disease comprises asthma, chronic obstructive pulmonary disease, chronic bronchitis, emphysema, acute bronchitis, cystic fibrosis, pneumonia, tuberculosis, pulmonary edema, acute respiratory distress syndrome, pneumoconiosis, interstitial lung disease, pulmonary embolism, or pulmonary hypertension; the diabetes comprises Type 1 diabetes, Type 2 diabetes, or gestational diabetes; the heart disease comprises coronary artery disease, enlarged heart (cardiomegaly), heart attack, irregular heart rhythm, atrial fibrillation, heart rhythm disorders, heart valve disease, sudden cardiac death, congenital heart disease, heart muscle disease (cardiomyopathy), dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, pericarditis, pericardial effusion, marfan syndrome, or heart

murmurs; the liver disease comprises fascioliasis, hepatitis, alcoholic liver disease, fatty liver disease (hepatic steatosis), hereditary diseases, Gilbert's syndrome, cirrhosis, primary biliary cirrhosis, primary sclerosing cholangitis, or Budd–Chiari syndrome; the gastric disease comprises gastritis, gastric polyp, gastric ulcer, benign tumor of stomach, acute gastric mucosa lesion, antral gastritis, or gastric stromal tumors; the biliary disease comprises calculus of bile duct, cholecystolithiasis, cholecystitis, cholangiectasis, cholangitis, or gallbladder polyps; the cardiovascular disease comprises coronary artery disease, peripheral arterial disease, cerebrovascular disease, renal artery stenosis, aortic aneurysm, cardiomyopathy, hypertensive heart disease, heart failure, pulmonary heart disease, cardiac dysrhythmias, endocarditis, inflammatory cardiomegaly, myocarditis, valvular heart disease, congenital heart disease, rheumatic heart disease, coronary artery disease, peripheral arterial disease, cerebrovascular disease, or renal artery stenosis.

[021] In some embodiments, the apparatus further comprises a sensor positioned to be partially inside the chamber and capable of detecting a property of the biological subject at the microscopic level.

[022] In some embodiments, the apparatus further comprises a read-out circuitry which is connected to at least one sensor and transfers data from the sensor to a recording device. In some examples, the connection between the read-out circuit and the sensor is digital, analog, optical, thermal, piezo-electrical, piezo-photronic, piezo-electrical photronic, opto-electrical, electro-thermal, opto-thermal, electric, electromagnetic, electromechanical, or mechanical.

[023] In some embodiments, the sensor is positioned on the interior surface of the chamber.

[024] In some other embodiments, each sensor is independently a thermal sensor, optical sensor, acoustical sensor, biological sensor, chemical sensor, electro-mechanical sensor, electro-chemical sensor, electro-optical sensor, electro-thermal sensor, electro-chemical-mechanical sensor, bio-chemical sensor, bio-mechanical sensor, bio-optical sensor, electro-optical sensor, bio-electro-optical sensor, bio-thermal optical sensor, electro-chemical optical sensor, bio-thermal sensor, bio-physical sensor, bio-electro-mechanical sensor, bio-electro-chemical sensor, bio-electro-optical sensor, bio-electro-thermal sensor, bio-mechanical-optical sensor, bio-mechanical thermal sensor, bio-thermal-optical sensor, bio-electro-chemical-optical sensor, bio-electro-mechanical optical sensor, bio-electro-thermal-optical sensor, bio-electro-chemical-mechanical sensor, physical sensor, mechanical sensor, piezo-electrical sensor, piezo-electro

photronic sensor, piezo-photronic sensor, piezo-electro optical sensor, bio-electrical sensor, bio-marker sensor, electrical sensor, magnetic sensor, electromagnetic sensor, image sensor, or radiation sensor.

[025] In some other embodiments, the thermal sensor comprises a resistive temperature micro-sensor, a micro-thermocouple, a thermo-diode and thermo-transistor, and a surface acoustic wave (SAW) temperature sensor; the image sensor comprises a charge coupled device (CCD) or a CMOS image sensor (CIS); the radiation sensor comprises a photoconductive device, a photovoltaic device, a pyro-electrical device, or a micro-antenna; the mechanical sensor comprises a pressure micro-sensor, micro-accelerometer, flow meter, viscosity measurement tool, micro-gyrometer, or micro flow-sensor; the magnetic sensor comprises a magneto-galvanic micro-sensor, a magneto-resistive sensor, a magneto diode, or magneto-transistor; the biochemical sensor comprises a conductimetric device, a bio-marker, a bio-marker attached to a probe structure, or a potentiometric device.

[026] In some embodiments, at least one sensor is a probing sensor and applies a probing or disturbing signal to the biological subject.

[027] In some other embodiments, at least another sensor, different from the probing sensor, is a detection sensor and detects a response from the biological subject upon which the probing or disturbing signal is applied.

[028] In some embodiments, the chamber of the apparatus of this invention has a length ranging from 1 micron to 50,000 microns, from 1 micron to 15,000 microns, from 1 micron to 10,000 microns, from 1.5 microns to 5,000 microns, or from 3 microns to 1,000 microns.

[029] In some embodiments, the chamber of the apparatus of this invention has a width or height ranging from 0.1 micron to 100 microns; from 0.1 micron to 25 microns, from 1 micron to 15 microns, or from 1.2 microns to 10 microns.

[030] In some embodiments, the apparatus of this invention includes at least four sensors which are located on one side, two opposite sides, or four sides of the interior surface of the chamber. For example, the two sensors in the micro-cylinder can be apart by a distance ranging from 0.1 micron to 500 microns, from 0.1 micron to 50 microns, form 1 micron to 100 microns, from 2.5 microns to 100 microns, or from 5 microns to 250 microns. For some examples, at least one of the panels comprises at least two sensors that are arranged in at least two arrays each separated by at least a micro sensor in a cylinder.

[031] In some embodiments, at least one array of the sensors in the panel of the apparatus of this invention comprises two or more sensors.

[032] In some embodiments, the sorting unit or the detection unit of the apparatus of this invention further includes an application specific integrated circuit chip which is internally bonded to or integrated into one of the panels or a micro-cylinder. For example, the sorting unit or the detection unit further comprises a memory unit, a logic processing unit, an optical device, imaging device, camera, viewing station, acoustic detector, piezo-electrical detector, piezo-photronic detector, piezo-electro photronic detector, electro-optical detector, electro-thermal detector, bio-electrical detector, bio-marker detector, bio-chemical detector, chemical sensor, thermal detector, ion emission detector, photo-detector, x-ray detector, radiation material detector, electrical detector , or thermal recorder, each of which is integrated into the a panel or a micro cylinder.

[033] In some embodiments, the biological subject is a blood sample, a urine sample, or a sweat sample of a mammal.

[034] In some other embodiments, one signal contains information related to the disease's location or where the disease is present in the source of the biological subject.

[035] In still some other embodiments, one signal contains information related to the occurrence or type of the disease.

[036] In yet still come other embodiments, the apparatus of this invention is able to detect the presence of at least two different diseases at the same time or to determine the status or progression of a disease.

[037] One aspect of this invention provides an apparatus for detecting presence or monitoring progression of a disease in a biological subject. The biological subject can be a blood sample, a urine sample, or a sweat sample of a mammal. The apparatus comprises a chamber in which the biological subject passes through, and at least one detection transducer placed partially or completely in the chamber; wherein at least two types of information about the biological subject selected from the group consisting of chemical composition, cellular classification, molecular classification, and any combination thereof, are detected by the detection transducer and collected for analysis to determine whether the disease is likely to be present with the biological subject or to determine the status of the disease, therefore providing the ability to continuously determine or monitor progression of the disease.

[038] In some embodiments, the detection transducer detects at least one selected from the group consisting of a chemical composition, a cellular classification, a molecular classification, and any combination thereof; and the detected information is collected for analysis to as to whether the disease is likely to be present with the biological subject.

[039] An example of the chemical composition includes protein (such as a sugar-based protein, an embryonic protein, a protein-based antigen, and a carbohydrate antigen). Examples of the molecular classification include DNA, RNA, or a biomarker.

[040] As used herein, the term "biomarker" means a measurable indicator of the severity or presence of some disease state, but more generally a biomarker is anything that can be used as an indicator of a particular disease state or some other physiological state of an organism. A biomarker can be a substance that is introduced into an organism as a means to examine organ function or other aspects of health. For example, rubidium chloride is used in isotopic labeling to evaluate perfusion of heart muscle. It can also be a substance whose detection indicates a particular disease state, for example, the presence of an antibody may indicate an infection. More specifically, a biomarker indicates a change in expression or state of a protein that correlates with the risk or progression of a disease, or with the susceptibility of the disease to a given treatment. Biomarkers can be specific cells, molecules, or genes, gene products, enzymes, or hormones.

[041] Examples of the cellular classification include circulating tumor cells, cell surface properties, cell signaling properties, and cell geometrical properties.

[042] In some embodiments, the chemical composition, cellular classification, or molecular classification includes a property of the biological subject at microscope level selected from the group consisting of a thermal, optical, acoustical, biological, chemical, physical-chemical, electro-mechanical, electro-chemical, electro-chemical-mechanical, bio-physical, bio-chemical, bio-mechanical, bio-electrical, bio-physical-chemical, bio-electro-physical, bio-electro-mechanical, bio-electro-chemical, bio-chemical-mechanical, bio-electro-physical-chemical, bio-electro-physical-mechanical, bio-electro-chemical-mechanical, physical, an electric, magnetic, electro-magnetic, and mechanical property. The thermal property can be temperature or vibrational frequency; the optical property is optical absorption, optical transmission, optical reflection, optical-electrical property, brightness, or fluorescent emission; the radiation property is radiation emission, signal triggered by radioactive material, or information probed by radioactive material; the chemical property is pH value, chemical reaction, bio-chemical

reaction, bio-electro-chemical reaction, reaction speed, reaction energy, speed of reaction, oxygen concentration, oxygen consumption rate, ionic strength, catalytic behavior, chemical additives to trigger enhanced signal response, bio-chemical additives to trigger enhanced signal response, biological additives to trigger enhanced signal response, chemicals to enhance detection sensitivity, bio-chemicals to enhance detection sensitivity, biological additives to enhance detection sensitivity, or bonding strength; the physical property is density, shape, volume, or surface area; the electrical property is surface charge, surface potential, resting potential, electrical current, electrical field distribution, surface charge distribution, cell electronic properties, cell surface electronic properties, dynamic changes in electronic properties, dynamic changes in cell electronic properties, dynamic changes in cell surface electronic properties, dynamic changes in surface electronic properties, electronic properties of cell membranes, dynamic changes in electronic properties of membrane surface, dynamic changes in electronic properties of cell membranes, electrical dipole, electrical quadruple, oscillation in electrical signal, electrical current, capacitance, three-dimensional electrical or charge cloud distribution, electrical properties at telomere of DNA and chromosome, capacitance, or impedance; the biological property is surface shape, surface area, surface charge, surface biological property, surface chemical property, pH, electrolyte, ionic strength, resistivity, cell concentration, or biological, electrical, physical or chemical property of solution; the acoustic property is frequency, speed of acoustic waves, acoustic frequency and intensity spectrum distribution, acoustic intensity, acoustical absorption, or acoustical resonance; the mechanical property is internal pressure, hardness, flow rate, viscosity, fluid mechanical properties, shear strength, elongation strength, fracture stress, adhesion, mechanical resonance frequency, elasticity, plasticity, or compressibility.

[043] The disease that can be detected or monitor for progress can be a cancer, an inflammatory disease, diabetes, a lung disease, a heart disease, a liver disease, a gastric disease, a biliary disease, or a cardiovascular disease. Examples of cancer comprise breast cancer, lung cancer, esophageal cancer, intestine cancer, cancer related to blood, liver cancer, stomach cancer, cervical cancer, ovarian cancer, rectum cancer, colon cancer, nasopharyngeal cancer, cardiac carcinoma, uterine cancer, oophoroma, pancreatic cancer, prostate cancer, brain tumor, and circulating tumor cells; examples of the inflammatory disease include acne vulgaris, asthma, autoimmune diseases, autoinflammatory diseases, celiac disease, chronic prostatitis,

diverticulitis, glomerulonephritis, hidradenitis suppurativa, hypersensitivities, inflammatory bowel diseases, interstitial cystitis, otitis, pelvic inflammatory disease, reperfusion injury, rheumatic fever, rheumatoid arthritis, sarcoidosis, transplant rejection, and tasculitis; examples of the lung disease include asthma, chronic obstructive pulmonary disease, chronic bronchitis, emphysema, acute bronchitis, cystic fibrosis, pneumonia, tuberculosis, pulmonary edema, acute respiratory distress syndrome, pneumoconiosis, interstitial lung disease, pulmonary embolism, and pulmonary hypertension; examples of the diabetes include Type 1 diabetes, Type 2 diabetes, and gestational diabetes; examples of the heart disease include coronary artery disease, enlarged heart (cardiomegaly), heart attack, irregular heart rhythm, atrial fibrillation, heart rhythm disorders, heart valve disease, sudden cardiac death, congenital heart disease, heart muscle disease (cardiomyopathy), dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, pericarditis, pericardial effusion, marfan syndrome, and heart murmurs; examples of the liver disease include fascioliasis, hepatitis, alcoholic liver disease, fatty liver disease (hepatic steatosis), hereditary diseases, Gilbert's syndrome, cirrhosis, primary biliary cirrhosis, primary sclerosing cholangitis, and Budd–Chiari syndrome; examples of the gastric disease include gastritis, gastric polyp, gastric ulcer, benign tumor of stomach, acute gastric mucosa lesion, antral gastritis, and gastric stromal tumors; examples of the biliary disease include calculus of bile duct, cholecystolithiasis, cholecystitis, cholangiectasis, cholangitis, and gallbladder polyps; the cardiovascular disease comprises coronary artery disease, peripheral arterial disease, cerebrovascular disease, renal artery stenosis, aortic aneurysm, cardiomyopathy, hypertensive heart disease, heart failure, pulmonary heart disease, cardiac dysrhythmias, endocarditis, inflammatory cardiomegaly, myocarditis, valvular heart disease, congenital heart disease, rheumatic heart disease, coronary artery disease, peripheral arterial disease, cerebrovascular disease, and renal artery stenosis.

[044] In some other embodiments, the apparatus can further include a sensor positioned to be partially inside the chamber and capable of detecting a property of the biological subject at the microscopic level.

[045] In some other embodiments, the apparatus can further include a read-out circuitry which is connected to at least one sensor and transfers data from the sensor to a recording device.

[046] The connection between the read-out circuit and the sensor can be digital, analog, optical, thermal, piezo-electrical, piezo-photronic, piezo-electrical photronic, opto-electrical, electro-thermal, opto-thermal, electric, electromagnetic, electromechanical, or mechanical.

[047] The sensor can be positioned on the interior surface of the chamber.

[048] In some other embodiments, each sensor is independently a thermal sensor, optical sensor, acoustical sensor, biological sensor, chemical sensor, electro-mechanical sensor, electro-chemical sensor, electro-optical sensor, electro-thermal sensor, electro-chemical-mechanical sensor, bio-chemical sensor, bio-mechanical sensor, bio-optical sensor, electro-optical sensor, bio-electro-optical sensor, bio-thermal optical sensor, electro-chemical optical sensor, bio-thermal sensor, bio-physical sensor, bio-electro-mechanical sensor, bio-electro-chemical sensor, bio-electro-optical sensor, bio-electro-thermal sensor, bio-mechanical-optical sensor, bio-mechanical thermal sensor, bio-thermal-optical sensor, bio-electro-chemical-optical sensor, bio-electro-mechanical optical sensor, bio-electro-thermal-optical sensor, bio-electro-chemical-mechanical sensor, physical sensor, mechanical sensor, piezo-electrical sensor, piezo-electro photronic sensor, piezo-photronic sensor, piezo-electro optical sensor, bio-electrical sensor, bio-marker sensor, electrical sensor, magnetic sensor, electromagnetic sensor, image sensor, or radiation sensor. For example, the thermal sensor comprises a resistive temperature micro-sensor, a micro-thermocouple, a thermo-diode and thermo-transistor, and a surface acoustic wave (SAW) temperature sensor; the image sensor comprises a charge coupled device (CCD) or a CMOS image sensor (CIS); the radiation sensor comprises a photoconductive device, a photovoltaic device, a pyro-electrical device, or a micro-antenna; the mechanical sensor comprises a pressure micro-sensor, micro-accelerometer, flow meter, viscosity measurement tool, micro-gyrometer, or micro flow-sensor; the magnetic sensor comprises a magneto-galvanic micro-sensor, a magneto-resistive sensor, a magneto diode, or magneto-transistor; the biochemical sensor comprises a conductimetric device, a bio-marker, a bio-marker attached to a probe structure, or a potentiometric device.

[049] In some other embodiments, at least one sensor is a probing sensor and applies a probing or disturbing signal to the biological subject.

[050] In some other embodiments, at least another sensor, different from the probing sensor, is a detection sensor and detects a response from the biological subject upon which the probing or disturbing signal is applied.

[051] The chamber can have a length ranging from 1 micron to 50,000 microns, from 1 micron to 15,000 microns, from 1 micron to 10,000 microns, from 1.5 microns to 5,000 microns, or from 3 microns to 1,000 microns. On the other hand, the chamber can have a width or height ranging from 0.1 micron to 100 microns; from 0.1 micron to 25 microns, from 1 micron to 15 microns, or from 1.2 microns to 10 microns.

[052] In some other embodiments, the apparatus comprises at least four sensors which are located on one side, two opposite sides, or four sides of the interior surface of the chamber. For example, the two sensors in the micro-cylinder can be apart by a distance ranging from 0.1 micron to 500 microns, from 0.1 micron to 50 microns, form 1 micron to 100 microns, from 2.5 microns to 100 microns, or from 5 microns to 250 microns; at least one of the panels comprises at least two sensors that are arranged in at least two arrays each separated by at least a micro sensor in a cylinder; or at least one array of the sensors in the panel comprises two or more sensors.

[053] In some other embodiments, the sorting unit or the detection unit further comprises an application specific integrated circuit chip which is internally bonded to or integrated into one of the panels or a micro-cylinder.

[054] In still some other embodiments, the sorting unit or the detection unit further comprises a memory unit, a logic processing unit, an optical device, imaging device, camera, viewing station, acoustic detector, piezo-electrical detector, piezo-photronic detector, piezo-electro photronic detector, electro-optical detector, electro-thermal detector, bio-electrical detector, bio-marker detector, bio-chemical detector, chemical sensor, thermal detector, ion emission detector, photo-detector, x-ray detector, radiation material detector, electrical detector , or thermal recorder, each of which is integrated into the a panel or a micro cylinder.

[055] In some other embodiments, one signal contains information related to the disease's location or where the disease is present in the source of the biological subject.

[056] In still some other embodiments, one signal contains information related to the occurrence or type of the disease.

[057] The apparatus of this invention is able to detect the presence of at least two different diseases at the same time or to determine the status or progression of a disease.

[058] In another aspect, the present invention provides a method for detecting the presence or progression of a disease in a biological subject, comprising detecting at least two types of

information selected from the group consisting of chemical composition, cellular classification, molecular classification and any combination thereof of the biological subject, and analyzing the collected information to determine if the likely presence or progression of the status of the disease with the biological subject. Examples of the disease include cancer, an inflammatory disease, diabetes, lung diseases, liver diseases, gastric diseases, biliary diseases, or a cardiovascular disease. Specifically, the cancer can be breast cancer, lung cancer, esophageal cancer, intestine cancer, cancer related to blood, liver cancer, stomach cancer, cervical cancer, ovarian cancer, rectum cancer, colon cancer, nasopharyngeal cancer, cardiac carcinoma, uterine cancer, oophoroma, pancreatic cancer, prostate cancer, brain tumor, or circulating tumor cells; the inflammatory disease can be acne vulgaris, asthma, autoimmune diseases, autoinflammatory diseases, celiac disease, chronic prostatitis, diverticulitis, glomerulonephritis, hidradenitis suppurativa, hypersensitivities, inflammatory bowel diseases, interstitial cystitis, otitis, pelvic inflammatory disease, reperfusion injury, rheumatic fever, rheumatoid arthritis, sarcoidosis, transplant rejection, or tasculitis; the cardiovascular disease can be coronary artery disease, peripheral arterial disease, cerebrovascular disease, renal artery stenosis, aortic aneurysm, cardiomyopathy, hypertensive heart disease, heart failure, pulmonary heart disease, cardiac dysrhythmias, endocarditis, inflammatory cardiomegaly, myocarditis, valvular heart disease, congenital heart disease, rheumatic heart disease, coronary artery disease, peripheral arterial disease, cerebrovascular disease, or renal artery stenosis.

[059] The biological subject can be cells, a sample of an organ or tissue, DNA, RNA, virus, or protein. For example, the cells are circulating tumor cells or cancer cells, e.g., breast cancer, lung cancer, esophageal cancer, cervical cancer, ovarian cancer, rectum cancer, colon cancer, nasopharyngeal cancer, cardiac carcinoma, uterine cancer, oophoroma, pancreatic cancer, prostate cancer, brain tumor, intestine cancer, cancer related to blood, liver cancer, stomach cancer, or circulating tumor cells. In some other embodiments, the biological subject is contained in a media and transported into the first intra-unit channel.

[060] In yet another aspect, the invention provides a method for detecting presence or progression of a disease in a biological subject, which includes testing at least two types of information in the biological subject, with one of the at least two types of information indicating the disease's presence or progression in status and the other type of information indicating the disease's location.

[061] In some embodiments, the two levels of information each comprise protein level information, molecular level information, cellular level information, genetic-level information, or any combination thereof.

[062] In yet another aspect, the invention provides a method for detecting presence or progression of a disease in a biological subject, which comprising measuring at least one parameter correlated to a property at the protein, cellular, molecular, or genetic level.

[063] For instance, the property is a thermal, optical, acoustical, biological, chemical, physical-chemical, electro-mechanical, electro-chemical, electro-chemical-mechanical, bio-physical, bio-chemical, bio-mechanical, bio-electrical, bio-physical-chemical, bio-electro-physical, bio-electro-mechanical, bio-electro-chemical, bio-chemical-mechanical, bio-electro-physical-chemical, bio-electro-physical-mechanical, bio-electro-chemical-mechanical, physical, an electric, magnetic, electro-magnetic, or mechanical property of the biologic subject. Specifically, for example, the thermal property is temperature or vibrational frequency; the optical property is optical absorption, optical transmission, optical reflection, optical-electrical property, brightness, or fluorescent emission; the radiation property is radiation emission, signal triggered by radioactive material, or information probed by radioactive material; the chemical property is pH value, chemical reaction, bio-chemical reaction, bio-electro-chemical reaction, reaction speed, reaction energy, speed of reaction, oxygen concentration, oxygen consumption rate, ionic strength, catalytic behavior, chemical additives to trigger enhanced signal response, bio-chemical additives to trigger enhanced signal response, biological additives to trigger enhanced signal response, chemicals to enhance detection sensitivity, bio-chemicals to enhance detection sensitivity, biological additives to enhance detection sensitivity, or bonding strength; the physical property is density, shape, volume, or surface area; the electrical property is surface charge, surface potential, resting potential, electrical current, electrical field distribution, surface charge distribution, cell electronic properties, cell surface electronic properties, dynamic changes in electronic properties, dynamic changes in cell electronic properties, dynamic changes in cell surface electronic properties, dynamic changes in surface electronic properties, electronic properties of cell membranes, dynamic changes in electronic properties of membrane surface, dynamic changes in electronic properties of cell membranes, electrical dipole, electrical quadruple, oscillation in electrical signal, electrical current, capacitance, three-dimensional electrical or charge cloud distribution, electrical properties at telomere of DNA and chromosome,

DNA static electrical force, DNA surface charge, DNA surrounding media electrical properties, quantum mechanical effects, capacitance, or impedance; the biological property is surface shape, surface area, surface charge, surface biological property, surface chemical property, pH, electrolyte, ionic strength, resistivity, cell concentration, or biological, electrical, physical or chemical property of solution; the acoustic property is frequency, speed of acoustic waves, acoustic frequency and intensity spectrum distribution, acoustic intensity, acoustical absorption, or acoustical resonance; the mechanical property is internal pressure, hardness, flow rate, viscosity, fluid mechanical properties, shear strength, elongation strength, fracture stress, adhesion, mechanical resonance frequency, elasticity, plasticity, or compressibility.

[064] In some other embodiments, the parameter can be simultaneously correlated to at least two levels of information each independently selected from the group consisting of chemical composition, cellular classification, molecular classification, genetic classification, and any combination thereof.

[065] For example, the parameter is a function of at least two levels of information each independently selected from the group consisting of chemical composition, cellular classification, molecular classification, genetic classification, and any combination thereof.

[066] In some other embodiments, the at least two levels of information interact with each other to amplify the measured parameter of the biological subject.

[067] For instance, the measured parameter can include a property at the protein level, cellular level, molecular level, or genetic level.

[068] In yet still another aspect of this invention is a method for detecting presence or monitoring progression of a disease in a biological subject, comprising testing at least two parameters of the biological subject for at least two different levels of information, processing the at least two different levels of information to result in a new parameter that has a stronger signal intensity than the sum of the signal intensities of the at least two levels of information.

[069] In some embodiments, the at least two levels parameters comprise information selected from the group consisting of chemical composition, cellular classification, molecular classification, and any combination thereof of the biological subject. For example, one testing parameter contains two biological levels of information, and its signal intensity is greater than the sum of the two signal intensities of the testing parameters with each containing one of the two biological levels. For another example, one signal has information related to the disease's

location or where the disease is present in the biological subject. For still another example, one signal contains information related to the presence or type of the disease.

[070] The invention also provides a method for detecting presence or monitoring progression of a disease in a biological subject, which comprises tested one parameter containing at least two levels of signal, wherein the tested parameter's signal intensity is greater than the sum of the intensity of the at least two levels of signal.

[071] In some embodiments, the at least two levels of signal comprise information selected from the group consisting of chemical composition, cellular classification, molecular classification, and any combination thereof of the biological subject.

[072] The present invention also provides methods for detecting the presence or progression of a disease in a biological subject, comprising measuring a biophysical property at a microscopic level of cells in the biological subject with an apparatus described above, wherein information related to the measured biological property of the cells in the biological subject is detected by the detection transducer and collected for analysis to determine whether the disease is likely to be present with the biological subject or to determine the status of the disease, thereby providing the ability to continuously determine or monitor progression of the disease.

[073] In some embodiments, the determination is by comparing the biophysical information of the detected biological subject with the same biological information of a confirmed disease-free or diseased biological subject.

[074] In some other embodiments, the biophysical property is an electric property at the microscopic level. Examples of the electronic property include surface charge, surface potential, resting potential, electrical current, electric conductance, electrical resistance, capacitance, quantum mechanical effects, electrical field distribution, surface charge distribution, cell electronic properties, cell surface electronic properties, dynamic changes in electronic properties, dynamic changes in cell electronic properties, dynamic changes in cell surface electronic properties, dynamic changes in surface electronic properties, electronic properties of cell membranes, dynamic changes in electronic properties of membrane surface, dynamic changes in electronic properties of cell membranes, electrical dipole, electrical quadruple, oscillation in electrical signal, electrical current, capacitance, three-dimensional electrical or charge cloud distribution, electrical properties at telomere of DNA and chromosome, capacitance, and impedance.

[075] Yet another aspect of the present invention is methods for treating or slowing progression of a disease in a biological subject, comprising administering to the biological subject thereof a therapeutic agent that enhances or increase the level of a biophysical property at the microscopic level of the biological subject. For example, the therapeutic agent is administered orally or by intravenous injection. As another example, the biophysical property is an electronic property which can be surface charge, surface potential, resting potential, electrical current, electrical field distribution, surface charge distribution, cell electronic properties, cell surface electronic properties, dynamic changes in electronic properties, dynamic changes in cell electronic properties, dynamic changes in cell surface electronic properties, dynamic changes in surface electronic properties, electronic properties of cell membranes, dynamic changes in electronic properties of membrane surface, dynamic changes in electronic properties of cell membranes, electrical dipole, electrical quadruple, oscillation in electrical signal, electrical current, capacitance, three-dimensional electrical or charge cloud distribution, electrical properties at telomere of DNA and chromosome, capacitance, or impedance.

[076] Also within the scope of this invention is a therapeutic agent for treating or slowing progression of a disease in a biological subject, which agent includes at least a component that alters or enhances electronic property-of the biological subject. Examples of such a component include electrolytes. Such a component enhances electrical current and/or electrical conductance, reduces electrical resistance, and/or adjusts or alters quantum mechanical effects.

[077] In yet another aspect, this invention provides a method for detecting a disease in a biological subject, comprising using a micro-fluidic device to detect at least one physical or bio-physical property of the biological subject with a reagent. For instance, the physical or bio-physical property may be measured by using a liquid sample.

[078] In some embodiments, the bio-physical property comprises a mechanical property, an acoustical property, an optical property, an electrical property, an electro-magnetic property, or an electro-mechanical property. In some further embodiments, the electrical property comprises electrical current, electrical conductance, capacitance, electrical resistance, or quantum mechanical effect. For instance, the bio-physical property comprises quantum mechanical effects that affect gene replications and mutations. The quantum mechanical effects may be detected either directly or indirectly in the measured sample.

[079] Examples of the bio-physical property also include, but are not limited to, trans-

membrane potential, a membrane voltage, a membrane potential, a zeta potential, an impedance, an optical reflective index, an optical refractive index, potassium ions, sodium ions, chloride ions, nitride ions, calcium ions, an electro-static force, an electro-static force acting on cells, an electro-static force acting on DNA double helix, an electro-static force acting on RNA, an electrical charge on cell membrane, an electrical charge on DNA double helix, an electrical charge on RNA, quantum effects, near-field electrical properties, near-field electro-magnetic properties, membrane bilayer properties, ion permeability, electrical current, electrical conductance, capacitance, and electrical resistance.

[080] In some embodiments, the micro-fluidic device directly or indirectly measures ions or ion levels in a liquid sample of the biological subject. For instance, the micro-fluidic device may measure ion levels or concentrations by a bio-chemistry or electrode method.

[081] In some embodiments, the micro-fluidic device directly or indirectly measures potassium ions. In some embodiments, the micro-fluidic device directly or indirectly measures the concentration of potassium ions.

[082] In some embodiments, the micro-fluidic device directly or indirectly measures one or more of the following ions: potassium ions, sodium ions, chloride ions, nitride ions and calcium ions. In some embodiments, the micro-fluidic device directly or indirectly measures the concentration(s) of one or more ions selected from the group consisting of potassium ions, sodium ions, chloride ions, nitride ions and calcium ions.

[083] In some embodiments, the micro-fluidic device directly or indirectly measures ion permeability.

[084] In some embodiments, the biophysical physical property is related to and responsible for cell to cell interactions, cell signal, cell surface properties, cell electro-static force, cell repulsive force, DNA surface properties, DNA surface charge, DNA surrounding media electrical properties, quantum mechanical effects, gene mutation frequencies, or quantum mechanical effects.

[085] In some embodiments, the biophysical property may be a predictor of immunity, infection, disease, pre-cancer or cancer; or a predictor of disease progress from healthy state to disease state, from disease state to pre-cancer state, and from pre-cancer state to cancer state. wherein the bio-physical property is measured by using liquid sample.

[086] In some embodiments, a further device is used for adjusting the bio-physical properties in

the biological subject such as blood. The bio-physical property may be first measured and then adjusted. In some embodiments, such bio-physical property comprises a mechanical property, an acoustical property, an optical property, an electrical property, an electro-magnetic property, or an electro-mechanical property. More specifically, the electrical property may comprise electrical current, electrical conductance, capacitance, electrical resistance, or quantum mechanical effect. In some embodiments, the further device adjusts the current to a higher value, adjusts the electrical conductance to a higher value, adjusts the electrical resistance to a lower value, or alters the quantum mechanical effect.

[087] In some embodiments, a reagent is injected into blood to adjust bio-physical properties in the blood. For instance, the reagent contains ions, oxidizers, and components to impacting electrical properties of the blood. Examples of the electrical property include, but are not limited to, electrical current, electrical conductance, capacitance, electrical resistance, and quantum mechanical effect.

[088] In some further embodiments, the reagent is a drug capable of adjusting the biological properties in the blood. For instance, the drug may be capable of releasing, upon intake, ions and charged components and capable of adjusting electrical properties of the blood. Such electrical property may comprise electrical current, electrical conductance, capacitance, electrical resistance, or quantum mechanical effect.

[089] In some embodiments, wherein at least one bio-marker is added to the liquid sample for physical or bio-physical property and related properties to be measured. For instance, the bio-marker may provide at least some indicative information of risks of cancer occurrence at a given organ and location. In some embodiments, the obtained information and data are analyzed in conjunction with information and data obtained from test(s) comprising of bio-marker tests, genomics tests, and circulating tumor cell tests, and overall cancer risks and location(s) of possible cancer occurrence are obtained.

[090] Still in another aspect, this invention provides a medical device for treating a biological subject, comprising a channel in which the biological subject passes through, and at least one transducer placed partially or completely in the channel; wherein the transducer is configured to transmit at least one bio-physical property, or material or element onto the biological subject. The invention also provides a medical device for treating a biological subject, comprising a channel in which the biological subject passes through, and at least one transducer placed

partially or completely in the channel; wherein the transducer is configured to transmit at least one bio-physical energy, or material or element onto the biological subject.

[091] Preferably, the biological subject is a liquid of a mammal. For instance, the biological subject is a blood sample, a urine sample, or a sweat sample of the mammal. The biological subject may comprise blood, proteins, red blood cells, while blood cells, T cells, other cells, gene mutations, quantum mechanical effects, DNA, RNA, or other biological entities.

[092] In some embodiments, the bio-physical property, bio-physical energy, material or element comprises a mechanical property or energy, an acoustical property or energy, an optical property or energy, an electrical property or energy, an electro-magnetic property or energy, or an electro-mechanical property or energy. For instance, the electrical property or energy comprises electrical current, electrical conductance, capacitance, electrical resistance, net electrical charge in extracellular region, membrane potential, membrane polarization, ion concentrations, electro-static force and charge on DNA double helix and RNA double helix, or quantum mechanical effect.

[093] In some embodiments, a medical device with channel(s) with at least one transducer placed along its side wall is fabricated. In some embodiments, a pulsed electrical voltage is applied to the sample through the said transducer. For instance, the sample can be a blood sample. With the blood sample from a patient circulating through the medical device, the applied pulsed electrical voltage can impact electrical field, charge distribution and/or possibly membrane potential of the blood. In some further embodiments, a medical device with channel(s) and transducer(s) on its side wall(s) and small opening(s) connecting to the channel(s) is used to treating the blood sample passing through the channel(s). For instance, a bio-physical energy (e.g., an electrical pulse) may be applied to the transducer and transmitted to the blood sample, while a desired amount of ions (e.g., potassium ions) are added to the blood through the small opening(s) in the channel(s). The purpose of these embodiments is to enhance the electrical conductivity of the blood, net electrical charge in the blood (particularly in the extracellular region of the blood), and/or polarization of the membrane potential.

[094] Examples of the bio-physical property, bio-physical energy, material or element also include, but are not limited to a trans-membrane potential, a membrane voltage, a membrane potential, a zeta potential, an impedance, an optical reflective index, an optical refractive index, potassium ions, sodium ions, chloride ions, nitride ions, calcium ions, an electro-static force, an

electro-static force acting on cells, an electro-static force acting on DNA double helix, an electro-static force acting on RNA, an electrical charge on cell membrane, an electrical charge on DNA double helix, an electrical charge on RNA, quantum effects, near-field electrical properties, near-field electro-magnetic properties, membrane bilayer properties, ion permeability, electrical current, electrical conductance, capacitance, and electrical resistance.

[095] In some embodiments, the transmitted bio-physical property or energy adjusts the current of the biological subject to a higher value, adjusts the electrical conductance of the biological subject to a higher value, adjusts the electrical resistance of the biological subject to a lower value, or alters the quantum mechanical effect of the biological subject.

[096] As used herein, the term "or" is meant to include both "and" and "or". It may be interchanged with "and/or."

[097] As used herein, a singular noun is meant to include its plural meaning. For instance, a micro device can mean either a single micro device or multiple micro-devices.

[098] As used herein, the term "patterning" means shaping a material into a certain physical form or pattern, including a plane (in which case "patterning" would also mean "planarization").

[099] As used herein, the term "a biocompatible material" refers to a material that is intended to interface with a living organism or a living tissue and can function in intimate contact therewith. When used as a coating, it reduces the adverse reaction a living organism or a living tissue has against the material to be coated, e.g., reducing the severity or even eliminating the rejection reaction by the living organism or living tissue. As used herein, it encompasses both synthetic materials and naturally occurring materials. Synthetic materials usually include biocompatible polymers, made either from synthetic or natural starting materials, whereas naturally occurring biocompatible materials include, e.g., proteins or tissues.

[0100] As used herein, the term "a biological subject" or "a biological sample" for analysis or test or diagnosis refers to the subject to be analyzed by a disease detection apparatus. It can be a single cell, a single biological molecular (e.g., DNA, RNA, or protein), a single biological subject (e.g., a single cell or virus), any other sufficiently small unit or fundamental biological composition, or a sample of a subject's organ or tissue that may having a disease or disorder.

[0101] As used herein, the term "disease" is interchangeable with the term "disorder" and generally refers to any abnormal microscopic property or condition (e.g., a physical condition) of a biological subject (e.g., a mammal or biological species).

[102] As used herein, the term "subject" generally refers to a mammal, e.g., a human person.

[103] As used herein, the term "microscopic level" refers to the subject being analyzed by the disease detection apparatus of this invention is of a microscopic nature and can be a single cell, a single biological molecular (e.g., DNA, RNA, or protein), a single biological subject (e.g., a single cell or virus), and other sufficiently small unit or fundamental biological composition.

[104] As used herein, an "apparatus" or a "micro-device" or "micro device" can be any of a wide range of materials, properties, shapes, and degree of complexity and integration. The term has a general meaning for an application from a single material to a very complex device comprising multiple materials with multiple sub units and multiple functions. The complexity contemplated in the present invention ranges from a very small, single particle with a set of desired properties to a fairly complicated, integrated unit with various functional units contained therein. For example, a simple micro-device could be a single spherical article of manufacture of a diameter as small as 100 angstroms with a desired hardness, a desired surface charge, or a desired organic chemistry absorbed on its surface. A more complex micro device could be a 1 millimeter device with a sensor, a simple calculator, a memory unit, a logic unit, and a cutter all integrated onto it. In the former case, the particle can be formed via a fumed or colloidal precipitation process, while the device with various components integrated onto it can be fabricated using various integrated circuit manufacturing processes. In some places, a micro-device or micro device represents a sub-equipment unit.

[105] As used herein, the term "parameter" refers to a particular detection target (e.g., a property of microscopic level, physical property such as hardness, viscosity, current, or voltage, or chemical property such as pH value) of the biological subject to be detected, and can include micro-level property.

[106] As used herein, the term "level" refers to chemical composition (including biochemical composition such as protein, genetic materials, e.g., DNA and RNA), cellular classification, or molecular classification of the biological subject to be detected.

[107] As used herein, the term "component" refers a lower division or building block of a level described above. For instance, a protein level can include such components as alpha-feto protein or sugar protein; and the level of a cellular classification can include such components as surface voltage and membrane composition.

[108] As used herein, if not specifically defined, a "channel" or "chamber" can be either an inter-unit channel or an intra-unit channel.

[109] Biological subjects that can be detected by the apparatus include, e.g., blood, urine, saliva, tear, and sweat. The detection results can indicate the possible occurrence or presence of a disease (e.g., one in its early stage) in the biological subject.

[110] As used herein, the term "absorption" typically means a physical bonding between the surface and the material attached to it (absorbed onto it, in this case). On the other hand, the word "adsorption" generally means a stronger, chemical bonding between the two. These properties are very important for the present invention as they can be effectively used for targeted attachment by desired micro devices for measurement at the microscopic level.

[111] As used herein, the term "contact" (as in "the first micro-device *contacts* a biologic entity") is meant to include both "direct" (or physical) contact and "non-direct" (or indirect or non-physical) contact. When two subjects are in "direct" contact, there is generally no measurable space or distance between the contact points of these two subjects; whereas when they are in "indirect" contact, there is a measurable space or distance between the contact points of these two subjects.

[112] As used herein, the term "probe" or "probing," in addition to its dictionary meaning, could mean applying a signal (e.g., an acoustic, optical, magnetic, chemical, electrical, electro-magnetic, bio-chemical, bio-physical, or thermal signal) to a subject and thereby stimulating the subject and causing it to have some kind of intrinsic response.

[113] As used herein, the term "thermal property" refers to temperature, freezing point, melting point, evaporation temperature, glass transition temperature, or thermal conductivity.

[114] As used herein, the term "optical property" refers to reflection, optical absorption, optical scattering, wave length dependent properties, color, luster, brilliance, scintillation, or dispersion.

[115] As used herein, the term "electrical property" refers to surface charge, surface potential, electrical field, charge distribution, electrical field distribution, resting potential, action potential, or impedance of a biological subject to be analyzed.

[116] As used herein, the term "magnetic property" refers to diamagnetic, paramagnetic, or ferromagnetic.

[117] As used herein, the term "electromagnetic property" refers to property that has both electrical and magnetic dimensions.

[118] As used herein, the term "acoustical property" refers to the characteristics found within a structure that determine the quality of sound in its relevance to hearing. It can generally be measured by the acoustic absorption coefficient. See, e.g., United States Patent No. 3,915,016, for means and methods for determining an acoustical property of a material; T.J. Cox et al., *Acoustic Absorbers and Diffusers*, 2004, Spon Press.

[119] As used herein, the term "biological property" is meant to generally include chemical and physical properties of a biological subject.

[120] As used herein, the term "chemical property" refers to pH value, ionic strength, or bonding strength within the biological sample.

[121] As used herein, the term "physical property" refers to any measurable property the value of which describes a physical system's state at any given moment in time. The physical properties of a biological sample may include, but are not limited to absorption, albedo, area, brittleness, boiling point, capacitance, color, concentration, density, dielectrical, electrical charge, electrical conductivity, electrical impedance, electrical field, electrical potential, emission, flow rate, fluidity, frequency, inductance, intrinsic impedance, intensity, irradiance, luminance, luster, malleability, magnetic field, magnetic flux, mass, melting point, momentum, permeability, permittivity, pressure, radiance, solubility, specific heat, strength, temperature, tension, thermal conductivity, flow rate, velocity, viscosity, volume, surface area, shape, and wave impedance.

[122] As used herein, the term "mechanical property" refers to strength, hardness, flow rate, viscosity, toughness, elasticity, plasticity, brittleness, ductility, shear strength, elongation strength, fracture stress, or adhesion of the biological sample.

[123] As used herein, the term "disturbing signal" has the same meaning as "probing signal" and "stimulating signal."

[124] As used herein, the term "disturbing unit" has the same meaning as "probing unit" and "stimulating unit."

[125] As used herein, the term "conductive material" (or its equivalent "electrical conductor") is a material which contains movable electrical charges. A conductive material can be a metal (e.g., copper, silver, or gold) or non-metallic (e.g., graphite, solutions of salts, plasmas, or conductive polymers). In metallic conductors, such as copper or aluminum, the movable charged particles are electrons (see electrical conduction). Positive charges may also be mobile in the

form of atoms in a lattice that are missing electrons (known as holes), or in the form of ions, such as in the electrolyte of a battery.

[126] As used herein, the term "electrically insulating material" (also known as "insulator" or "dielectric") refers to a material that resists the flow of electrical current. An insulating material has atoms with tightly bonded valence electrons. Examples of electrically insulating materials include glass or organic polymers (e.g., rubber, plastics, or Teflon).

[127] As used herein, the term "semiconductor" (also known as "semiconducting material") refers to a material with electrical conductivity due to electron flow (as opposed to ionic conductivity) intermediate in magnitude between that of a conductor and an insulator. Examples of inorganic semiconductors include silicon, silicon-based materials, and germanium. Examples of organic semiconductors include such aromatic hydrocarbons as the polycyclic aromatic compounds pentacene, anthracene, and rubrene; and polymeric organic semiconductors such as poly(3-hexylthiophene), poly(p-phenylene vinylene), polyacetylene and its derivatives. Semiconducting materials can be crystalline solids (e.g., silicon), amorphous (e.g., hydrogenated amorphous silicon and mixtures of arsenic, selenium and tellurium in a variety of proportions), or even liquid.

[128] As used herein, the term "biological material" has the same meaning of "biomaterial" as understood by a person skilled in the art. Without limiting its meaning, biological materials or biomaterials can generally be produced either in nature or synthesized in the laboratory using a variety of chemical approaches utilizing organic compounds (e.g., small organic molecules or polymers) or inorganic compounds (e.g., metallic components or ceramics). They generally can be used or adapted for a medical application, and thus comprise whole or part of a living structure or biomedical device which performs, augments, or replaces a natural function. Such functions may be benign, like being used for a heart valve, or may be bioactive with a more interactive functionality such as hydroxyl-apatite coated hip implants. Biomaterials can also be used every day in dental applications, surgery, and drug delivery. For instance, a construct with impregnated pharmaceutical products can be placed into the body, which permits the prolonged release of a drug over an extended period of time. A biomaterial may also be an autograft, allograft, or xenograft which can be used as a transplant material. All these materials that have found applications in other medical or biomedical fields can also be used in the present invention.

[129] As used herein, the term "microelectronic technology or process" generally encompasses the technologies or processes used for fabricating micro-electronic and optical-electronic components. Examples include lithography, etching (e.g., wet etching, dry etching, or vapor etching), oxidation, diffusion, implantation, annealing, film deposition, cleaning, direct-writing, polishing, planarization (e.g., by chemical mechanical polishing), epitaxial growth, metallization, process integration, simulation, or any combinations thereof. Additional descriptions on microelectronic technologies or processes can be found in, e.g., Jaeger, Introduction to Microelectronic Fabrication, 2nd Ed., Prentice Hall, 2002; Ralph E. Williams, Modern GaAs Processing Methods, 2nd Ed., Artech House, 1990; Robert F. Pierret, Advanced Semiconductor Fundamentals, 2nd Ed., Prentice Hall, 2002; S. Campbell, The Science and Engineering of Microelectronic Fabrication, 2nd Ed., Oxford University Press, 2001, the contents of all of which are incorporated herein by reference in their entireties.

[130] As used herein, the term "selective" as included in, e.g., "patterning material B using a microelectronics process *selective* to material A", means that the microelectronics process is effective on material B but not on material A, or is substantially more effective on material B than on material B (e.g., resulting in a much higher removal rate on material B than on material A and thus removing much more material B than material A).

[131] As used herein, the term "carbon nano-tube" generally refers to as allotropes of carbon with a cylindrical nanostructure. See, e.g., Carbon Nanotube Science, by P.J.F. Harris, Cambridge University Press, 2009, for more details about carbon nano-tubes.

[132] Through the use of a single micro-device or a combination of micro-devices integrated into a disease detection apparatus, the disease detection capabilities can be significantly improved in terms of sensitivity, specificity, speed, cost, apparatus size, functionality, and ease of use, along with reduced invasiveness and side-effects. A large number of micro-device types capable of measuring a wide range of microscopic properties of biological sample for disease detection can be integrated and fabricated into a single detection apparatus using micro-fabrication technologies and novel process flows disclosed herein. While for the purposes of demonstration and illustration, a few novel, detailed examples have been shown herein on how microelectronics or nano-fabrication techniques and associated process flows can be utilized to fabricate highly sensitive, multi-functional, and miniaturized detection devices, the principle and general approaches of employing microelectronics and nano-fabrication technologies in the

design and fabrication of high performance detection devices have been contemplated and taught, which can and should be expanded to various combination of fabrication processes including but not limited to thin film deposition, patterning (lithography and etch), planarization (including chemical mechanical polishing), ion implantation, diffusion, cleaning, various materials, and various process sequences and flows and combinations thereof.

Brief Descriptions of the Figures

[133] Fig. 1 (a) illustrates a set of traditional detection apparatus each of which detects and relies on a single detection technology. Fig. 1 (b) and Fig. (c) are illustration of a detection apparatus of this invention where multiple sub-equipment units are integrated.

[134] Fig. 2 is a schematic illustration of a detection apparatus of this invention which comprises multiple sub-equipment units, a delivery system, and a central control system.

[135] Fig. 3 is a perspective illustration of a detection apparatus of this invention in which a biological sample placed in it or moving through it can be tested.

[136] Fig. 4 illustrates an apparatus of the present invention which comprises two slabs each of which is fabricated with one or more detection or probing units.

[137] Fig. 5 is a perspective, cross-sectional illustration of a detection apparatus of this invention with multiple micro-devices placed at a desired distance for time of flight measurements with enhanced sensitivity, specificity, and speed, including time dependent or dynamic information.

[138] Fig. 6 is a perspective illustration of a novel set of microscopic probes, included in a detection apparatus of this invention, for detecting various electronic or magnetic states, configurations, or other properties of a biological sample (e.g., a cell).

[139] Fig. 7 is a perspective illustration of a novel four-point probe, included in a detection apparatus of this invention, for detecting weak electronic signal in a biological sample (e.g., a cell).

[140] Fig. 8 illustrates a fluid delivery system, which is a pretreatment part for the detection apparatus, and it delivers a sample or auxiliary material at a desired pressure and speed into a device.

[141] Fig. 9 illustrates how a micro-device in a disease detection apparatus of this invention can communicate, probe, detect, and optionally treat and modify biological subjects at a microscopic level.

[142] Fig. 10 illustrates another micro-device or sub-equipment that can detect the optical properties of the biological subject with a set of optical sensors.

[143] Fig. 11 illustrates another micro-device or sub-equipment that can separate biological subjects of different geometric size and detect their properties respectively.

[144] Fig. 12 illustrates a micro-device or sub-equipment that can measure the acoustic property of a biological subject.

[145] Fig. 13 illustrates a micro-device or sub-equipment that can measure the internal pressure of a biological subject.

[146] Fig. 14 illustrates a micro-device or sub-equipment that has concaves between the probe couples, in the bottom or ceiling of the channel.

[147] Fig. 15 illustrates another micro-device or sub-equipment that has concaves of a different shape from those illustrated in Fig. 14.

[148] Fig. 16 illustrates a micro-device or sub-equipment that has a stepped channel.

[149] Fig. 17 illustrates a micro-device or sub-equipment that has a set of thermal meters.

[150] Fig. 18 illustrates a micro-device or sub-equipment that includes a carbon nano-tube as the channel with DNA contained therein.

[151] Fig. 19 illustrates a micro-device or sub-equipment that includes a detecting device and an optical sensor.

[152] Fig. 20 illustrates an integrated apparatus of this invention that includes a detecting device and a logic circuitry.

[153] Fig. 21 illustrated a micro-device or sub-equipment that includes a detecting device and a filter.

[154] Fig. 22 illustrates how apparatus of this invention can be used to measure a DNA' geometric factors.

[155] Fig. 23 illustrates an apparatus of this invention with a cover atop the trench to form a channel.

[156] Fig. 24 is a diagram of sub-equipment unit for detecting a disease in a biological subject.

[157] Fig. 25 shows an example of a sample filtration unit.

[158] Fig. 26 shows another example of a sample filtration unit.

[159] Fig. 27 is a diagram of a pre-processing unit of an apparatus of this invention.

[160] Fig. 28 is a diagram of an information processing unit of an apparatus of this invention.

[161] Fig. 29 shows the integration of multiple signals which results in cancellation of noise and enhancement of signal to noise ratio.

[162] Fig. 30 shows a novel disease detection method in which at least one probe object is launched at a desired speed and direction toward a biological subject, resulting in a collision.

[163] Fig. 31 shows a process of this invention for detecting a biological subject using disease detection apparatus.

[164] Fig. 32 shows another embodiment of disease detection process wherein diseased and healthy biological subjects are separated and the diseased biological subjects are delivered to further test.

[165] Fig. 33 shows an arrayed biological detecting device wherein a series of detecting devices fabricated into an apparatus.

[166] Fig. 34 shows another embodiment of a disease detection device of the current invention including inlet and outlet of the device, the channel where the biological subject passes through, and detection devices aligned along the walls of the channel.

[167] Fig. 35 shows an example of the apparatus of this invention packaged and ready for use.

[168] Fig. 36 shows another example of the apparatus of this invention that is packaged and ready for use.

[169] Fig. 37 shows yet another example of the apparatus of this invention that is packaged and ready for use.

[170] Fig. 38 shows an apparatus of this invention that has a channel (trench) and an array of micro sensors.

[171] Fig. 39 shows another apparatus of this invention comprising several "sub-devices."

[172] Fig. 40 shows an example of the apparatus of this invention which includes an application specific integrated circuit (ASIC) chip with I/O pads.

[173] Fig. 41 is a diagram of the underlying principal of the apparatus of this invention which functions by combining various pre-screening and detection methods in unobvious ways.

[174] Fig. 42 shows cross-sectional and outside views of a channel into which a biological subject can flow.

[175] Fig. 43 shows a biological subject to be detected passing through a channel aligned with detectors along its passage in an apparatus of this invention.

[176] Fig. 44 is a view of the apparatus of this invention showing one or two sorting units therein.

[177] Fig. 45 shows an apparatus of this invention with a high number of desired structures fabricated simultaneously on the same chip.

[178] Fig. 46 shows another novel device layout for sorting, screening, separating, probing and detecting diseased biological entities, in which a desired component or multiple components through the middle channel into the middle chamber can play a wide range of roles.

[179] Fig. 47 shows that, compared with multiple stand-alone detection apparatuses, an apparatus of this invention with multiple sub-units of different functions and technologies assembled or integrated has a significantly reduced apparatus volume or size, therefore reduced costs since many common hardware (e.g., a sample handling unit, a sample measurement unit, a data analysis unit, a display, a printer, etc.) can be shared in an integrated apparatus.

[180] Fig. 48 shows that when multiple sub-units with different functions and technologies are assembled into one apparatus, a more diverse functionality, improved detection functionality, sensitivity, detection versatility, and reduced volume and cost can be achieved, where a number of common utilities including, e.g., input hardware, output hardware, sample handling unit, sample measurement unit, data analysis unit and data display unit can be shared.

[181] Fig. 49 shows a number of different classifications of biological information are collected in a device and processed in the novel technology.

[182] Fig. 50 shows measured information in this novel technology includes protein, cellular and molecular level information, or combination of them.

[183] Fig. 51 shows signals from different biological classifications may interact, combine, and/or amplify to enhance signal in this novel technology.

[184] Fig. 52 shows detected signal in this novel technology as a function of cancer cell concentration. Signal increases with increasing amount of cancer cells.

[185] Fig. 53 shows detected signal in this novel technology as a function of a bio-marker level. Signal increases with increasing level of bio-marker.

[186] Fig. 54 shows Advantage of this novel technology compared with traditional bio-marker (AFP) for liver cancer. Using 58 confirmed liver cancer samples, sensitivity of this novel technology is 79.3%, while that of AFP is 55.9%.

[187] Fig. 55 shows the results of detected signal CDA before and after adding molecular level reaction triggering agent.

[188] Fig. 56 shows the numbers of actual samples tested by this invention and the unexpected results achieved or shown by these tests.

[189] Fig. 57 shows the results of a multi-level detection system of this invention.

[190] Fig. 58 shows the CDA values of the control group, non-cancer disease group and cancer group.

[191] Fig. 59 shows the relationship between disease state and detected cell signaling properties and/or cell media properties.

[192] Fig. 60 shows a scheme of cells, proteins, and genetic components (DNA, RNA, etc.) and their surrounding liquid media (e.g., blood).

[193] Fig. 61 shows scanning curves of control (healthy) and lung cancer cell lines.

[194] Fig. 62 shows a typical scanning curve for control (healthy) whole blood sample.

[195] Fig. 63 shows scanning curves for control (healthy) whole blood sample and liver cancer whole blood sample.

[196] Fig. 64 shows scanning curves for control (healthy), disease, and liver cancer whole blood samples.

[197] Fig. 65 shows comparison of claimed technology in this application versus circulating tumor cell (CTC) and circulating tumor (cancer) DNA (ctDNA). In this technology, signal exists for all groups starting with healthy group and rises rapidly with disease group, pre-cancer group and cancer group, with a high signal to noise ratio (schematically each dot represents signal, the higher the signal, the more dots), while CTC and ctDNA technologies only have signal in cancer stage II, with a very weak signal, and expected poor signal to noise ratio).

[198] Fig. 66 shows that CDA technology is a multi-level and multi-parameter test that can also be carried out in conjunction with other tests including bio-markers (protein level), CTC (cellular level), and/or ct-DNA and other DNA based tests (genetic tests).

[199] Fig. 67 shows a schematic of a proposed model, in which shift in bio-physical properties such as electrical properties cause changes at cellular, protein, and molecular (gene) levels which

result in changes at immunity and inflammation, and likelihood (or less likelihood) of diseases and cancer occurrence.

[200] Fig. 68 shows that as CDA increases and electrical current, conductance, ion level, membrane potential and polarization decrease, a number of cellular level (cell signaling, cell repulsion, resting potential and cell surface charge decrease) and molecular level (DNA surface charge decrease, quantum mechanical effect change, and DNA mutation increases) properties degrade, resulting in increased disease and cancer occurrence.

[201] Fig. 69 shows the CDA value (a value based on the measured properties claimed in this patent application and after data analysis) for control (healthy) group, non-cancer disease group, and cancer group. The DCA value becomes progressively higher from healthy stage, to non-cancer disease group, and to cancer group.

[202] Fig. 70 shows that as electrical current and conductance decrease (ion (e.g., potassium, chloride, sodium, and calcium) concentration or net ion concentration or charge decreases), a number of cellular level (cell signaling, cell repulsion, resting potential, membrane potential and cell surface charge decrease) properties change and degrade.

[203] Fig. 71 shows the changes in electrical properties of DNA surrounding media and/or DNA surface charge between health and cancer cases.

[204] Fig. 72 shows that the CDA technology has higher sensitivity and specificity than traditional CT imaging.

[205] Fig. 73 shows that the CDA values appear to correlate with mutation frequency for (a) healthy, (b) lung cancer just after diagnosis and before surgery, and (c) after surgery and treatment individuals / groups.

[206] Fig. 74 shows use of the CDA technology for prognosis of a targeted drug treatment of small cell lung cancer at three stages, i.e., after diagnosis, after phase 1 treatment, and after phase 2 treatment.

[207] Fig. 75 shows a schematic of cell membranes with intracellular and extracellular regions, with decreasing membrane potential and net charge Q in extracellular region.

[208] Fig. 76 shows a schematic of membranes of two cells showing membrane potential, intracellular space, and extracellular space.

Detailed Description of the Invention

[0209] One aspect of the present invention relates to apparatus for detecting a disease in a biological subject *in vivo* or *in vitro* (e.g., human being, an organ, a tissue, or cells in a culture). Each apparatus comprises a delivery system, at least two sub-equipment units, and optionally a central control system. Each sub-equipment is capable of measuring at least a microscopic property of a biological sample. Accordingly, the apparatus of this invention can detect different parameters of the biological subject and provide accuracy, sensitivity, specificity, efficiency, non-invasiveness, practicality, conclusive, and speed in early-stage disease detection at reduced costs. In addition, the apparatus of this invention has some major advantages, such as reducing effective foot print (e.g., defined as function per unit space), reducing space for the medical devices, reducing overall cost, and providing conclusive and effective diagnosis by one device.

[0210] The delivery system can be a fluid delivery system. By the constant pressure fluid delivery system, microscopic biological subjects can be delivered onto or into one or more desired sub-equipment units of the apparatus.

[0211] As a key component of the apparatus, the micro-device should include means to perform at least the function of addressing, controlling, forcing, receiving, amplifying, or storing information from each probing address. As an example, the apparatus can further include a central control system for controlling the biological subject matter to be transported to one or more desired sub-equipment units and reading and analyzing a detected data from each sub-equipment unit. The central control system includes a controlling circuitry, an addressing unit, an amplifier circuitry, a logic processing circuitry, a memory unit, an application specific chip, a signal transmitter, a signal receiver, or a sensor.

[0212] In some embodiments, the fluid delivering system comprises a pressure generator, a pressure regulator, a throttle valve, a pressure gauge, and distributing kits. As examples of these embodiments, the pressure generator can include a motor piston system and a bin containing compressed gas; the pressure regulator (which can consist of multiple regulators) can down-regulate or up-regulate the pressure to a desired value; the pressure gauge feeds back the measured value to the throttle valve which then regulates the pressure to approach the target value.

[0213] The biological fluid to be delivered can be a sample of a biological entity to be detected for disease or something not necessarily to be detected for disease. In some embodiments, the fluid to be delivered is liquid (e.g., a blood sample or a lymph sample). The pressure regulator

can be a single pressure regulator or multiple pressure regulators which are placed in succession to either down-regulate or up-regulate the pressure to a desired level, particularly when the initial pressure is either too high or too low for a single regulator to adjust to the desired level or a level that is acceptable for an end device or target.

[0214] Optionally, the apparatus includes additional features and structures to deliver a second liquid solution containing at least an enzyme, protein, oxidant, reducing agent, catalyst, radio-active component, optical emitting component, or ionic component. This second liquid solution can be added to the sample to be measured before or during sorting of the biological subject sample to be measured, or before or during the measurement (i.e., detection) of the biological subject sample, for the purposes of further enhancing the apparatus' measurement sensitivity.

[0215] In some other embodiments, the system controller includes a pre-amplifier, a lock-in amplifier, an electrical meter, a thermal meter, a switching matrix, a system bus, a nonvolatile storage device, a random access memory, a processor, or a user interface. The interface can include a sensor which can be a thermal sensor, a flow meter, an optical sensor, an acoustic detector, a current meter, an electrical sensor, a magnetic sensor, an electro-magnetic sensor, a pH meter, a hardness measurement sensor, an imaging device, a camera, a piezo-electrical sensor, a piezo-photronic sensor, a piezo-electro photronic sensor, an electro-optical sensor, an electro-thermal sensor, a bio-electrical sensor, a bio-marker sensor, a bio-chemical sensor, a chemical sensor, an ion emission sensor, a photo-detector, an x-ray sensor, a radiation material sensor, an electrical sensor, a voltage meter, a thermal sensor, a flow meter, or a piezo- meter..

[0216] In still some other embodiments, apparatus of this invention further includes a biological interface, a system controller, a system for reclaiming or treatment medical waste. The reclaiming and treatment of medical waste can be performed by the same system or two different systems.

[0217] Another aspect of this invention provides apparatus for interacting with a cell, which include a device for sending a signal to the cell and optionally receiving a response to the signal from the cell.

[0218] In some embodiments, the interaction with the cell can be probing, detecting, sorting, communicating with, treating, or modifying with a coded signal that can be a thermal, optical, acoustical, biological, chemical, electro-mechanical, electro-chemical, electro-optical, bio-electro-optical, bio-thermal optical, electro-chemical optical, electro-chemical-mechanical, bio-

chemical, bio-mechanical, bio-electro-mechanical, bio-electro-chemical, bio-electro-chemical-mechanical, electric, magnetic, electro-magnetic, physical, or mechanical signal, or a combination thereof.

[0219] In some other embodiments, the device or the sub-equipment unit contained in the apparatus can include multiple surfaces coated with one or more elements or combinations of elements, and a control system for releasing the elements. In some instances, the control system can cause release of the elements from the device surface via an energy including but not limited to thermal energy, optical energy, acoustic energy, electrical energy, electro-magnetic energy, magnetic energy, radiation energy, or mechanical energy in a controlled manner. The energy can be in the pulsed form at desired frequencies.

[0220] In some other embodiments, the device or the sub-equipment unit contained in the apparatus includes a first component for storing or releasing one element or a combination of elements onto the surface of the cell or into the cell; and a second component for controlling the release of the elements (e.g., a circuitry for controlling the release of the elements). The elements can be a biological component, a chemical compound, ions, catalysts, Ca, C, Cl, Co, Cu, H, I, Fe, Mg, Mn, N, O, P, F, K, Na, S, Zn, or a combination thereof. The signal, pulsed or constant, can be in the form of a released element or combination of elements, and it can be carried in a liquid solution, gas, or a combination thereof. In some instances, the signal can be at a frequency ranging from about $1x10^{-4}$ Hz to about 100 MHz or ranging from about $1x10^{-4}$ Hz to about 10 Hz, or at an oscillation concentration ranging from about 1.0 nmol/L to about 10.0 mmol/L. Also, the signal comprises the oscillation of a biological component, a chemical compound, Ca, C, Cl, Co, Cu, H, I, Fe, Mg, Mn, N, O, P, F, K, Na, S, Zn, or a combination thereof, e.g., at desired oscillating frequencies.

[0221] In some embodiments, the signal to be sent to the cell can be in the form of oscillating element, compound, or an oscillating density of a biological component, and a response to the signal from the cell is in the form of oscillating element, compound, or an oscillating density of a biological component.

[0222] In some embodiments, the device or the sub-equipment unit can be coated with a biological film, e.g., to enhance compatibility between the device and the cell.

[0223] In some other embodiments, the device or the sub-equipment unit can include components for generating a signal to be sent to the cell, receiving a response to the signal from

the cell, analyzing the response, processing the response, and interfacing between the device and the cell.

[0224] Still another aspect of this invention provides devices or sub-equipment units each including a micro-filter, a shutter, a cell counter, a selector, a micro-surgical kit, a timer, and a data processing circuitry. The micro-filter can discriminate abnormal cells by a physical property (e.g., dimension, shape, or velocity), mechanical property, electric property, magnetic property, electro-magnetic, thermal property (e.g., temperature), optical property, acoustical property, biological property, chemical property, electro-chemical property, bio-chemical property, bio-electro-chemical property, bio-electro-mechanical property, or electro-mechanical property. The devices each can also include one or more micro-filters. Each of these micro-filters can be integrated with two cell counters, one of which is installed at the entrance of each filter well, while the other is installed at the exit of each filter well. The shape of the micro-filter's well is rectangle, ellipse, circle, or polygon; and the micro-filter's dimension ranges from about 0.1 μm to about 500 μm or from about 5 um to about 200 um. As used herein, the term "dimension" means the physical or feature size of the filter opening, e.g., diameter, length, width, or height. The filter can be coated with a biological or bio-compatible film, e.g., to enhance compatibility between the device and the cell.

[0225] In addition to separation of biological entity by its size and other physical features, the filter can also contain additional features and functions to perform biological entity separation via other properties, which comprise of mechanical property, electric property, magnetic property, electro-magnetic, thermal property (e.g., temperature), optical property, acoustical property, biological property, chemical property, electro-chemical property, bio-chemical property, bio-electro-chemical property, bio-electro-mechanical property, and electro-mechanical property.

[0226] In some embodiments of these devices, the shutter sandwiched by two filter membranes can be controlled by a timer (thus time shutter). The timer can be triggered by the cell counter. For instance, when a cell passes through the cell counter of the filter entrance, the clock is triggered to reset the shutter to default position, and moves at a preset speed towards the cell pathway, and the timer records the time as the cell passes through the cell counter at the exit.

[0227] Still a further aspect of this invention provides methods for fabricating a micro-device with micro-trench and probe embedded in the micro-trench's sidewalls. A micro-trench is an

unclosed tunnel (see, e.g., Fig. 2(i), **2030**), which can be coupled with another upended symmetric trench (see, e.g., Fig. 2(k), **2031**) to form a closed channel (see, e.g., Fig. 2(l), **2020**). The method may include chemical vapor deposition, physical vapor deposition, or atomic layer deposition to deposit various materials on a substrate (where the substrate can be a semiconductor material such as silicon, or an insulating material such as glass or silicon dioxide material); patterning the deposited layer(s) utilizing methods comprising of lithography, etch, and chemical mechanical polishing to form desired features (such as a trench); chemical mechanical planarization for surface planarization; chemical cleaning for particle removal; diffusion or ion implantation for doping elements into specific layers; or thermal anneal to reduce the crystal defects and activate diffused ions. An example of such method includes: depositing a first material onto a substrate; depositing a second material onto the first material and patterning the second material by a microelectronic process (e.g., lithography, etch) to form a detecting tip; depositing a third material on the second material and then planarize the third material by a polishing process; depositing a fourth material on the third material and patterning the fourth material first by a microelectronic process (e.g., lithography, etch) and then by a microelectronic process (e.g., another etch) to remove a portion of the third material and optionally a portion of the first material while this etch is typically selective to the second material (lower etch rate for the second material), in which the fourth material serves as a hardmask. A hardmask generally refers to a material (e.g., inorganic dielectric or metallic compound) used in semiconductor processing as an etch mask in lieu of polymer or other organic "soft" materials. In one embodiment, a channel is formed in the substrate layer (such as a silicon or a silicon dioxide or glass layer) or in the layer(s) above the substrate layer, with at least one probe (such as gold, tungsten, aluminum, silver, copper, or nickel conductive probing tip) being formed on the wall of the channel to probe desired biological sample properties (such as physical, bio-physical, or bio-chemical properties).

[0228] In some embodiments, the method further includes coupling two devices or sub-equipment units that are thus fabricated and symmetric (i.e., a flipped mirror) to form a detecting device with channels. The entrance of each channel can be optionally bell-mouthed, e.g., such that the size of channel's opening end (the entrance) is larger than the channel's body, thereby making it easier for a cell to enter the channel. The shape of each channel's cross-section can be rectangle, ellipse, circle, or polygon. The micro-trenches of the coupled two micro-devices can

be aligned by the module of alignment marks designed on the layout of the micro-device. The dimension of the micro-trench can range from about 0.1 um to about 500 um.

[0229] Alternatively, the method can also include covering the micro-trench of the micro-device with a flat panel. Such a panel can comprise or be made with silicon, SiGe, SiO_2, Al_2O_3, quartz, low optical loss glasses, or other optical materials. Examples of other potentially suitable optical materials include acrylate polymer, AgInSbTe, synthetic alexandrite, arsenic triselenide, arsenic trisulfide, barium fluoride, CR-39, cadmium selenide, caesium cadmium chloride, calcite, calcium fluoride, chalcogenide glass, gallium phosphide, GeSbTe, germanium, germanium dioxide, glass code, hydrogen silsesquioxane, Iceland spar, liquid crystal, lithium fluoride, lumicera, METATOY, magnesium fluoride, agnesium oxide, negative index metamaterials, neutron supermirror, phosphor, picarin, poly(methyl methacrylate), polycarbonate, potassium bromide, sapphire, scotophor, spectralon, speculum metal, split-ring resonator, strontium fluoride, yttrium aluminum garnet, yttrium lithium fluoride, yttrium orthovanadate, ZBLAN, zinc selenide, and zinc sulfide.

[0230] In other embodiments, the method can further include integrating three or more sub-equipment units or devices thus fabricated to yield an enhanced device with an array of the channels.

[0231] Another aspect of this invention relates to a set of novel process flows for fabricating micro-devices (including micro-probes and micro-indentation probes) for their applications in disease detection by measuring microscopic properties of a biological sample. The micro-devices can be integrated into detection apparatus of this invention as sub-equipment units to measure one or more properties at microscopic levels. For example, a cancerous cell may have a different hardness (harder), density (denser), and elasticity than a normal cell.

[0232] Another aspect of this invention is to involve in cellular communications and regulate cellular decision or response (such as differentiation, dedifferentiation, cell division and cell death) with fabricated signals generated by the micro-devices disclosed herein. This could be further employed to detect and treat diseases.

[0233] Another aspect of the current application is that the inventive method or measured parameter in the method is a function of at least two levels F (level 1, level 2), where level 1 can be a biological entity such as protein and level 2 can be another biological entity such as genetics, where the measured signal strength of F (level 1, level 2) is greater than the sum of the

signal containing only level 1 information f (level 1) and the signal containing only level 2 information f (level 2):

Signal strength of F (level 1, level 2) >
signal strength of f (level 1) + signal strength of f (level 2)

[0234] The above novel feature and property can be extended to a measured parameter which is a function containing many levels F (level 1, level 2, level 3 ..... level n). One novel and unobvious feature of this innovation is that the measured signal in a parameter containing multiple biological levels is synergistically enhanced over the measured signals with each signal containing a single biological level only. With this approach, the typically weak detection signal in disease detection such as cancer detection (especially in early stage cancer detection) can be effectively enhanced or magnified, making early disease detection possible and more effective.
[0235] To further enhance measurement capabilities, multiple micro-devices can be implemented into a piece of detection apparatus as sub-equipment units employing the time of flight technique, in which at least one probing micro-device and one sensing micro-device placed at a preset, known distance. The probing micro-device can apply a signal (e.g., a voltage, a charge, an electrical field, a laser beam, a thermal pulse, a train of ions, or an acoustic wave) to the biological sample to be measured, and the detection (sensing) micro-device can measure response from or of the biological sample after the sample has traveled a known distance and a desired period of time. For instance, a probing micro-device can apply an electrical charge to a cell first, and then a detection (sensing) micro-device subsequently measures the surface charge after a desired period of time (**T**) has lapsed and the cell has traveled a certain distance (**L**).
[0236] The micro-devices or the sub-equipment units contained in the apparatus of this invention can have a wide range of designs, structures, functionalities, flexibilities, and applications due to their diverse properties, high degree of flexibilities, and ability of integration, miniaturization, and manufacturing scalability. They include, e.g., a voltage comparator, a four point probe, a calculator, a logic circuitry, a memory unit, a micro cutter, a micro hammer, a micro shield, a micro dye, a micro pin, a micro knife, a micro needle, a micro thread holder, micro tweezers, a micro laser, a micro optical absorber, a micro mirror, a micro wheeler, a micro filter, a micro chopper, a micro shredder, micro pumps, a micro absorber, a micro signal detector, a micro

driller, a micro sucker, a micro tester, a micro container, a signal transmitter, a signal generator, a friction sensor, an electrical charge sensor, a temperature sensor, a hardness detector, an acoustic wave generator, an optical wave generator, a heat generator, a micro refrigerator and a charge generator.

[0237] Further, it should be noted that advancements in manufacturing technologies have now made fabrications of a wide range of micro-devices and integration of various functions onto the same device highly feasible and cost effective. The typical human cell size is about 10 microns. Using state-of-the-art integrated circuit fabrication techniques, the minimum feature size defined on a micro-device can be as small as 0.1 micron or below. Thus, it is ideal to utilize the disclosed micro-devices for biological applications.

[0238] In terms of materials for the micro-devices in the apparatus of this invention, the general principle or consideration is the material's compatibility with a biological entity. Since the time in which a micro-device is in contact with a biological sample (e.g., a cell) may vary, depending on its intended application, a different material or a different combination of materials may be used to make the micro-device. In some special cases, the materials may dissolve in a given pH in a controlled manner and thus may be selected as an appropriate material. Other considerations include cost, simplicity, ease of use and practicality. With the significant advancements in micro fabrication technologies such as integrated circuit manufacturing technology, highly integrated devices with minimum feature size as small as 0.1 micron can now be made cost-effectively and commercially. One good example is the design and fabrication of micro electro mechanical devices (MEMS), which now are being used in a wide variety of applications in the electronics industry and beyond.

[0239] Good disease (cancer and non-cancer) detection results in terms of measurement sensitivity and specificity have been obtained on multiple types of cancer tested, demonstrating validity of the apparatus of this invention for improved ability to detect diseases (e.g., cancers), particularly in their early stages. The present invention provides novel "Cancer Differentiation Analysis" (CDA) liquid biopsy technology. The experimental results have also shown that multiple cancer types can be detected using the disclosed apparatus, which itself is an improvement over many existing detection apparatuses.

[0240] Specifically, studies utilizing the apparatus of this invention have been carried out on multiple types of cancer and non-cancer diseases (including an inflammatory disease, diabetes, a

lung disease, a heart disease, a liver disease, a gastric disease, a biliary disease, or a cardiovascular disease). In these studies, whole blood samples were used within 5 days after being obtained and/or properly transported/stored in a 0.5-20 ºC refrigerated environment. The samples of the control group were obtained from healthy people confirmed by physical examinations with normal AFP and CEA values (in normal ranges).

Table 1. Data from the Test for Lung Diseases

Group		Samples	Gender (Male %)	Age Range	Age Mean	Age Median	CDA Mean (rel. units)	CDA Median (rel. units)	CDA STDEV (rel. units)
CDA	Control	981	54	22 - 91	59	61	36.55	36.20	7.18
	Lung Disease	95	71	21 - 90	65	67	45.75	45.66	22.67
	Pulmonary infection	75	67	21 - 85	65	66	45.78	45.83	9.08
	Pneumonia	14	79	22 - 87	61	63	44.49	45.25	9.21
	Chronic obstructive pulmonary disease	4	100	73 - 90	81	81	45.63	43.55	6.56
	Tuberculosis	2	100	65 - 66	66	66	53.87	53.87	11.92

Table 2. Data from Tests for Diabetes

Group		Samples	Gender (Male %)	Age Range	Age Mean	Age Median	CDA Mean (rel. units)	CDA Median (rel. units)	CDA STDEV (rel. units)
CDA (rel. units)	Control	981	54	22 - 91	59	61	36.55	36.20	7.18

	Diabetes	62	55	37 - 86	62	62	44.31	45.01	12.47
	Type-2 Diabetes	39	49	37 - 86	61	62	47.08	46.45	13.34
	Unclear types	23	65	43 - 86	63	62	39.62	41.92	9.32

Table 3. Data from Tests for Heart Diseases

Group		Samples	Gender (Male%)	Age Range	Age Mean	Age Median	CDA Mean (rel. units)	CDA Median (rel. units)	CDA STDEV (rel. units)
CDA (rel. units)	Control	981	54	22 - 91	59	61	36.55	36.20	7.18
	Heart Disease	54	45	21 - 105	73	75	44.24	44.43	11.97
	Coronary disease	26	38	50 - 94	71	70	41.99	42.70	13.39
	Other heart disease	14	57	61 - 91	76	76	46.88	47.73	6.86
	Heart failure	9	44	74 - 105	82	80	48.60	45.41	14.58
	Arrhythmia	5	20	21 – 85	62	70	40.69	44.18	9.11

Table 4. Data from Tests for Liver Diseases

Group		Samples	Gender (Male%)	Age Range	Age Mean	Age Median	CDA Mean (rel. units)	CDA Median (rel. units)	CDA STDEV (rel. units)
CDA (rel. units)	Control	981	54	22 – 91	59	61	36.55	36.20	7.18
	Liver Disease	160	68	24 – 87	55.56	53.50	44.29	44.75	8.32

	Cirrhosis	88	78	30 – 87	57.68	55.00	43.68	43.72	8.62
	Hepatitis	56	63	24 – 76	54.27	52.50	43.32	43.84	7.74

Table 5. Data from Tests for Gastric Diseases

Group		Samples	Gender (Male%)	Age Range	Age Mean	Age Median	CDA Mean (rel. units)	CDA Median (rel. units)	CDA STDEV (rel. units)
CDA (rel. units)	Control	981	54	22 - 91	59	61	36.55	36.20	7.18
	Gastric Disease	47	60	29 - 89	60.81	63.00	44.24	44.90	9.29
	Gastritis	28	61	29 - 89	60.29	62.00	45.16	45.01	9.37
	Gastric polyp	12	67	33 - 71	61.00	66.00	41.70	44.37	8.17
	Gastric ulcer	2	50	59 - 79	69.00	69.00	36.76	36.76	11.12

Table 6. Summary of Descriptive Statistics

Group		Samples	Gender (Male%)	Age Range	Age Mean	Age Median	CDA Mean (rel. units)	CDA Median (rel. units)	CDA STDEV (rel. units)
CDA (rel. units)	Control	981	54	22 - 91	59	61	36.55	36.20	7.18
	Lung Disease	95	71	21 - 90	65	67	45.75	45.66	22.67
	Diabetes	62	55	37 - 86	62	62	44.31	45.01	12.47
	Heart Disease	54	45	21 - 105	73	75	44.24	44.43	11.97

	Liver Disease	160	68	24 - 87	55.56	53.50	44.29	44.75	8.32
	Gastric Disease	47	60	29 - 89	60.81	63.00	44.24	44.90	9.29
	Biliary Disease	28	57	21 - 85	60.11	60.50	45.75	46.57	11.82

Table 7. Results of ROC Curve Analysis

Group	Area Under the Curve (rel. units)	Cut-off Value (rel. units)	Sensitivity	Specificity
Lung Disease	0.788	41	74.7%	73.9%
Diabetes	0.727	41	72.6%	72.3%
Heart Disease	0.736	41	74.1%	74.3%
Liver Disease	0.758	41	70.0%	73.8%
Gastric Disease	0.740	41	74.5%	74.3%
Biliary Disease	0.779	41	82.1%	74.4%

[0241] CDA value is obtained from an algorithm using calculation based on tested values from the studies. CDA value increases with risks of diseases. In other words, the higher the CDA values, the higher the risks of diseases.

[0242] As the above tables show, the CDA values are higher for various diseases (mid 40s) than those of control (healthy) group (around 36). Statistical analysis of CDA values for those two groups shows that there was a statistically significant difference in CDA values between those two groups. Accordingly, the studies above show that the apparatus and methods of this invention were able to distinguish some major diseases from control group, with sensitivity and

specificity likely higher than existing technologies.

[0243] Set forth below are several illustrations or examples of apparatus of this invention containing a class of innovative micro-devices that are integrated as sub-equipment units.

[0244] Fig. 1 (a) illustrates a set of traditional detection apparatus each of which relies on a single detection technology. As shown in Fig. 1 (a), current diagnosis devices detect a disease on a narrow focus and typically by one single technology (e.g., x-ray machine or NMR machine).

[0245] Fig. 1 (b) and Fig. (c) are an illustration of a detection apparatus of this invention where multiple sub-equipment units are integrated into one piece of apparatus. As a result, the novel apparatus has a smaller size comparing to traditional devices.

[0246] Fig. 2 is a schematic illustration of a detection apparatus of this invention which comprises multiple sub-equipment units, a delivery system, and a central control system. The central control system comprises multiple processing units each of which can be a computer, data analysis unit, or display unit. The central control system is interacted with and used by multiple sub-equipment units. This resource sharing process can effectively reduce cost and size of the apparatus. The biological subject (e.g. a fluid sample) can flows to each sub-equipment units via the delivery system. The delivery system can also transport the biological subject to one or more desired sub-equipments for specific diagnosis purposes.

[0247] To enhance detection speed and sensitivity, a large number of micro-devices can be integrated into a single apparatus of this invention. Each micro-device can be a independent sub-equipment unit in the apparatus. To achieve the above requirements, the detection apparatus should be optimized with its surface area maximized to contact the biological sample and with large number of micro-devices integrated on the maximized surface.

[0248] Instead of measuring a single property of a biological subject for disease diagnosis, various micro-devices can be integrated into a detection apparatus to detect multiple properties. Various micro-devices can constitute different sub-equipment units. Fig. 3 is a perspective, cross-sectional illustration of a disease detection apparatus of this invention **133** with multiple micro-devices **311**, **312**, **313**, **314**, and **315**, of different detection probes in which a sample **211** such as a blood sample placed in it or moving through it can be tested for multiple properties including but not limited to mechanical properties (e.g., density, hardness and adhesion), thermal properties (e.g., temperature), biological properties, chemical properties (e.g., pH), physical

properties, acoustical properties, electrical properties (e.g., surface charge, surface potential, and impedance), magnetic properties, electromagnetic properties, and optical properties.

[0249] As illustrated herein, it is desirable to optimize the detection apparatus design to maximize measurement surface area, since the greater the surface area, the greater number of micro-devices that can be placed on the detection apparatus to simultaneously measure the sample, thereby increasing detection speed and also minimizing the amount of sample needed for the test.

[0250] Fig. 4 is a perspective illustration of an apparatus or a sub-equipment unit of this invention. It includes two slabs separated by a narrow spacing with a sample such as a blood sample to be measured placed between the slabs, with multiple micro-devices placed at the inner surfaces of the slabs to measure one or more properties of the sample at microscopic levels.

[0251] Yet another aspect of this invention relates to a set of novel fabrication process flows for making micro-devices or sub-equipment units for disease detection purposes. Thus, a micro-device with two probes capable of measuring a range of properties (including mechanical and electrical properties) of biological samples is fabricated, using the above novel fabrication process flow.

[0252] Detection apparatus integrated with micro-devices disclosed in this application is fully capable of detecting pre-chosen properties on a single cell, a single DNA, a single RNA, or an individual, small sized biological matter level. In another further aspect, the invention provides the design, integration, and fabrication process flow of micro-devices capable of making highly sensitive and advanced measurements on very weak signals in biological systems for disease detection under complicated environment with very weak signal and relatively high noise background. Those novel capabilities using the class of micro-devices disclosed in this invention for disease detection include but not limited to making dynamic measurements, real time measurements (such as time of flight measurements, and combination of using probe signal and detecting response signal), phase lock-in technique to reduce background noise, and 4-point probe techniques to measure very weak signals, and unique and novel probes to measure various electronic, electromagnetic and magnetic properties of biological samples at the single cell (e.g., a telomere of DNA or chromosome), single molecule (e.g., DNA, RNA, or protein), single biological subject (e.g., virus) level.

[0253] For example, in a time of flight approach to obtain dynamic information on the biological

sample (e.g., a cell, a substructure of a cell, a DNA, a RNA, or a virus), a first micro-device is first used to send a signal to perturb the biological subject to be diagnosed, and then a second micro-device is employed to accurately measure the response from the biological subject. In one embodiment, the first micro-device and the second micro-device are positioned with a desired or pre-determined distance **L** apart, with a biological subject to be measured flowing from the first micro-device towards the second micro-device. When the biological subject passes the first micro-device, the first micro-device sends a signal to the passing biological subject, and then the second micro-device detects the response or retention of the perturbation signal on the biological subject. From the distance between the two micro-devices, time interval, the nature of perturbation by the first micro-device, and measured changes on the biological subject during the time of flight, microscopic and dynamic properties of the biological subject can be obtained. In another embodiment, a first micro-device is used to probe the biological subject by applying a signal (e.g., an electronic charge) and the response from the biological subject is detected by a second micro-device as a function of time.

[0254] To further increase detection sensitivity, a novel detection process for disease detection is used, in which time of flight technique is employed. Fig. 5 is a perspective, cross-sectional illustration of detection apparatus **155** with multiple micro-devices **321** and **331** placed at a desired distance **700** for time of flight measurements to attain dynamic information on biological sample **211** (e.g., a cell) with enhanced measurement sensitivity, specificity, and speed. In this time of flight measurement, one or more properties of the biological sample **211** are first measured when the sample **211** passes the first micro-device **321**. The same properties are then measured again when the sample **211** passes the second micro-device **331** after it has travelled the distance **700**. The change in properties of sample **211** from at micro-device **321** to at micro-device **331** indicates how it reacts with its surrounding environment (e.g., a particular biological environment) during that period. It may also reveal information and provide insight on how its properties evolve with time. Alternatively, in the arrangement shown in Fig. 5, micro-device **321** could be used first as a probe to apply a probe signal (e.g., an electrical charge) to sample **211** as the sample passes the micro-device **321**. Subsequently, the response of the sample to the probe signal can be detected by micro-device **331** as the sample passes it (e.g., change in the electrical charge on the sample during the flight). Measurements on biological sample **211** can be done via contact or non-contact measurements. In one embodiment, an array of micro-devices can be

deployed at a desired spacing to measure properties of the biological subject over time.

[0255] The utilization of micro-devices (e.g., made by using the fabrication process flows of this invention) as discussed above and illustrated in Fig. 5 can be helpful for detecting a set of new, microscopic properties of a biological sample (e.g., a cell, a cell substructure, or a biological molecule such as DNA or RNA or protein) that have not been considered in existing detection technologies. Such microscopic properties can be thermal, optical, acoustical, biological, chemical, electro-mechanical, electro-chemical, electro-chemical-mechanical, bio-chemical, bio-mechanical, bio-electro-mechanical, bio-electro-chemical, bio-electro-chemical-mechanical, electrical, magnetic, electromagnetic, physical, or mechanical properties, or a combination thereof, of a biological sample that is a single biological subject (such as a cell, a cell substructure, a biological molecule – e.g., DNA, RNA, or protein – or a sample of a tissue or organ). It is known that biological matters includes from basic bonding such as OH, CO, and CH bonding, to complex, three dimensional structures such as DNA and RNA. Some of them have a unique signature in terms of its electronic configuration. Some of them may have unique thermal, optical, acoustical, biological, chemical, electro-mechanical, electro-chemical, electro-chemical-mechanical, bio-chemical, bio-mechanical, bio-electro-mechanical, bio-electro-chemical, bio-electro-chemical-mechanical, electrical, magnetic, electromagnetic, physical, or mechanical properties and configurations, or a combination thereof. Normal biological subject and diseased biological subject may carry different signatures with respective to the above said properties. However, none of the above stated parameters or properties have been routinely used as a disease detection property. Using a disease detection apparatus including one or more apparatus of this invention, those properties can be detected, measured, and utilized as useful signals for disease detection, particularly for early stage detection of serious diseases such as cancer.

[0256] Fig. 6 is a perspective illustration of a novel set of microscopic probes **341**, **342**, **343**, **344**, **345**, **346**, and **347** designed and configured to detect various electronic, magnetic, or electromagnetic states, configurations, or other properties at microscopic level on biological samples **212**, **213**, **214**, and **215**, which can be a single cell, DNA, RNA, and tissue or sample. As an example, in terms of measuring electronic properties, the shapes of biological samples **212**, **213**, **214**, and **215** in Fig. 10 may represent electronic monopole (sample **212**), dipole (samples **213** and **214**), and quadruple (sample **215**). The micro-devices **341**, **342**, **343**, **344**, **345**,

346, and **347** are optimized to maximize measurement sensitivity of those said parameters including but not limited to electronic states, electronic charge, electronic cloud distribution, electrical field, and magnetic and electromagnetic properties, and the micro-devices can be designed and arranged in three dimensional configurations. For some diseases such as cancer, it is likely that electronic states and corresponding electronic properties differ between normal and cancerous cells, DNA, RNA, and tissue. Therefore, by measuring electronic, magnetic and electromagnetic properties at microscopic levels including at cell, DNA, and RNA levels, disease detection sensitivity and specificity can be improved.

[0257] In addition to the above examples in measuring electrical properties (e.g., charge, electronic states, electronic charge, electronic cloud distribution, electrical field, current, and electrical potential, and impedance), mechanical properties (e.g., hardness, density, shear strength, and fracture strength) and chemical properties (e.g., pH) in a single cell, and in Fig. 6 for measuring electrical, magnetic or electromagnetic states or configurations of biological samples at cell and biological molecular (e.g., DNA, RNA, and protein) levels, other micro-devices are disclosed in this application for sensitive electrical measurements.

[0258] Fig. 7 is a perspective illustration of a four-point probe for detecting weak electronic signal in a biological sample such as a cell, where a four point probe **348** is designed to measure electrical properties (impedance and weak electrical current) of a biological sample **216**.

[0259] One of the key aspects of this invention is the design and fabrication process flows of micro-devices and methods of use the micro-devices for catching and/or measuring biological subjects (e.g., cells, cell substructures, DNA, and RNA) at microscopic levels and in three dimensional space, in which the micro-devices have micro-probes arranged in three dimensional manner with feature sizes as small as a cell, DNA, or RNA, and capable of trapping, sorting, probing, measuring, and modifying biological subjects. Such micro-devices can be fabricated using state-of-the-art microelectronics processing techniques such as those used in fabricating integrated circuits. Using thin film deposition technologies such as molecular epitaxy beam (MEB) and atomic layer deposition (ALD), film thickness as thin as a few monolayers can be achieved (e.g., 4 A to 10 A). Further, using electron beam or x-ray lithography, device feature size on the order of nanometers can be obtained, making micro-device capable of trapping, probing, measuring, and modifying a biological subject (e.g., a single cell, a single DNA or RNA molecule) possible.

[0260] Another aspect of this invention relates to micro-indentation probes and micro-probes for measuring a range of physical properties (such as mechanical properties) of biological subjects. Examples of the mechanical properties include hardness, shear strength, elongation strength, fracture stress, and other properties related to cell membrane which is believed to be a critical component in disease diagnosis.

[0261] Another novel approach provided by this invention is the use of phase lock-in measurement for disease detection, which reduces background noise and effectively enhances signal to noise ratio. Generally, in this measurement approach, a periodic signal is used to probe the biological sample and response coherent to the frequency of this periodic probe signal is detected and amplified, while other signals not coherent to the frequency of the probe signal is filtered out, which thereby effectively reduces background noise. In one of the embodiments in this invention, a probing micro-device can send a periodic probe signal (e.g., a pulsed laser team, a pulsed thermal wave, or an alternating electrical field) to a biological subject, response to the probe signal by the biological subject can be detected by a detecting micro-device. The phase lock-in technique can be used to filter out unwanted noise and enhance the response signal which is synchronized to the frequency of the probe signal. The following two examples illustrate the novel features of time of flight detection arrangement in combination with phase lock-in detection technique to enhance weak signal and therefore detection sensitivity in disease detection measurements.

[0262] Fig. 8 illustrates a fluid delivery system that includes a pressure generator, a pressure regulator, a flow meter, a flow regulator, a throttle valve, a pressure gauge, and distributing kits. The pressure generator **805** sustains fluid with desired pressure, and the pressure is further regulated by the regulator **801** and then accurately manipulated by the throttle valve **802**. Meanwhile, the pressure is monitored at real time and fed back to the throttle valve **802** by the pressure gauge **803**. The regulated fluid is then in parallel conducted into the multiple devices where a constant pressure is needed to drive the fluid sample.

[0263] Fig. 9 illustrates how a micro-device in a disease detection apparatus of this invention can communicate, probe, detect, and optionally treat and modify biological subjects at a microscopic level. Fig. 9(a) illustrates the sequence of cellular events from signal recognition to cell fates determination. First, as the signals **901** are detected by receptors **902** on the cell surface, the cell will integrate and encode the signals into a biologically comprehensible message, such as

calcium oscillation **903**. Consequently, corresponding proteins **904** in the cell will interact with the message, then be modified and transform into ion-interacted proteins **905** accordingly. Through the translocation, these modified proteins **905** will pass the carried message to the nuclear proteins, and the controlled modification on nuclear proteins will modulate the expression of gene **907** which includes transcription, translation, epigenetic processes, and chromatin modifications. Through messenger RNA **909**, the message is in turn passed to specific proteins **910**, thereby changing their concentration – which then determines or regulates a cell's decision or activities, such as differentiation, division, or even death.

[0264] Fig. 9(b) illustrates a micro-device or sub-equipment of this invention which is capable of detecting, communicating with, treating, modifying, or probing a single cell, by a contact or non-contact means. The apparatus is equipped with micro-probes and micro-injectors which are addressed and modulated by the controlling circuitry **920**. Each individual micro-injector is supplied with a separate micro-cartridge, which carries designed chemicals or compounds.

[0265] To illustrate how a micro-device can be used to simulate an intracellular signal, calcium oscillation is taken as an example mechanism. First, a Ca^{2+}-release-activated channel (CRAC) has to be opened to its maximal extent, which could be achieved by various approaches. In an example of the applicable approaches, a biochemical material (e.g., thapsigargin) stored in the cartridge **924** is released by an injector **925** to the cell, and the CRAC will open at the stimulus of the biological subject. In another example of the applicable approaches, the injector **924** forces a specific voltage on cell membrane, which causes the CRAC to open as well.

[0266] The Ca^{2+} concentration of a solution in the injector **928** can be regulated as it is a desirable combination of a Ca^{2+}-containing solution **926**, and a Ca^{2+} free solution **927**. While the injector **930** contains a Ca^{2+} free solution, then injectors **928** and **930** are alternately switched on and off at a desired frequency. As such, the Ca^{2+} oscillation is achieved and the content inside the cell membrane are then exposed to a Ca^{2+} oscillation. Consequently, the cell's activities or fate is being manipulated by the regulated signal generated by the apparatus.

[0267] Meanwhile, the cell's response (e.g., in the form of a thermal, optical, acoustical, mechanical, electrical, magnetic, electromagnetic property, or a combination thereof) can be monitored and recorded by the probes integrated in this apparatus.

[0268] Fig. 9(c) illustrates another design of a micro-device or sub-equipment which is able to setup communication with a single cell. The apparatus is equipped with micro-probes which are

coated with biologically compatible compounds or elements, e.g., Ca, C, Cl, Co, Cu, H, I, Fe, Mg, Mn, N, O, P, F, K, Na, S, or Zn. These probes can generate oscillating chemical signals with such an element or compound to interact with the cell, and results into a response that affects the cell's activities or eventual fate as describe above. Likewise, this apparatus can probe and record the cell's response (e.g., in the form of an electrical, magnetic, electromagnetic, thermal, optical, acoustical, biological, chemical, electro-mechanical, electro-chemical, electro-chemical-mechanical, bio-chemical, bio-mechanical, bio-electro-mechanical, bio-electro-chemical, bio-electro-chemical-mechanical, physical, mechanical property, or a combination thereof) as well.

[0269] As surface charge will affect the shape of a biological subject, by using novel and multiple plates, information on the shape and charge distribution of biological subjects can be obtained. The general principle and design of the micro-device can be extended to a broader scope, thereby making it possible to obtain other information on the biological subject via separation by applying other parameters such as ion gradient, thermal gradient, optical beam, or another form of energy.

[0270] Fig. 10 illustrates another micro-device or sub-equipment of this invention for detecting or measuring microscopic properties of a biological subject **1010** by utilizing a micro-device that includes a channel, a set of probes **1020**, and a set of optical sensors **1032** (see, Fig. 10(a)). The detected signals by probes **1020** can be correlated to information including images collected by the optical sensors **1032** to enhance detection sensitivity and specificity. The optical sensors can be, e.g., a CCD camera, a florescence light detector, a CMOS imaging sensor, or any combination.

[0271] Alternatively, a probe **1020** can be designed to trigger optical emission such as florescence light emission **1043** in the targeted biological subject such as diseased cells, which can then be detected by an optical probe **1032** as illustrated in **Fig. 10(c).** Specifically, biological subjects can be first treated with a tag solution which can selectively react to diseased cells. Subsequently, upon reacting (contact or non-contact) with probe **1020**, optical emissions from diseased cells occur and can be detected by optical sensors **1032**. This novel process using the apparatus of this invention is more sensitive than such conventional methods as traditional florescence spectroscopy as the emission trigger point is directly next to the optical probe and the triggered signal **1043** can be recorded in real time and on-site, with minimum loss of signal.

[0272] Fig. 11 illustrates another embodiment of the apparatus of this invention, which can be used to separate biological subjects of different geometric size and detect their properties respectively. It includes at least an entrance channel **1110**, a disturbing fluid channel **1120**, an accelerating chamber **1130**, and two selecting channels **1140** and **1150**. The angle between **1120** and **1110** is between 0^{o} and 180^{o}. The biological subject **1101** flows in the x-direction from **1110** to **1130**. The biocompatible distribution fluid **1102** flows from **1120** to **1130**. Then the fluid **1102** will accelerate **1101** in y-direction. However, the acceleration correlates with the radius of the biological subjects and the larger ones are less accelerated than the small ones. Thus, the larger and smaller subjects are separated into different channels. Meanwhile, probes can be optionally assembled aside the sidewall of **1110**, **1120**, **1130**, **1140**, and **1150**. They could detect electrical, magnetic, electromagnetic, thermal, optical, acoustical, biological, chemical, physical, mechanical properties, or combinations thereof at the microscopic level. In the mean time, if desired, a cleaning fluid can also be injected into the system for dissolving and/or cleaning biological residues and deposits (e.g., dried blood and protein) in the narrow and small spaces in the apparatus, and ensuring smooth passage of a biological subject to be tested through the apparatus.

[0273] The channel included in the apparatus of this invention can have a width of, e.g., from 1 nm to 1 mm. The apparatus should have at least one inlet channel and at least two outlet channels.

[0274] Fig. 12 shows another micro-device or sub-equipment of this invention with an acoustic detector **1220** for measuring the acoustic property of a biological subject **1201**. This device includes a channel **1210**, and at least an ultrasonic emitter and an ultrasonic receiver installed along the sidewall of the channel. When the biological subject **1201** passes through the channel **1210**, the ultrasonic signal emitted from **1220** will be received after carrying information on **1201** by the receiver **1230**. The frequency of the ultrasonic signal can be, e.g., from 2 MHz to 10 GHz, and the trench width of the channel can be, e.g., from 1 nm to 1 mm. The acoustic transducer (i.e., the ultrasonic emitter) can be fabricated using a piezo-electrical material (e.g., quartz, berlinite, gallium, orthophosphate, $GaPO_4$, tourmalines, ceramics, barium, titanate, $BatiO_3$, lead zirconate, titanate PZT, zinc oxide, aluminum nitride, and polyvinylidene fluorides).

[0275] Fig. 13 shows another apparatus of this invention that includes a pressure detector for biological subject **1301**. It includes at least one channel **1310** and whereon at least one piezo-

electrical detector **1320**. When the biologic subject **1301** passes through the channel, the piezo-electrical detector **1320** will detect the pressure of **1301**, transform the information into an electrical signal, and send it out to a signal reader. Likewise, the trench width in the apparatus can be, e.g., from 1 nm to 1 mm, and the piezo-electrical material can be, e.g., quartz, berlinite, gallium, orthophosphate, $GaPO_4$, tourmalines, ceramics, barium, titanate, $BatiO_3$, lead zirconate, titanate PZT, zinc oxide, aluminum nitride, or polyvinylidene fluorides.

[0276] Fig. 14 shows another apparatus of this invention that include a concave groove **1430** between a probe couple, in the bottom or ceiling of the channel. When a biological subject **1410** passes through, the concave **1430** can selectively trap the biological subject with particular geometric characteristics and makes the probing more efficiently. The shape of concave's projection can be rectangle, polygon, ellipse, or circle. The probe could detect electrical, magnetic, electromagnetic, thermal, optical, acoustical, biological, chemical, physical, mechanical properties, or combinations thereof. Similarly, the trench width can be, e.g., from 1 nm to 1 mm. Fig. 14(a) is an up-down view of this apparatus, Fig. 14(b) is a side view, whereas Fig. 14(c) is a perspective view.

[0277] Fig. 15 is another apparatus of this invention that also includes concave grooves **1530** (of a different shape from those shown in Fig. 14) on the bottom or ceiling of the channel. When a biological subject **1510** passes through, the concave grooves **1530** will generate a turbulent fluidic flow, which can selectively trap the micro-biological subjects with particular geometric characteristics. The probe could detect, e.g., electrical, magnetic, electromagnetic, thermal, optical, acoustical, biological, chemical, physical, mechanical properties, or a combination thereof. The depth of the concave groove can be, e.g., from 10 nm to 1 mm, and the channel width can be, e.g., from 1 nm to 1 mm.

[0278] Fig. 16 illustrated a micro-device with a stepped channel **1610**. When a biological subject **1601** passes through the channel **1610**, probe couples of different distances can be used to measure different microscopic properties, or even the same microscopic at different sensitivity at various steps (**1620**, **1630**, **1640**) with probe aside each step. This mechanism can be used in the phase lock-in application so that signal for the same microscopic property can be accumulated. The probes can detect or measure microscopic electrical, magnetic, electromagnetic, thermal, optical, acoustical, biological, chemical, physical, mechanical properties, or combinations thereof.

[0279] Fig. 17 illustrates another apparatus of this invention with thermal meters **1730**. It includes a channel, a set of probes **1720**, and a set of thermal meters **1730**. The thermal meters **1730** can be an infrared sensor, a transistor sub-threshold leakage current tester, or thermister.

[0280] Fig. 18 illustrates a specific apparatus of this invention which includes carbon a nano-tube **1820** with a channel **1810** inside, probes **1840** which can detect at the microscopic level an electrical, magnetic, electromagnetic, thermal, optical, acoustical, biological, chemical, physical, or mechanical property, or a combination thereof. The carbon nano-tube **1820** as shown contains a double-helix DNA molecule **1830**. The carbon nano-tube can force and sense electrical signals by the probes **1840** aside. The diameter of the carbon nano tube diameter can be, e.g., from 0.5 nm to 50 nm, and its length can range from, e.g., 5 nm to 10 mm.

[0281] Fig. 19 shows an integrated apparatus of this invention that includes a detecting device (shown in Fig. 19(a)) and an optical sensor (shown in Fig. 19(b)) which can be, e.g., a CMOS image sensor (CIS), a Charge-Coupled Device (CCD), a florescence light detector, or another image sensor. The detecting device comprises at least a probe and a channel, and the image device comprises at least 1 pixel. Fig. 19(c-1) and Fig. 19(c-2) illustrate the device with the detecting device and optical sensor integrated. As illustrated in Fig. 19(d), when biological subjects **1901**, **1902**, **1903** pass through, the probe **1910** in the channel **1920**, its electrical, magnetic, electromagnetic, thermal, optical, acoustical, biological, chemical, physical, mechanical property or a combination thereof could be detected by the probe **1910** (see Fig. 19(e)), meanwhile its image could be synchronously recorded by the optical sensor (Fig. 19(f)). Both the probed signal and image are combined together to provide a diagnosis and enhanced detection sensitivity and specificity. Such a detecting device and an optical sensing device can be designed in a system-on-chip or be packaged into one chip.

[0282] Fig. 20 shows a micro-device or sub-equipment with a detecting micro-device (Fig. 20(a)) and a logic circuitry (Fig 20(b)). The detecting device comprises at least a probe and a channel, and the logic circuitry comprises an addressor, an amplifier, and a RAM. When a biological subject **2001** passes through the channel, its property could be detected by the probe **2030**, and the signal can be addressed, analyzed, stored, processed, and plotted in real time. Fig. 20(c-1) and Fig. 20(c-2) illustrate the device with detecting device and Circuitry integrated. Similarly, the detecting device and the integrated circuit can be designed in a System-on-Chip or be packaged into one chip.

[0283] Fig. 21 shows a micro-device or sub-equipment of this invention that comprises a detecting device (Fig. 21(a)) and a filter (Fig. 21(b)). When a biological subject **2101** passes through the device, a filtration is performed in the filter, and irrelevant objects can be removed. The remaining subjects' property can then be detected by the probe device (Fig. 20(a)). The filtration before probing will enhance the precision of the device. The width of the channel can also range, e.g., from 1 nm to 1 mm.

[0284] Fig. 22 shows the geometric factors of DNA **2230** such as spacing in DNA's minor groove (**2210**) have an impact on spatial distribution of electrostatic properties in the region, which in turn may impact local biochemical or chemical reactions in the segment of this DNA. By probing, measuring, and modifying spatial properties of DNA (such as the spacing of minor groove) using the disclosed detector and probe **2220**, one may detect properties such as defect of DNA, predict reaction/process at the segment of the DNA, and repair or manipulate geometric properties and therefore spatial distribution of electrostatic field/charge, impacting biochemical or chemical reaction at the segment of the DNA. For example, tip **2220** can be used to physically increase spacing of minor groove **2210**.

[0285] Fig. 23 shows the fabrication process for an apparatus of this invention that has a flat cover atop of trench to form a channel. This will eliminate the need for coupling two trenches to form a channel, which can be tedious for requiring perfect alignment. The cover can be transparent and allow observation with a microscope. It can comprise or be made of silicon, SiGe, SiO_2, various types of glass, or Al_2O_3.

[0286] Fig. 24 is a diagram of an apparatus of this invention for detecting a disease in a biological subject. This apparatus includes a pre-processing unit, a probing and detecting unit, a signal processing, and a disposal processing unit.

[0287] Fig. 25 shows an example of a sample filtration sub-unit in the pre-processing unit, which can separate the cells with different dimensions or sizes. This device comprises at least one entrance channel **2510**, one disturbing fluid channel **2520**, one accelerating chamber **2530**, and two selecting channels (**2540** and **2550**). The angle **2560** between **2520** and **2510** ranges from 0^o to 180^o.

[0288] The biological subject **2501** flows in the **x** direction from the entrance channel **2510** to the accelerating chamber **2530**. A bio-compatible fluid **2502** flows from disturbing fluid channel **2520** to the accelerating chamber **2530**, it then accelerates the biological subject **2501** in the **y**-

direction. The acceleration correlates with the radius of the biological subject and the larger ones are less accelerated than the smaller ones. Then, the larger and smaller subjects are separated into different selecting channels. Meanwhile, probes can be optionally assembled on the sidewalls of the channels **2510**, **2520**, **2530**, **2540**, and **2550**. The probes could detect, at the microscopic level, electrical, magnetic, electromagnetic, thermal, optical, acoustical, biological, chemical, biochemical, electro-mechanical, electro-chemical, electro-chemical-mechanical, physical, mechanical properties, or combinations thereof.

[0289] Fig. 26 is a diagram of another example of a sample filtration unit in the apparatus of this invention. **2601** represents small cells, while **2602** represents large cells. When a valve **2604** is open and another valve **2603** is closed, biological subjects (**2601** and **2602**) flow towards exit A. Large cells that have larger size than the filtration hole are blocked against exit A, while small cells are flushed out through exit A. The entrance valve **2604** and exit A valve **2607** are then closed, and a bio-compatible fluid is injected through the fluid entrance valve **2606**. The fluid carries big cells are flushed out from exit B. The larger cells are then analyzed and detected in the detection part of the invention.

[0290] Fig. 27 is a diagram of a pre-processing unit of an apparatus of this invention. This unit includes a sample filtration unit, a recharging unit or system for recharging nutrient or gas into the biological subject, a constant pressure delivery unit, and a sample pre-probing disturbing unit.

[0291] Fig. 28 is a diagram of an information or signal processing unit of an apparatus of this invention. This unit includes an amplifier (such as a lock-in amplifier) for amplifying the signal, an A/D converter, and a micro-computer (e.g., a device containing a computer chip or information processing sub-device), a manipulator, a display, and network connections.

[0292] Fig. 29 shows the integration of multiple signals which results in cancellation of noise and enhancement of signal/noise ratio. In this Figure, a biological **2901** is tested by Probe 1 during Δt between **t1** and **t2**, and by Probe 2 during Δt between **t3** and **t4**. **2902** is **2901**'s tested signal from Probe 1, and **2903** is from Probe 2. Signal **2904** is the integration result from signal **2902** and **2903**. The noise cancels out each other in certain extent and results in an improved signal strength or signal/noise ratio. The same principle can be applied to data collected from more than more than 2 micro-devices or probing units.

[0293] Fig. 30 shows a novel disease detection method of this invention in which at least one

probe object is launched at a desired speed and direction toward a biological subject, resulting in a collision. The response(s) by the biological subject during and/or after the collision is detected and recorded, which can provide detailed and microscopic information on the biological subject such as weight, density, elasticity, rigidity, structure, bonding (between different components in the biological subject), electrical properties such as electrical charge, magnetic properties, structural information, and surface properties. For example, for a same type of cell, it is expected that a cancerous cell will experience a smaller traveling distance after the collision than that of a normal cell due to its denser, greater weight, and possibly larger volume. As shown in Fig. 30(a), a probe object **3011** is launched towards a biological subject **3022**. After the collision with the probe object **3011**, the biological subject **3022** may be pushed (scattered) out a distance depending on its properties as shown Fig. 30(b).

[0294] Fig. 30(c) shows a schematic of a novel disease detection device with a probe object launch chamber **3044**, an array of detectors **3033**, a probe object **3022** and a biological subject to be tested **3011**. In general, a test object can be an inorganic particle, an organic particle, a composite particle, or a biological subject itself. The launch chamber comprises a piston to launch the object, a control system interfaced to an electronic circuit or a computer for instructions, and a channel to direct the object.

[0295] Fig. 31 illustrates a method for detecting a disease in a biological subject. A biological subject **3101** passes through the channel **3131** at a speed v, and probe **3111** is a probe which can grossly detect the properties of the biological subject at high speed.

[0296] Probe **3112** is a fine probing device which is coated by a piezo-electrical material. There is a distance ΔL between probe **3111** and probe **3112**.

[0297] When the biological subjects are tested when getting through **3111**, if the entity is identified to be a suspected abnormal one, the system would trigger the piezo-electrical probe **3112** to stretch into the channel and probe particular properties after a time delay of Δt. And probe **3112** retracts after the suspected entity passed through.

[0298] The probing device is capable of measuring at the microscopic level an electrical, magnetic, electromagnetic, thermal, optical, acoustical, biological, chemical, electro-mechanical, electro-chemical, electro-chemical-mechanical, bio-chemical, bio-mechanical, bio-electro-mechanical, bio-electro-chemical, bio-electro-chemical-mechanical, physical or mechanical property, or a combination thereof, of the biological subject.

[0299] The width of the micro-channel can range from about 1 nm to about 1 mm.

[0300] Fig. 32 shows a process of detecting a disease in a biological subject. A biological subject **3201** passes through the channel **3231** at a speed **v**. Probe **3211** is a probe which can grossly detect the properties of the biological subject at high speed. **3221** and **3222** are piezo-electrical valves to control the micro-channel **3231** and **3232**. **3212** is a fine probing device which can probe biological properties more particularly. **3231** is flush channel to rush out normal biological subjects. **3232** is detection channel where the suspected entities are fine detected in this channel.

[0301] When a biological subject is tested while getting through **3211**, if it is normal, the valve **3221** of the flush channel is open, while the detection channel valve **3222** is closed, the biological subject is flushed out without a time-consuming fine detection.

[0302] When the biological subject is tested while getting through **3211**, if it is suspected to be abnormal or diseased, the valve **3221** of the flush channel is closed, while the detection channel valve **3222** is open, the biological subject is conducted to the detection channel for a more particular probing.

[0303] The width of the micro-channel can range from about 1 nm to about 1 mm.

[0304] The probing device is capable of measuring at the microscopic level an electrical, magnetic, electromagnetic, thermal, optical, acoustical, biological, chemical, electro-mechanical, electro-chemical, electro-chemical-mechanical, bio-chemical, bio-mechanical, bio-electro-mechanical, bio-electro-chemical, bio-electro-chemical-mechanical, physical or mechanical property, or a combination thereof, of the biological subject.

[0305] Fig. 33 illustrates an arrayed biological detecting device. As shown in Fig. 33(a), **3301** are arrayed micro-channels which can get through the fluidics and biological subjects. **3302** are probing devices embedded aside the channels. The sensors are wired by bit-lines **3321** and word-lines **3322**. The signals are applied and collected by the decoder R\row-select **3342** and decoder column select **3341**. As illustrated in Fig. 33(b), the micro-channel arrayed biological detecting device **3300** can be embedded in a macro-channel **3301**. The micro-channel's dimension ranges from about 1 um to about 1 mm. The shape of the micro-channel can be rectangle, ellipse, circle, or polygon.

[0306] The probing device is capable of measuring at the microscopic level an electrical, magnetic, electromagnetic, thermal, optical, acoustical, biological, chemical, electro-mechanical,

electro-chemical, electro-chemical-mechanical, bio-chemical, bio-mechanical, bio-electro-mechanical, bio-electro-chemical, bio-electro-chemical-mechanical, physical or mechanical property, or a combination thereof, of the biological subject.

[0307] Fig. 34 illustrates a device of the current invention for disease detection. **3401** is inlet of the detecting device, and **3402** is the outlet of the device. **3420** is the channel where the biological subjects pass through. **3411** is the optical component of the detecting device.

[0308] As illustrated in Fig. 34(b), the optical component **3411** consists of an optical emitter **3412** and an optical receiver **3413**. The optical emitter emits an optical pulse (e.g. laser beam pulse), when the biological subject **3401** passing through the optical component, and the optical sensor detects the diffraction of the optical pulse, then identify the morphology of the entity.

[0309] Fig. 35 shows an example of the apparatus of this invention packaged and ready for integration with a sample delivery system and data recording device. As illustrated in Fig. 35(a), the device **3501** is fabricated by micro-electronics processes described herein and has at least a micro-trench **3511**, a probe **3522**, and a bonding pad **3521**. The surface of the device's top layer can include SixOyNz, Si, SixOy, SixNy, or a compound containing the elements of Si, O, and N. Component **3502** is a flat glass panel. In Fig. 35(b), the flat panel **3502** is shown to be bonded with micro-device **3501** on the side of micro-trench. The bonding can be achieved by a chemical, thermal, physical, optical, acoustical, or electrical means, or any combination thereof. Fig. 35(c) shows a conductive wire being bonded with the bonding pad from the side of the pads. As illustrated in Fig. 35(d), the device **3501** is then packaged in a plastic cube with only conducting wires exposed. In Fig. 35(e), a conical channel **3520** is carved through packaging material and connecting the internal channel of the device. As illustrated in Fig. 35(f), the larger opening mouth of the conical channel makes it operational and convenient to mount a sample delivery injector with the device, thereby better enabling the delivery of sample from an injector with relatively large size of injector needle into device with relatively small channels.

[0310] Fig. 36 shows another example of the apparatus of this invention packaged and ready for integration with a sample delivery system and data recording device. As shown in Fig. 36(a), a micro-device **3600** is fabricated by one or more micro-electronics processes as described in International Application No. PCT/US2011/042637, entitled "Apparatus for Disease Detection." The micro-device **3600** has at least a micro-trench **3604**, a probe **3603**, a connecting port **3602**, and a bonding pad **3605**. On the top of the micro-device **3600**, the surface layer comprises

SixOyNz, Si, SixOy, SixNy, or a compound consisting of Si, O, and N. The surface layer can be covered, and thus the micro-device **3600** is mounted, with a flat glass panel **3601**. See Fig. 36(b). The mounting can be by a chemical, thermal, physical, optical, acoustical, or electrical means. As shown in Fig. 36(c), the conductive wire is bonded with bonding pad from the side of the pads. Fig. 36(d) illustrates that the micro-device **3600** can then be packaged in a cube with only conducting wires exposed. The packaging cube can comprise a packaging material such as plastic, ceramic, metal, glass, or quartz. As shown in Fig. 36(e), a tunnel **3641** is then drilled into the cube until the tunnel reaches the connecting port **3602**. Further, as shown in Fig. 36(f), the tunnel **3641** is then being connected to other pipes which can delivery a sample to be tested into the micro-device **3600**, and flush out the sample after the sample is tested.

[0311] Fig. 37 shows yet another example of the apparatus of this invention packaged and ready for integration with a sample delivery system and data recording device. As illustrated in Fig. 37(a), device **3700** is a micro-fluidic device which has at least one micro-channel **3701**. **3703** is a pipe that conducts a fluidic sample. The micro-channel **3701** and the conducting pipe **3703** are aligned and submerged in a liquid, for example, water. Fig. 37(b) illustrates that, when the temperature of the liquid in which the micro-device and conducting pipe are submerged, is decreased to its freezing point or lower, the liquid solidifies into a solid **3704**. As illustrated in Fig. 37(c), while the temperature of the liquid is maintained below the freezing point, the combination (including the solid **3704**, the conducting pipe **3703**, and the device **3700**) is enclosed into a packaging material **3705** whose melting temperature is higher than that of the solid **3704**, with only the conducting pipe exposed. Fig. 37(d) shows that, after the temperature is increased above the melting point of the solid **3704**, the solid material **3704** melts and becomes a liquid and is then exhausted from the conducting pipe **3703**. The space **3706** wherein the solid material **3704** once filled is now available or empty, and the channel **3701** and the conducting pipe **3703** are now connected through and sealed in the space **3706**.

[0312] Fig. 38 shows an apparatus of this invention that has a channel (trench) and an array of micro sensors. In Fig. 38(a), **3810** is a device fabricated by microelectronics techniques; **3810** comprises micro-sensor array **3801** and addressing and read-out circuitry **3802**. The micro-sensor array can include thermal sensors, piezo-electrical sensors, piezo-photronic sensors, piezo-optical electronic sensors, image sensors, optical sensors, radiation sensors, mechanical sensors, magnetic sensors, bio-sensors, chemical sensors, bio-chemical sensors, acoustic sensors,

or a combination of them. Examples of thermal sensors include resistive temperature micro-sensors, micro-thermocouples, thermo-diodes and thermo-transistors, and SAW (surface acoustic wave) temperature sensor. Examples of image sensors include CCD (Charge Coupled Device) and CIS (CMOS image sensor). Examples of radiation sensors include photoconductive devices, photovoltaic devices, pyro-electrical devices, and micro-antennas. Examples of mechanical sensors include pressure micro-sensors, micro-accelerometers, micro-gyrometers, and micro flow-sensors. Examples of magnetic sensors include magneto-galvanic micro-sensors, magneto-resistive sensors, magneto diodes and magneto-transistors. Examples of biochemical sensors comprise conductimetric devices and potentiometric devices. Fig. 38(b) shows a micro-device **3820** that includes a micro-trench **3821**. As illustrated in Fig. 38(c), **3810** and **3820** are bonded together to form the new micro-device **3830** which include a trench or channel **3831**. The micro-sensor array **3801** is exposed in the channel **3831**.

[0313] Fig. 39 shows another apparatus of this invention comprising several "sub-devices." Particularly, as illustrated in Fig. 39(a), the device **3910** composes "sub-devices" **3911**, **3912**, **3913**, and **3914**, among which **3911** and **3913** are devices which can apply disturbing signals, and **3912** and **3914** are micro-sensor arrays. Fig. 39(b) illustrates the functioning diagram of the device **3910**, when biological samples **3921** under the test are passing through the channel **3910**, they are disturbed by signal A applied by **3911**, then being tested and recorded by detecting sensor array **1** of **3912**. These biological samples are then disturbed by disturb probe **3913** of array **2**, and being tested by detecting sensor **3914** of array **2**. Disturbing probe **3911** of array **1** and disturbing probe **3913** of array **2** can apply the same or different signals. Likewise, detecting sensor **3912** of array **1** and detecting sensor **3914** of array **2** can sense or detect the same or different properties.

[0314] Fig. 40 shows an example of the apparatus of this invention which includes an application specific integrated circuit (ASIC) chip with I/O pads. Specifically, as illustrated in Fig. 40, **4010** is a micro-device with a micro-fluidic channel **4012** and I/O pads **4011**. **4020** is an Application Specific Integrated Circuit (ASIC) chip with I/O pads **4021**. **4020** and **4010** can be wired together through the bonding of I/O pads. As such, with an ASIC circuitry **4020**, the micro-fluidic detecting device **4010** can perform more complicated computing and analytical functions.

[0315] Fig. 41 is a diagram of the underlying principal of the apparatus of this invention which

functions by combining various pre-screening and detection methods in unobvious ways. In Fig. 41(a), a biological subject is first pre-screened for diseased biological entities, and then the diseased biological entities are separated from the normal (healthy or non-diseased) biological entities. The biological subject containing the diseased biological entities separated from the normal biological entities is detected using a desired disease detection method. In Fig. 41(b), a biological sample has gone through multiple, successive cell separation steps to concentrate diseased cells (or biological entities). In Fig. 41(c), after pre-screening to concentrate diseased biological entities, bio-marker is used to detect diseased biological entities. In Fig. 41(d), bio-marker is first used to separate out diseased biological entities and then the sorted out, diseased biological entities are further detected by various detection methods. In short, this process includes initial screening, initial separation, further screening, further separation, probing with one or more disturbing signals or disturbing parameters (e.g., physical, mechanical, chemical, biological, bio-chemical, bio-physical, optical, thermal, acoustical, electrical, electro-mechanical, piezo-electrical, micro-electro-mechanical, or a combination thereof), and finally detection. This sequence can repeat one or more times. The effect of this process is concentrating the diseased entities for improved detection sensitivity and specificity, particularly for a biological subject with a very low concentration of diseased entities, such as circulating tumor cell (CTC).

[0316] In Fig. 41(e) through Fig. 41(g), a set of novel processes include (a) pre-screening, pre-separation and initial separation for diseased biological entities, (b) further separation of diseased biological entities, (c) optionally carry out initial detection, and (d) detection using various processes and detection methods. In the pre-separation process, one of the embodiments utilizes nano-particles or nano- magnetic particles attached with bio-markers to sort out diseased biological entities. During pre-separation process, the diseased biological entities are concentrated for higher concentration, which will make further separation and/or following detection easier. The biological sample following pre-separation process can go through further separation process to further enhance the concentration of diseased biological entities. Finally, the biological sample gone through the pre-separation and follow-up separation steps will go through detection step(s), in which various detection techniques and processes can be used to determine diseased biological entities and their types. In some embodiments, multiple detection steps can be utilized to detect diseased biological entities.

[0317] Fig. 42(a) shows a cross-sectional view of a channel **(4211)** into which a biological

subject can flow. Fig. 42(b) shows an outside view of the channel, along which an array of detectors (**4222**) are installed along the path of the flow of the biological subject. Alternatively, both probes and detectors can be installed to both disturb the biological subject to be detected and detect response signals from such disturb signals. Fig. 42(c) shows a cross-section of the wall of the channel, where detectors (**4222**) are mounted through to contact the biological subject to be detected and also are making contact with the outside world (e.g., to connect to a detection circuitry).

[0318] Fig. 43(a) shows a biological subject (**4333**) to be detected passing through a channel (**4311**) aligned with detectors (**4322**) along its passage. The detectors can be the same type of detectors, or a combination of various detectors. Further, probes capable of sending out probing or disturbing signals to the biological subject to be detected can also be implemented along the channels, along with detectors which can detect response from the biological subject which has been probed or disturbed by the probe. The detected signals can be acoustical, electrical, optical (e.g., imaging), biological, bio-chemical, bio-physical, mechanical, bio-mechanical, electro-magnetic, electro-mechanical, electro-chemical-mechanical, electro-chemical-physical, thermal, and thermal-mechanical property related signals, or a combination of them. Fig. 43(b) shows an example of a set of detected signals (e.g., images, pressures, or electrical voltages) (**4344**) along the path of the biological subject, which recorded its behavior and properties as it passes through the channel. For example, for an optical detector, the size of the circle shown in the Fig. 43(b) could mean the optical emission from the biological subject (such as an optical emission from a florescence component attached to the biological subject), the strength of a strain (pressure) acting on the side wall of the channel detected by a piezo-electric detector or a piezo-photronic detector, or thermal emission from the biological subject detected by a thermal detector or an IR sensor. Such detected signals can be solely from the biological subject as it passes through the channel, or responses from the biological subject to a disturbing or probing signal by the probe.

[0319] Like Fig. 43(b), Fig. 43(c) through Fig. 43(e) show additional examples of various detected signal patterns (**4344**) as the biological subject passes through the channel and is detected by the novel detectors and processes disclosed in the application.

[0320] To effectively sorting, separating, screening, probing, or detecting of diseased biological entities, a chamber (or chambers) integrated with various channels can be deployed as shown Fig. 44(a), where incoming sample flowing into a chamber (**4411**) first. In the chamber, various

techniques such as bio-markers and nano-technology (magnetic beads or nano-particles with bio-markers attached to them) based processes can be used to sort out, screen, and separate out the diseased biological entities. For example, a biological sample flowing from the left into the chamber can have its diseased entities separated out in the chamber, and passed downward through the bottom channel, while its normal entities can continue to flow from the chamber in the right hand direction, through the channel in the right side of the chamber. Depending upon the design, the diseased entities, having entered into the chamber on the left, can also be separated out in the chamber, and continue on towards right and flow into the channel on the right side of the chamber, while normal entities will continue to flow down toward and through the channel at the bottom of the chamber. Fig. 44(b) shows multiple chambers integrated with channels in which biological entities can be sorted, screened, separated, probed or detected. In the application of screening and separation, the multiple chambers can carry out multiple screening and separation steps. As shown in Fig. 44(b), for a biological sample flowing from the left toward the right direction, it will enter into the first chamber on the left (**4433**) and undergo a first screening and separation. The biological sample can continue to flow towards the right, enter into the second chamber, the chamber on the right (**4444**), and undergo a second screening and further separation. In this way, through a multi-staged screening and separation process, the concentration of a diseased entity can be successively enhanced which can be helpful for a sensitive final or late stage detection. This type of device design and process could be very useful for defection of a biological sample with an initially very low concentration of diseased entity population, such as for the detection of circulating tumor cell (CTC) which is typically in the concentration of one part in one billion cells or 10 billion cells.

[0321] To significantly speed up the sorting, screening, probing and detection operations using the disclosed device and process, a high number of desired structures such as those discussed in Fig. 45 can be fabricated simultaneously on the same chip as shown in Fig. 45.

[0322] Fig. 46 shows another novel device layout for sorting, screening, separating, probing and detecting diseased biological entities, in which a desired component or multiple components through the middle channel into the middle chamber **4611** can play a wide range of roles. For example, the component flowing into the middle chamber could be a bio-marker which can be freshly added into the top chamber **4622** and bottom chamber **4633** when its (bio-marker) concentration needs to be adjusted. The timing, flow rate, and amount of component in the

middle chamber **4611** need to be added into the top and bottom chambers (**4622** and **4633**) can be pre-programmed or controlled via a computer or software in real time. The component into the middle chamber **4612** could also be nano-particles or magnetic beads attached to bio-markers. In another novel embodiment, the component into the middle chamber **4611** could be a disturbing agent which will disturb the biological subject or samples to be detected in the top and bottom chambers.

[0323] Fig. 47 shows that, compared with multiple stand alone detection apparatuses (see Fig. 47(a), **4711**, **4722**, **4733**, and **4744**), an apparatus (**4755**) with multiple sub-units of different functions and technologies (**4766**) assembled or integrated has a significantly reduced apparatus volume or size (see Fig. 47(b)), therefore reduced costs since many common hardware (e.g., a sample handling unit, a sample measurement unit, a data analysis unit, a display, a printer, etc.) can be shared in an integrated apparatus. For example, such a multi-functional, integrated apparatus can include a bio-marker detector, an imaging based detector, a photo-detector, an x-ray detector, a nuclear magnetic resonance imaging detector, an electrical detector, and an acoustic detector all of which are assembled and integrated into the single apparatus, so that the apparatus can have improved detection functionality, sensitivity, detection versatility, and reduced volume and cost.

[0324] Fig. 48 shows that when multiple sub-units with different functions and technologies **(2055)** are assembled into one apparatus, a more diverse functionality, improved detection functionality, sensitivity, detection versatility, and reduced volume and cost can be achieved, where a number of common utilities including, e.g., input hardware, output hardware, sample handling unit, sample measurement unit, data analysis unit and data display unit (**4811**, **4833**, and **4844**) can be shared. For example, when a range of detection units utilizing various detection technologies are assembled into one apparatus, many functions and hardware such as sample handling unit, sample measurement unit, data transmission unit, data analysis unit, computer, and display unit can be shared, thereby significantly reducing the apparatus' equipment volume or size, costs, and complexity while improving measurement functionality and sensitivity.

[0325] One of the key aspects of the present invention relates to a novel technology for detecting disease, in which a number of different classifications of biological information are collected in a device and processed or analyzed. For instance, Fig. 49 shows a number of different

classifications of biological information (e.g., protein, cellular, and/or molecular) can be collected in a device according to the present invention, and processed in the novel technology according. As shown in Fig. 50, the measured information according to the present invention includes protein, cellular and molecular level information, or combination of them.

[0326] Tests were carried out in the laboratory with the apparatus of this invention on certain cancerous tissue samples (with multiple samples for each type of cancer) although the apparatus of this invention can be used for detection of other types of cancer or other types of treatment. In the tests, healthy control samples were obtained from animals with no known cancer disease at the time of collection and no history of malignant disease. Both cancerous samples and healthy control samples were collected and cultured in the same type of culture solution. The cultured samples were then mixed with a dilution buffer and diluted to the same concentration. The diluted samples were maintained at the room temperature for different time intervals and processed within a maximum of 6 hours after being recovered. The diluted samples were tested at the room temperature (20~23 oC) and in the humidity of 30%~40%. The samples were tested with an apparatus of this invention under the same conditions and stimulated by the same pulse signal.

[0327] The tests show that, in general, the control groups' tested (measured) values (i.e., measured values in relative units for the testing parameter) were lower than the cancerous or diseased groups. Under the same stimulation (in terms of stimulation type and level) with a stimulating or probing signal applied by a probing unit of the tested apparatus of this invention, the difference shown in the measured values between the control groups and the cancerous groups became much more significant, e.g., ranging from 1.5 times to almost 8 times in terms of level of increase in such difference, compared with that without simulation. In other words, the cancerous groups' response to the stimulating signal was much higher than that of the control groups. Thus, the apparatus of this invention has been proven to be able to significantly enhance the relative sensitivity and specificity in the detection and measurement of diseased cells, in comparison to the control or healthy cells.

[0328] Further, the test results show that in terms of the novel parameter utilized by the apparatus of this invention, the cancerous group and the control group showed significantly different response. Such difference is significantly greater than the measurement noise. There

was a large window to separate the control groups from the cancerous groups, showing a high degree of sensitivity of the novel measurement method and apparatus.

[0329] Fig. 51 shows signals from different biological classifications may interact, combine, and/or amplify to enhance signal in this novel technology. Compared with the traditional technology, signal and information collected by the apparatus and methods of this invention is linearly and can even be non-linearly amplified; and additional two-factor and three-factor (or higher order) interactions between various levels (cellular, protein, molecular or other levels) and components/parameters (exemplified in the following table) are not only just novel, unique, but also exhibited unexpected reliable and sensitive results when compared to the traditional technology.

Traditional technology		This invention
P - protein based (bio-marker, AFP, CEA, PSA, etc.)		P
	+	
C - cellular based (CTC, ctDNA)		C
	+	
M - Molecular (genomics, DNA, RNA)		M
	+	
=> one-dimensional information		P-C
	+	
		P-M
	+	
		M-C
	+	
	=> seven dimensional info	M-C-P
Other level/parameter (O)	+	M-C-P-O
	=> more dimensional info	

[0330] Fig. 52 shows detected signal in this novel technology as a function of cancer cell concentration. The results provided in Fig. 52 show that the signal increases with increasing amount of cancer cells.

[0331] Fig. 53 shows detected signal in this novel technology as a function of a bio-marker level. The results provided in Fig. 52 show that the signal increases with increasing level of bio-marker.

[0332] Fig. 54 shows test results proving an advantage of this novel technology compared with traditional bio-marker (AFP) for liver cancer. As shown in Fig. 54, using 58 confirmed liver cancer samples, sensitivity of this novel technology is 79.3%, which is significantly higher than

that of AFP (i.e., 55.9%).

[0333] Studies were also undertaken to examine the effect of adding molecular level reaction triggering agent on the efficacy of the apparatus and methods for detecting disease of this invention. The results provided in Fig. 55 show that the difference in signal between the control (healthy) group and cancer group was increased, indicating the detection system did detect molecular level information.

[0334] The apparatus and methods of this invention has been used in test of more than 20 different types of cancer in all stages of development and showed expectedly high sensitivity and specificity. Fig. 56 shows that to validate the usefulness and sensitivity of this invention, over 60,000 samples were collected, with 30,000 samples in retrospective investigation, and 30,000 samples in general screening, and remarkable sensitivity and selectivity of this invention was demonstrated from testing those samples.

[0335] Fig. 57 shows in a multi-level detection system of this invention, one biological level (for example, protein) can interact with another biological level(s) (such as genetic level), resulting in synergistic reactions and resultant amplification in signal.

[0336] Fig. 58 shows the CDA values of the control group, non-cancer disease group and cancer group. As detected by the apparatus and methods of this invention, the cancer group always has a higher CDA value than that of a non-cancer disease group, and this difference in CDA value between the cancer group and non-cancer disease group is statistically significant particularly for monitoring the progression of a disease state, e.g., from an inflammatory disease to a pre-cancer condition to a malignant cancer or tumor and then to a late stage cancer. In other words, CDA values can be used in a disease and cancer-differentiating analysis with the help of the apparatus and methods of this invention.

[0337] Fig. 59 shows the relationship between disease state and detected cell signaling properties and/or cell media properties. Traditional cancer screening and prognosis IVD methods such as bio-markers and genomics (e.g., circuiting tumor-DNA (ct-DNA)) are unable to detect cancer early, and have relatively lower signals. Bio-markers are not effective for early stage cancer detection (as shown in Fig. 59), but also lack markers for a number of cancer types. In the case of CTC and ct-DNA, as also shown in Fig. 59, signals occur only after solid tumor has been formed, making early stage cancer detection relatively. Compared with those traditional methods, the novel CDA technology according to the present invention can directly or indirectly

measures cell and cell media properties, cell signaling, cell interactions, and/or DNA mutation frequencies, thereby resulting in significantly higher signals, which are available for even pre-cancer or early stage cancer detections.

[0338] Another major novel aspect of this application relates to an effective method to probe and track ability (including immune system) to detect and prevent potential diseases, ability to flight diseases, and the state of a life body, including but not limited to healthy state, non-cancer disease state, pre-cancer state, and cancer state.

[0339] Using a novel microfluidic device equipped with sensitive sensors and a fully automated testing machine developed in this work, the method of this invention has been demonstrated on about 100,000 samples which included control (healthy group), disease group, pre-cancer disease group, and cancer group individuals. The test results showed statistically significant blood micro-electrical current level decreasing from healthy group to disease group, and further decreasing to cancer group, signifying potential importance of this new detection technology for early stage cancer detection. In early stage non-small cell lung cancer (NSCLC) tests, sensitivity and specificity reached ~ 85% and 93%, respectively. It has also shown that it is capable to detect over 20 types of cancer, including esophageal cancer and brain tumor which do not have other effective screening methods. As the class of electrical properties is a fundamental bio-physical sub-field and impacting many aspects of human blood, it has multi-level effects at cellular, protein, and even molecular levels. Data appear to reveal that this novel technology provides a potentially powerful insight into how cancer evolves and can be highly valuable for pre-cancer and early stage cancer detection. Its mechanism, potential significance, and ramifications will be presented.

[0340] Since the liquid media (for example, blood) is interfacing, connecting and communicating with both cells, proteins, and genetic components (DNAs, RNAs, etc.), it plays a critical role in the interfacing, interactions, and communications (for example, cell signaling) between cells, proteins, and genetic components (DNAs, RNAs, etc.) and other biological entities, and the occurrence and progression of diseases including but not limited to non-cancer diseases, pre-cancer diseases and cancer. On the other hand, in the transition from a heathy individual to a disease state, immune system is degraded and disease detection and killing agents such as T cell lost function. In this invention, it is believed that immune system degradation (decrease) and loss in disease detection and disease fighting will and action is caused by changes

in properties in the said liquid media surrounding cells, proteins, genetic components (DNAs, RNAs, etc.) and other biological entities. Specifically, those properties can be biological properties (protein concentration, protein types, DNA sequence, DNA static electrical force, DNA surface charge, DNA surrounding media electrical properties, quantum mechanical effects, etc.), bio-chemistry properties, physical properties (thermal, mechanical, electrical, and electro-magnetic properties), bio-physical properties, properties. For example, the shift in the above property (for example, reduction in the above said physical properties) may affect (for example, reduction in effectiveness and efficiency, and transduction degradation) cell signaling and communications by cells and between cells and other biological entities, resulting in the compromise of immune system, loss of detection capability of cells such as T cells to detect cancer cells and ability to kill cancer cells. Therefore, by measuring the above properties including physical and bio-physical properties, one is able to detect the onset of disease and track disease from one stage to the next stage, making early detection and prevention of disease possible.

[0341] Fig. 60 shows that in a blood sample, among other components, there are cells, proteins, and genetic components (DNAs, RNAs, etc.) which are surrounded by a liquid media interacting with the just-mentioend components. In addition, cell interacts and communicates (for example, one cell through its surface signaling interacts and communicates with the surface of another cell via acoustical, optical, electro-magnetic and electrical means) with other cells and other biological entities including but not limited to proteins and genetic components (DNA, RNA, etc.) via cell signaling. At the same time, proteins and genetic components (DNAs, RNAs, etc.) can interact with other protein components and genetic components (DNAs, RNAs, etc.). Since the liquid media around which the cells, proteins, and genetic components (DNAs, RNAs, etc.) are within interacts and interfaces with all the above said biological entities, the media plays a critical role and function in signal transmission, interactions, and functions of the above said biological components which may (a) affect healthy or disease states of the biological body, (b) progression of diseases such as non-cancer diseases, pre-cancer diseases, and cancer, and (c) ability for diseases such as cancer to evade/escape detection and/or elimination by immune system and/or disease killing agents such as T cells. Through measuring physical, bio-physical, chemical, biological, and bio-chemical properties of the said media and cell signaling, one is expected to be able to detect and track immune system, resistance to diseases, ability to detect

diseases, ability to flight diseases, and the state of life body, including but not limited to healthy state, non-cancer disease state, pre-cancer state, and cancer state. The above said physical properties include but not limited to acoustical, optical, mechanical, chemical, bio-chemical, electrical, electro-magnetic, and thermal properties.

Exemplary Test

Mechanism

[0342] A micro fluidic device was fabricated by an integrated circuit method in which micro-channels were formed along which sample fluid can be passed, and on whose sides detection transducers (i.e., sensors) were formed to probe the fluid. During dada collection, a voltage meter with automated data recording capabilities was used. When fluid sample arrives at a micro-channel, sensors in the channel can probe the sample via applying a constant voltage while recording micro- electrical current response as a function of time dependent behavior (time sweep) as shown Figure 61 for control (healthy) and cancer cell line samples, in which a typical micro- electrical current curve is shown, with Y axis being current and X axis being time. The characteristic current versus time curve collected is dependent upon the properties of the samples measured and reveals the state of the individual tested. Fully automated test machine consisting of sample transport units, mixing chamber, and testing unit with micro-fluidic device is designed and assembled for data collection.

Cell Line Characteristics

[0343] Four cell lines were utilized in the preliminary research. Human non-small cell lung cancer cell line A-549 (Cat. No. TCHu150), human embryonic lung cell line MRC-5 (Cat. No. GNHu41), human hepatoma cell line QGY (Cat. No. TCHu 42) and human hepatocyte cell line HL-7702 (Cat. No. GNHu 6), which were purchased from Cell Bank of Typical Culture Preservation Committee of Chinese Academy of Sciences/Cell Resource Center of Shanghai Academy of Life Sciences, Chinese Academy of Sciences, were cultured in complete growth medium of RPMI-1640 medium which contain 10% FBS (fetal bovine serum) and 1% penicillin-streptomycin in atmosphere of 95% air and 5% carbon dioxide in 37°C. Cell suspension solutions were prepared for testing.

Blood Sample Characteristics

[0344] Samples used in a CDA test were whole blood or serum samples, with whole blood

typically used.

[0345] Whole blood was drawn into an EDTA tube with anticoagulant agent. In addition, cell lines for both control (healthy) and cancer samples were also used in initial development phase of the work to test and validate signals of the technology.

Algorithm

[0346] With a large data base from retrospective studies, an algorithm has been built with a CVD test numbers along with cut-off values as a test outcome which is correlated to cancer risk, which (CDA value) is proportional to cancer risk. Based on CDA values, three regions were divided, healthy, medium risk, and high risk.

Results

[0347] Both retrospective studies and population screenings were carried out. For both medium risk and high risk groups, a follow-up was carried out on randomly selected 3,000 individuals. For the 3,000 individuals, feedback on 2,000 was obtained.

[0348] Fig. 61 shows scanning curves of control (healthy) and lung cancer cell lines, indicating that the electronic current for lung cancer is much lower than that of the control group. Specifically, it shows a typical curve for a control cell line sample (healthy cell line) and lung cancer cell line, with electrical current decreasing overtime and reaching a stable value in both cases. The two curves showed clearly different values at multiple points on the curves, especially significant difference in electrical current values between the two curves at their respective resting positions (60 seconds), indicating that this novel technology could distinguish normal cells and cancerous cells.

[0349] Furthermore, there is noticeable difference between control, disease and liver cancer samples (Figs. 62-64), with decreasing electrical current from control state to disease state, and from disease state to cancer state, demonstrating potential viability of this novel approach to detect disease and cancer, and ability to track disease progression.

[0350] Fig. 62 shows a typical scanning curve for control (healthy) whole blood sample, indicating a similar profile as that for a control cell line sample.

[0351] Data for a typical control whole blood sample and a liver cancer whole blood sample are shown in Fig. 63, showing again ability to differentiate a normal sample from a cancer sample.

[0352] Fig. 64 are a set of scan traces for whole blood samples of control, disease and liver cancer. Fig. 64 shows noticeable difference between control, disease and liver cancer samples,

with decreasing electrical current from control state to disease state, and from disease state to cancer state, demonstrating potential viability of this novel approach to detect disease and cancer, and ability to track disease progression.

[0353] Having initially confirmed feasibility of this new technology for disease detection, multiple retrospective clinical studies have been carried out. Data on over 20 types of cancer have been collected, and an algorithm has been built based upon a large data base. A set of test parameters have been built around the above-mentioned algorithm. The key parameter calculated from this algorithm based on raw data is CDA indicator, whose value is proportional to the cancer risk, and inversely proportional micro- electrical current value of the sample tested.

[0354] Table 8 shows significance test of difference – non-parametric test of various types of cancer. In Table 8, the distribution of CDA is the same across the categories of Group. Asymptotic significances are displayed. The significance level is 0.05. Table 8 shows that the difference in CDA values between control group and various cancer types are of statistical significance.

Table 8. Hypothesis Test Summary

Null Hypothesis	Test	Sig.	Decision
Control (1717) vs. Cancer (10078)	Independent Samples MannWhitney U Test	0.000	Reject the null Hypothesis
Control (1717) vs. Lung Cancer (1907)		0.000	Reject the null Hypothesis
Control (1717) vs. Colon Cancer (710)		0.000	Reject the null Hypothesis
Control (1717) vs. Esophageal Cancer (1590)		0.000	Reject the null Hypothesis
Control (1717) vs. Gastric Cancer (1117)		0.000	Reject the null Hypothesis
Control (1717) vs. Rectal Cancer (522)		0.000	Reject the null Hypothesis
Control (1717) vs. Cardia Cancer (135)		0.000	Reject the null Hypothesis
Control (1717) vs. Liver Cancer (738)		0.000	Reject the null Hypothesis
Control (1717) vs. Pancreatic Cancer (134)		0.000	Reject the null Hypothesis
Control (1717) vs. Ovarian Cancer (337)		0.000	Reject the null Hypothesis
Control (1717) vs. Breast Cancer (348)		0.000	Reject the null Hypothesis

Control (1717) vs. Cervical Cancer (318)		0.000	Reject the null Hypothesis
Control (1717) vs. Uterine Cancer (105)		0.000	Reject the null Hypothesis
Control (1717) vs. Prostatic Cancer (31)		0.000	Reject the null Hypothesis
Control (1717) vs. Brain Tumor (50)		0.000	Reject the null Hypothesis
Control (1717) vs. Lymphoma (322)		0.000	Reject the null Hypothesis
Control (1717) vs. Nasopharyngeal Cancer (121)		0.000	Reject the null Hypothesis
Control (1717) vs. Other Cancer (1593)		0.000	Reject the null Hypothesis

[0355] A summary of cancer screening sensitivity and specificity for control group and a number of cancer types from retrospective study is given in Table 9. Table 9 showed that overall, both sensitivity and specificity of CDA technology of various cancer types are relatively high, demonstrating CDA technology is potentially suited for a large number of cancer types. In addition, statistical analysis of the data Table 8 showed that P values for each two groups (each cancer group and control group) are all less than 0.001, also meaning that the difference in CDA values between control group and various cancer types listed in Table 8 are of statistical significance.

Table 9. CDA technology demonstrates high sensitivity and specificity for cancer screening of various types of cancer

Control (1717) vs.	**Sensitivity**	**Specificity**
Cancer (10078)	86.6%	86.9%
Lung Cancer (1907)	88.4%	88.4%
Colon Cancer (710)	87.7%	87.4%
Esophageal Cancer (1590)	86.9%	86.8%
Gastric Cancer (1117)	82.4%	86.8%
Rectal Cancer (522)	83.1%	86.8%
Cardia Cancer (135)	79.3%	87.0%
Liver Cancer (738)	89.7%	88.8%

Pancreatic Cancer (134)	82.8%	88.0%
Ovarian (337)	85.5%	86.9%
Breast Cancer (348)	86.2%	87.1%
Cervical Cancer (318)	84.0%	87.4%
Uterine Cancer (105)	84.8%	87.3%
Prostatic Cancer (31)	80.6%	87.5%
Brain Tumor (50)	82.0%	87.1%
Lymphoma (322)	87.6%	87.7%
Nasopharyngeal Cancer (121)	81.0%	87.1%
Other cancer (1593)	85.6%	86.8%

[0356] Table 10 shows CDA values of non-small lung cancer samples at various stages and control sample, and corresponding sensitivity and specificity, which are higher than traditional methods, particularly at stage I.

Table 10. CDA technology demonstrates high sensitivity and specificity for early stage screening of NSCLC

Group		Sample Size	Average CDA (rel. units)	Median CDA (rel. units)	SD of CDA (rel. units)	Sensitivity	Specificity
Control		248	33.98	34.72	5.50	/	/
NSCLC	Stage I	108	49.49	50.63	9.03	85.2%	90.7%
	Stage II	90	52.38	53.66	7.21	93.3%	91.1%
	Stage III	246	53.66	53.87	5.26	98.0%	95.6%
	Stage IV	388	52.45	52.96	6.11	95.1%	95.2%

[0357] Esophageal cancer is a cancer which still does not have a bio-marker and IVD screening method. In this investigation, CDA technology has been evaluated for esophageal cancer screening. Esophageal cancer results are summarized in Table 11. Results showed even at stage I, sensitivity and specificity are above 80%, far better than those by other technologies, which will have significant clinical meaning in catching esophageal cancer early.

Table 11. CDA technology demonstrates high sensitivity and specificity for early stage

screening of esophageal cancer

Group		Sample Size	Average CDA (rel. units)	Median CDA (rel. units)	SD of CDA (rel. units)	Sensitivity	Specificity
Control		248	33.98	34.72	5.50	/	/
Esophageal Cancer	Stage Ⅰ	38	47.38	48.47	6.78	81.6%	84.7%
	Stage Ⅱ	88	45.63	44.96	10.28	80.7%	84.7%
	Stage Ⅲ	95	47.37	46.03	9.66	80.0%	84.7%
	Stage Ⅳ	63	54.37	53.22	16.04	85.7%	85.1%

[0358] CDA technology was utilized to screen ~ 70,000 general populations. Based on CDA values, screened individuals were divided into three groups: low risk, medium risk, and high risk. Follow-up was carried out on about 3600 individuals with medium to high risk values, out of which 2240 individuals were able to have made contact and willing to share results from follow-up tests and diagnosis. Table 12A shows cancer cases screened out by CDA technology (based on follow-up on 2240 individuals initially tested with medium and high CDA values and later confirmed by oncologists). Table 12B shows pre-cancer cases screened out by CDA technology (based on follow-up on 2240 individuals initially tested with medium and high CDA values and later confirmed by oncologists). As shown in Table 12A and Table 12B, at the time of the follow-up contact, 73 individuals were diagnosed by oncologists having cancer, and 113 individuals were confirmed with pre-cancer diseases. Follow-up is no-going with remaining individuals. CDA test results on Caucasian group showed comparable sensitivity and specificity as those on Chinese Han ethnic group.

Table 12A

Cancer Cases	**Number**	**Percent**
Lung cancer	14	19%
Colorectal cancer	14	19%
Prostate cancer	9	12%
Gastric cancer	6	8%
Breast cancer	5	7%
Esophageal cancer	3	4%
Lymphoma cancer	3	4%

Cutaneum carcinoma	3	4%
Renal carcinoma	3	4%
Liver cancer	2	4%
Pancreatic cancer	2	3%
Cancerous goiter	2	3%
Cervical cancer	2	3%
Bladder cancer	1	1%
Tonsillar Carcinoma	1	1%
Osteocarcinoma	1	1%
Leukaemia	1	1%

Table 12B

Non-Cancerous Disease	**Number**	**percent**
Pulmonary nodule	27	24%
Gastroduodenal diseases	25	22%
Thyroid nodule	17	15%
Hysteromyoma	11	10%
Liver disease	8	7%
Colorectal polyp	7	6%
Renal cyst	5	4%
Breast disease	5	4%
Prostatic cyst	2	2%
Gallbladder polyps	2	2%
Oophoritic cyst	2	2%
Enteric adenoma	1	1%
Meningioma	1	1%

[0359] Fig. 65 shows a schematic comparing CDA technology with other cancer detection technologies, in which number of dots are proportional to detection signal. Unlike traditional cancer detection technologies which have relatively low signal to noise ratio, and some of them have signals starting when solid tumor has been formed. In contrast, signal at a CDA technology starts with health group and increases statistically significantly with disease progression, indicating that CDA technology is potentially a viable technology for pre-cancer and early stage cancer detection.

[0360] While the functions and properties of bio-physics have played a critical role in physiology, they have not been extensively utilized in the field of IVD of cancer, which has traditionally been more heavily replied upon bio-chemistry, immunology, and genomics. This work represents a novel approach and breakthrough in the field cancer detection. Results demonstrated that this technology has unique advantage to detect cancer early, and can be an

effective approach to track disease progression, as it showed statistical difference between healthy group and disease group, and between disease group and cancer group. Compared with traditional approaches, the current approach detects a signal which is much more foundational and it is in existence in all human being including healthy individuals. Therefore, its signal is much earlier in nature in detecting occurrence of cancer. Further, micro- electrical current has shown to decrease significantly from healthy group to disease group and from disease to cancer group, making it ideal for early stage cancer detection and tracking diseases leading to cancer.

[0361] Results from tests (a) using samples with increasing amount of cancer cells, (b) using samples with increasing amount of bio-marker concentration CEA, and (c) with samples with and without an assay which is known to cause a molecular level reaction showed that CDA values are proportional to increasing amount of cancer cells and bio-marker CEA concentrations. In addition, CDA values are dependent on with and without molecular level reactions. Based on the above observations, it can be stated that CDA values are a function of cellular, protein, and molecular levels (as shown in Fig. 50).

[0362] Fig. 66 shows that CDA technology is a multi-level and multi-parameter test that can also be carried out in conjunction with other tests including bio-markers (protein level), CTC (cellular level), and/or ct-DNA and other DNA based tests (genetic tests). While CDA is a function of multiple levels as stated above, it is also an advantage sometimes to perform CDA tests in conjunction with other cancer tests to obtain additional combined test results such as combined tests with bio-markers, CTCs, and genomics tests as shown in Fig. 66, where additional dimensional information can be obtained.

[0363] Fig. 67 shows a schematic of a proposed model, in which shift in bio-physical properties such as electrical properties cause changes at cellular, protein, and molecular (gene) levels which result in changes at immunity and inflammation, and likelihood (or less likelihood) of diseases and cancer occurrence.

[0364] Fig. 68 shows that as CDA increases and electrical current, conductance, ion level, membrane potential and polarization decrease, a number of cellular level (cell signaling, cell repulsion, resting potential and cell surface charge decrease) and molecular level (DNA surface charge decrease, quantum mechanical effect change, and DNA mutation increases) properties degrade, resulting in increased disease and cancer occurrence.

[365] Having demonstrated viability of this new technology for pre-cancer and early stage cancer detection, possible mechanism can be further proposed. A scheme of cells, proteins, and genetic components (DNA, RNA, etc.) and their surrounding liquid media (e.g., blood) is described above and provided in Fig. 60. First of all, as one of the important bio-physical parameters, electrical properties (which include but not limited to electrical current, conductance, quantum mechanical effects, electrical field, resting potential of cells, capacitance, cell surface charge, and electro-static force) affect at cellular, protein, and molecular levels. Specifically, electrical properties including micro-electrical current, conductance, and quantum mechanical effects not only impact cell surface properties, they also affect how cells interact each other (for example, repulsion and attraction between cells) as well as possibly cell signaling and shifting resting potential of cells. Also, electrical properties modify protein surface phase and structure. In addition, shift in micro-electrical current (and accordingly conductance) confirmed in the work in blood and/or change of quantum mechanical effects may possibly affect functioning and replications of DNA (increased mistakes in gene replications), and even causing increased frequency of DNA mutations. This conjuncture is directly and indirectly support by: (a) recent bio-physics work in mechanical stress studies indicated correlations between mechanical aspects in cellular structure and nuclear and chromatin organization including altered genomic program, (b) a shift in electrical property likely impacts surface charge of and electro-static force exerting on three dimensional DNA double helix structures and, (c) bio-physics work in this study in the area of electrical properties also indicated correlation between electrical property shift and occurrence of cancer which is often a result of increased gene mutation; (d) quantum mechanical effects affect gene replications and mutations. Based on experimental data presented in this work and above direct and indirect evidences, a hypothesis on cancer occurrence is proposed as follows. As micro- electrical current is reduced, at cellular level, cell surface charge as well as repulsive force between cells is reduced, cell signaling also is reduced and likely becomes less efficient and effective, and resting potential is shifted. All of the above stated developments at cellular level are not desirable. At molecular level, with reduction in micro-electrical current and/or change of quantum mechanical effects, mutation frequency may increase due to likely reduced electro-static force and surface charge on double helix three dimensional structures and amino acids surfaces, and possibly impacting quantum mechanical effects at DNA microscopic level, resulting in increased replication errors. The above hypothesis on the negative effects at

multi-biological levels caused by reduced micro- electrical current (and conductance) of blood match our experimental observations and data in retrospective investigations on healthy group, disease group, and cancer group samples, and also agree with results from initial follow-up studies on general population screening. Since this model is based upon electrical properties of blood, it is named electrical model of cancer (EMOC).

[0366] Compared with other traditional cancer detection technologies, CDA technology has many unique features and clear advantages. First, many existing technologies detect cancer signals after cancer has already formed which make those technologies ineffective for early stage cancer detection, while CDA technology detects a bio-physical parameter which exists in healthy individuals and rises as the risk of cancer increases (as shown in Fig. 69), where CDA values for healthy group, disease group and cancer group showed statistical difference ($P < 0.001$). Such rise in CDA values is statistically significant before and during early stage of cancer, making CDA technology far more suited for early stage cancer detection. Secondly, unlike most of existing cancer detection technologies which are based on detecting a single level (for example, bio-marker at protein level and CTC at cellular level) and even a single parameter, CDA technology is a multi-level and multi-parameter technology which is much more comprehensive and contains much more information, making it more accurate. Thirdly, CDA technology detects micro- electrical current signal which is more fundamental with a high signal to noise ratio, and decrease in micro- electrical current likely to be the cause for loss of immunity and increasing occurrence of cancer which can be detected well before cancer is formed, in contrast to most of the exiting detection technologies which pick up signal when cancer has already occurred and in many cases are already at late stage cancer.

[0367] In addition, based on CDA value dependent disease progression behavior (disease progresses with decreasing micro- electrical current of the blood sample); based on the above proposed hypothesis, new model for cancer occurrence is proposed as follows. In this new model, as a major bio-physical parameter, the shift in electrical properties of blood, specifically, decreasing in micro- electrical current and/or changing quantum mechanical effects (which affect gene replications and mutations) is causing negative effects at multi-levels which include (1) reduced surface charge, cell repulsion, and cell signaling efficiency at cellular level, and (2) reduced electrostatic force, DNA surface charge, and possibly increased mutation at DNA level. Further, it is hypothesized that reduced micro-electrical current (and conductance) also causes

reduced surveillance capability of T cells for cancer cell detection and reduced immunity which increase occurrence of cancer. The above hypothesis is supported by data collected in this work showing that decreasing (increasing CDA values) in micro- electrical current is correlated with disease progress from healthy group to disease group, from disease group to pre-cancer group, and from pre-cancer group to cancer group.

[0368] Fig. 70 shows that as electrical current and conductance decrease (ion (e.g., potassium, chloride, sodium, and calcium) concentration or net ion concentration or charge decreases), a number of cellular level (cell signaling, cell repulsion, resting potential, membrane potential and cell surface charge decrease) properties change and degrade. For example, cell surface charge decreases, resulting in reduction in repulsive force between cells and decreased distance between cells. Finally, in cancer stage, cells lose concept of space and boundary, and collapse to each other (sticking/stacking to each other), in which repulsive force between cells are reduced due to reduced cell surface charge. Therefore, repulsive force between cells due to surface charge on cell surfaces are very important.

[0369] In this invention, changes in electrical properties in blood and DNA level can be used as a tool for disease detection. As electrical current and conductance decrease, a number of molecular level (DNA surface charge decreases, quantum mechanical effect change, and DNA mutation increases) properties degrade, resulting in increased disease and cancer occurrence. As shown in Fig. 71, in a sample from a health case (a), both surrounding and DNA surface have higher charge, while in a sample from a cancer case (b), both surrounding and DNA surface have less charge, possibly overall negative charge. Since for DNA double helix structures, DNA surface charge and electrical properties of the media may affect its electro-static force and hence 3-dimensional structures, as well as quantum mechanical effects (at atomic level, and with spacing between adjacent amino acids is only at a few angstrom), change at electrical properties of DNA surrounding media and/or DNA surface charge may affect DNA replications and cause increased replication error rate and gene mutations.

[0370] Furthermore, the new technology according to this invention can also be used in assisting in diagnosis, such as assisting in diagnosis of lung cancer. As shown in Fig. 72, compared with CT, this novel technology (parameters of CDA, CTF and PTF) has better and higher sensitivity and specificity. Additionally, its ROC is better than that of CT imaging.

[0371] As also shown in Fig. 73 that the CDA values appear to correlate with mutation

frequency for (a) healthy, (b) lung cancer just after diagnosis and before surgery, and (c) after surgery and treatment individuals / groups.

[0372] Initial clinical study results show that the novel technology according to this invention is capable of evaluating effectiveness of drug treatment of cancer. In this case (e.g., as shown in Fig. 74), this novel cancer detection technology is used for prognosis of a targeted drug treatment of small cell lung cancer at three stages — i.e., after diagnosis, after phase 1 treatment, and after phase 2 treatment. In Fig. 74, CTF is a parameter of this novel technology.

[0373] One of key aspects of this invention is that the bio-physical properties and its associated behaviors disclosed in this novel work are of common to a large number of cancer types, and can be used for detection of a large number of cancer types, making the disclosed method a viable technology for cancer screening, assisting in diagnosis, prognosis, therapy selection and reoccurrence detection.

[0374] Fig. 75 is a schematic of cell membranes with intracellular and extracellular regions, with decreasing membrane potential, net charge Q in extracellular region (and membrane polarization) from (a) to (b) to (c), and net charge $Qa < Qb < Qc$. Based on experimental data in this work in electrical conductivity in whole blood and serum, which showed decreasing electrical conductivity (decreasing electrical current and electrical charge) mainly due to properties in extracellular regions from healthy group to disease group to cancer group, it is claimed that schematic (a) corresponding to health condition, schematic (b) corresponding to disease condition, and schematic (c) corresponding to cancer condition.

[0375] Fig. 76 shows a schematic of membranes of two cells showing membrane potential, intracellular space, and extracellular space. As shown in Fig. 76, schematics (a), (b) and (c) represent healthy, disease and cancer cases including membrane potentials, ion distributions, and net charges, with decreasing blood conductivity (measured values), membrane potential and polarization, and net charge in extracellular region. Notably, the medical device according to the present invention can treat a biological subject (e.g., a blood sample) by reversing its situations presented in Figs. 76 and 77, e.g., from situation (c) to (b) and to (a) as shown in Fig. 76 or 77.

[0376] As shown in Fig. 76, high permeability of potassium ions into cells (and high concentrations of sodium and chloride ions in extracellular region) create differences in concentrations of ions on opposite sides of a cellular membrane and hence an electrical potential across membrane layer. In the local region or near field, it is not electrically neutral, while in a

larger scale, it is electrically neutral. By probing electrical properties at a local region or near field, information relating to cell properties including but not limited to electrical conductivity, electrical resistance, ion concentrations, ion levels, ion permeability, membrane potential, cell surface charge, electro-static force, electrical field, electro-magnetic field, and quantum mechanical effects can be obtained directly or indirectly.

[0377] In one embodiment, utilizing a micro-fluidic device with micro-channels and sensitive sensors, electrical properties of blood samples at near field of cells illustrated in above figure (schematic of cellular membranes) can be measured, and related electrical properties including electrical current across the region, trans-membrane potential, and ion levels (potassium ions, sodium ions, chloride ions, calcium ions, and nitride ions) can be directly and indirectly measured. Since disease state of mammals is related to the above-mentioned cellular bio-physical properties (and DNA, RNA and other biological entities in the cells), the above inventive measurement technology can be used to detect diseases including pre-cancer and cancer diseases. The membrane potential can regulate the balance between normal cellular activities including normal growth and replications, and carcinogenesis. As such, both ion level and concentration (potassium ions, sodium ions, chloride ions and calcium ions) and membrane potential could be used as a new, novel bio-marker for cancer prevention and early stage cancer detection.

[0378] The present invention provides a new cancer detection technology using a bio-physical approach based on electrical properties of liquid samples for IVD applications. In this new technology, a micro- electrical current is detected which has shown to be very effective in detecting pre-cancer and early stage cancer. This technology has the advantages of detecting cancer early, high sensitivity and specificity, covering a wide range of cancer types, and relatively simple and cost effective. Based on how CDA values are correlated to control, disease and cancer groups in this work, and possible effects of electrical properties in blood on disease progression, a new hypothesis on cancer occurrence model is proposed in which a reduction in blood micro electrical current (and conductance) and/or a change of quantum mechanical effects is proposed to cause a number of negative effects at cellular and molecular levels, resulting in reduced cell to cell signaling, cell to cell repulsion, and immunity, and increased gene mutation frequency, and hence increased occurrence of cancer.

[0379] While for the purposes of demonstration and illustration, the above cited novel, detailed

examples show how microelectronics and/or nano-fabrication techniques and associated process flows can be utilized to fabricate highly sensitive, multi-functional, powerful, and miniaturized detection devices, the principle and general approaches of employing microelectronics and nano-fabrication technologies in the design and fabrication of high performance detection devices have been contemplated and taught, which can and should be expanded to various combination of fabrication processes including but not limited to thin film deposition, patterning (lithography and etch), planarization (including chemical mechanical polishing), ion implantation, diffusion, cleaning, various materials, combination of processes and steps, and various process sequences and flows. For example, in alternative detection device design and fabrication process flows, the number of materials involved can be fewer than or exceed four materials (which have been utilized in the above example), and the number of process steps can be fewer or more than those demonstrated process sequences, depending on specific needs and performance targets. For example, in some disease detection applications, a fifth material such as a biomaterial-based thin film can be used to coat a metal detection tip to enhance contact between the detection tip and a biological subject being measured, thereby improving measurement sensitivity.

[0380] Applications for the detection apparatus and methods of this invention include detection of diseases (e.g., in their early stage), particularly for serious diseases like cancer. Since cancer cell and normal cell differ in a number of ways including differences in possible microscopic properties such as electrical potential, surface charge, density, adhesion, and pH, novel micro-devices disclosed herein are capable of detecting these differences and therefore applicable for enhanced capability to detect diseases (e.g., for cancer), particularly in their early stage. In addition to micro-devices for measuring electrical potential and electrical charge parameters, micro-devices capable of carrying out mechanical property measurements (e.g., density) can also be fabricated and used as disclosed herein. In mechanical property measurement for early stage disease detection, the focus will be on the mechanical properties that likely differentiate disease or cancerous cells from normal cell. As an example, one can differentiate cancerous cells from normal cells by using a detection apparatus of this invention that is integrated with micro-devices capable of carrying out micro-indentation measurements.

[0381] Although specific embodiments of this invention have been illustrated herein, it will be appreciated by those skilled in the art that any modifications and variations can be made without departing from the spirit of the invention. The examples and illustrations above are not intended

to limit the scope of this invention. Any combination of detection apparatus, micro-devices, fabrication processes, and applications of this invention, along with any obvious their extension or analogs, are within the scope of this invention. Further, it is intended that this invention encompass any arrangement, which is calculated to achieve that same purpose, and all such variations and modifications as fall within the scope of the appended claims.

[0382] All publications or patent applications referred to above are incorporated herein by reference in their entireties. All the features disclosed in this specification (including any accompanying claims, abstract and drawings) may be replaced by alternative features serving the same, equivalent or similar purpose, unless expressly stated otherwise. Thus, unless expressly stated otherwise, each feature disclosed is one example of a generic series of equivalent or similar features.

WHAT IS CLAIMED IS:

1. An apparatus for detecting presence or monitoring progression of a disease in a biological subject, comprising a chamber in which the biological subject passes through, and at least one detection transducer placed partially or completely in the chamber; wherein information related to properties of cells in the biological subject and of cell-surrounding media is detected by the detection transducer and collected for analysis to determine whether the disease is likely to be present with the biological subject or to determine the status of the disease, thereby providing the ability to continuously determine or monitor progression of the disease.

2. The apparatus of claim 1, wherein the properties of the cells and cell-surrounding media comprise cell signaling, cell surface properties, signal pathway affecting gene replication properties and processes, signal pathway affecting gene mutation properties and processes, signal pathway affecting protein fabrication and properties, signal pathway affecting cell replications and properties, communication pathway and signaling between proteins, cells and genes, cell surface hydrophobicity properties, cell surface hydrophobicity properties, cell surface transduction properties, cell surface signal transmission properties, cell surface geometrical properties, cell surface electrical properties, cell surface ion concentration, types and distribution properties, cell inner media electrical properties, cell inner signal transmission properties, cell inner media electrical charge properties, cell inner media ion concentrations, types, and distribution properties, cellular bulk electrical properties, cellular bulk electrical properties, cell-surrounding media signal transduction properties, cell-surrounding media electrical properties, cell-surrounding media signal transmission properties, cell-surrounding media electrical charge properties, cell-surrounding media transportation properties, cell, protein, DNA, RNA, ion, and micro vesicle transportation properties in cell-surrounding media, cell, protein, DNA, RNA, ion, and micro vesicle properties in cell-surrounding media, cell-surrounding media chemical properties, cell-surrounding media bio-physical properties, cell-surrounding media bio-chemistry properties, cell to cell-surrounding media interaction properties, cell to cell-surrounding media interface properties, cell to cell-surrounding media signaling properties, cell-surrounding media ion concentrations, types, and distribution properties, cell to cell signaling properties, cell to cell communication properties, cell-to-cell interaction properties or quantum mechanical effects; and the detected information is collected for analysis to as to whether the disease is likely to be present with or within the biological subject.

3. The apparatus of claim 1 or 2, wherein the cell surface properties comprise cell surface tension, cell surface area, cell surface charge, cell surface hydrophobicity, cell surface potential, cell surface protein types and compositions, cell surface bio-chemical components, cell surface signaling properties, cell surface mutations, or cell surface biological components.

4. The apparatus of claim 1 or 2, wherein the cell to cell interaction properties comprise cell to cell affinity, cell to cell repulsion, mechanical force, electrical force, gravitational force, chemical bonding, bio-chemical interactions, geometrical matching, bio-chemical matching, chemical matching, physical matching, biological matching, or cell to cell signaling properties.

5. The apparatus of claim 4, wherein the cell to cell signaling properties comprise signaling method, signaling strength, cell surrounding media its properties to which signal is transmitted, and signaling frequency.

6. The apparatus of claim 5, wherein the cell signaling comprises cell signal type, cell signal strength, cell signal frequency, cell interactions with cell media to which cell signal is transmitted, and cell interactions with other biological entities to which signal is transmitted.

7. The apparatus of any of claims 1-5, wherein the cell surrounding media comprises blood, proteins, red blood cells, while blood cells, T cells, other cells, gene mutations, quantum mechanical effects, DNA, RNA, or other biological entities.

8. The apparatus of claim 7, wherein the cell surrounding media properties comprise a thermal, optical, acoustical, biological, chemical, physical-chemical, electro-mechanical, electro-chemical, electro-chemical-mechanical, bio-physical, bio-chemical, bio-mechanical, bio-electrical, bio-physical-chemical, bio-electro-physical, bio-electro-mechanical, bio-electro-chemical, bio-chemical-mechanical, bio-electro-physical-chemical, bio-electro-physical-mechanical, bio-electro-chemical-mechanical, physical, an electric, magnetic, electro-magnetic, or mechanical property.

9. The apparatus of claim 8, wherein the thermal property is temperature or vibrational frequency; the optical property is optical absorption, optical transmission, optical reflection, optical-electrical property, brightness, or fluorescent emission; the radiation property is radiation emission, signal triggered by radioactive material, or information probed by radioactive material; the chemical property is pH value, chemical reaction, bio-chemical reaction, bio-electro-chemical reaction, reaction speed, reaction energy, speed of reaction, oxygen concentration, oxygen consumption rate, ionic strength, catalytic behavior, chemical additives to trigger

enhanced signal response, bio-chemical additives to trigger enhanced signal response, biological additives to trigger enhanced signal response, chemicals to enhance detection sensitivity, bio-chemicals to enhance detection sensitivity, biological additives to enhance detection sensitivity, or bonding strength; the physical property is density, shape, volume, or surface area; the electrical property is surface charge, surface potential, resting potential, electrical current, electrical field distribution, surface charge distribution, cell electronic properties, cell surface electronic properties, dynamic changes in electronic properties, dynamic changes in cell electronic properties, dynamic changes in cell surface electronic properties, dynamic changes in surface electronic properties, electronic properties of cell membranes, dynamic changes in electronic properties of membrane surface, dynamic changes in electronic properties of cell membranes, electrical dipole, electrical quadruple, oscillation in electrical signal, electrical current, capacitance, three-dimensional electrical or charge cloud distribution, electrical properties at telomere of DNA and chromosome, capacitance, or impedance; the biological property comprises protein, cell, genomics, quantum mechanical effects, cellular properties (which comprise chemical, physical, bio-chemical, bio-physical, and biological aspects of surrounding liquid, gas and solid of the said cell), surface shape, surface area, surface charge, surface biological property, surface chemical property, pH, electrolyte, ionic strength, resistivity, cell concentration, or biological, electrical, physical or chemical property of solution; the acoustic property is frequency, speed of acoustic waves, acoustic frequency and intensity spectrum distribution, acoustic intensity, acoustical absorption, or acoustical resonance; the mechanical property is internal pressure, hardness, flow rate, viscosity, fluid mechanical properties, shear strength, elongation strength, fracture stress, adhesion, mechanical resonance frequency, elasticity, plasticity, or compressibility.

10. The apparatus of any of claims 1-9, wherein the apparatus comprises a micro-electro-mechanical device, a semiconductor device, a micro-fluidic device, bio-chemistry machine, an immunology machine, a voltage meter, or a sequencing machine.

11. The apparatus of any of claims 1-10, wherein the collected information is in the physical, bio-physical, bio-chemical, biological, or chemical form.

12. The apparatus of claim 11, wherein the physical form of the collected information comprises mechanical, electrical, thermal, thermodynamic, optical, and acoustical properties of the cells or cell surrounding media.

13. The apparatus of any of claims 1-12, wherein the information is collected after a probe signal is applied to the cells or cell-surrounding media and a response signal is received.

14. The apparatus of claim 13, wherein the probe signal comprises a physical, bio-physical, bio-chemical, biological, or chemical signal.

15. The apparatus of claim 14, wherein the physical signal comprises a mechanical, electrical, thermal, thermodynamic, optical, or acoustical signal.

16. The apparatus of any of claims 1-15, wherein the disease is a cancer, an inflammatory disease, diabetes, a lung disease, a heart disease, a liver disease, a gastric disease, a biliary disease, or a cardiovascular disease.

17. The apparatus of claim 16, wherein the cancer comprises breast cancer, lung cancer, esophageal cancer, intestine cancer, cancer related to blood, liver cancer, stomach cancer, cervical cancer, ovarian cancer, rectum cancer, colon cancer, nasopharyngeal cancer, cardiac carcinoma, uterine cancer, oophoroma, pancreatic cancer, prostate cancer, brain tumor, or circulating tumor cells; the inflammatory disease comprises acne vulgaris, asthma, autoimmune diseases, autoinflammatory diseases, celiac disease, chronic prostatitis, diverticulitis, glomerulonephritis, hidradenitis suppurativa, hypersensitivities, inflammatory bowel diseases, interstitial cystitis, otitis, pelvic inflammatory disease, reperfusion injury, rheumatic fever, rheumatoid arthritis, sarcoidosis, transplant rejection, or tasculitis; the lung disease comprises asthma, chronic obstructive pulmonary disease, chronic bronchitis, emphysema, acute bronchitis, cystic fibrosis, pneumonia, tuberculosis, pulmonary edema, acute respiratory distress syndrome, pneumoconiosis, interstitial lung disease, pulmonary embolism, or pulmonary hypertension; the diabetes comprises Type 1 diabetes, Type 2 diabetes, or gestational diabetes; the heart disease comprises coronary artery disease, enlarged heart (cardiomegaly), heart attack, irregular heart rhythm, atrial fibrillation, heart rhythm disorders, heart valve disease, sudden cardiac death, congenital heart disease, heart muscle disease (cardiomyopathy), dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, pericarditis, pericardial effusion, marfan syndrome, or heart murmurs; the liver disease comprises fascioliasis, hepatitis, alcoholic liver disease, fatty liver disease (hepatic steatosis), hereditary diseases, Gilbert's syndrome, cirrhosis, primary biliary cirrhosis, primary sclerosing cholangitis, or Budd–Chiari syndrome; the gastric disease comprises gastritis, gastric polyp, gastric ulcer, benign tumor of stomach, acute gastric mucosa lesion, antral gastritis, or gastric stromal tumors; the biliary disease comprises

calculus of bile duct, cholecystolithiasis, cholecystitis, cholangiectasis, cholangitis, or gallbladder polyps; the cardiovascular disease comprises coronary artery disease, peripheral arterial disease, cerebrovascular disease, renal artery stenosis, aortic aneurysm, cardiomyopathy, hypertensive heart disease, heart failure, pulmonary heart disease, cardiac dysrhythmias, endocarditis, inflammatory cardiomegaly, myocarditis, valvular heart disease, congenital heart disease, rheumatic heart disease, coronary artery disease, peripheral arterial disease, cerebrovascular disease, or renal artery stenosis.

18. The apparatus of any of claims 1 to 17, further comprising a sensor positioned to be partially inside the chamber and capable of detecting a property of the biological subject at the microscopic level.

19. The apparatus of claim 18, further comprising a read-out circuitry which is connected to at least one sensor and transfers data from the sensor to a recording device.

20. The apparatus of claim 19, wherein the connection between the read-out circuit and the sensor is digital, analog, optical, thermal, piezo-electrical, piezo-photronic, piezo-electrical photronic, opto-electrical, electro-thermal, opto-thermal, electric, electromagnetic, electromechanical, or mechanical.

21. The apparatus of claim 20, wherein the sensor is positioned on the interior surface of the chamber.

22. The apparatus of claim 21, wherein each sensor is independently a thermal sensor, optical sensor, acoustical sensor, biological sensor, chemical sensor, electro-mechanical sensor, electro-chemical sensor, electro-optical sensor, electro-thermal sensor, electro-chemical-mechanical sensor, bio-chemical sensor, bio-mechanical sensor, bio-optical sensor, electro-optical sensor, bio-electro-optical sensor, bio-thermal optical sensor, electro-chemical optical sensor, bio-thermal sensor, bio-physical sensor, bio-electro-mechanical sensor, bio-electro-chemical sensor, bio-electro-optical sensor, bio-electro-thermal sensor, bio-mechanical-optical sensor, bio-mechanical thermal sensor, bio-thermal-optical sensor, bio-electro-chemical-optical sensor, bio-electro-mechanical optical sensor, bio-electro-thermal-optical sensor, bio-electro-chemical-mechanical sensor, physical sensor, mechanical sensor, piezo-electrical sensor, piezo-electro photronic sensor, piezo-photronic sensor, piezo-electro optical sensor, bio-electrical sensor, bio-marker sensor, electrical sensor, magnetic sensor, electromagnetic sensor, image sensor, or radiation sensor.

23. The apparatus of claim 22, wherein the thermal sensor comprises a resistive temperature micro-sensor, a micro-thermocouple, a thermo-diode and thermo-transistor, and a surface acoustic wave (SAW) temperature sensor; the image sensor comprises a charge coupled device (CCD) or a CMOS image sensor (CIS); the radiation sensor comprises a photoconductive device, a photovoltaic device, a pyro-electrical device, or a micro-antenna; the mechanical sensor comprises a pressure micro-sensor, micro-accelerometer, flow meter, viscosity measurement tool, micro-gyrometer, or micro flow-sensor; the magnetic sensor comprises a magneto-galvanic micro-sensor, a magneto-resistive sensor, a magneto diode, or magneto-transistor; the biochemical sensor comprises a conductimetric device, a bio-marker, a bio-marker attached to a probe structure, or a potentiometric device.

24. The apparatus of claim 20, wherein at least one sensor is a probing sensor and applies a probing or disturbing signal to the biological subject.

25. The apparatus of claim 24, wherein at least another sensor, different from the probing sensor, is a detection sensor and detects a response from the biological subject upon which the probing or disturbing signal is applied.

26. The apparatus of claim 1, wherein the chamber has a length ranging from 1 micron to 50,000 microns, from 1 micron to 15,000 micron, from 1 micron to 10,000 microns, from 1.5 microns to 5,000 microns, or from 3 microns to 1,000 microns.

27. The apparatus of claim 26, wherein the chamber has a width or height ranging from 0.1 micron to 100 microns; from 0.1 micron to 25 microns, from 1 micron to 15 microns, or from 1.2 microns to 10 microns.

28. The apparatus of claim 20, comprising at least four sensors which are located on one side, two opposite sides, or four sides of the interior surface of the chamber.

29. The apparatus of claim 28, wherein the two sensors in the micro-cylinder are apart by a distance ranging from 0.1 micron to 500 microns, from 0.1 micron to 50 microns, form 1 micron to 100 microns, from 2.5 microns to 100 microns, or from 5 microns to 250 microns.

30. The apparatus of claim 29, wherein at least one of the panels comprises at least two sensors that are arranged in at least two arrays each separated by at least a micro sensor in a cylinder.

31. The apparatus of claim 30, wherein at least one array of the sensors in the panel comprises two or more sensors.

32. The apparatus of claim 31 wherein the sorting unit or the detection unit further comprises an application specific integrated circuit chip which is internally bonded to or integrated into one of the panels or a micro-cylinder.

33. The apparatus of claim 32, wherein the sorting unit or the detection unit further comprises a memory unit, a logic processing unit, an optical device, imaging device, camera, viewing station, acoustic detector, piezo-electrical detector, piezo-photronic detector, piezo-electro photronic detector, electro-optical detector, electro-thermal detector, bio-electrical detector, bio-marker detector, bio-chemical detector, chemical sensor, thermal detector, ion emission detector, photo-detector, x-ray detector, radiation material detector, electrical detector , or thermal recorder, each of which is integrated into the a panel or a micro cylinder.

34. The apparatus of claim 1, wherein the biological subject is a blood sample, a urine sample, or a sweat sample of a mammal.

35. The apparatus of any of claims 1-34, wherein one signal contains information related to the disease's location or where the disease is present in the source of the biological subject.

36. The apparatus of any of claims 1-34, wherein one signal contains information related to the occurrence or type of the disease.

37. The apparatus of any of claims 1-36, wherein the apparatus is able to detect the presence of at least two different diseases at the same time or to determine the status or progression of a disease.

38. The apparatus of claim 37, wherein the apparatus is capable of detecting at least two different types of cancer simultaneously.

39. The apparatus of claim 1, wherein the disease comprises healthy stage, non-cancer disease stage, pre-cancer stage, early stage cancer stage, and mid to late stage cancer stage, with statistically significant detection or monitoring between any of the two stages.

40. The apparatus of any of claims 1-39, wherein the signal detected comprises cellular information, protein information, gene information, and any combination thereof.

41. The apparatus of claim 1, wherein the apparatus is capable of detecting biological, bio-chemistry, physical and bio-physical properties of liquid media surrounding cells, proteins, and genetic components, and shift in the said properties.

42. The apparatus of claim 41, wherein the liquid media comprises blood, urine, saliva, or sweat.

43. The apparatus of claim 41, wherein the biological properties comprise any one of protein concentrations, protein types, cellular properties, quantum mechanical effects, or genetic sequence.

44. The apparatus of claim 43, wherein the physical properties comprise any one of thermal properties, mechanical properties, electrical properties, or electro-magnetic properties.

45. The apparatus of claim 41, wherein the detected properties correlate with the immune system, disease detection capability or disease killing ability.

46. The apparatus of claim 1, wherein the disease to be detected or monitored comprises degradation in immune system, a non-cancer disease, a pre-cancer condition, or cancer.

47. The apparatus of claim 41, wherein the detected properties correlate to cell signaling, disease detection, disease killing, communications between cells, proteins, genetic components, or effectiveness and efficiency in the cell signaling and communications.

48. The apparatus of claim 47, wherein the detected properties correlate with and provide an early detection on immune system degradation, loss of ability to detect cancer, cancer killing ability, pre-cancer stage, or early stage cancer.

49. A method for detecting the presence or progression of a disease in a biological subject, comprising detecting information related to properties of cells in the biological subject and of cell-surrounding media, and analyzing the collected information to determine if the likely presence or progression of the status of the disease with the biological subject.

50. The method of claim 49, wherein the detection is conducted with an apparatus of any of claims 1-48.

51. The method of claim 49 or 50, wherein the properties of the cells and cell-surrounding media comprise cell signaling, cell surface properties, or cell-to-cell interaction properties; and the detected information is collected for analysis to as to whether the disease is likely to be present with or within the biological subject.

52. The method of claim 51, wherein the cell surface properties comprise cell surface tension, cell surface area, cell surface charge, cell surface hydrophobicity, cell surface potential, cell surface protein types and compositions, cell surface bio-chemical components, cell surface signaling properties, cell surface mutations, DNA surface charge, DNA surrounding media electrical properties, quantum mechanical effects, or cell surface biological components.

53. The method of claim 51, wherein the cell to cell interaction properties comprise cell to

cell affinity, cell to cell repulsion, mechanical force, electrical force, gravitational force, chemical bonding, bio-chemical interactions, geometrical matching, bio-chemical matching, chemical matching, physical matching, biological matching, or cell to cell signaling properties.

54. The method of claim 51, wherein the cell to cell signaling properties comprise signaling method, signaling strength, cell surrounding media its properties to which signal is transmitted, and signaling frequency.

55. The method of claim 51, wherein the cell signaling comprises cell signal type, cell signal strength, cell signal frequency, cell interactions with cell media to which cell signal is transmitted, and cell interactions with other biological entities to which signal is transmitted.

56. The method of claim 49, wherein the cell surrounding media comprises blood, proteins, red blood cells, while blood cells, T cells, other cells, DNA surface charge, DNA surrounding media electrical properties, quantum mechanical effects, gene mutations, DNA, RNA, or other biological entities.

57. The method of any of claims 49-56, wherein the method is able to detect the presence of at least two different diseases at the same time or to determine the status or progression of a disease.

58. The method of claim 57, wherein the method is capable of detecting at least two different types of cancer simultaneously.

59. The method of claim 57, wherein the disease comprises healthy stage, non-cancer disease stage, pre-cancer stage, early stage cancer stage, and mid to late stage cancer stage, with statistically significant detection or monitoring between any of the two stages.

60. A method for detecting the presence or progression of a disease in a biological subject, comprising measuring a biophysical property at a microscopic level of cells in the biological subject with an apparatus of any of claims 1-48, wherein information related to the measured biological property of the cells in the biological subject is detected by the detection transducer and collected for analysis to determine whether the disease is likely to be present with the biological subject or to determine the status of the disease, thereby providing the ability to continuously determine or monitor progression of the disease.

61. The method of claim 60, wherein the determination is by comparing the biophysical information of the detected biological subject with the same biological information of a confirmed disease-free or diseased biological subject.

62. The method of claim 60 or 61, wherein the biophysical property is an electric property at the microscopic level.

63. The method of any of claims 60-62, wherein the electronic property is surface charge, surface potential, resting potential, electrical current, electrical field distribution, surface charge distribution, cell electronic properties, cell surface electronic properties, dynamic changes in electronic properties, dynamic changes in cell electronic properties, dynamic changes in cell surface electronic properties, dynamic changes in surface electronic properties, electronic properties of cell membranes, dynamic changes in electronic properties of membrane surface, dynamic changes in electronic properties of cell membranes, electrical dipole, electrical quadruple, oscillation in electrical signal, electrical current, capacitance, three-dimensional electrical or charge cloud distribution, electrical properties at telomere of DNA and chromosome, DNA surface charge, DNA surrounding media electrical properties, quantum mechanical effects, capacitance, or impedance.

64. The method of claim 63, wherein the electronic property is electrical current, electric conductance, electrical resistance, capacitance, or quantum mechanical effect.

65. The method of any of claims 60-64, wherein the method is able to detect the presence of at least two different diseases at the same time or to determine the status or progression of a disease.

66. The method of claim 65, wherein the method is capable of detecting at least two different types of cancer simultaneously.

67. The method of claim 65, wherein the disease comprises healthy stage, non-cancer disease stage, pre-cancer stage, early stage cancer stage, and mid to late stage cancer stage, with statistically significant detection or monitoring between any of the two stages.

68. A method for treating or slowing progression of a disease in a biological subject, comprising administering to the biological subject thereof a therapeutic agent that enhances or increase the level of a biophysical property at the microscopic level of the biological subject.

69. The method of claim 68, wherein the therapeutic agent is administered orally or by intravenous injection.

70. The method of claim 68 or 69, wherein the biophysical property is an electronic property.

71. The method of claim 70, wherein the electronic property is surface charge, surface potential, resting potential, electrical current, electrical field distribution, surface charge

distribution, cell electronic properties, cell surface electronic properties, dynamic changes in electronic properties, dynamic changes in cell electronic properties, dynamic changes in cell surface electronic properties, dynamic changes in surface electronic properties, electronic properties of cell membranes, dynamic changes in electronic properties of membrane surface, dynamic changes in electronic properties of cell membranes, electrical dipole, electrical quadruple, oscillation in electrical signal, electrical current, capacitance, three-dimensional electrical or charge cloud distribution, electrical properties at telomere of DNA and chromosome, DNA surface charge, DNA surrounding media electrical properties, quantum mechanical effects, capacitance, or impedance.

72. The method of any of claims 68-71, wherein the method is able to detect the presence of at least two different diseases at the same time or to determine the status or progression of a disease.

73. The method of claim 72, wherein the method is capable of detecting at least two different types of cancer simultaneously.

74. The method of claim 72, wherein the disease comprises healthy stage, non-cancer disease stage, pre-cancer stage, early stage cancer stage, and mid to late stage cancer stage, with statistically significant detection or monitoring between any of the two stages.

75. A therapeutic agent for treating or slowing progression of a disease in a biological subject, comprising a component that alters or enhances electronic property of the biological subject.

76. The therapeutic agent of claim 75, wherein the component comprises electrolytes.

77. The therapeutic agent of claim 75 or 76, wherein the component enhances electrical current and/or electrical conductance, reduces electrical resistance, and/or alters quantum mechanical effect.

78. A method for detecting a disease in a biological subject, comprising using a micro-fluidic device of any of claims 1-48 to detect at least one physical or bio-physical property of the biological subject with a reagent.

79. The method of claim 78, wherein the bio-physical property comprises a mechanical property, an acoustical property, an optical property, an electrical property, an electro-magnetic property, or an electro-mechanical property.

80. The method of claim 79, wherein the electrical property comprises electrical current,

electrical conductance, capacitance, electrical resistance, or quantum mechanical effect.

81. The method of claim 79, wherein the bio-physical property comprises quantum mechanical effects that affect gene replications and mutations.

82. The method of claim 78, wherein the micro-fluidic device directly or indirectly measures the quantum mechanical effects.

83. The method of claim 79, wherein the bio-physical property comprises a trans-membrane potential, a membrane voltage, a membrane potential, a zeta potential, an impedance, an optical reflective index, an optical refractive index, potassium ions, sodium ions, chloride ions, nitride ions, calcium ions, an electro-static force, an electro-static force acting on cells, an electro-static force acting on DNA double helix, an electro-static force acting on RNA, an electrical charge on cell membrane, an electrical charge on DNA double helix, an electrical charge on RNA, quantum effects, near-field electrical properties, near-field electro-magnetic properties, membrane bilayer properties, ion permeability, electrical current, electrical conductance, capacitance, or electrical resistance.

84. The method of any of claims 78-83, wherein the micro-fluidic device directly or indirectly measures ions or ion levels in a liquid sample of the biological subject.

85. The method of claim 84, wherein the micro-fluidic device measures ion levels or concentrations by a bio-chemistry or electrode method.

86. The method of claim 84, wherein the micro-fluidic device directly or indirectly measures potassium ions.

87. The method of claim 84, wherein the micro-fluidic device directly or indirectly measures concentration of potassium ions.

88. The method of claim 84, wherein the micro-fluidic device directly or indirectly measures one or more ions selected from potassium ions, sodium ions, chloride ions, nitride ions and calcium ions.

89. The method of claim 84, wherein the micro-fluidic device directly or indirectly measures the concentration(s) of one or more ions selected from the group consisting of potassium ions, sodium ions, chloride ions, nitride ions and calcium ions.

90. The method of any of claims 78-89, wherein the micro-fluidic device directly or indirectly measures ion permeability.

91. The method of any of claims 78-90, wherein the biophysical physical property is related

to and responsible for cell to cell interactions, cell signal, cell surface properties, cell electro-static force, cell repulsive force, DNA surface properties, DNA surface charge, DNA surrounding media electrical properties, quantum mechanical effects, gene mutation frequencies, or quantum mechanical effects.

92. The method of any of claims 78-91, wherein the biophysical property is a predictor of immunity, infection, disease, pre-cancer or cancer.

93. The method of any of claims 92, wherein the biophysical property is a predictor of disease progress from healthy state to disease state, from disease state to pre-cancer state, and from pre-cancer state to cancer state.

94. The method of any of claims 78, wherein the physical or bio-physical property is measured by using liquid sample.

95. The method of any of claims 78-94, wherein a further device is used for adjusting the bio-physical properties in the biological subject such as blood.

96. The method of claim 95, wherein the bio-physical property is first measured and then adjusted.

97. The method of claim 96, wherein the bio-physical property comprises a mechanical property, an acoustical property, an optical property, an electrical property, an electro-magnetic property, or an electro-mechanical property.

98. The method of claim 97, wherein the electrical property comprises electrical current, electrical conductance, capacitance, electrical resistance, or quantum mechanical effect.

99. The method of claim 96 or 97, wherein the further device adjusts the current to a higher value, adjusts the electrical conductance to a higher value, adjusts the electrical resistance to a lower value, or alters the quantum mechanical effect.

100. The method of claim 96, wherein a reagent is injected into blood to adjust bio-physical properties in the blood.

101. The method of claim 100, wherein the reagent contains ions, oxidizers, and components to impacting electrical properties of the blood.

102. The method of claim 101, wherein the electrical property comprises electrical current, electrical conductance, capacitance, electrical resistance, or quantum mechanical effect.

103. The method of claim 100, wherein the reagent is a drug capable of adjusting the biological properties in the blood.

104. The method of claim 103, wherein the drug is capable of releasing, upon intake, ions and charged components and capable of adjusting electrical properties of the blood.

105. The method of claim 104, wherein the electrical property comprises electrical current, electrical conductance, capacitance, electrical resistance, or quantum mechanical effect.

106. The method of any of claims 78-105, wherein at least one bio-marker is added to the liquid sample for physical or bio-physical property and related properties to be measured.

107. The method of claim 106, wherein the bio-marker provides at least some indicative information of risks of cancer occurrence at a given organ and location.

108. The method of claim 107, wherein the obtained information and data are analyzed in conjunction with information and data obtained from test(s) comprising of bio-marker tests, genomics tests, and circulating tumor cell tests, and overall cancer risks and location(s) of possible cancer occurrence are obtained.

109. The method of any of claims 78-108, wherein the method is able to detect the presence of at least two different diseases at the same time or to determine the status or progression of a disease.

110. The method of claim 109, wherein the method is capable of detecting at least two different types of cancer simultaneously.

111. The method of claim 109, wherein the disease comprises healthy stage, non-cancer disease stage, pre-cancer stage, early stage cancer stage, and mid to late stage cancer stage, with statistically significant detection or monitoring between any of the two stages.

112. A medical device for treating a biological subject, comprising a channel in which the biological subject passes through, and at least one transducer placed partially or completely in the channel; wherein the transducer is configured to transmit at least one bio-physical property, bio-physical energy, material or element onto the biological subject.

113. The medical device of claim 112, wherein the biological subject is a liquid of a mammal.

114. The medical device of claim 112 or 113, wherein the biological subject is a blood sample, a urine sample, or a sweat sample of the mammal.

115. The medical device of any of claims 112-114, wherein the biological subject comprises blood, proteins, red blood cells, while blood cells, T cells, other cells, gene mutations, quantum mechanical effects, DNA, RNA, or other biological entities.

116. The medical device of any of claims 112-115, wherein the at least one bio-physical

property, bio-physical energy, material or element comprises a mechanical property or energy, an acoustical property or energy, an optical property or energy, an electrical property or energy, an electro-magnetic property or energy, or an electro-mechanical property or energy.

117. The medical device of claim116, wherein the at least one electrical property or energy comprises electrical current, electrical conductance, capacitance, electrical resistance, net electrical charge in extracellular region, membrane potential, membrane polarization, ion concentrations, electro-static force and charge on DNA double helix and RNA double helix, or quantum mechanical effect.

118. The medical device of claim 116, wherein the at least one bio-physical property, bio-physical energy, material or element comprises a trans-membrane potential, a membrane voltage, a membrane potential, a zeta potential, an impedance, an optical reflective index, an optical refractive index, potassium ions, sodium ions, chloride ions, nitride ions, calcium ions, an electro-static force, an electro-static force acting on cells, an electro-static force acting on DNA double helix, an electro-static force acting on RNA, an electrical charge on cell membrane, an electrical charge on DNA double helix, an electrical charge on RNA, quantum effects, near-field electrical properties, near-field electro-magnetic properties, membrane bilayer properties, ion permeability, electrical current, electrical conductance, capacitance, or electrical resistance.

119. The medical device of claim 118, wherein the transmitted bio-physical property or energy adjusts the current of the biological subject to a higher value, adjusts the electrical conductance of the biological subject to a higher value, adjusts the electrical resistance of the biological subject to a lower value, or alters the quantum mechanical effect of the biological subject.

120. The medical device of claim 116, wherein the at least one transducer is placed alongside a side wall of the channel, and is configured to apply a pulsed electrical voltage to the biological subject passing through the channel.

121. The medical device of claim 120, wherein the biological subject is the blood sample, and the applied voltage is configured to impact an electrical field, charge distribution, or membrane potential of the blood sample.

122. The medical device of any of claims 112-122, wherein the medical device comprises one or more channels, and the one or more channels comprise one or more transducers on sidewalls, and one or more small opening connecting to the one or more channels; wherein at least one transducer is configured to transmit a bio-physical energy to the biological subject, and the at

least one small opening is configured to add a desired amount of ions to the biological subject.

123. The medical device of claim 122, wherein the biological subject is the blood sample.

124. The medical device of claim 122 or 123, wherein the bio-physical energy is an electrical pulse.

125. The medical device of any of claims 122-124, wherein the added ions comprise potassium ions.

126. The medical device of any of claims 123-125, wherein the medical device enhances an electrical conductivity of the blood sample, a net electrical charge in the blood sample, or a polarization of membrane potential.

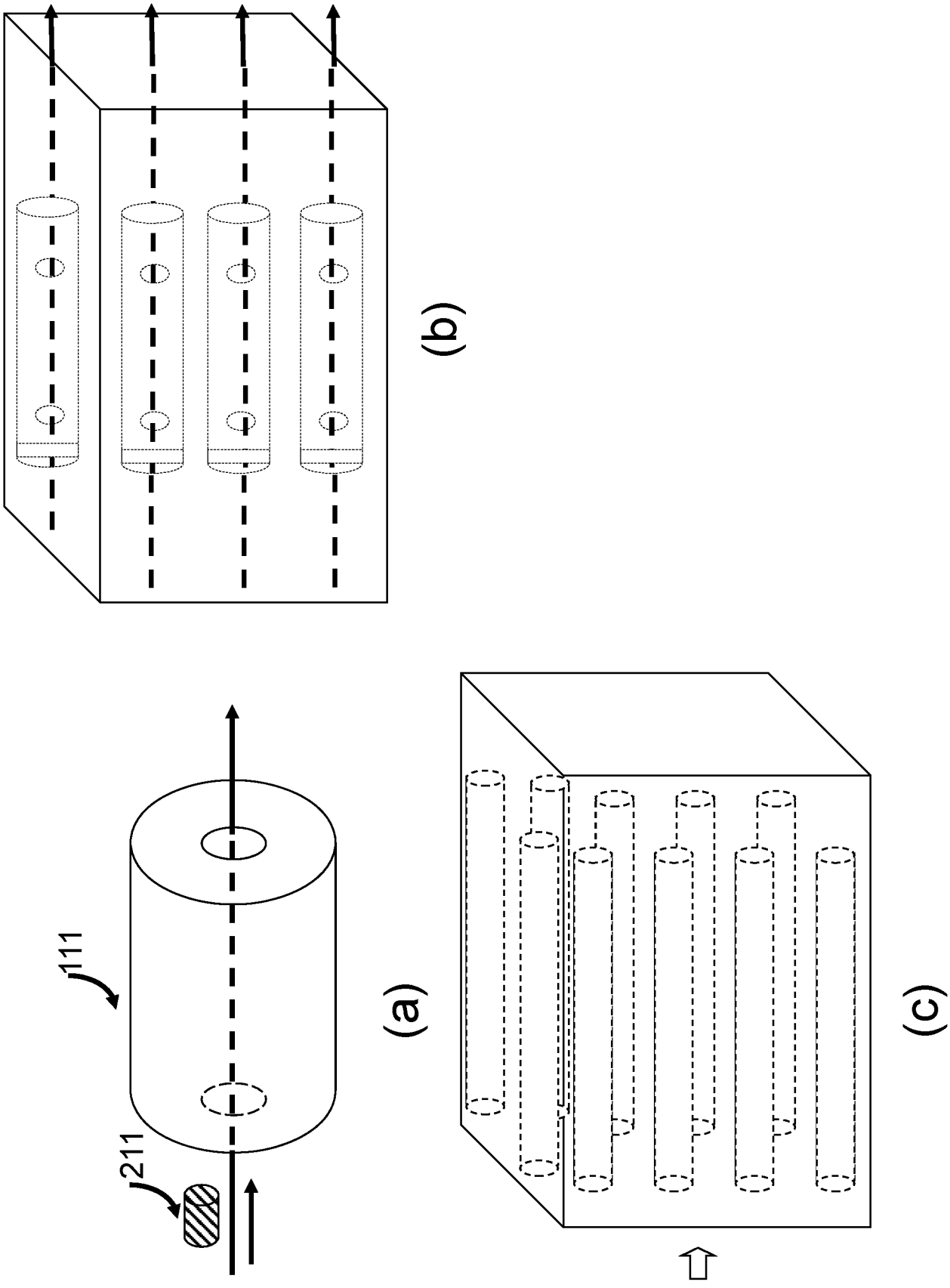


Fig. 1

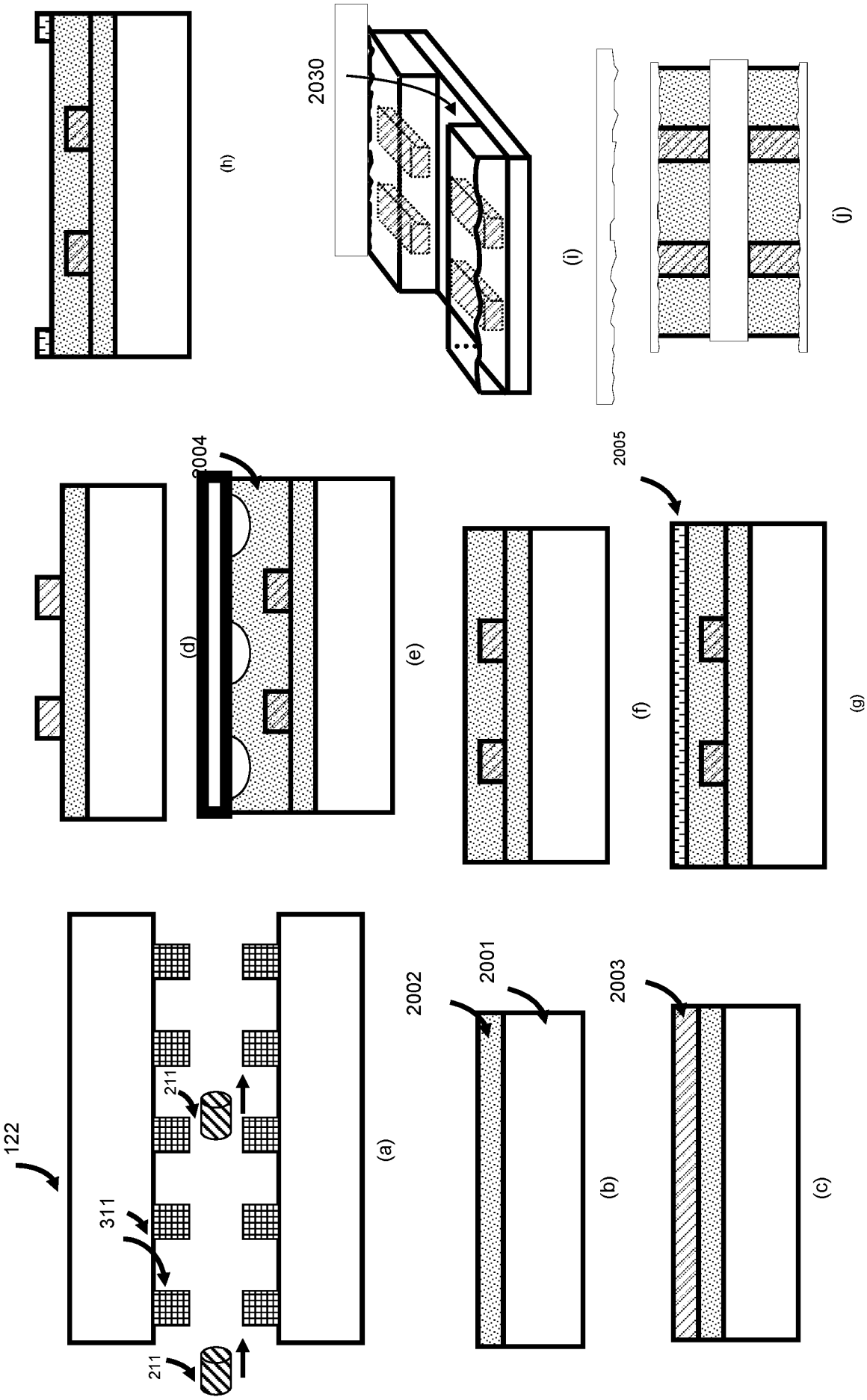


Fig. 2

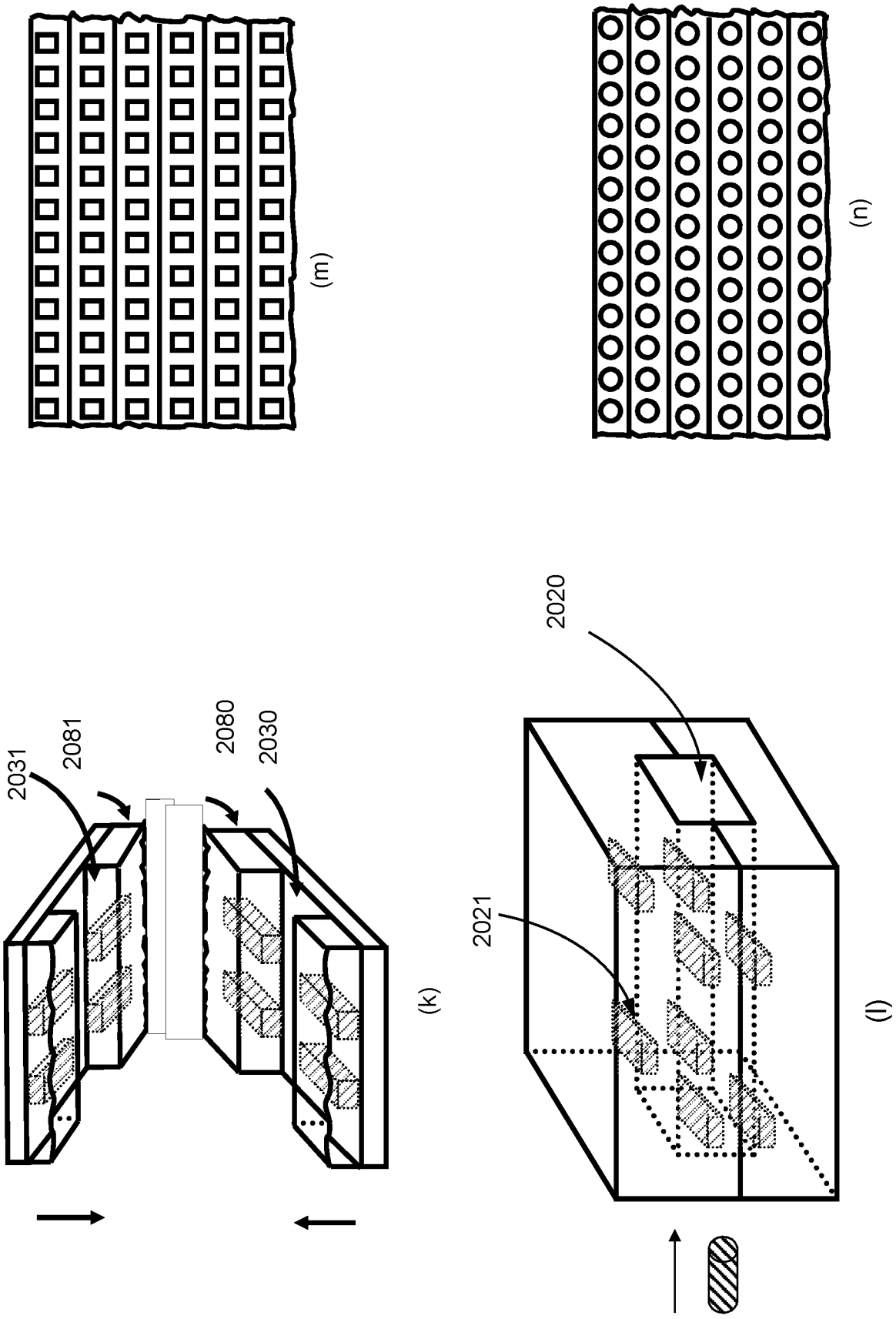


Fig. 2 (Cont.)

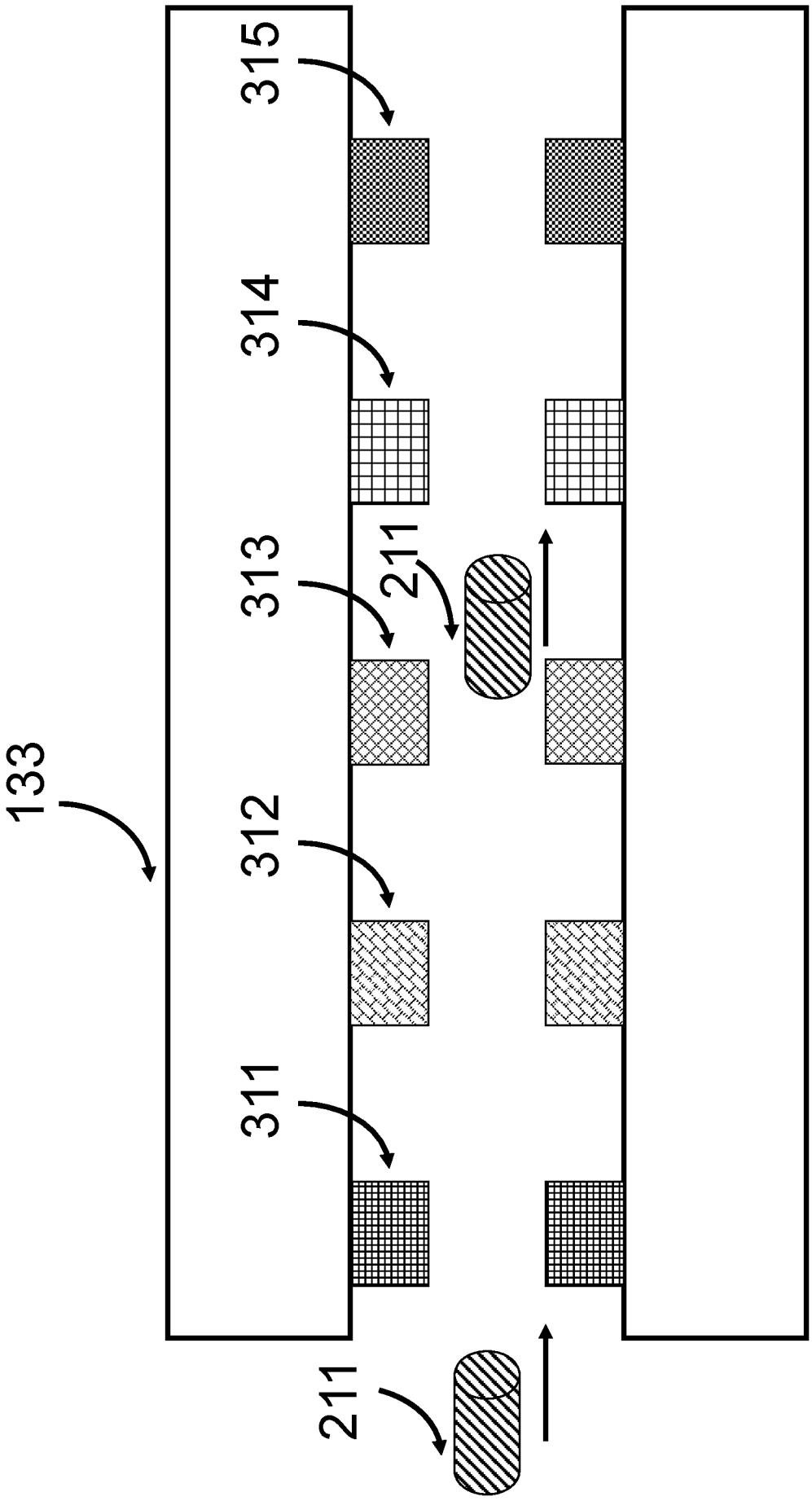


Fig. 3

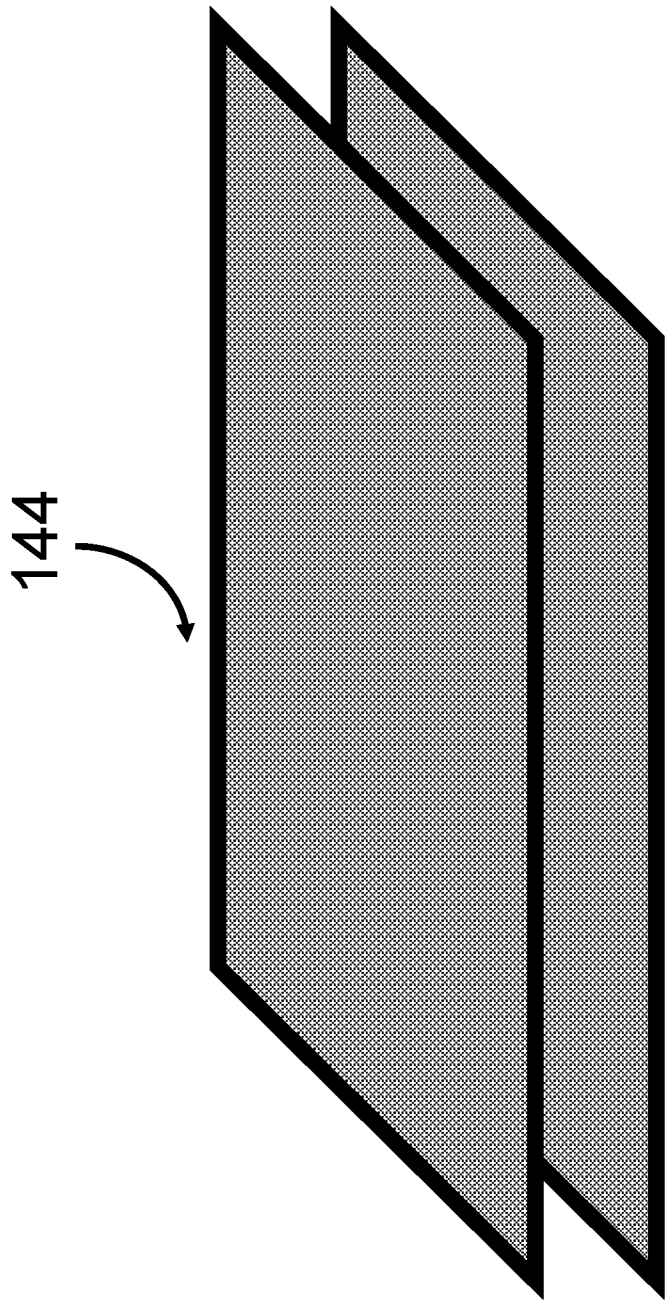


Fig. 4

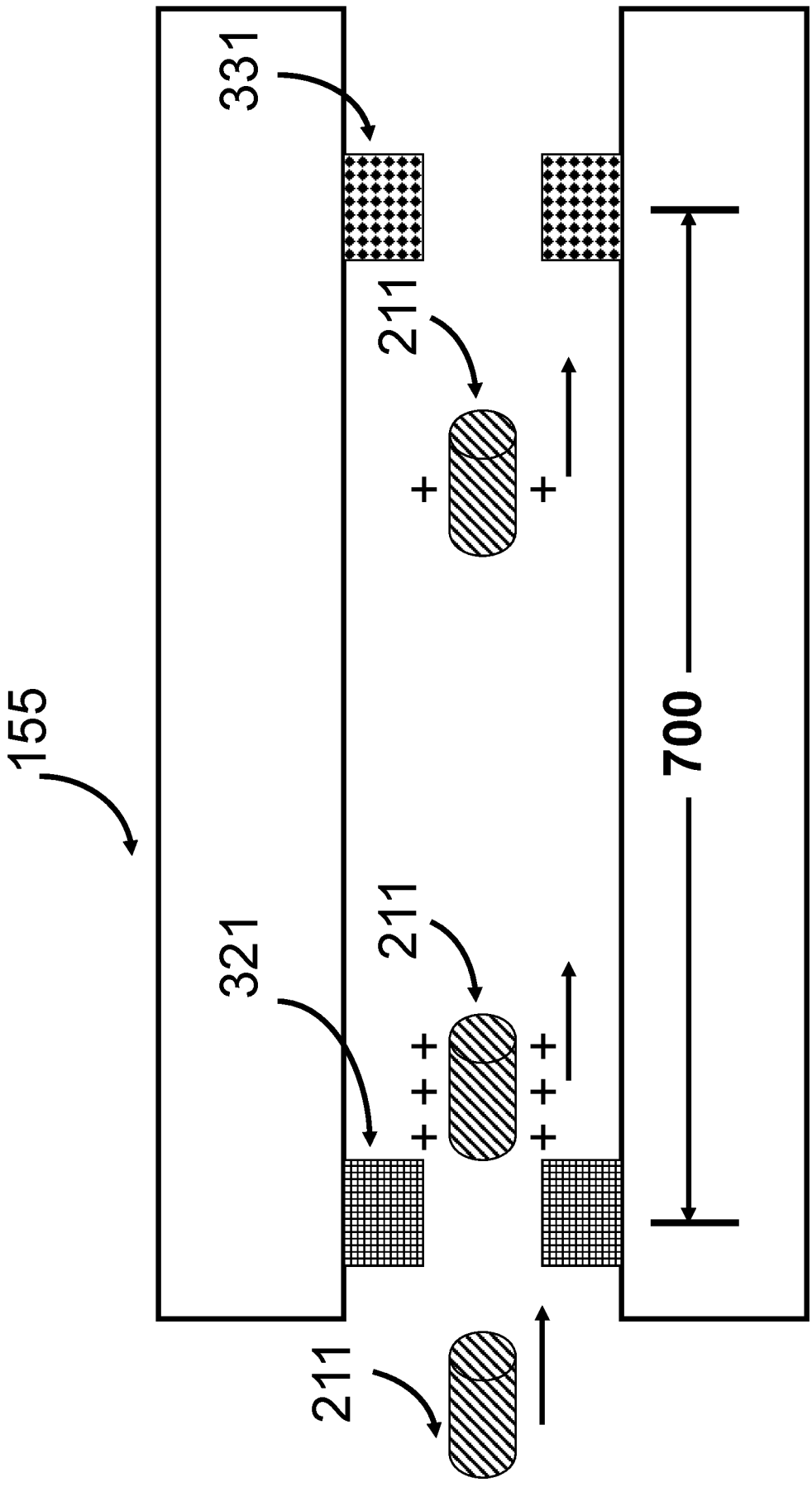


Fig. 5

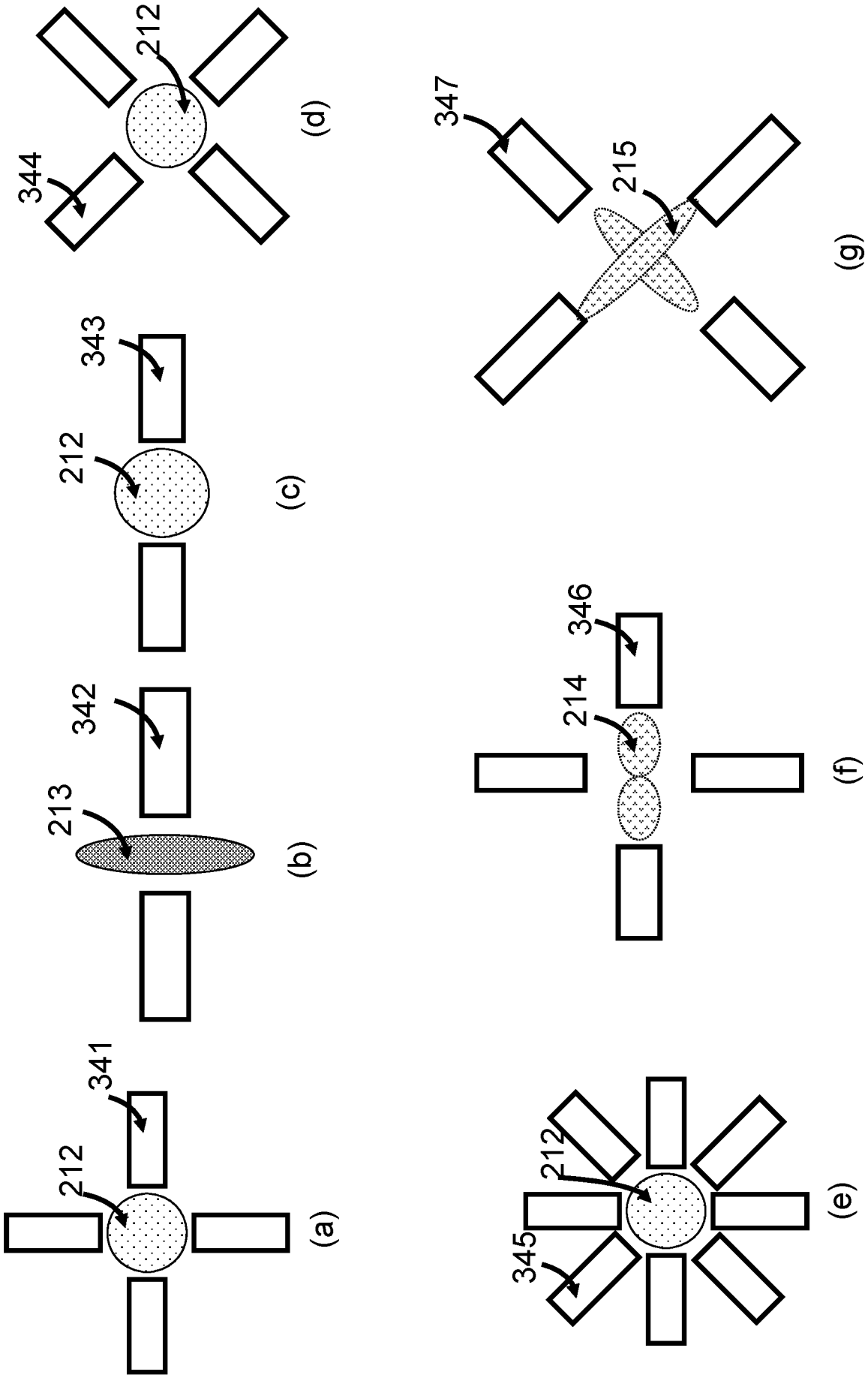


Fig. 6

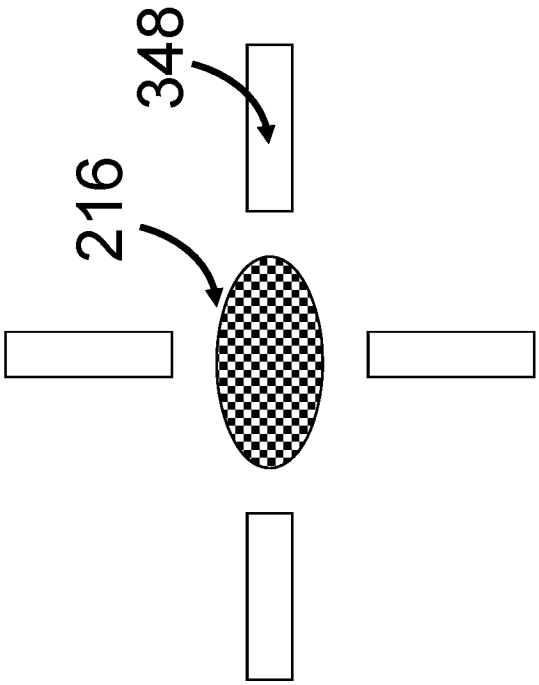


Fig. 7

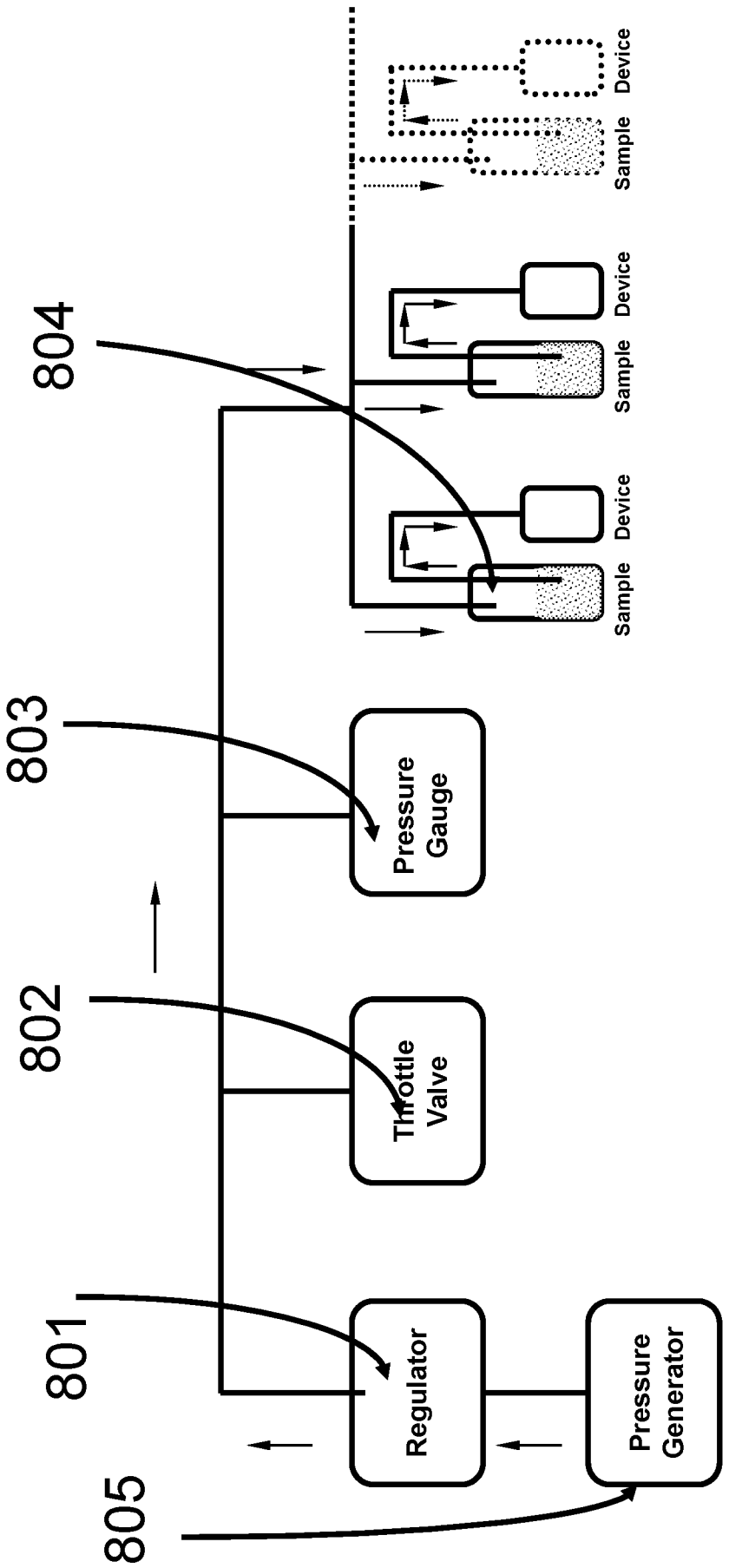

804
803
802
801
805
Regulator
Throttle Valve
Pressure Gauge
Pressure Generator
Sample
Device
Sample
Device
Sample
Device
Sample
Device

Fig. 8

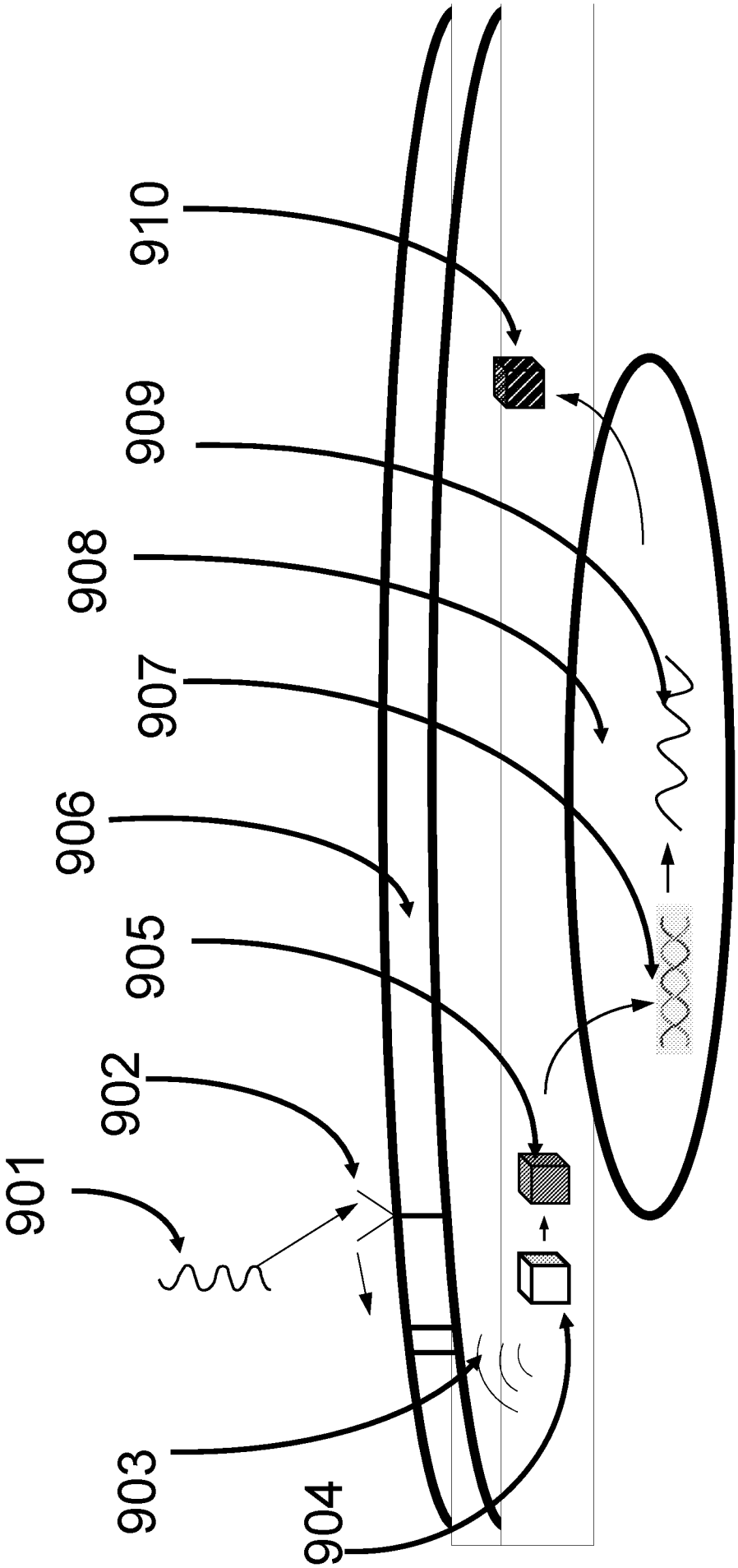


Fig. 9-a

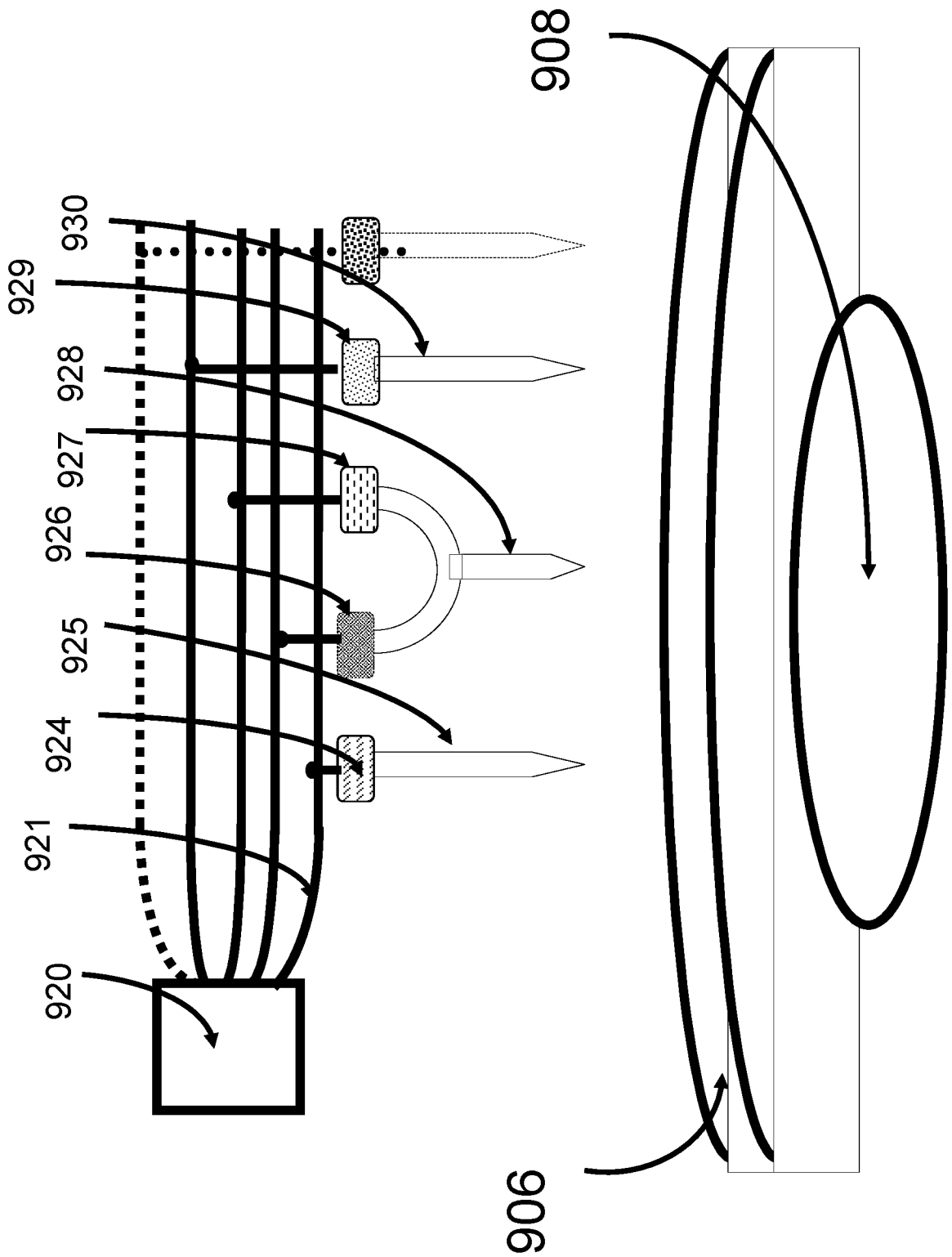


Fig. 9-b

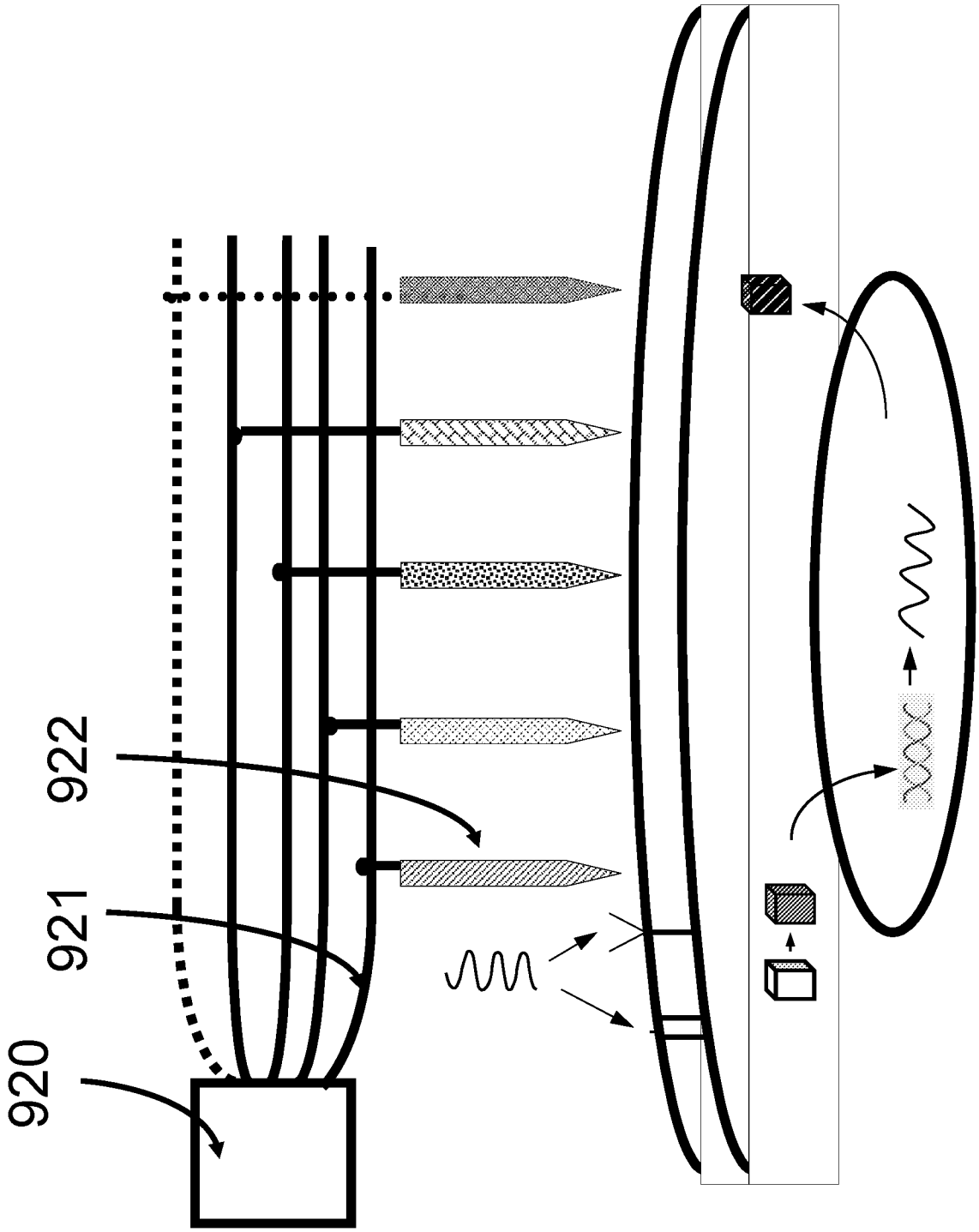


Fig. 9-c

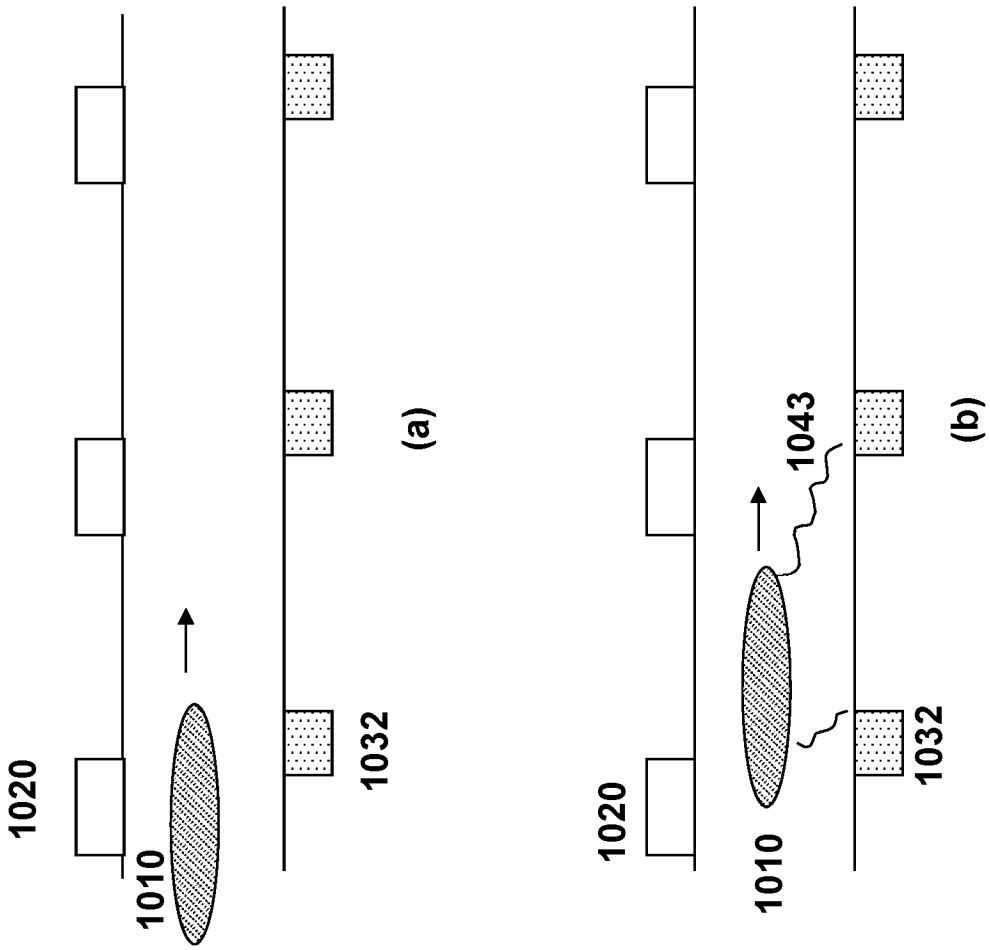


Fig. 10

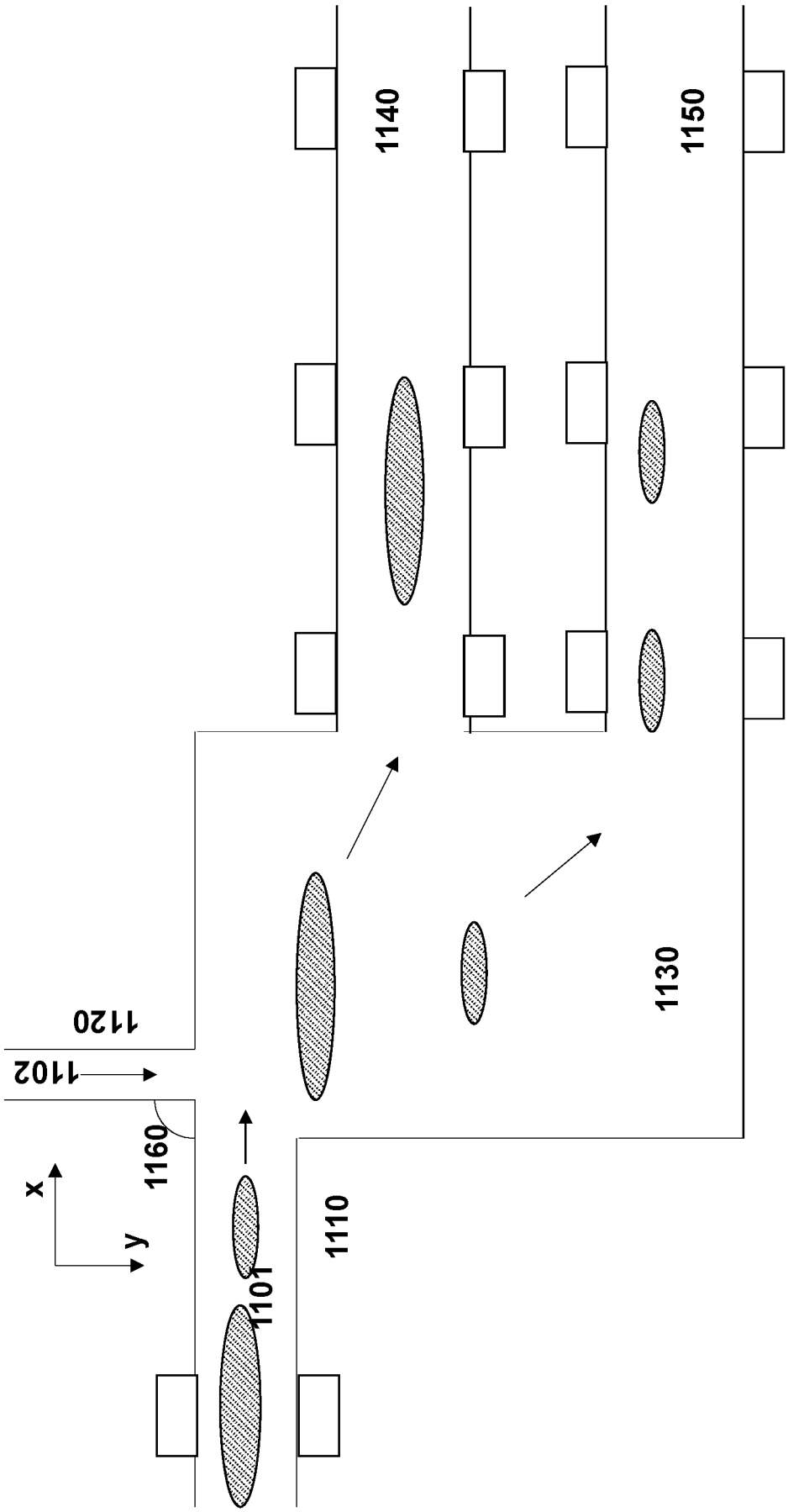


Fig. 11

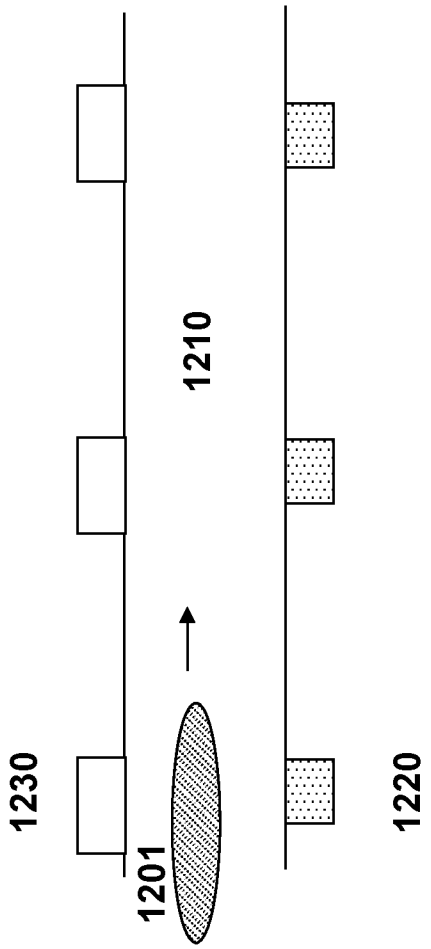


Fig. 12

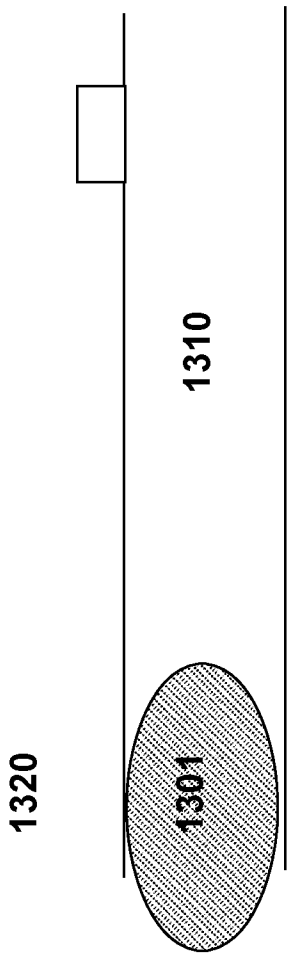


Fig. 13

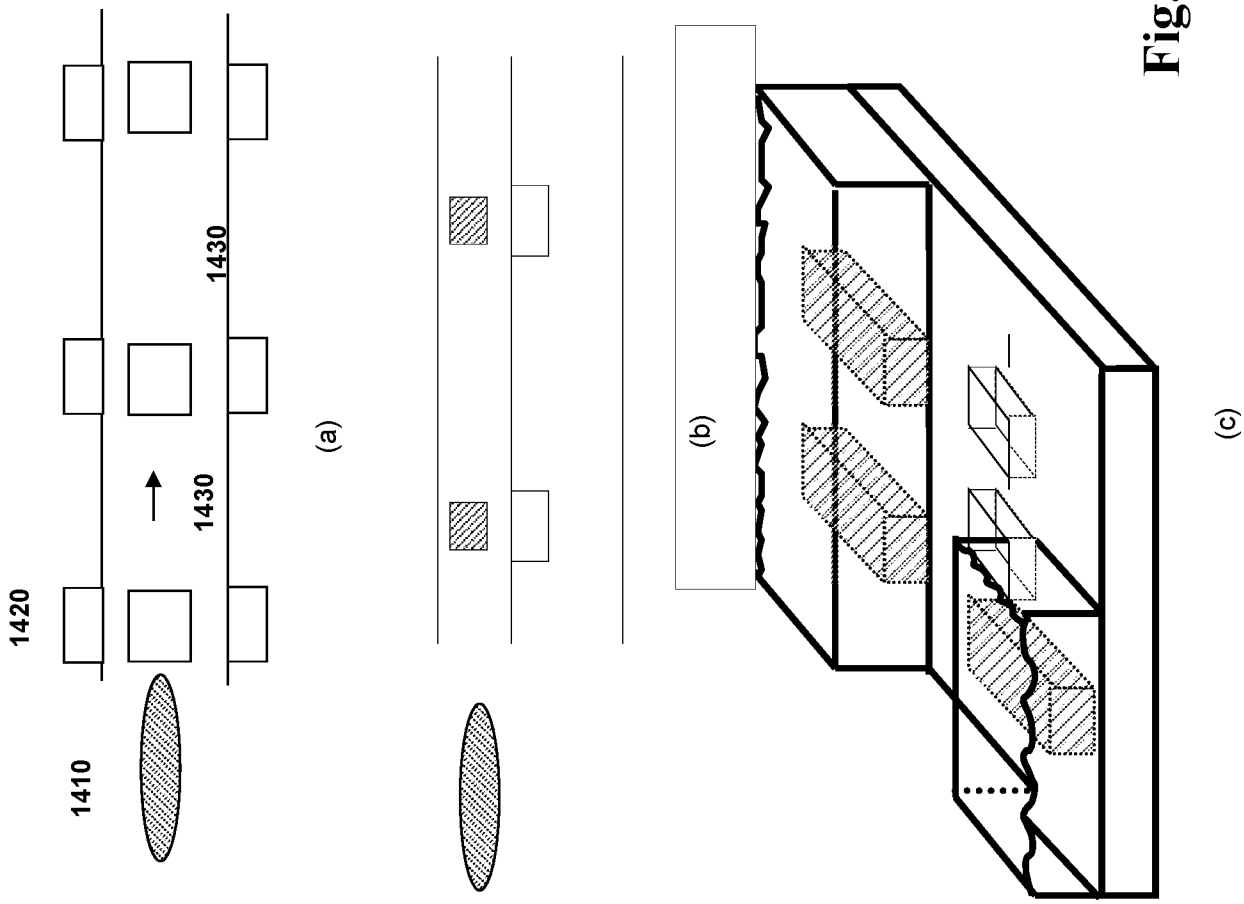


Fig. 14

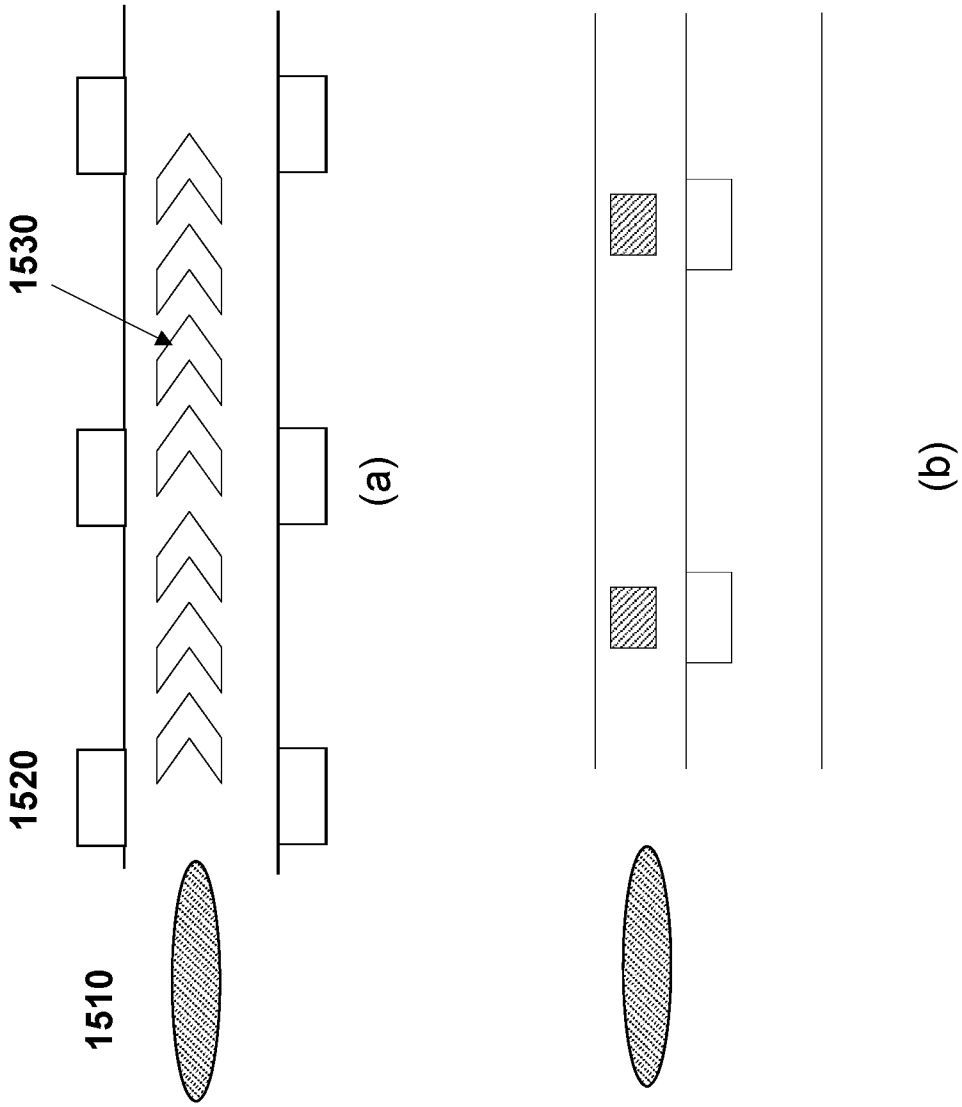


Fig. 15

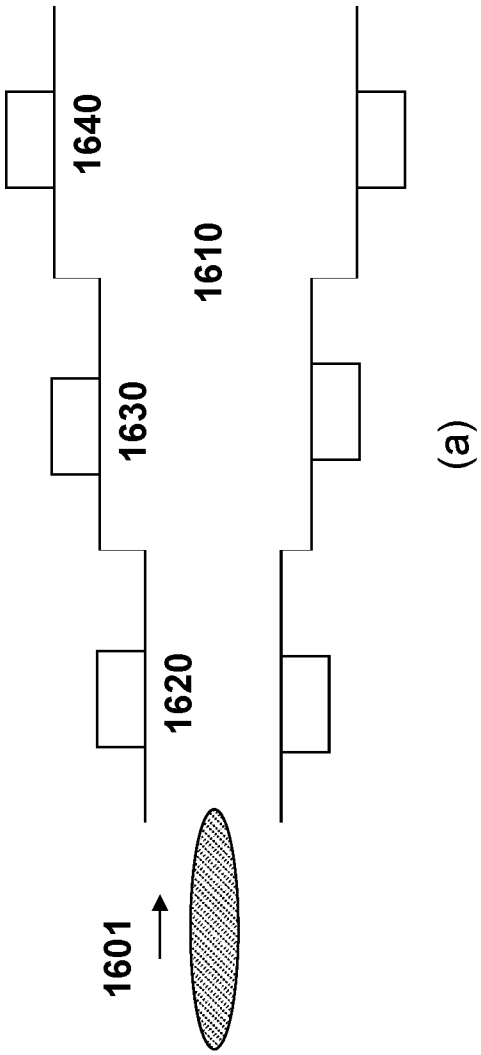


Fig. 16

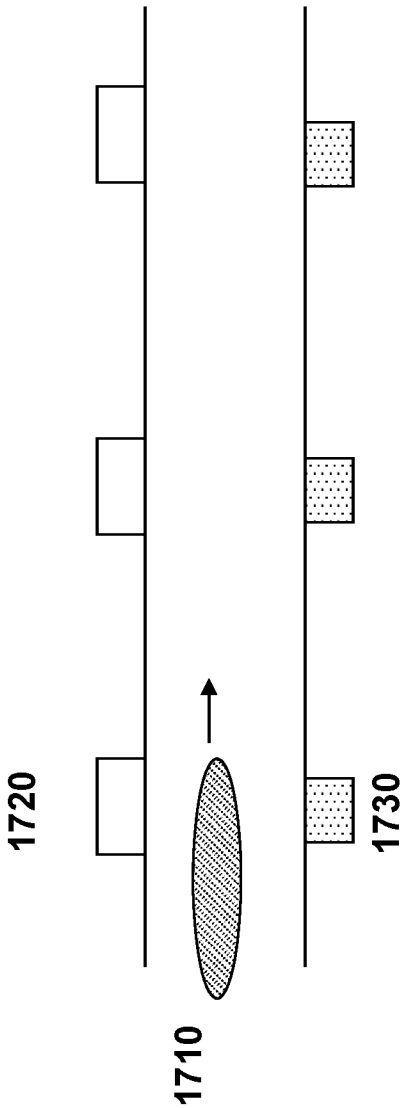


Fig. 17

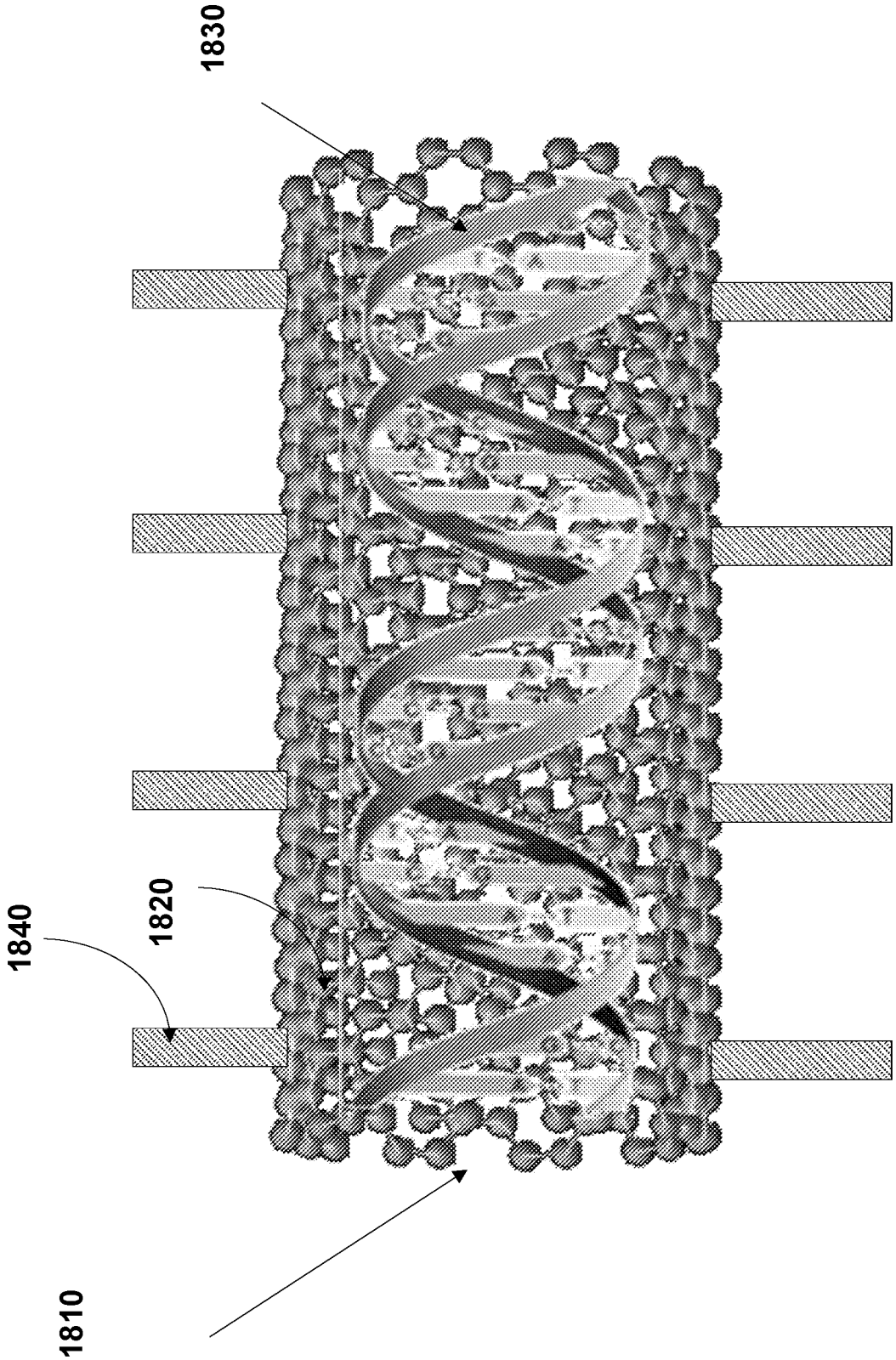


Fig. 18

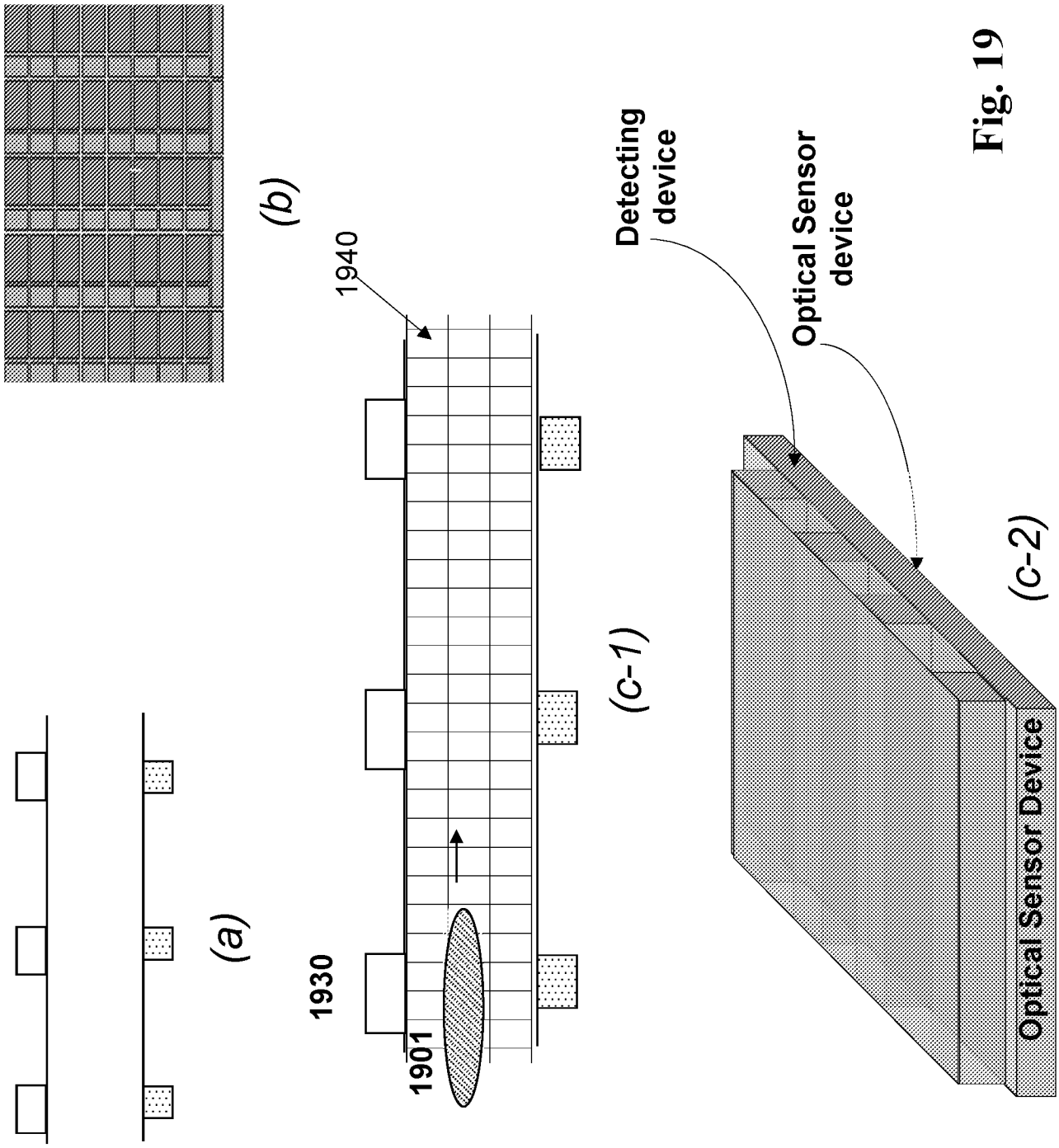

(a)
(b)
1940
1930
1901
(c-1)
Detecting device
Optical Sensor device
Optical Sensor Device
(c-2)

Fig. 19

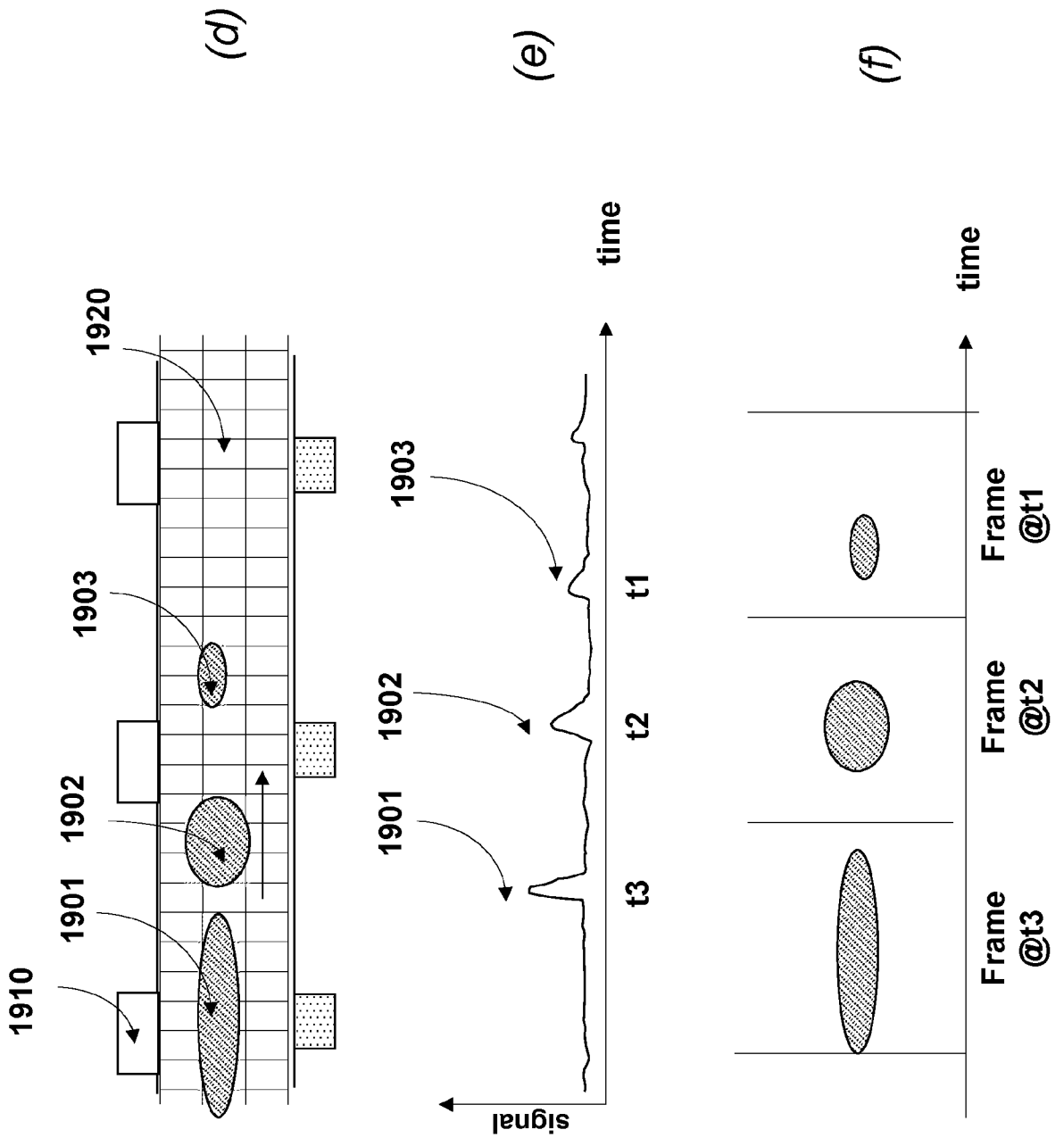

(d)
1920
1903
1902
1901
1910
(e)
time
1903
1902
1901
t1
t2
t3
signal
(f)
time
Frame @t1
Frame @t2
Frame @t3

Fig. 19 (Cont.)

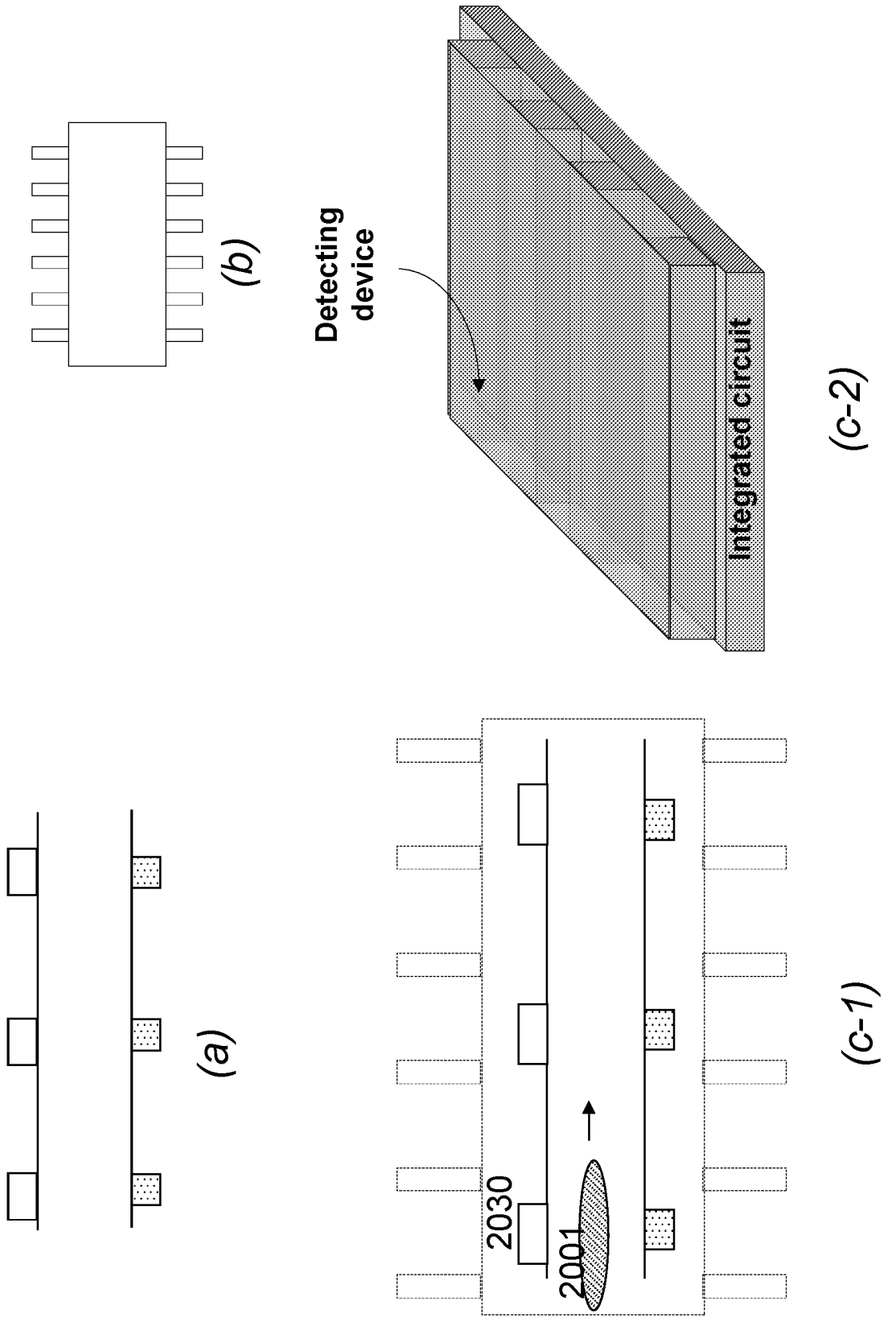


Fig. 20

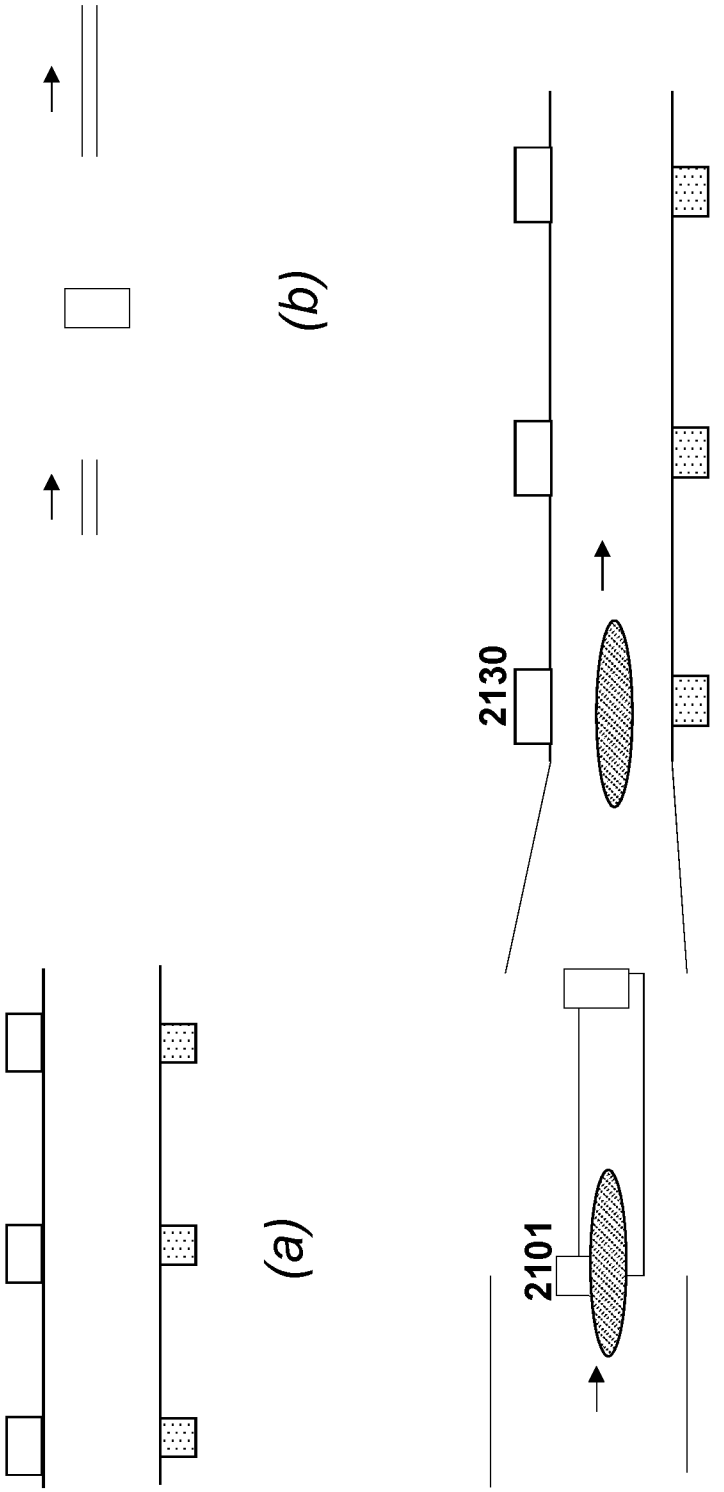


Fig. 21

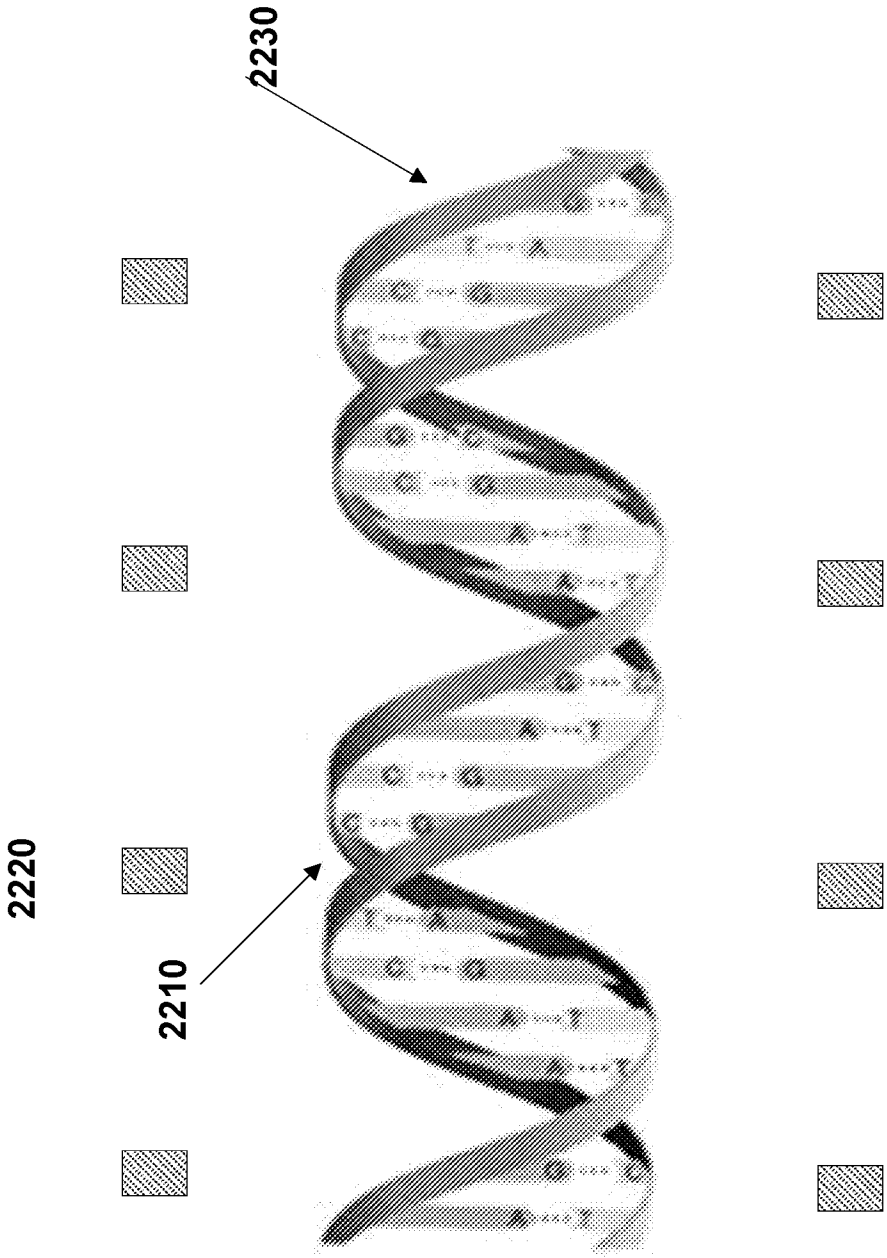
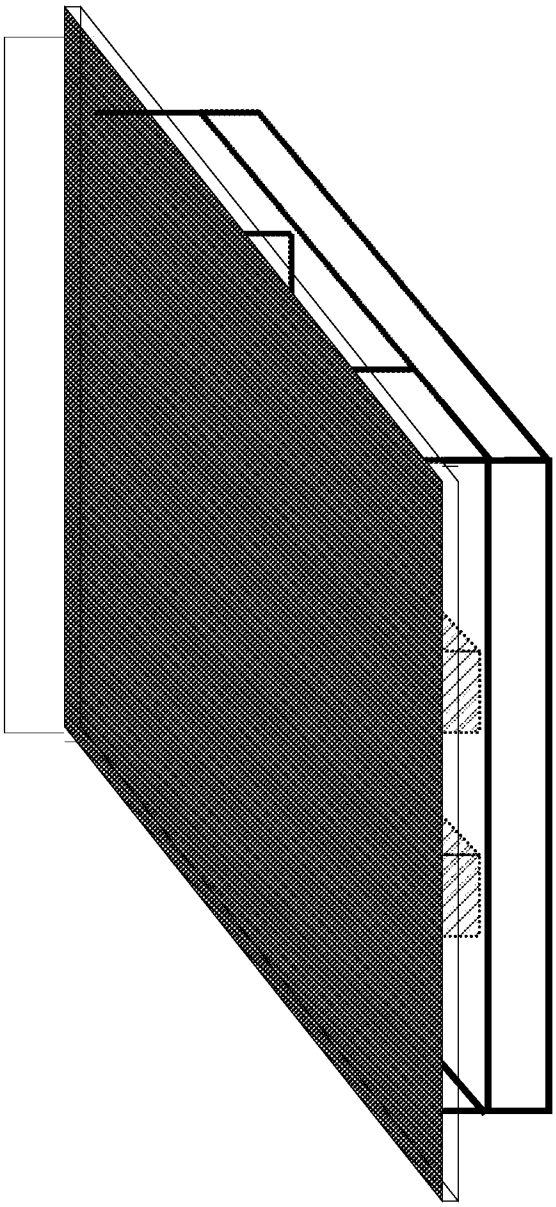


Fig. 22



Detecting device covered with transparent panel

Fig. 23

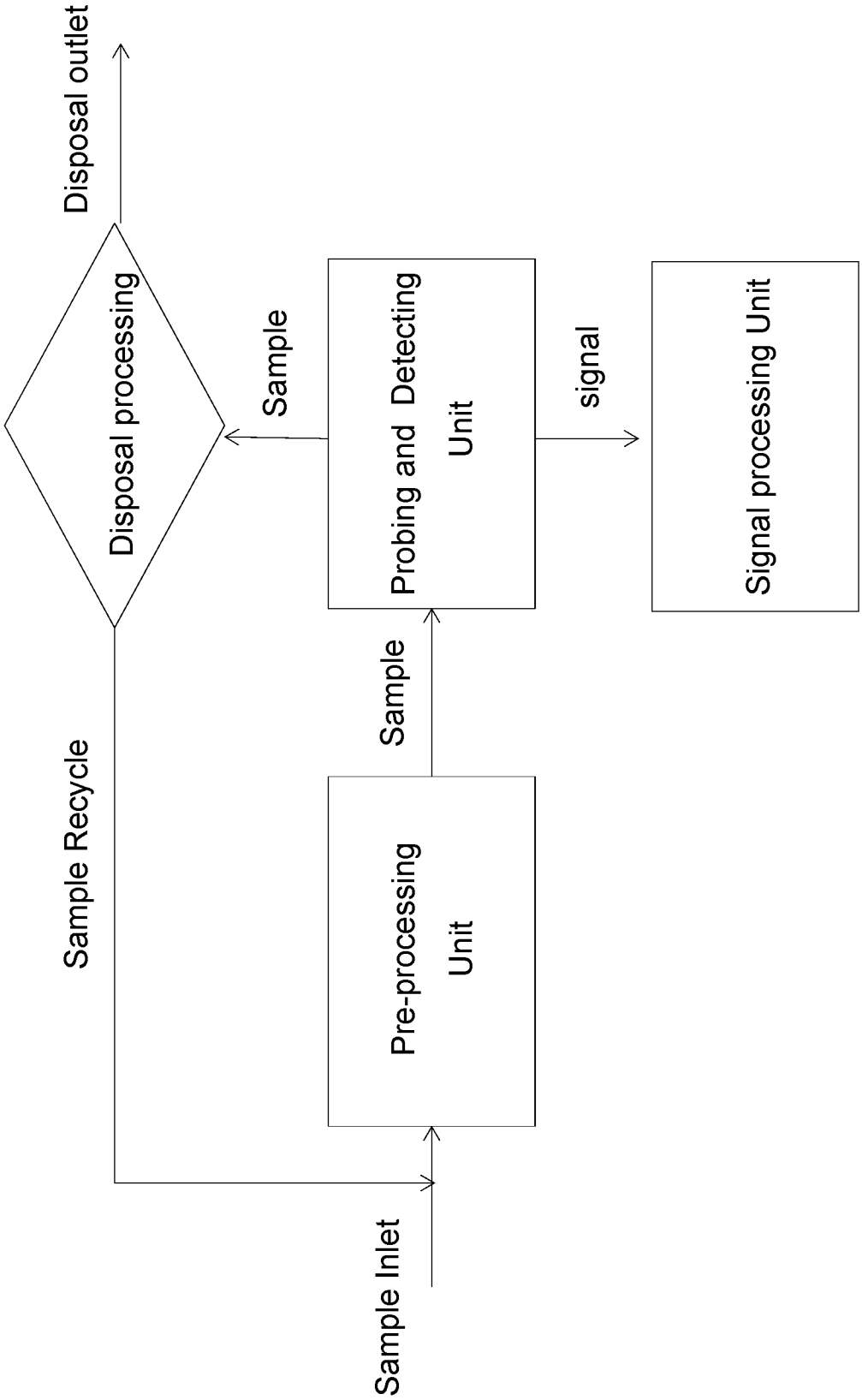

Disposal outlet
Disposal processing
Sample
Probing and Detecting Unit
signal
Signal processing Unit
Sample Recycle
Sample
Pre-processing Unit
Sample Inlet

Fig. 24

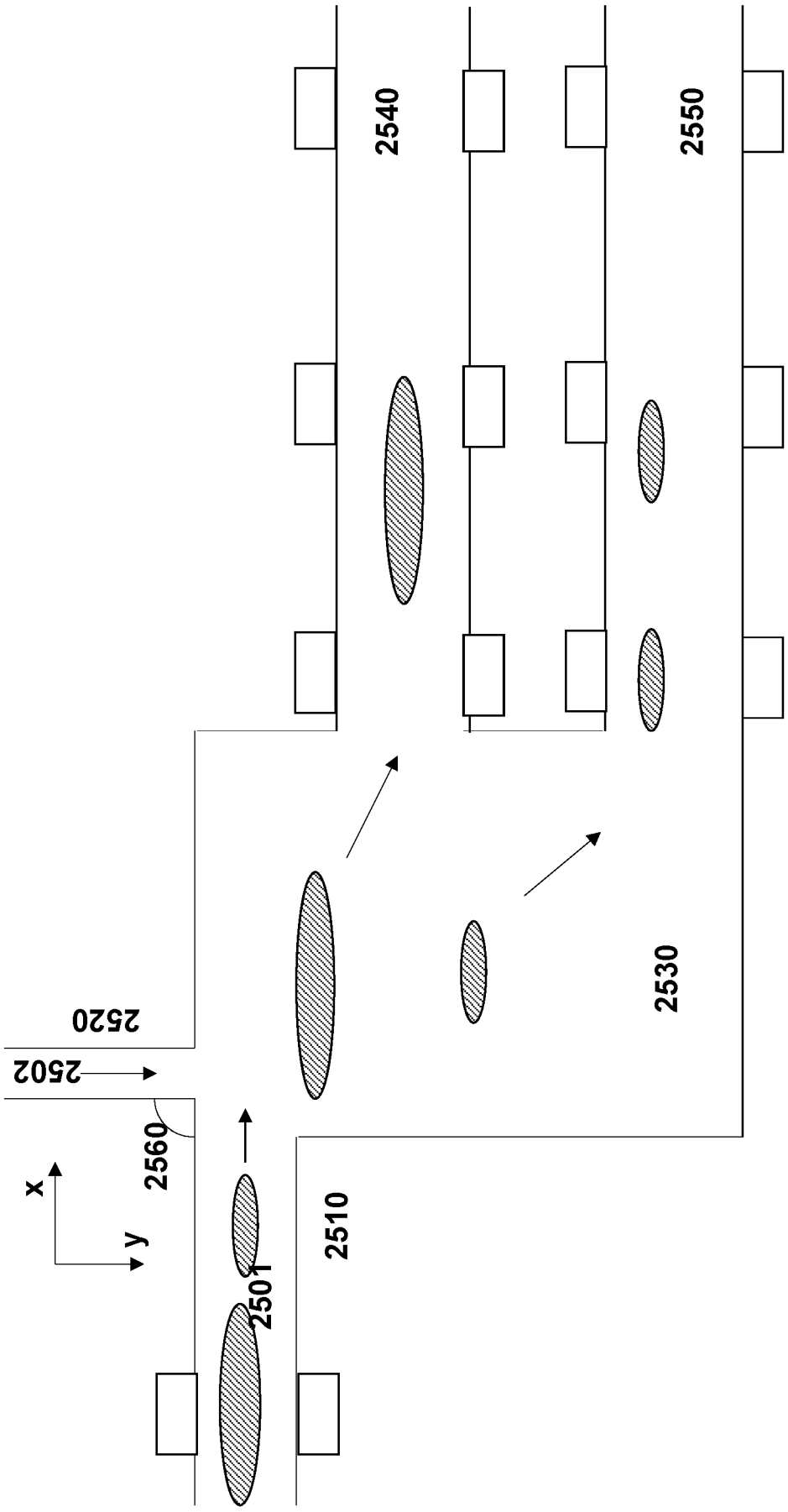


Fig. 25

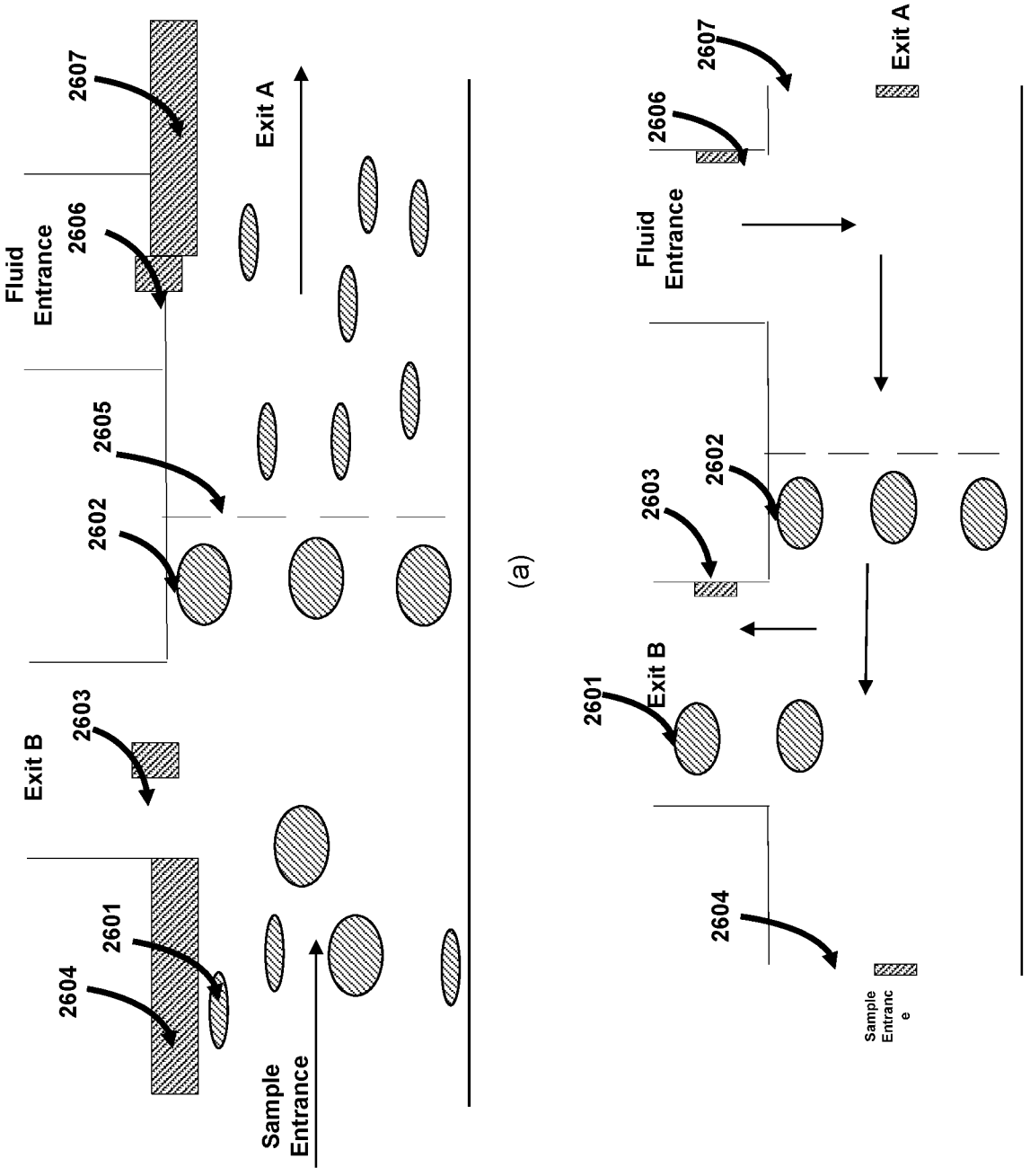

2607
Exit A
Fluid Entrance
2606
2605
2602
2603
Exit B
2601
2604
Sample Entrance
(a)
2607
2606
Exit A
Fluid Entrance
2603
2602
2601
Exit B
2604
Sample Entrance
(b)

Fig. 26

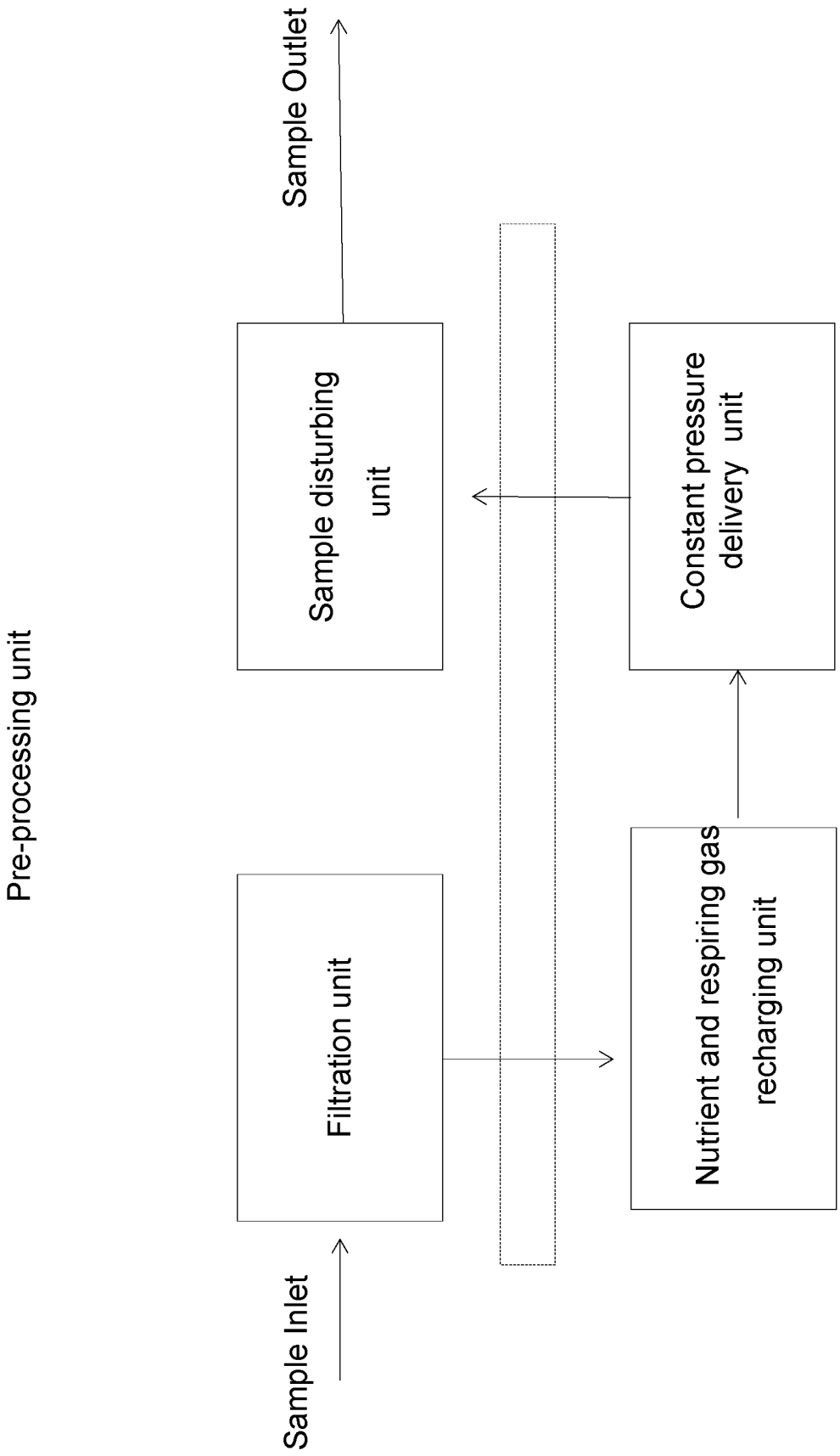

Pre-processing unit
Sample Inlet
Filtration unit
Nutrient and respiring gas recharging unit
Constant pressure delivery unit
Sample disturbing unit
Sample Outlet

Fig. 27

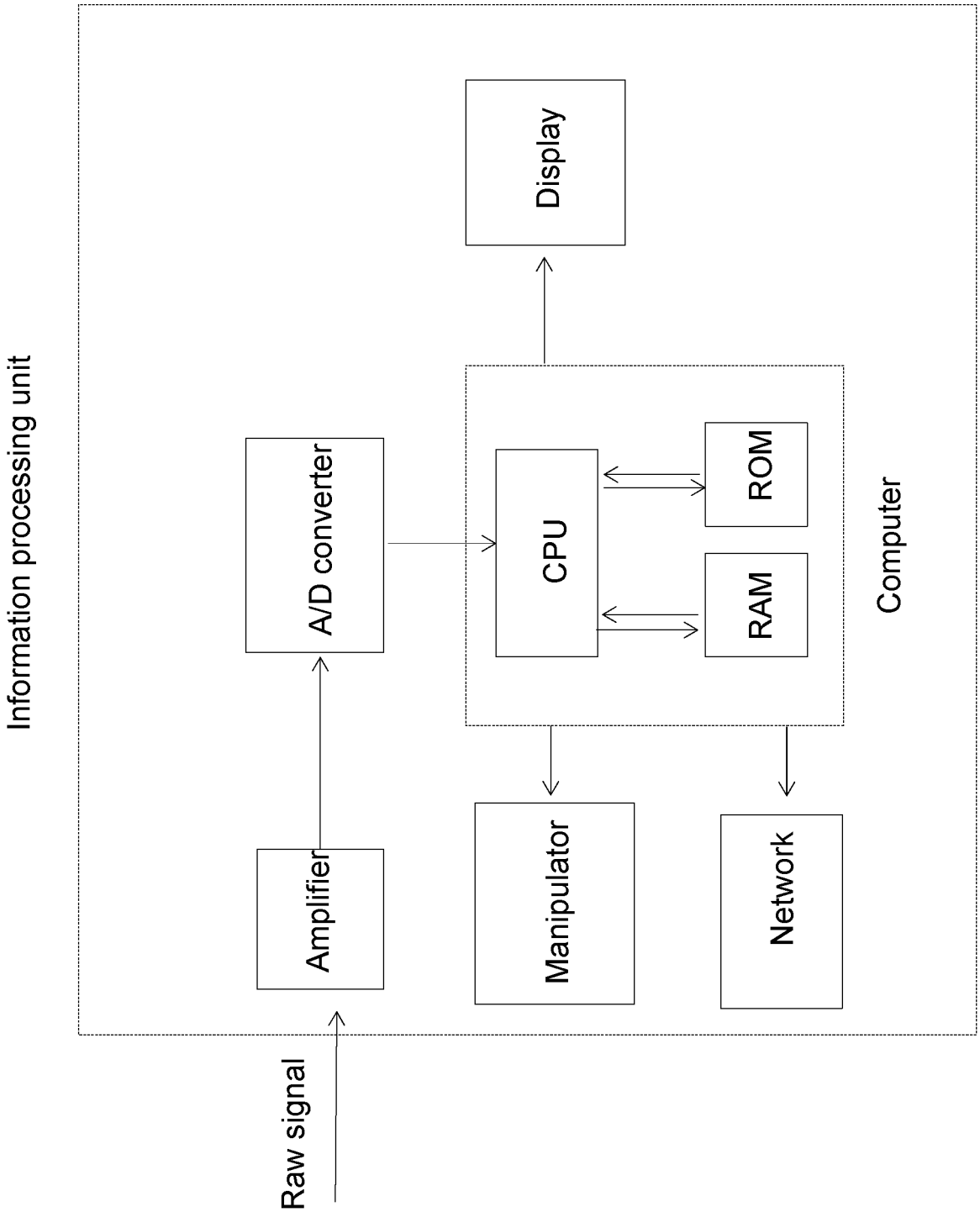

Information processing unit
Raw signal
Amplifier
A/D converter
Display
CPU
ROM
RAM
Computer
Manipulator
Network

Fig. 28

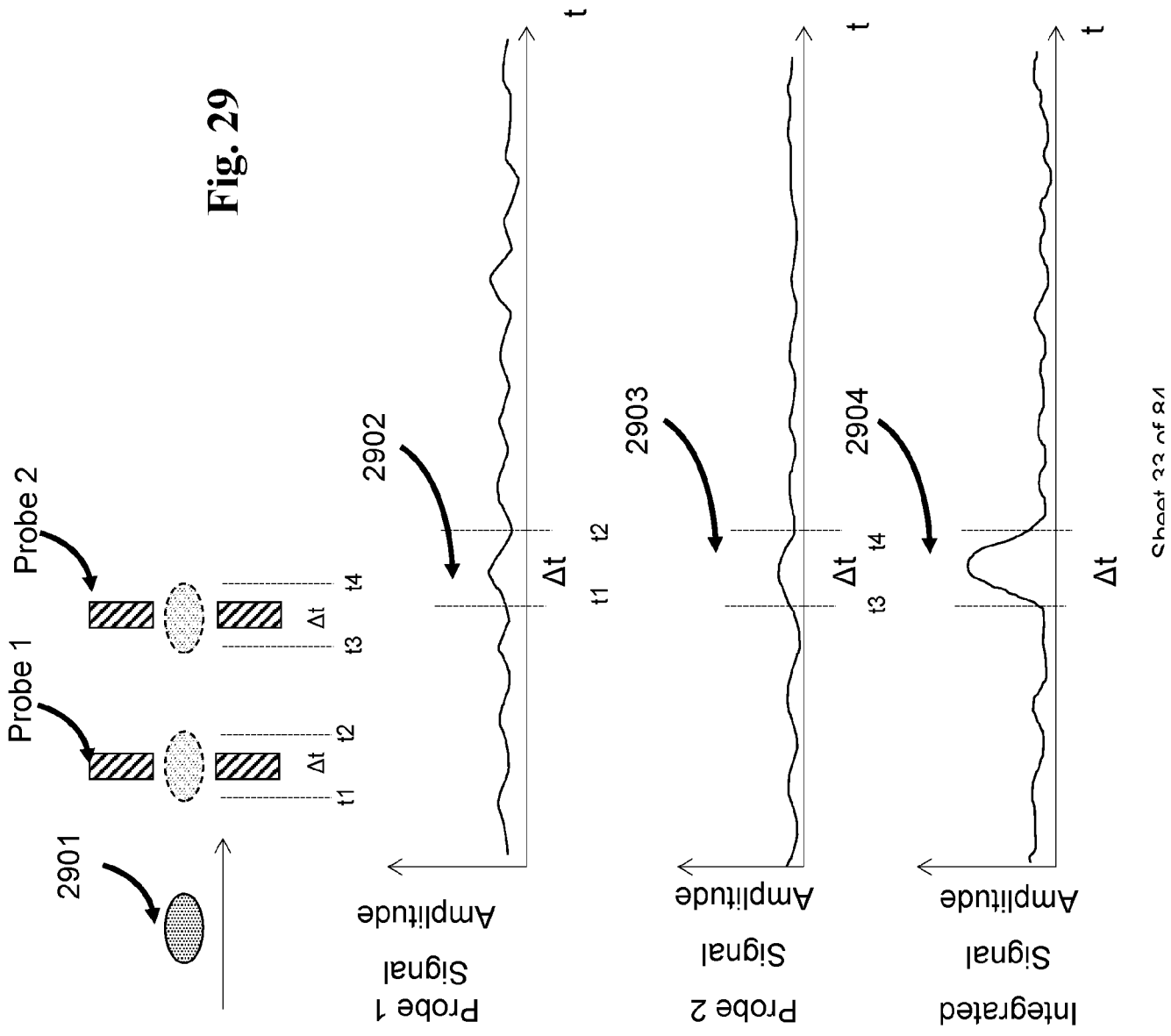

Probe 2
Probe 1
2901
Δt
t1
t2
t3
t4
2902
2903
2904
Probe 1 Signal
Probe 2 Signal
Integrated Signal
Amplitude
t

Fig. 29

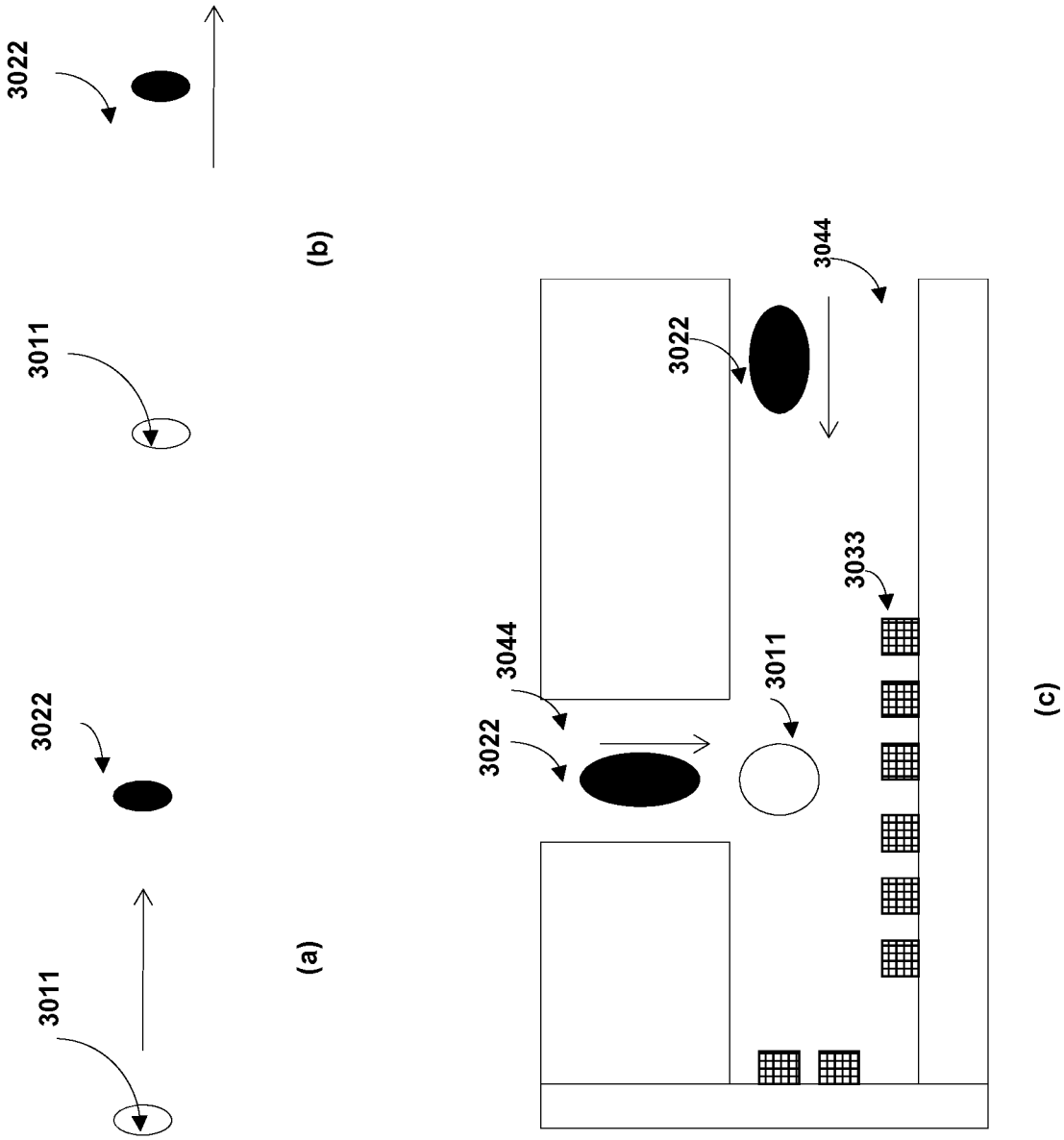


Fig. 30

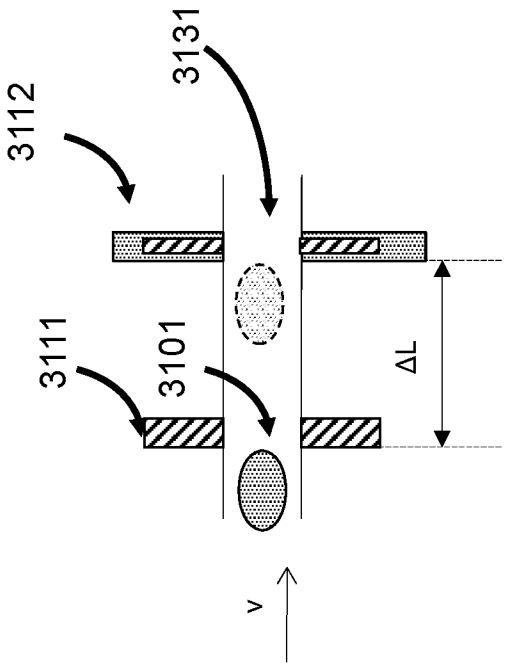


Fig. 31

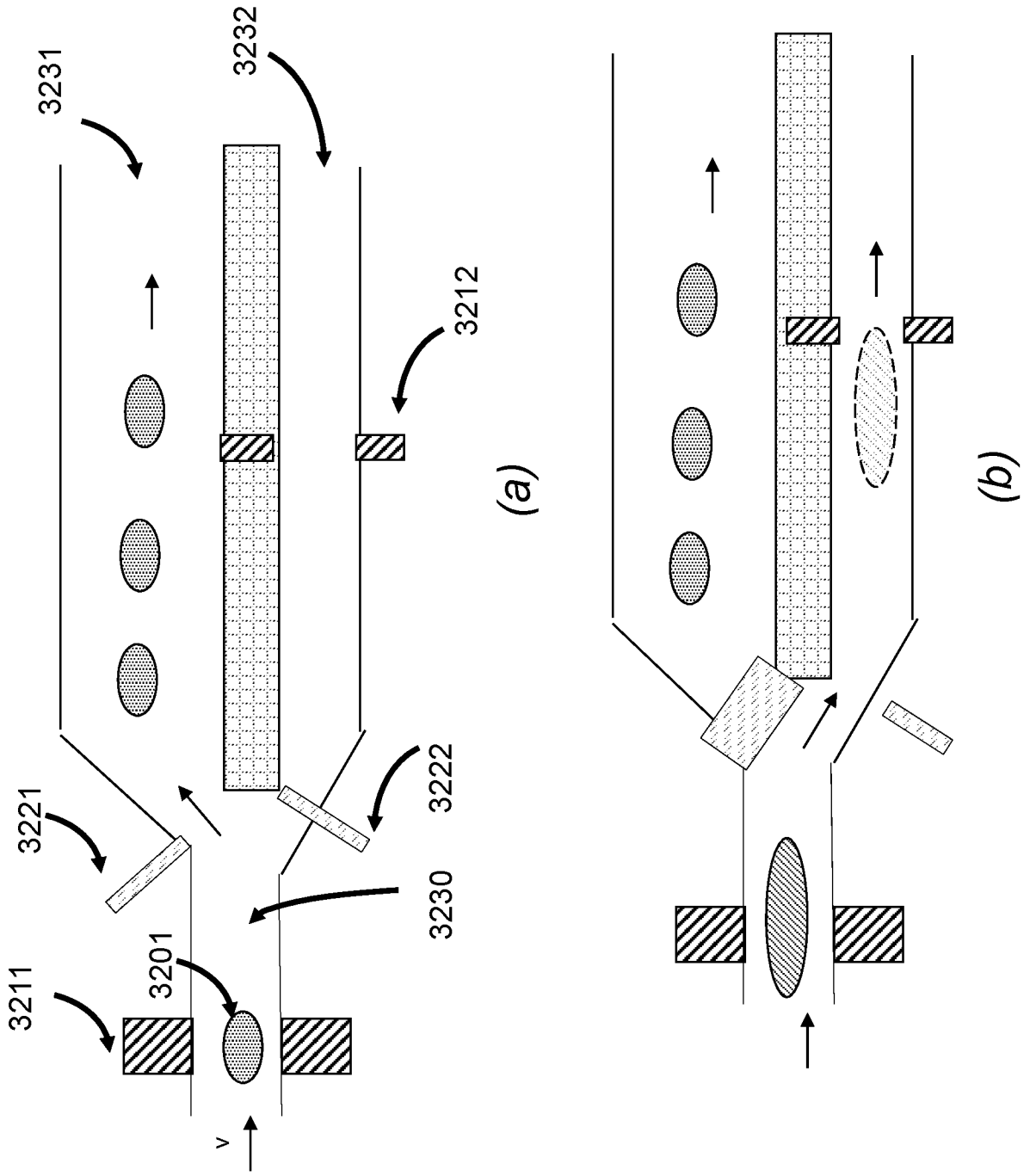


Fig. 32

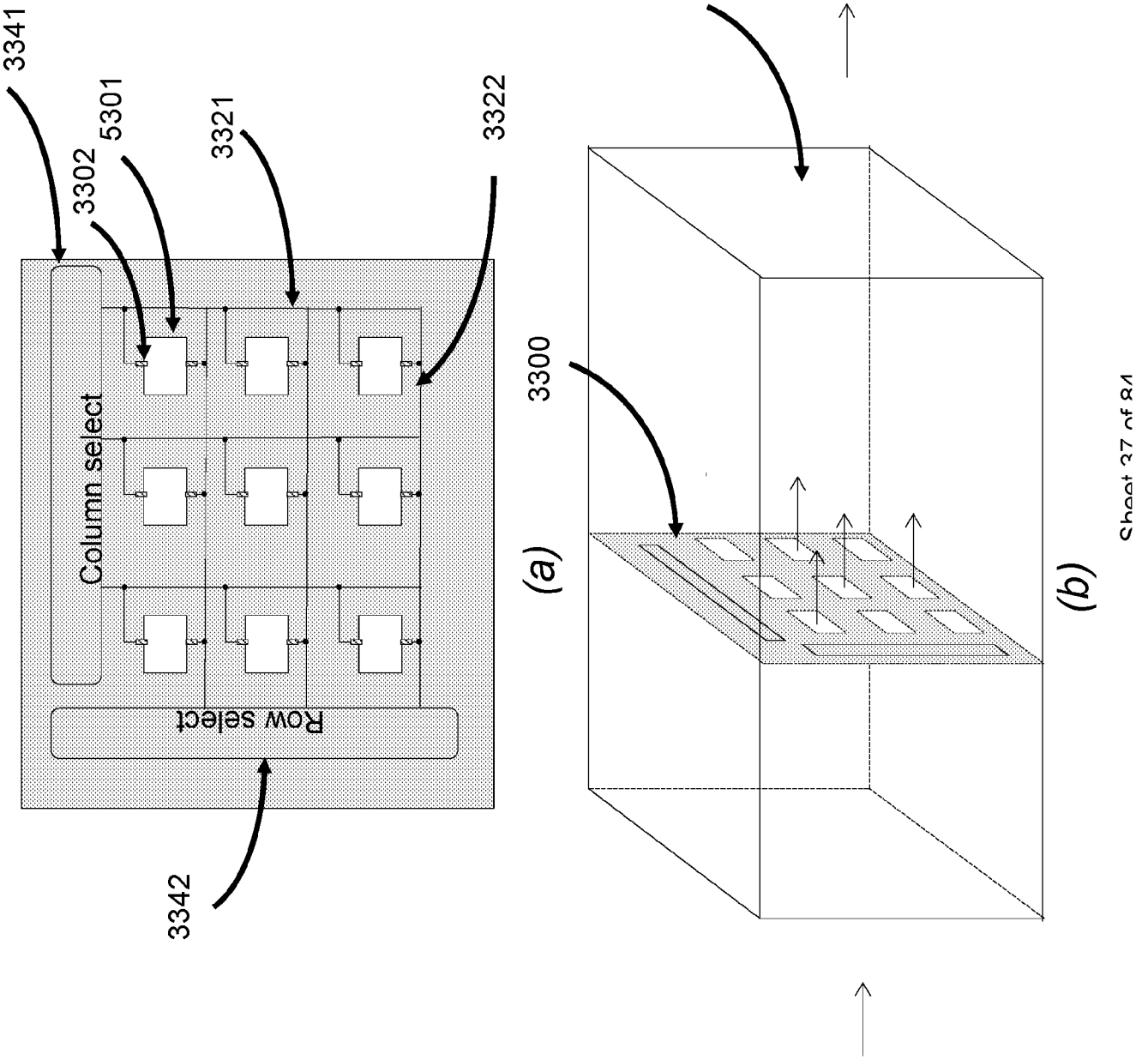

3341
5301
3302
3321
3322
Column select
Row select
3342
3301
3300
(a)
(b)

Fig. 33

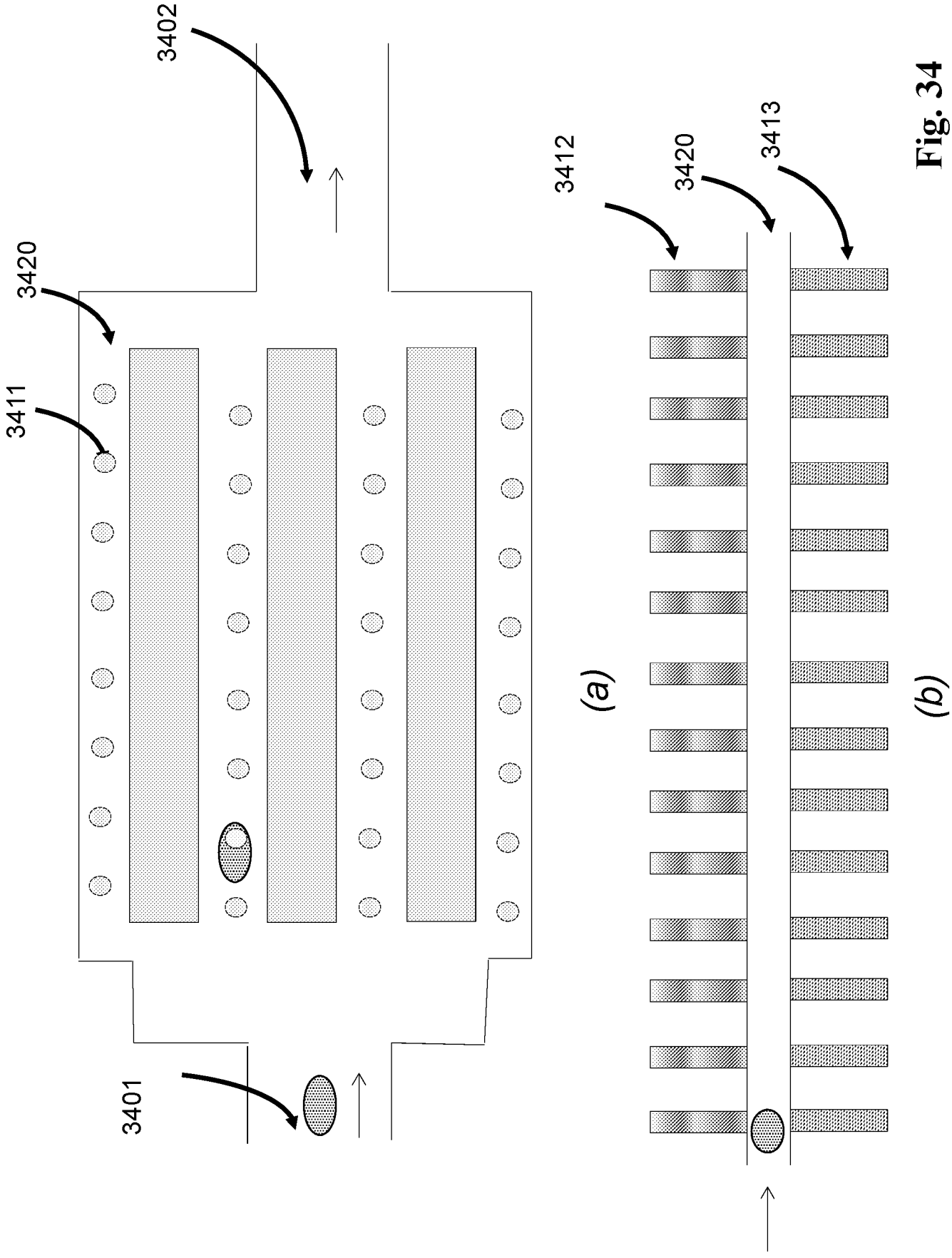


Fig. 34

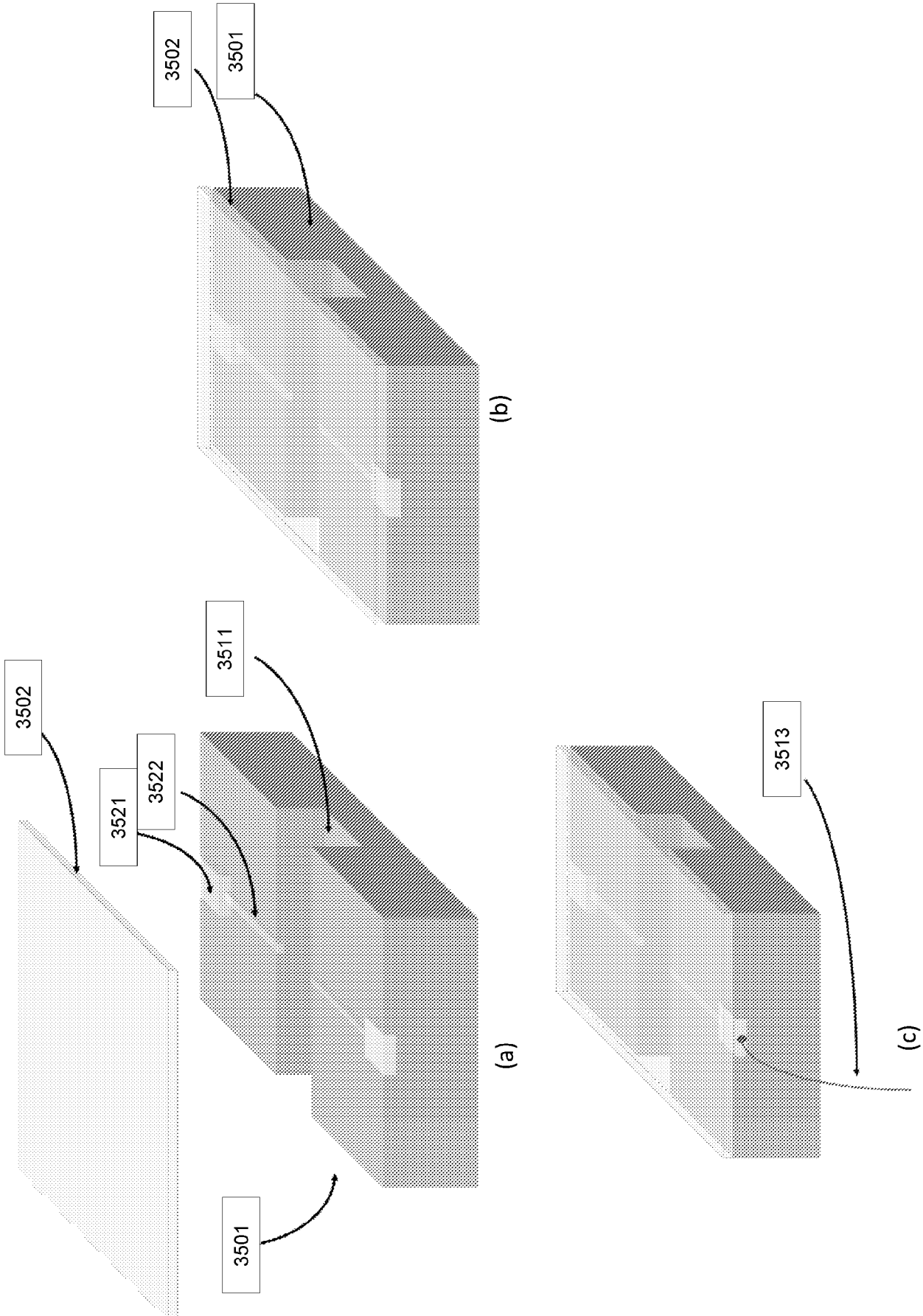


Fig. 35

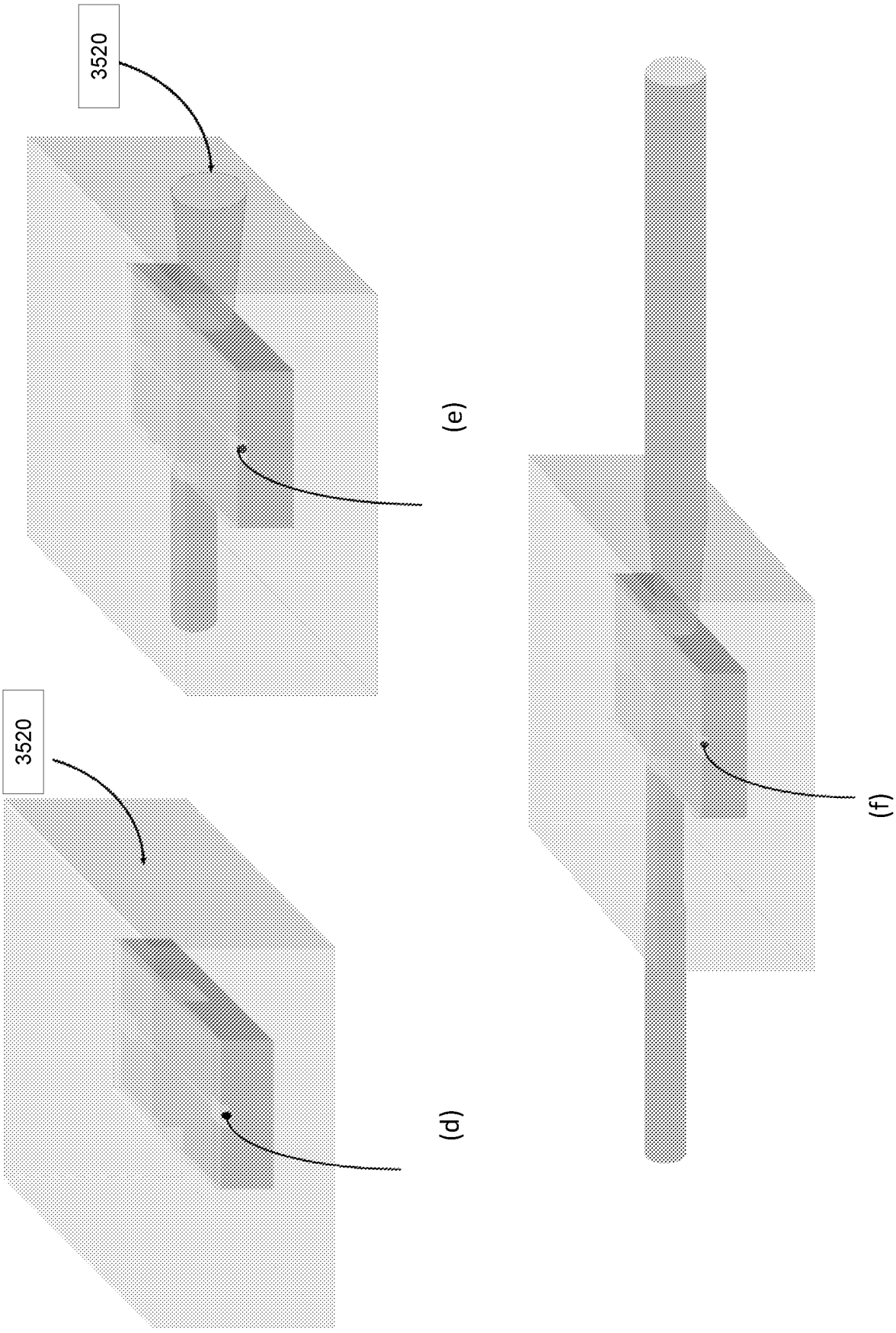


Fig. 35

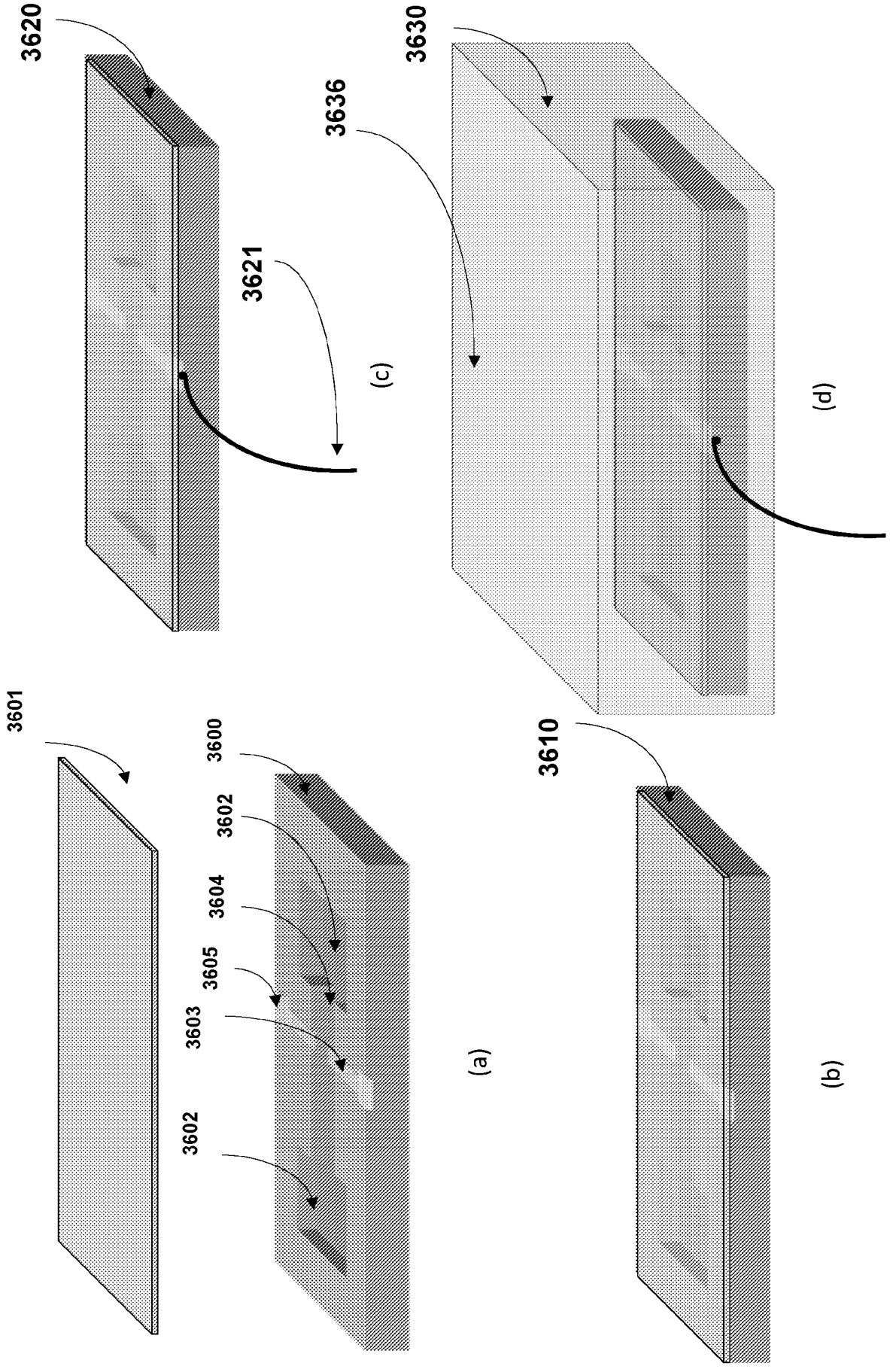

3620
3621
3636
3630
3601
3600
3602
3604
3605
3603
3602
3610
(a)
(b)
(c)
(d)

Fig. 36

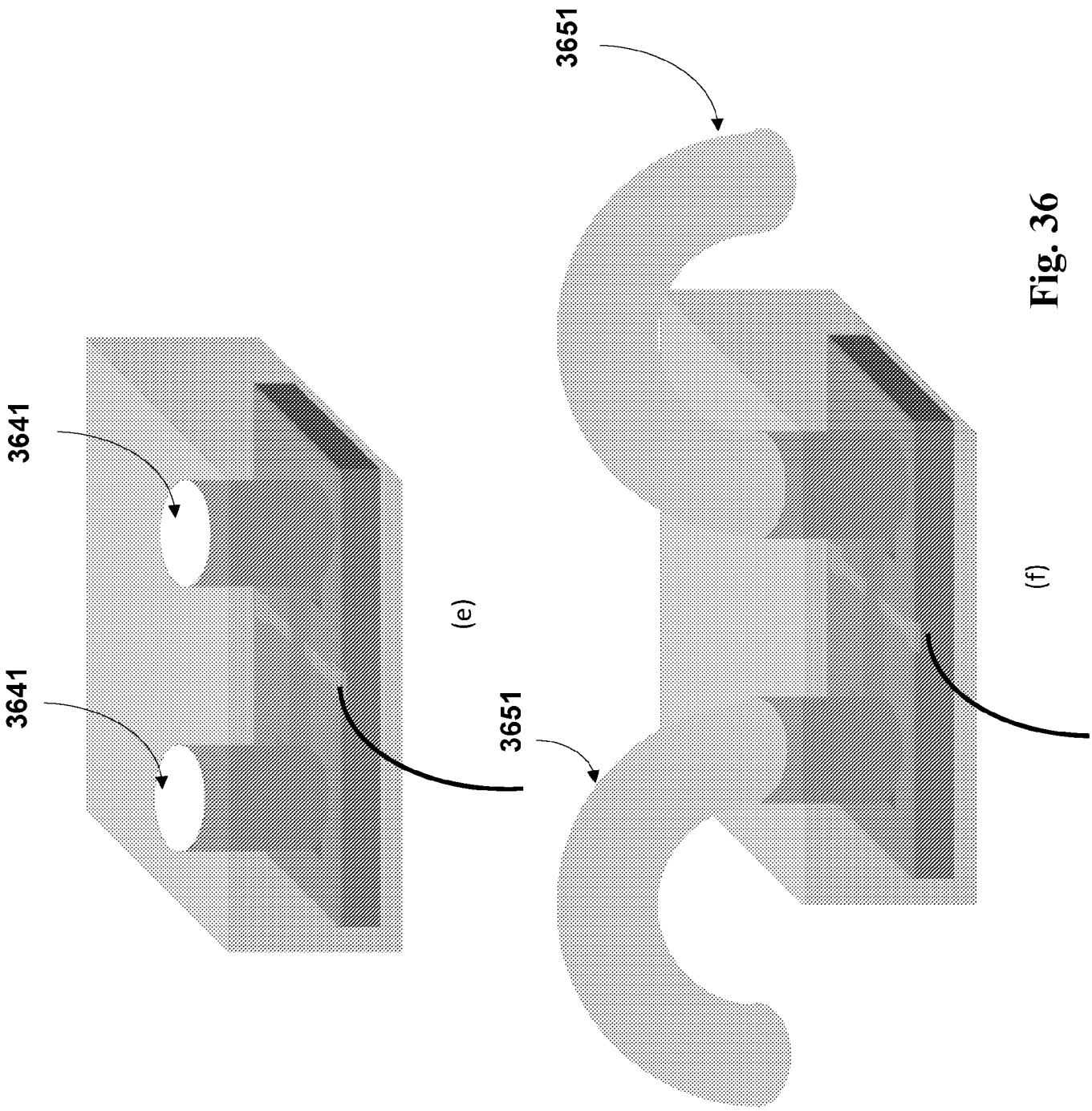


Fig. 36

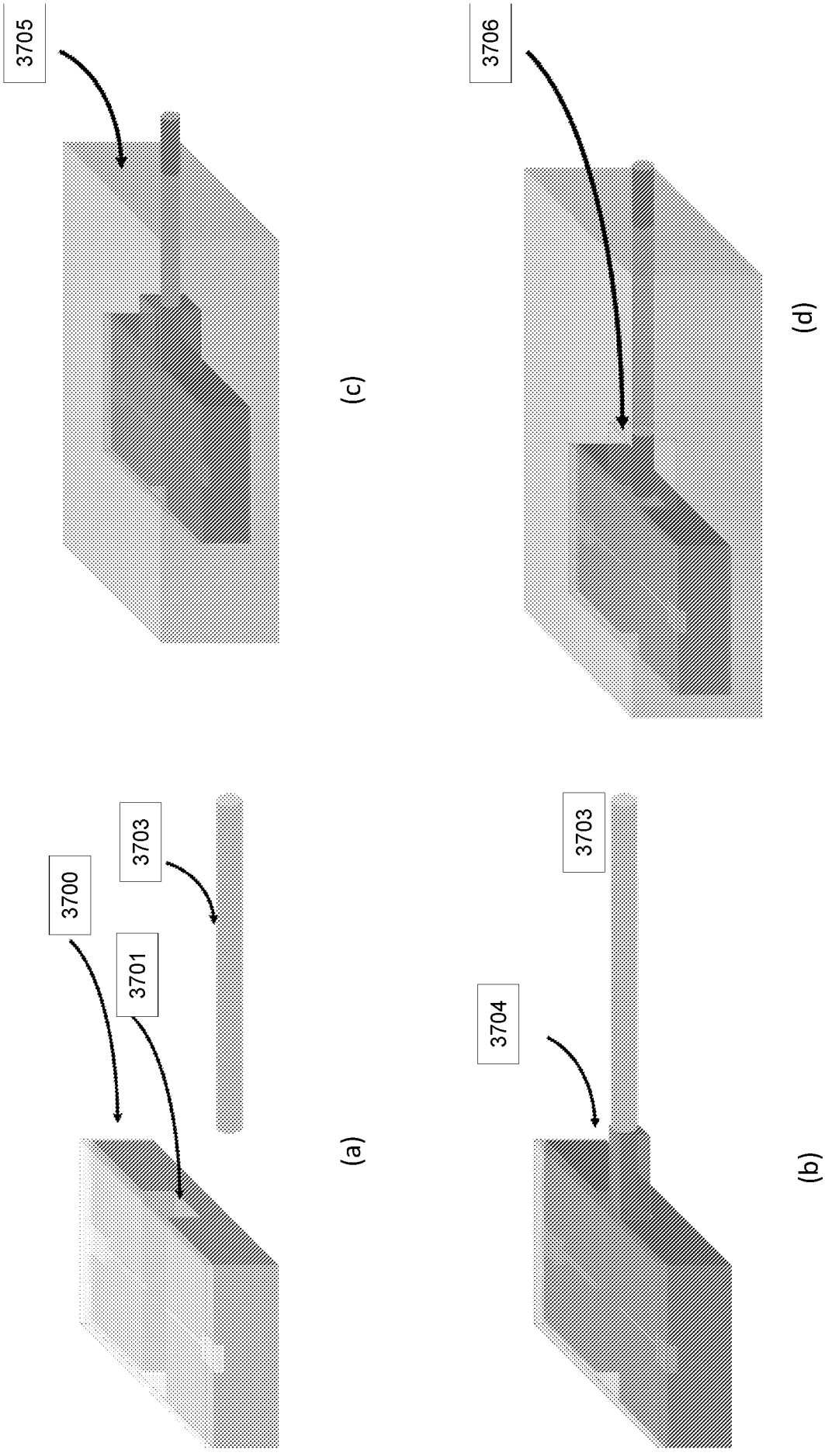


Fig. 37

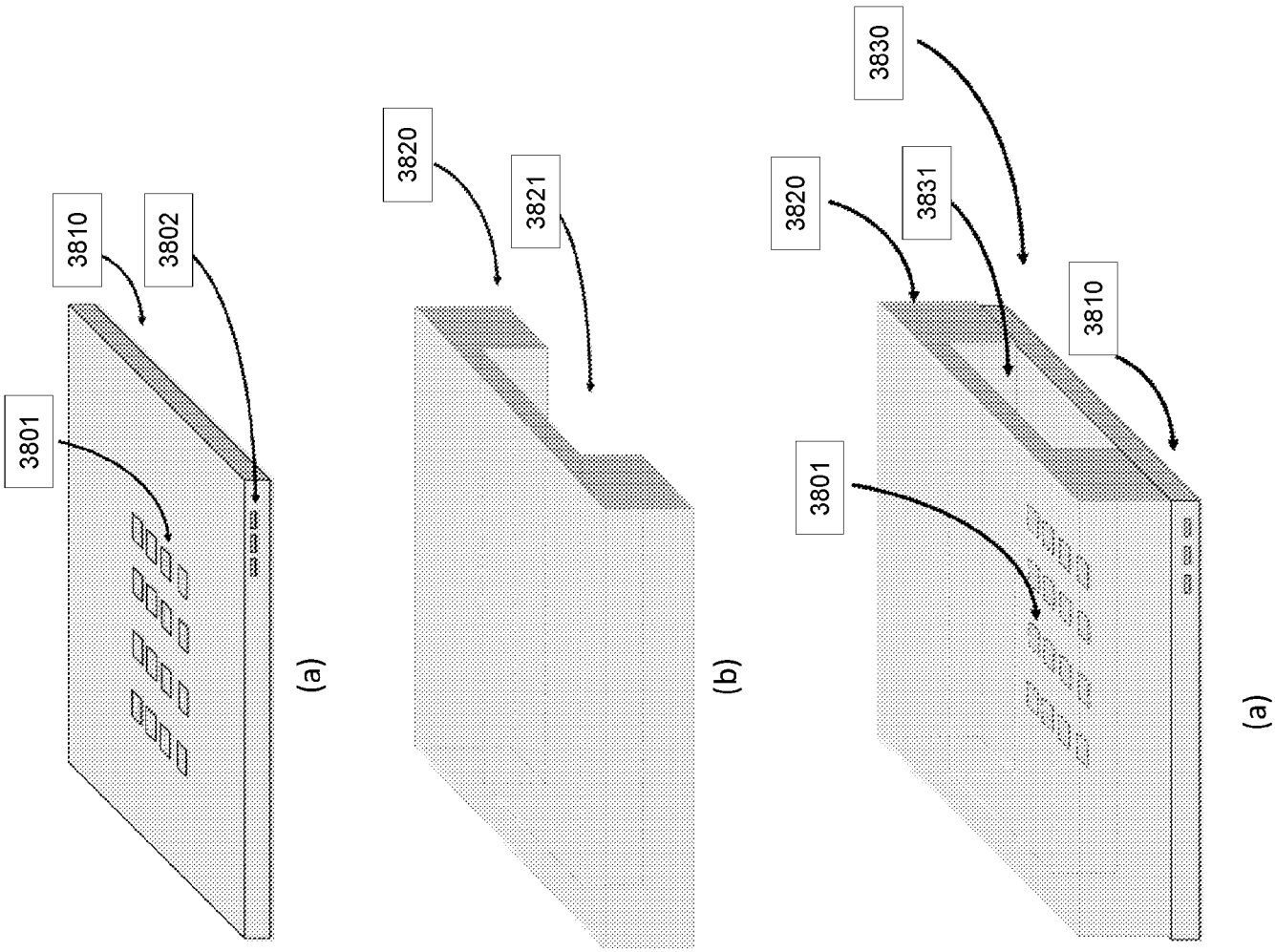
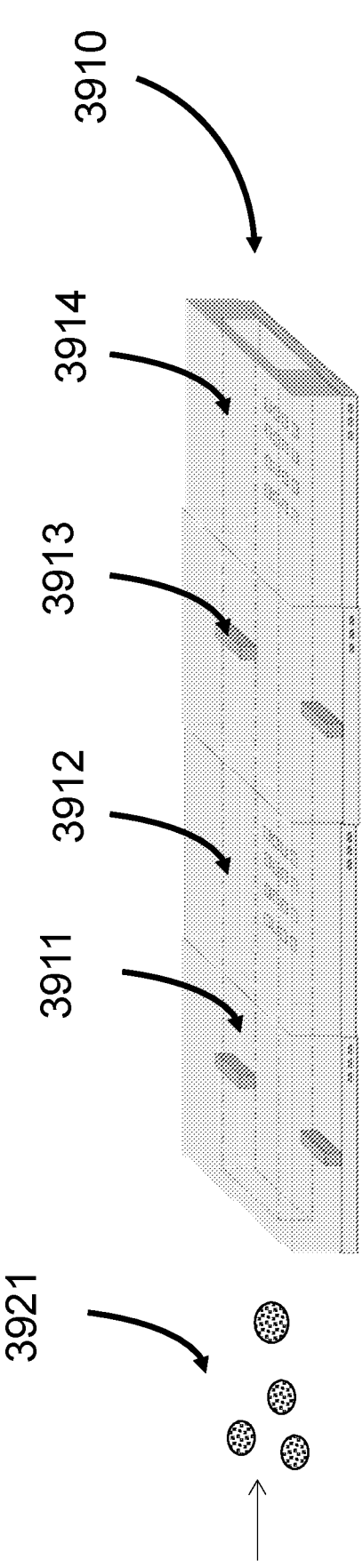
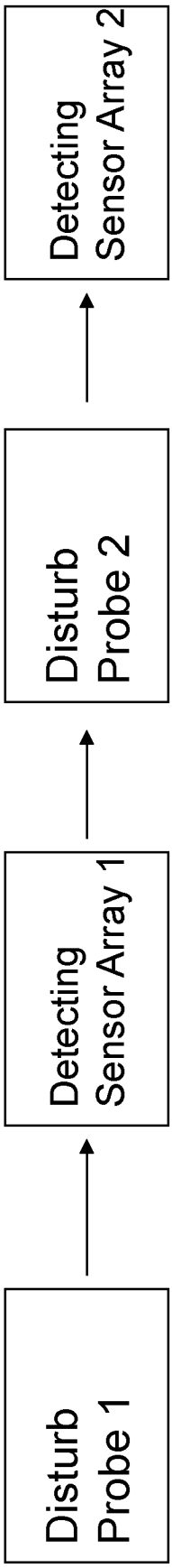


Fig. 38


3910
3914
3913
3912
3911
3921

(a)


Disturb Probe 1
Detecting Sensor Array 1
Disturb Probe 2
Detecting Sensor Array 2

(b)

Fig. 39

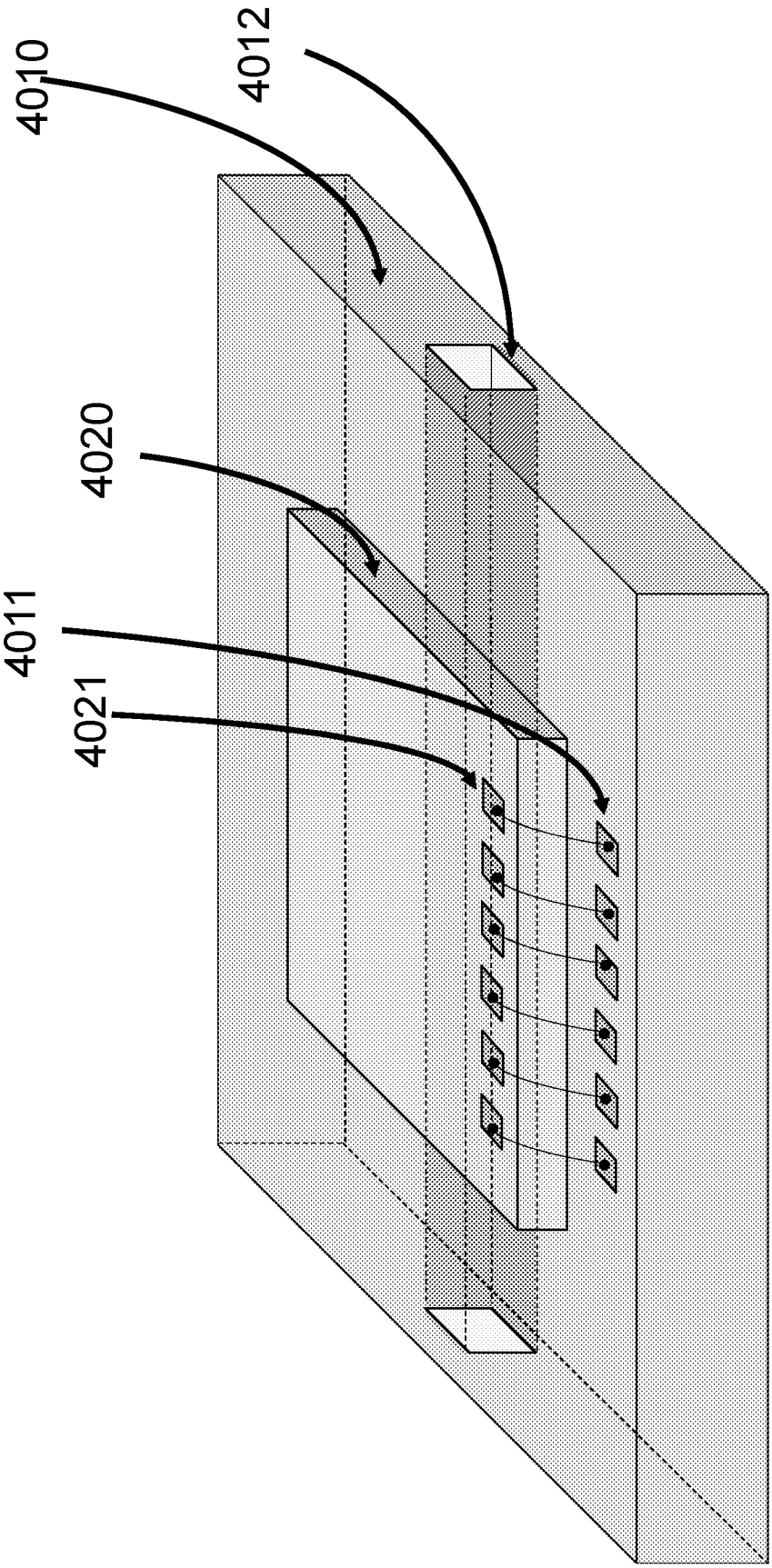


Fig. 40

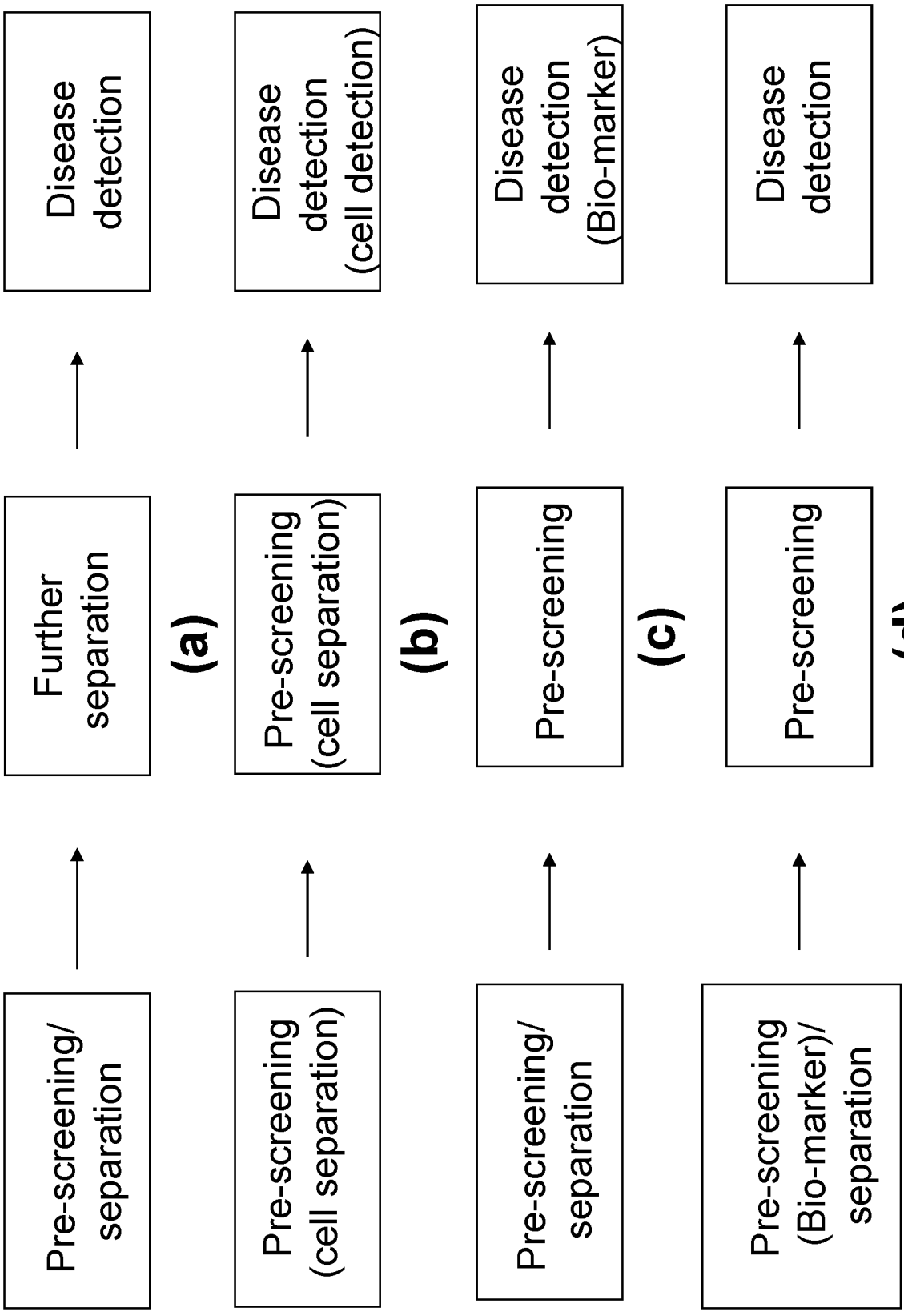

Pre-screening/ separation
Further separation
Disease detection
(a)
Pre-screening (cell separation)
Pre-screening (cell separation)
Disease detection (cell detection)
(b)
Pre-screening/ separation
Pre-screening
Disease detection (Bio-marker)
(c)
Pre-screening (Bio-marker)/ separation
Pre-screening
Disease detection
(d)

Fig. 41

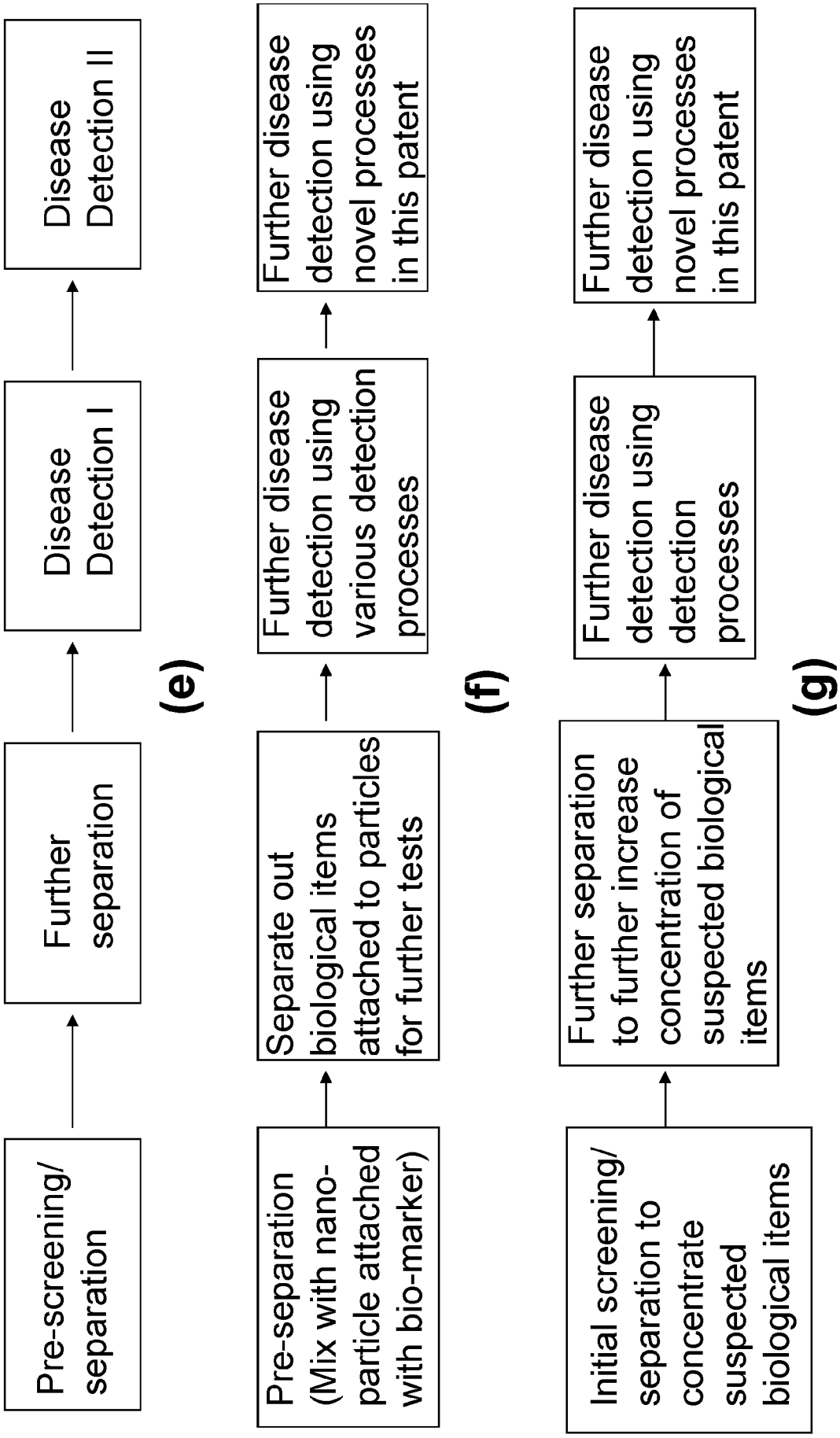

Pre-screening/ separation
Further separation
Disease Detection I
Disease Detection II
(e)
Pre-separation (Mix with nano-particle attached with bio-marker)
Separate out biological items attached to particles for further tests
Further disease detection using various detection processes
Further disease detection using novel processes in this patent
(f)
Initial screening/ separation to concentrate suspected biological items
Further separation to further increase concentration of suspected biological items
Further disease detection using detection processes
Further disease detection using novel processes in this patent
(g)

Fig. 41 (cont.)

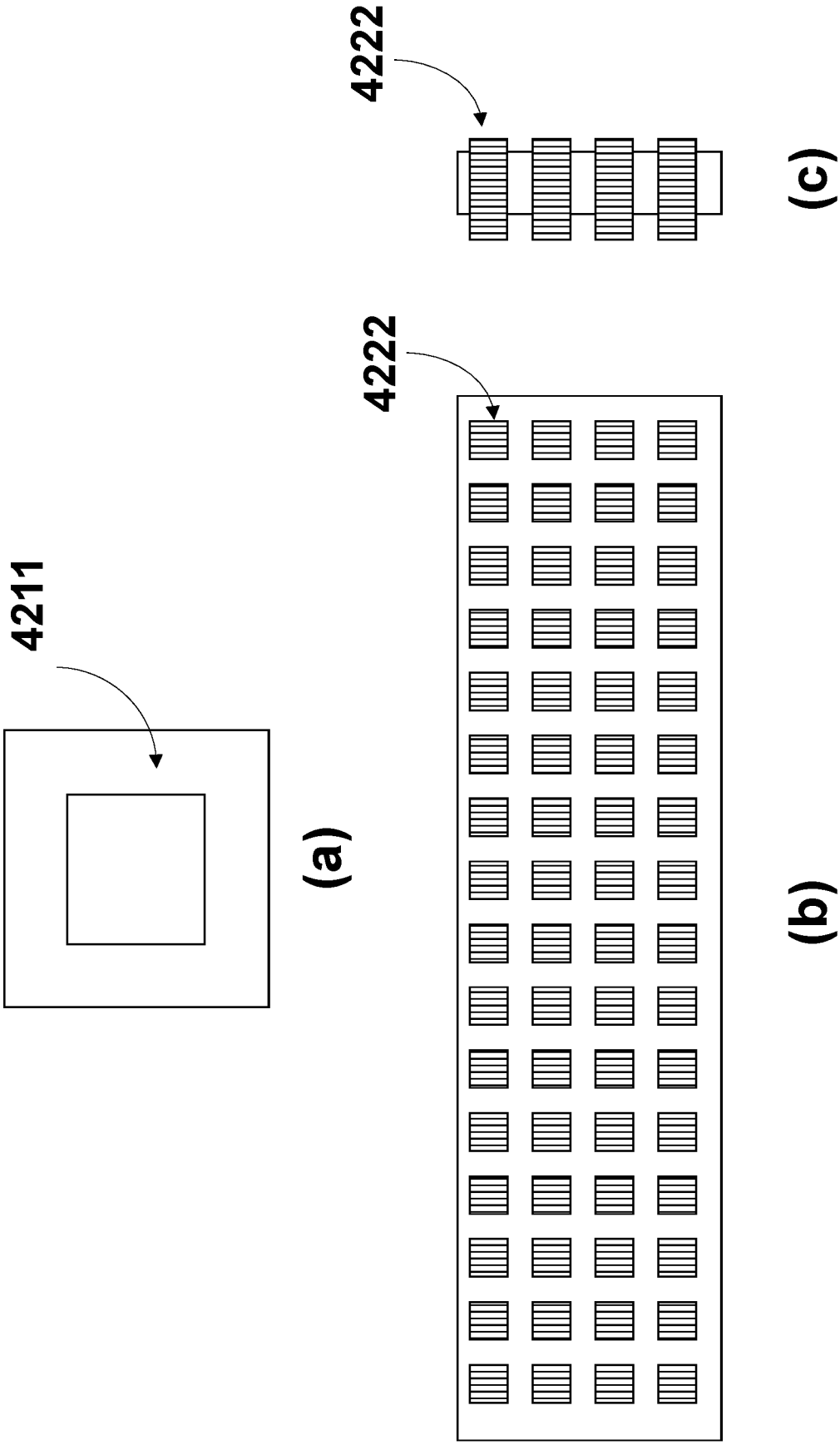


Fig. 42

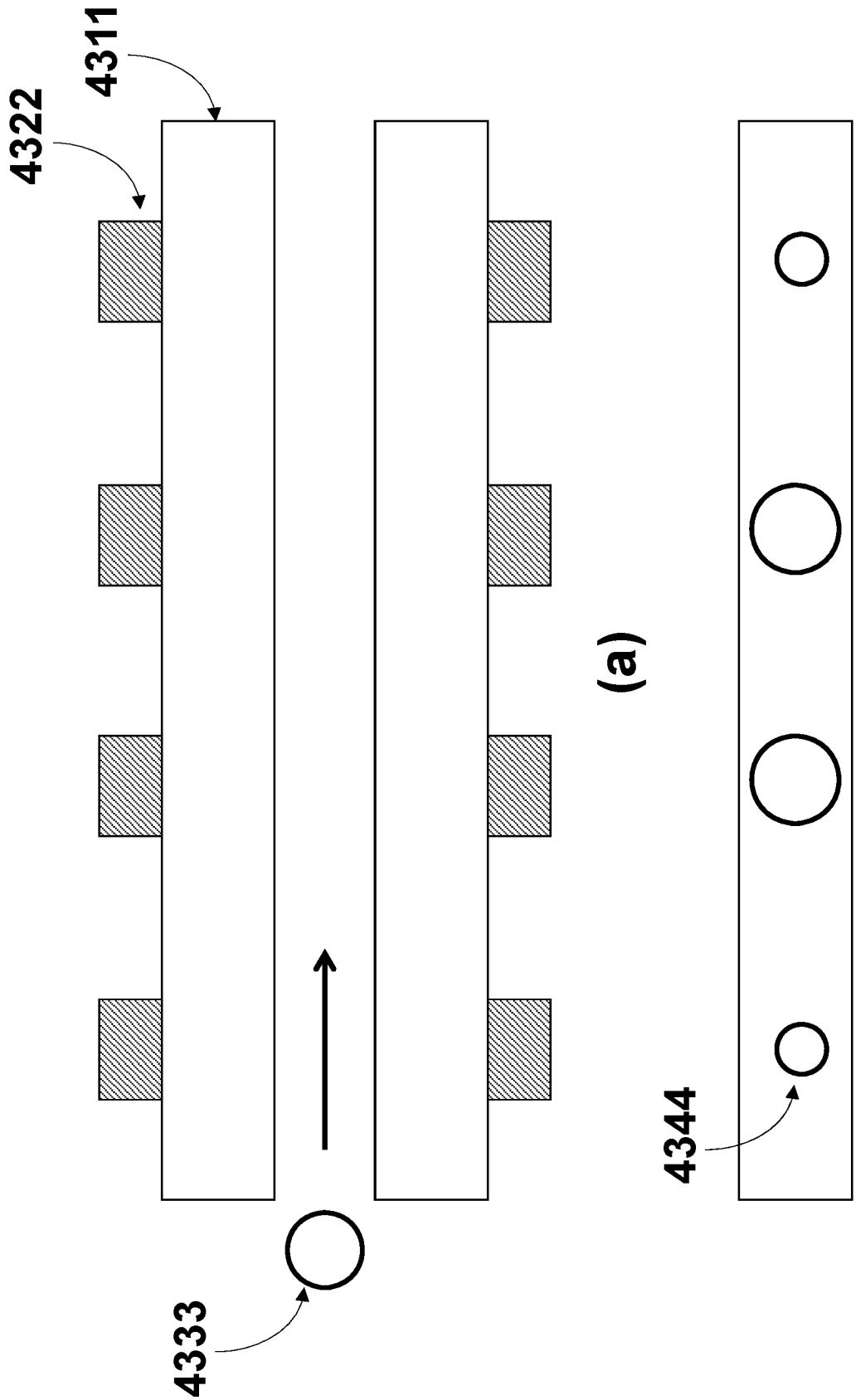


Fig. 43

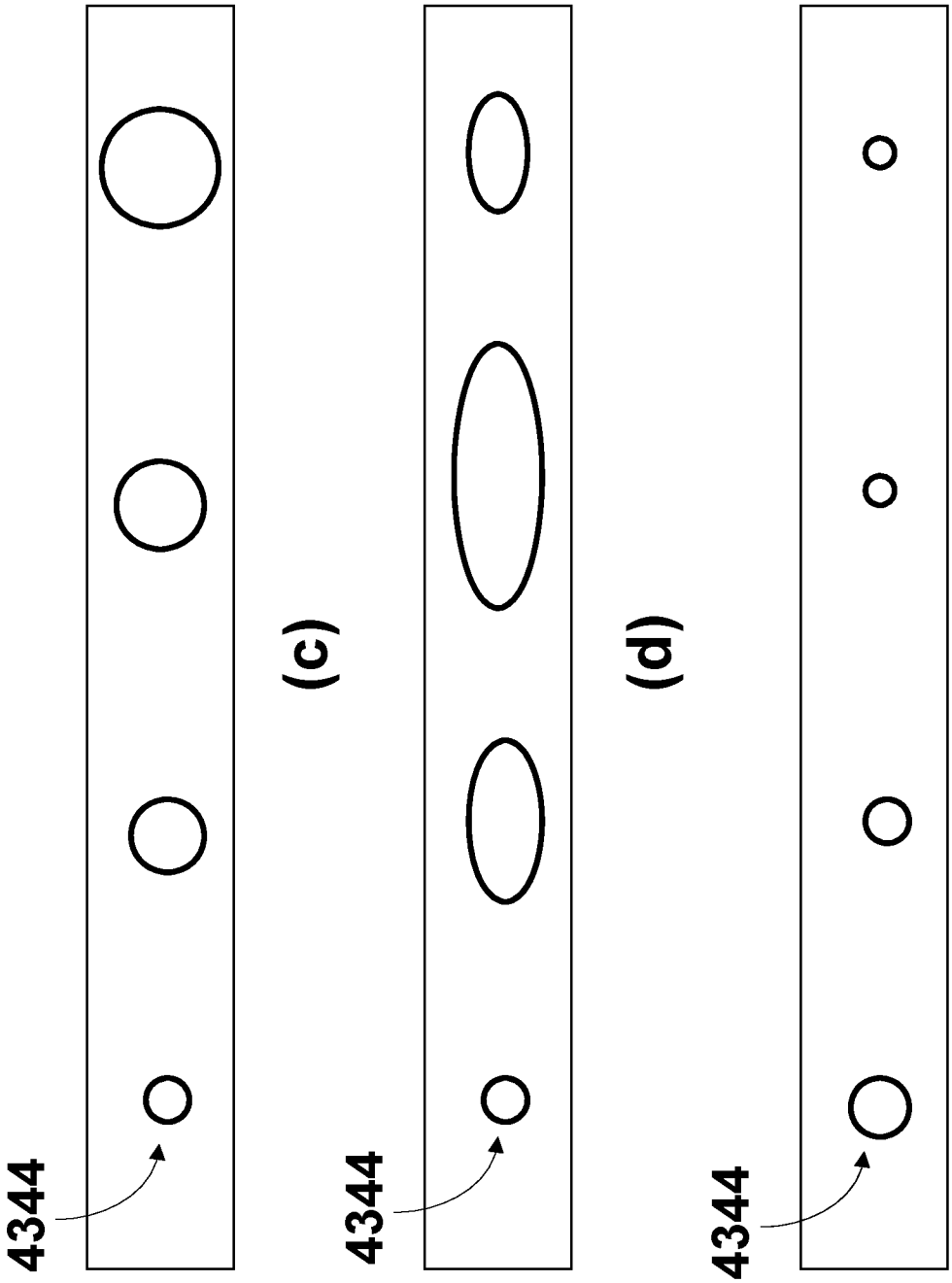


Fig. 43 (cont.)

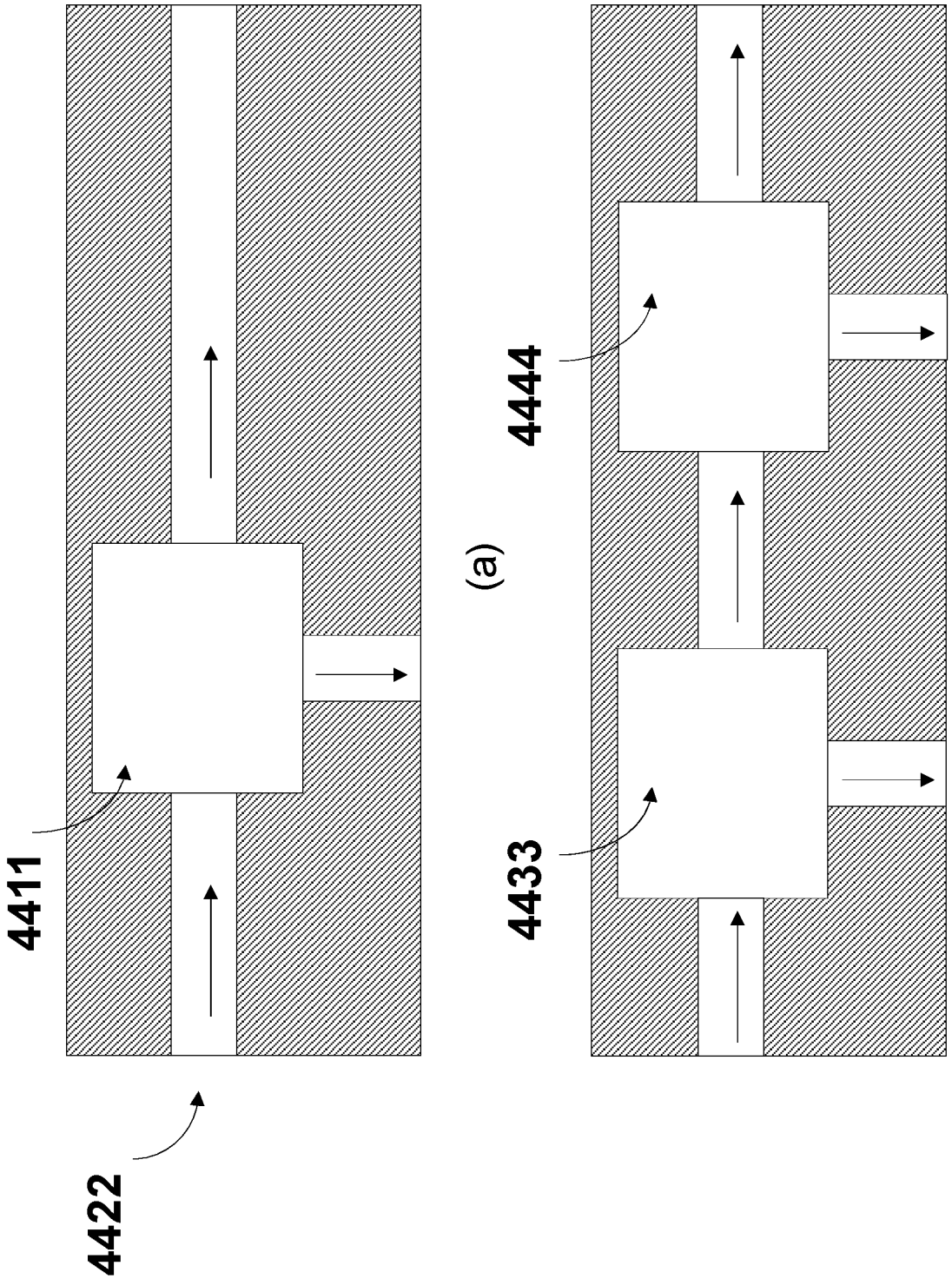


Fig. 44

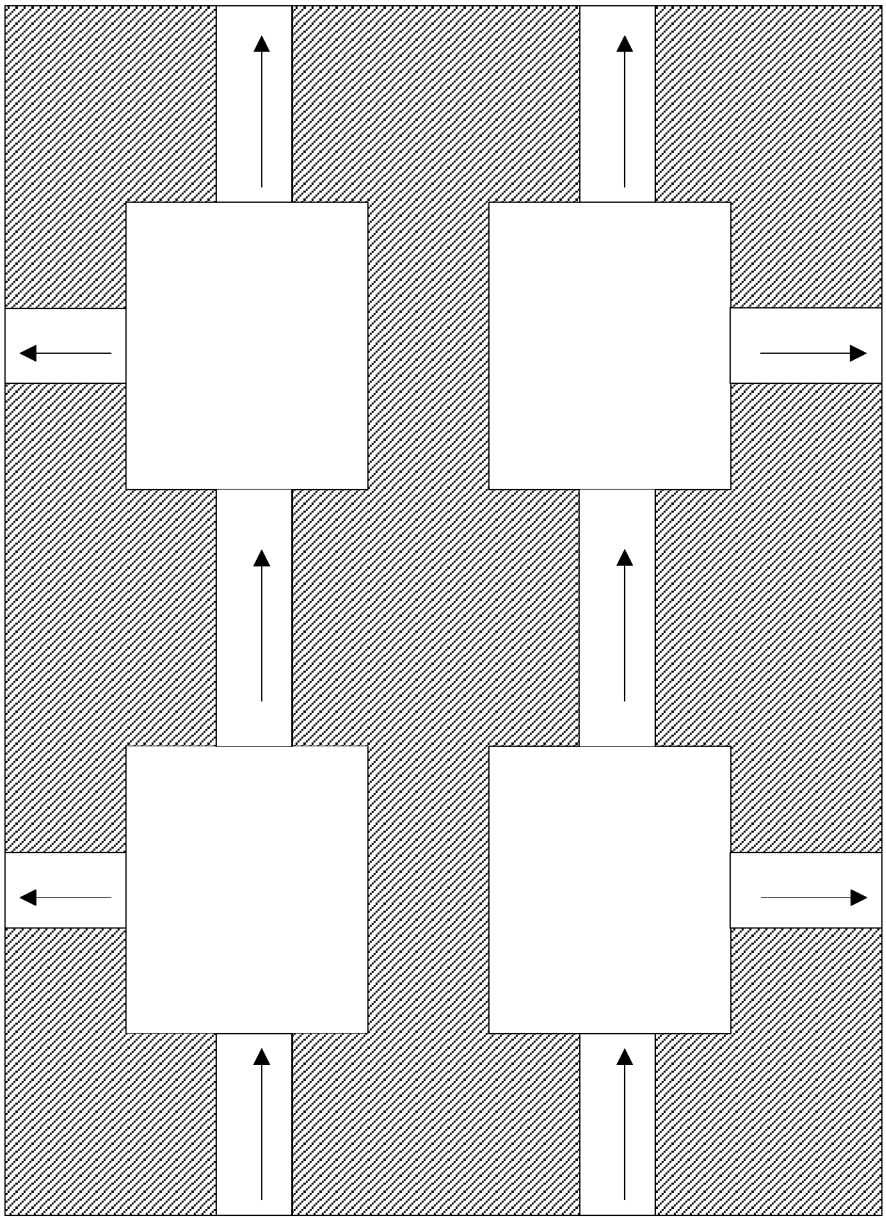


Fig. 45

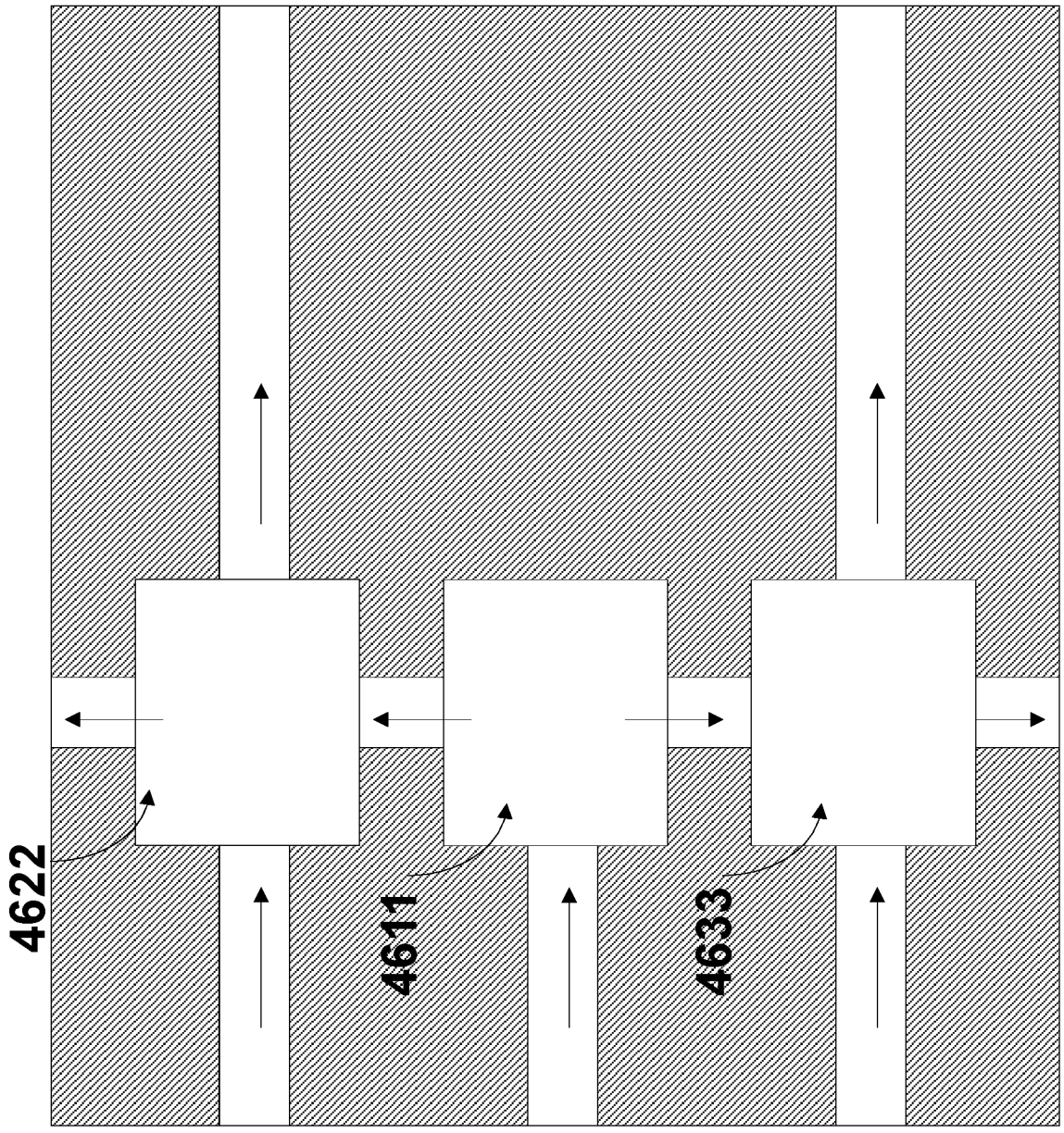


Fig. 46

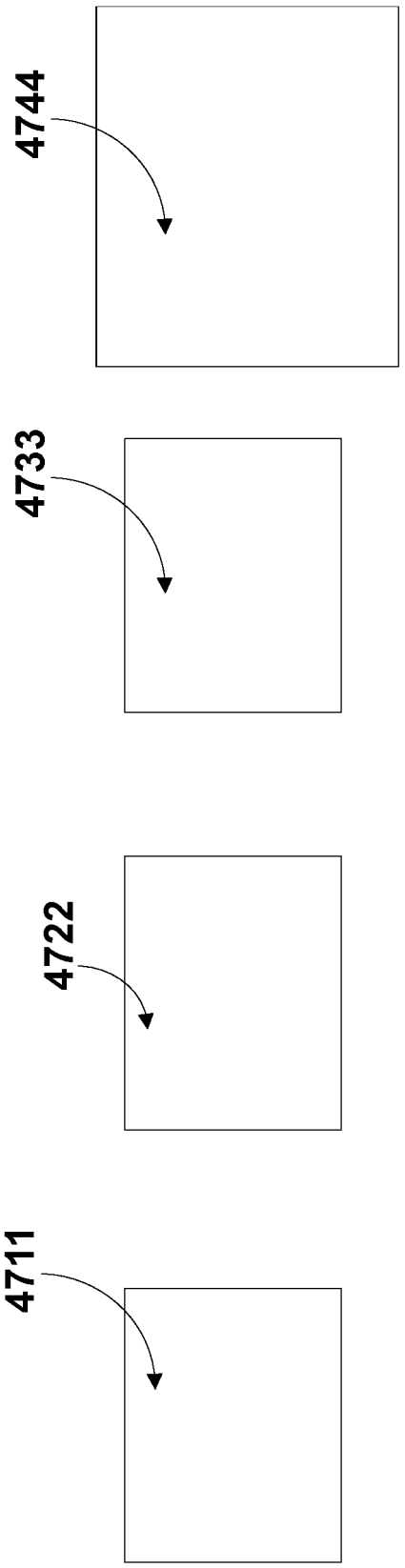


Fig. 47

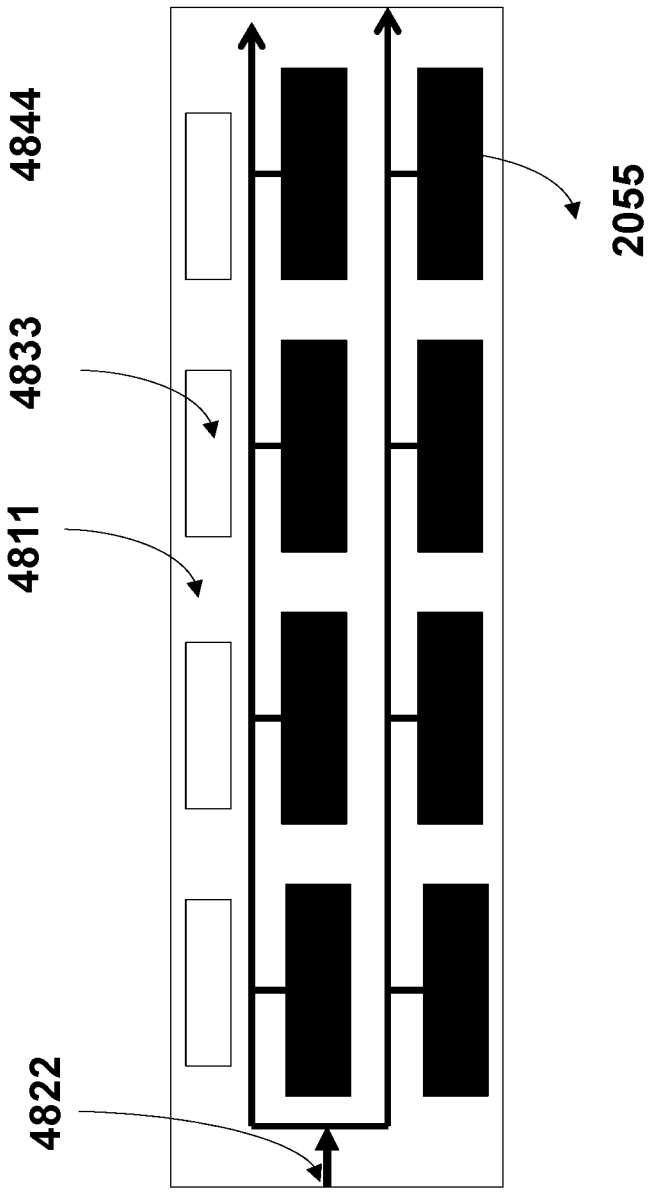


Fig. 48

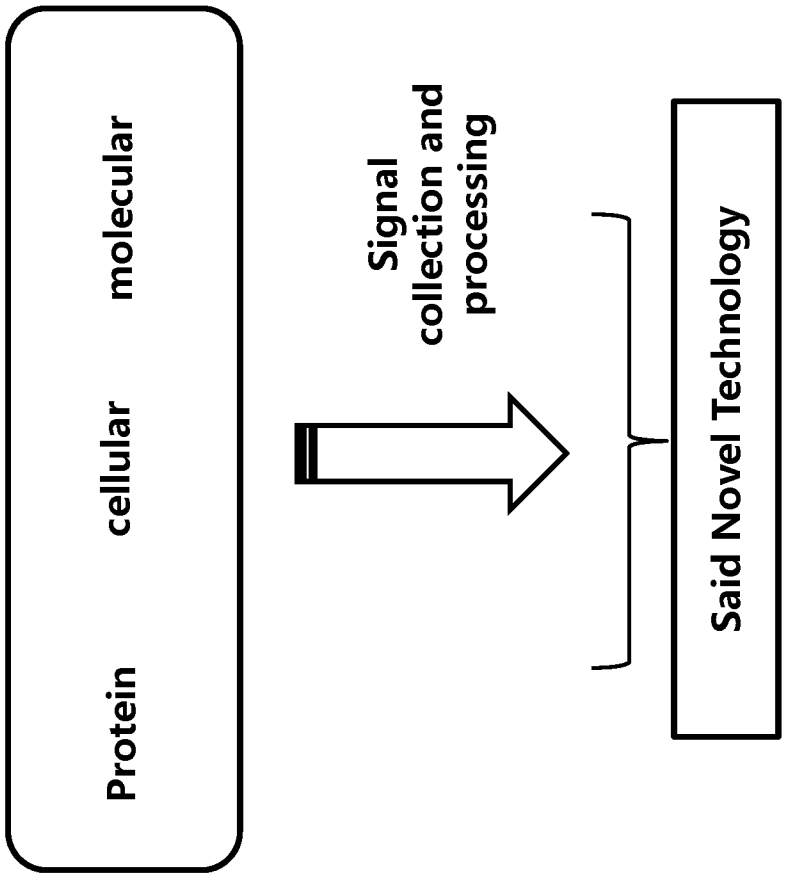

Protein
cellular
molecular
Signal collection and processing
Said Novel Technology

Fig. 49

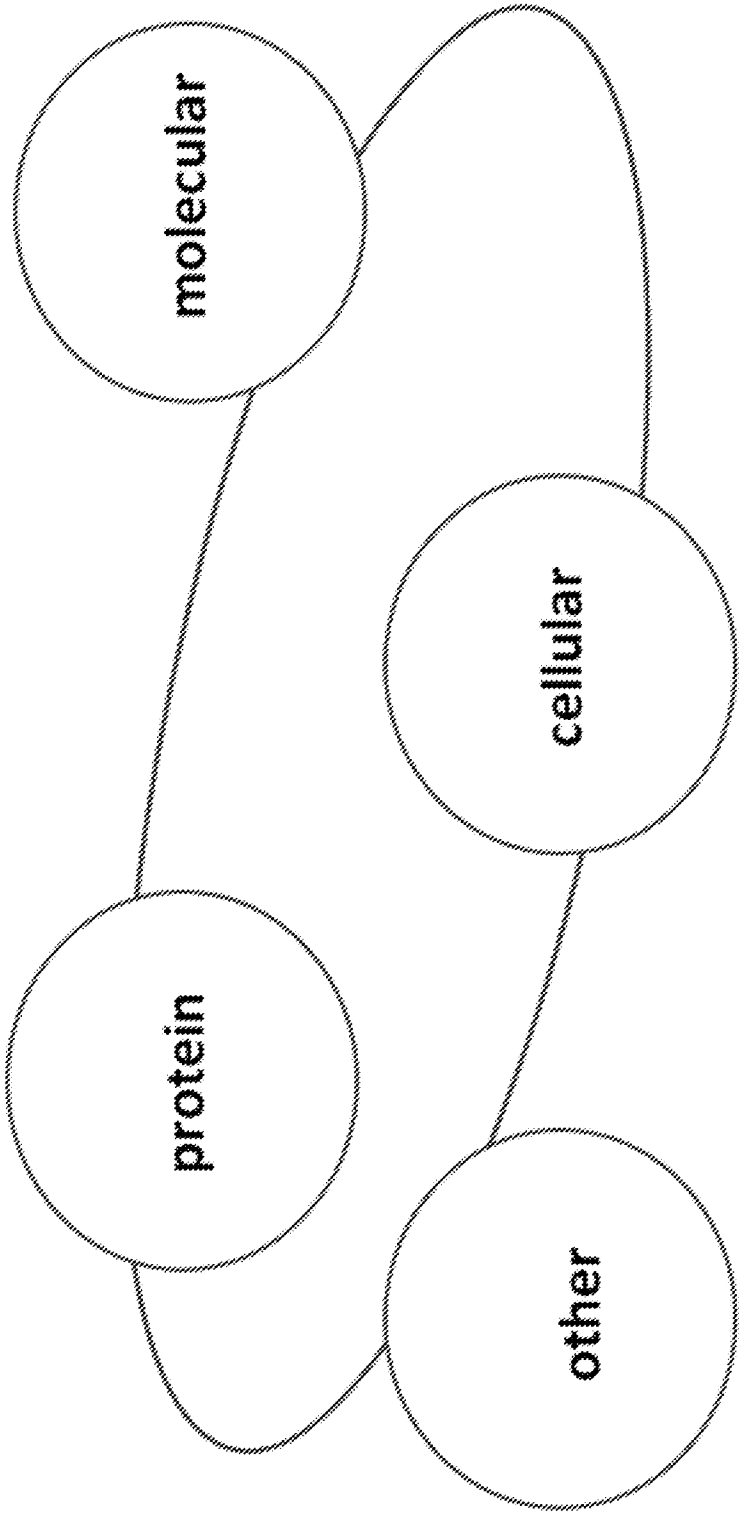


Fig. 50

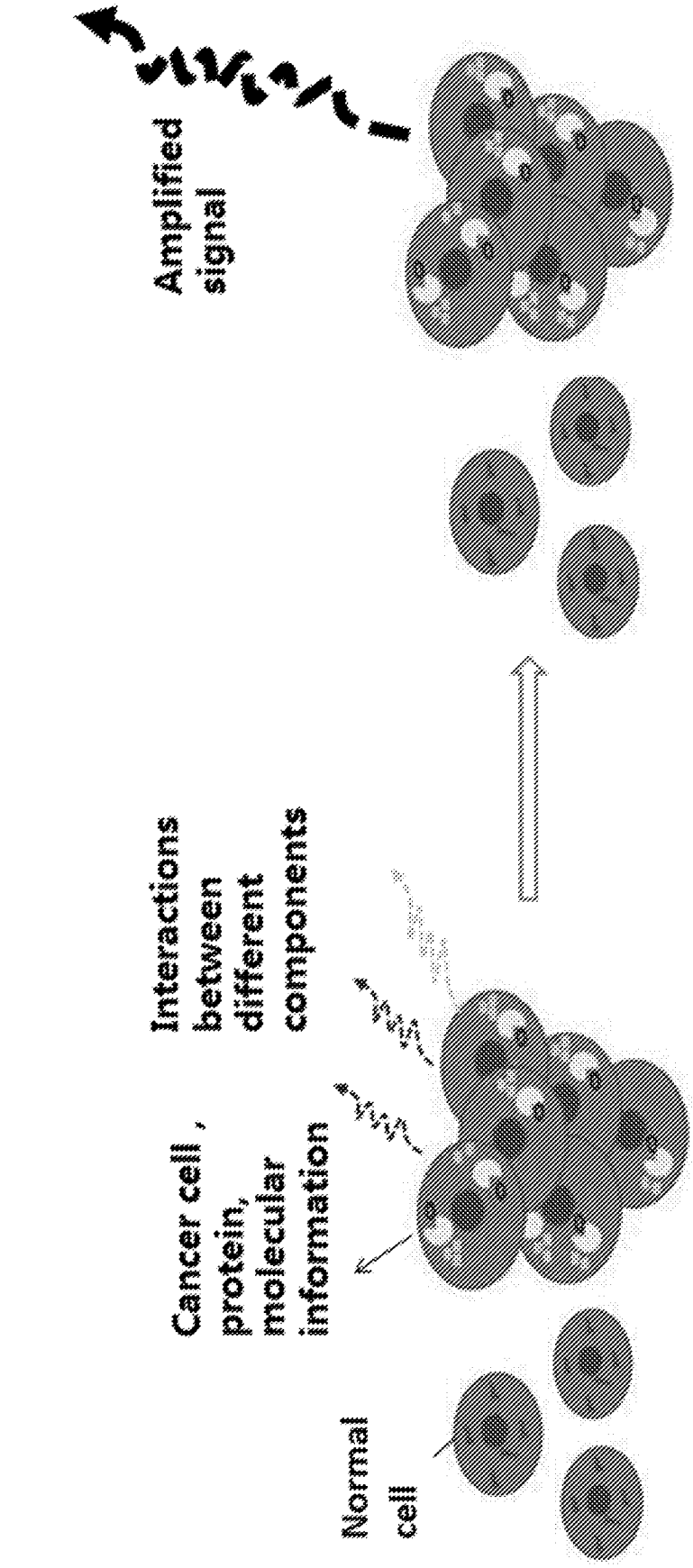

Amplified signal
Interactions between different components
Cancer cell, protein, molecular information
Normal cell

Fig. 51

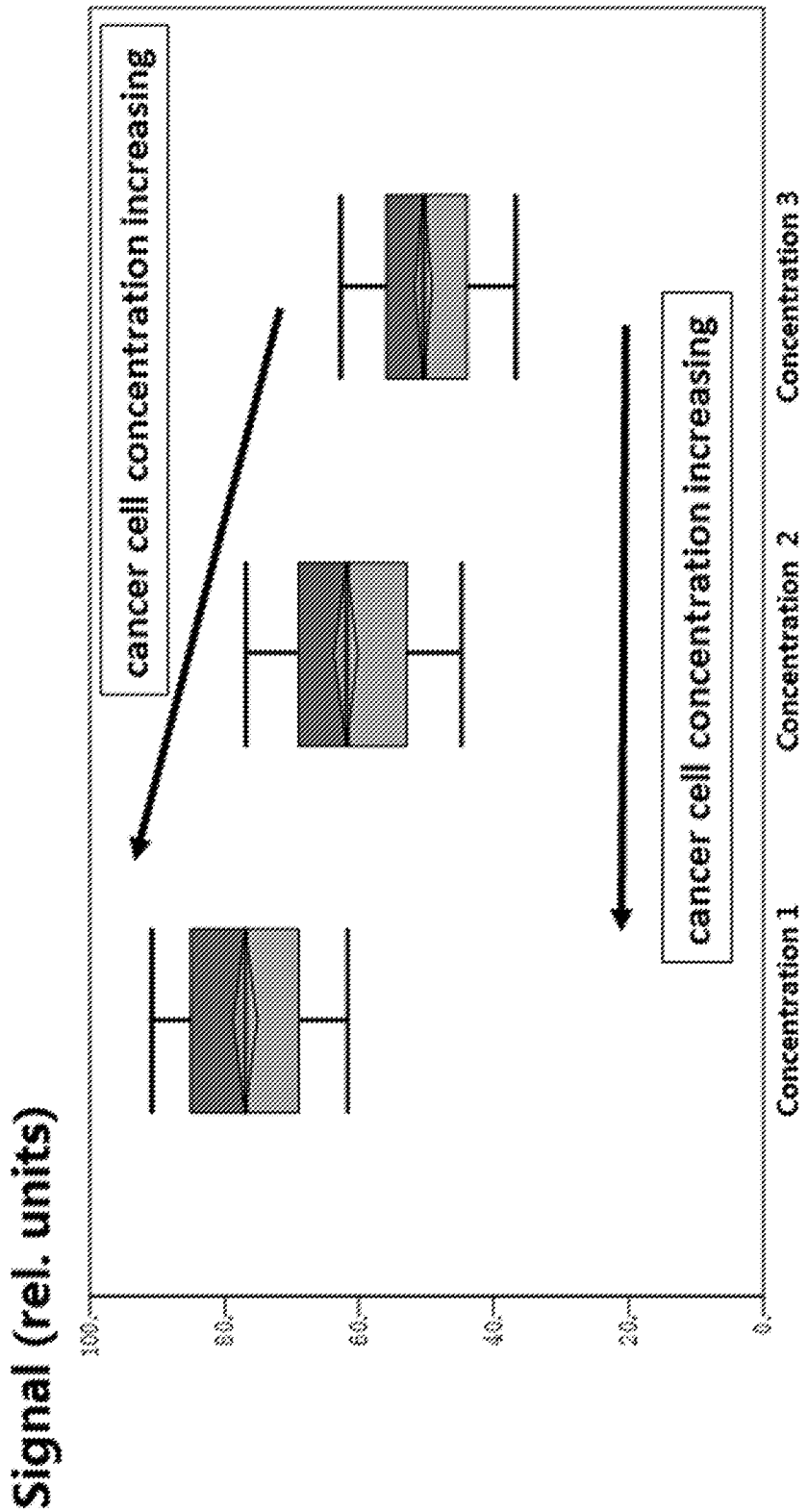

Signal (rel. units)
100
80
60
40
20
0
cancer cell concentration increasing
cancer cell concentration increasing
Concentration 1
Concentration 2
Concentration 3

Fig. 52

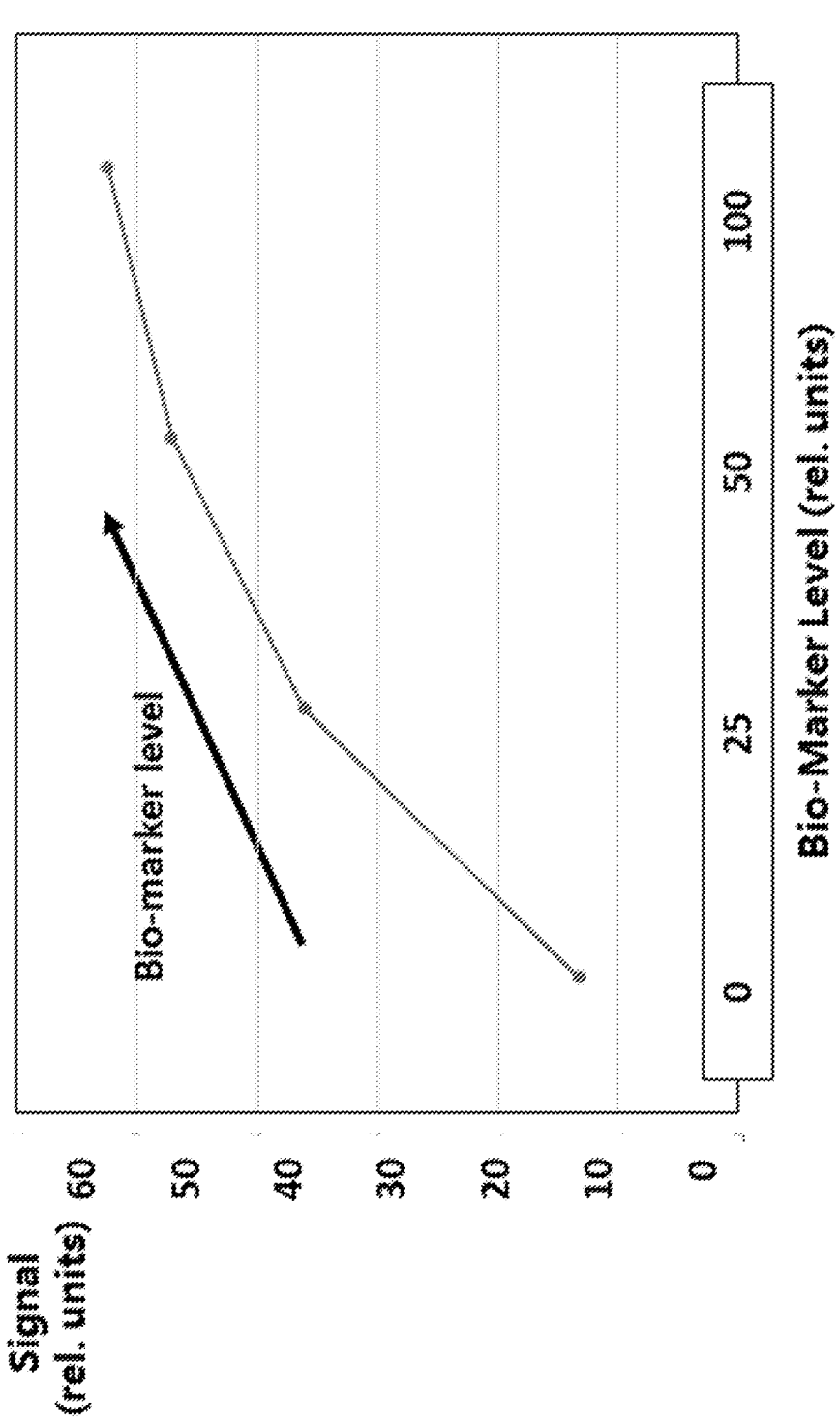

Signal (rel. units)
60
50
40
30
20
10
0
Bio-marker level
0
25
50
100
Bio-Marker Level (rel. units)

Fig. 53

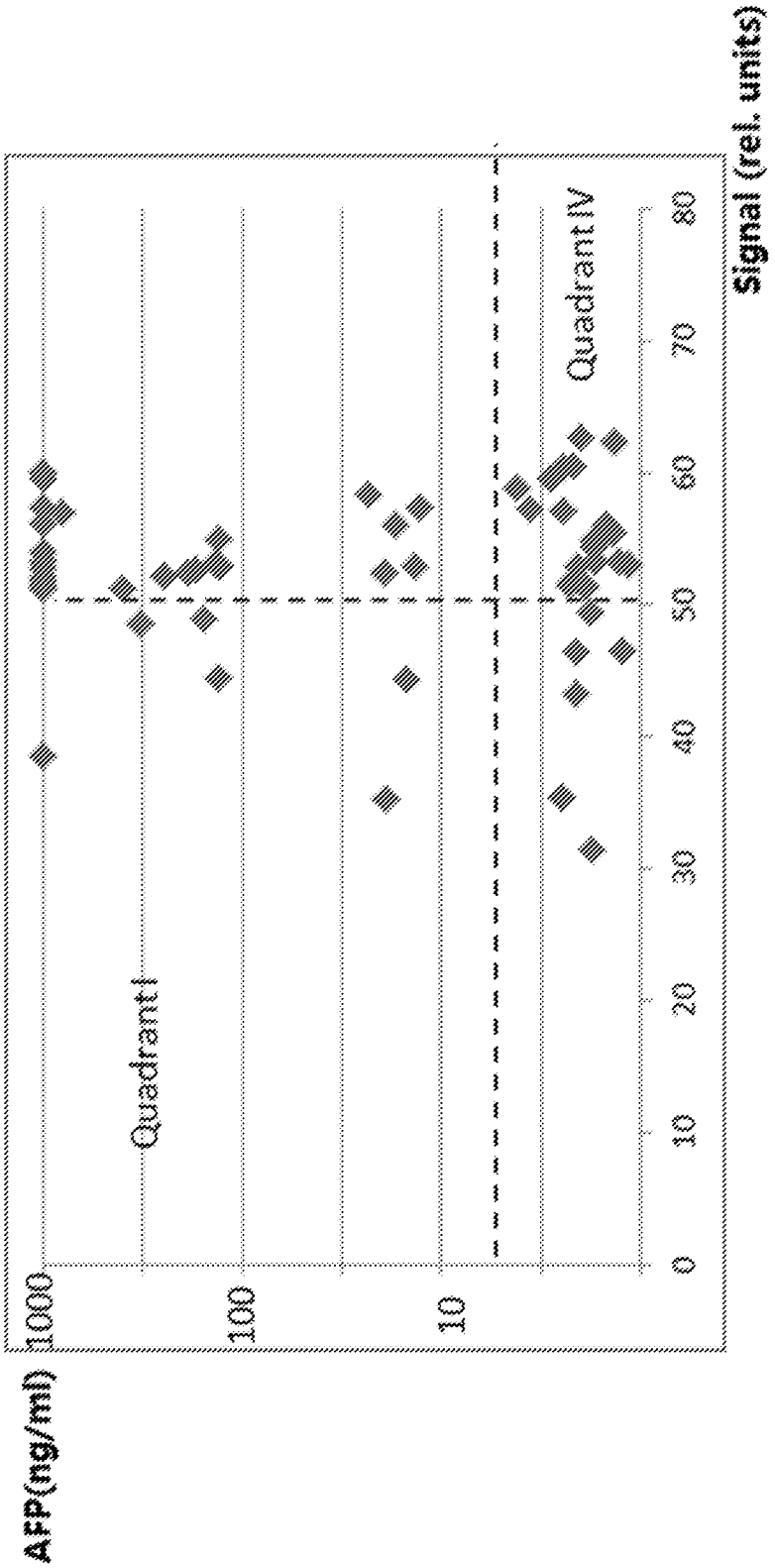

AFP(ng/ml)
1000
100
10
Quadrant I
Quadrant IV
0
10
20
30
40
50
60
70
80
Signal (rel. units)

Fig. 54

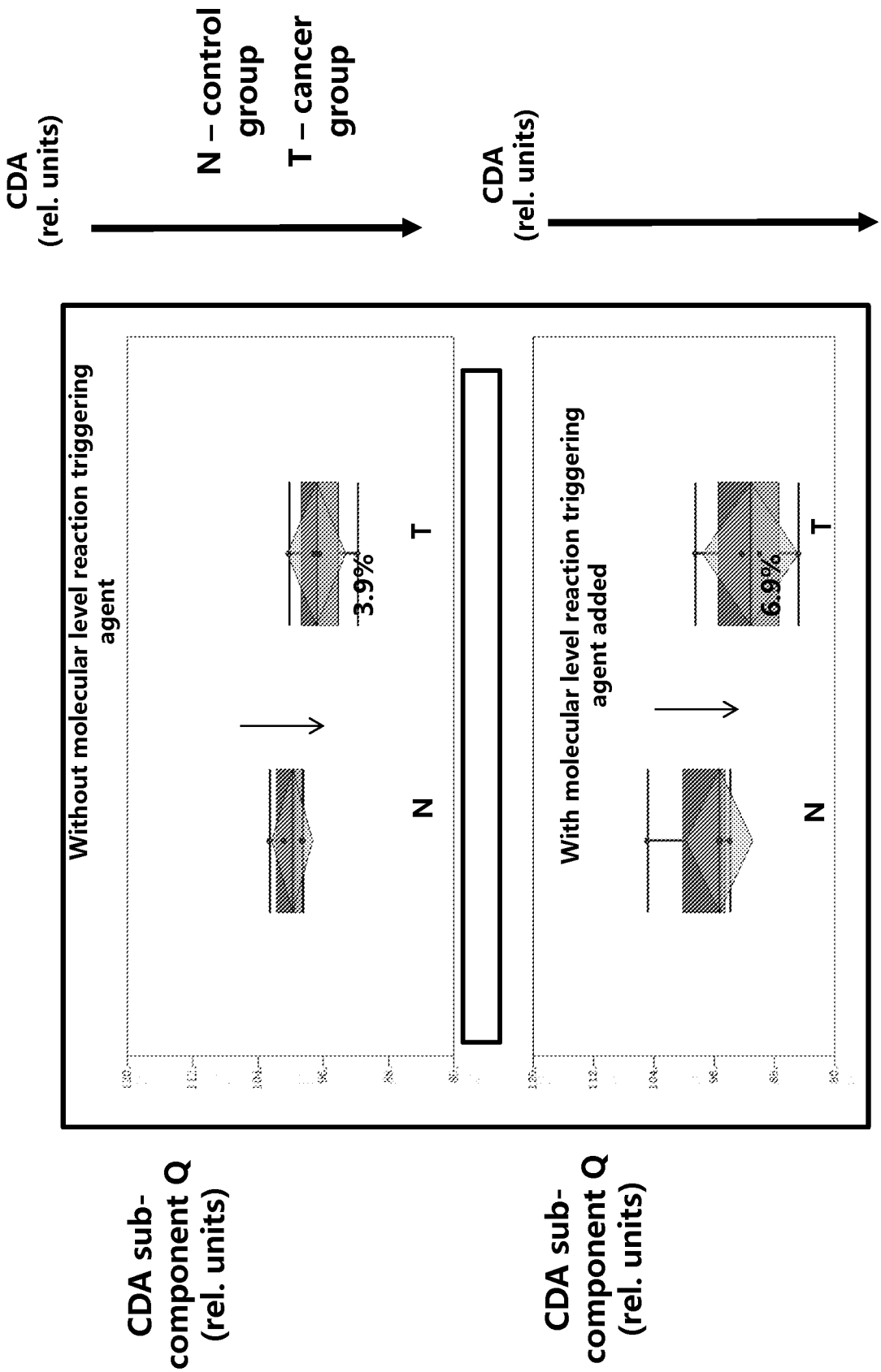

CDA (rel. units)
N – control group
T – cancer group
CDA (rel. units)
Without molecular level reaction triggering agent
3.9%
T
N
With molecular level reaction triggering agent added
6.9%
T
N
CDA sub-component Q (rel. units)
CDA sub-component Q (rel. units)

Fig. 55

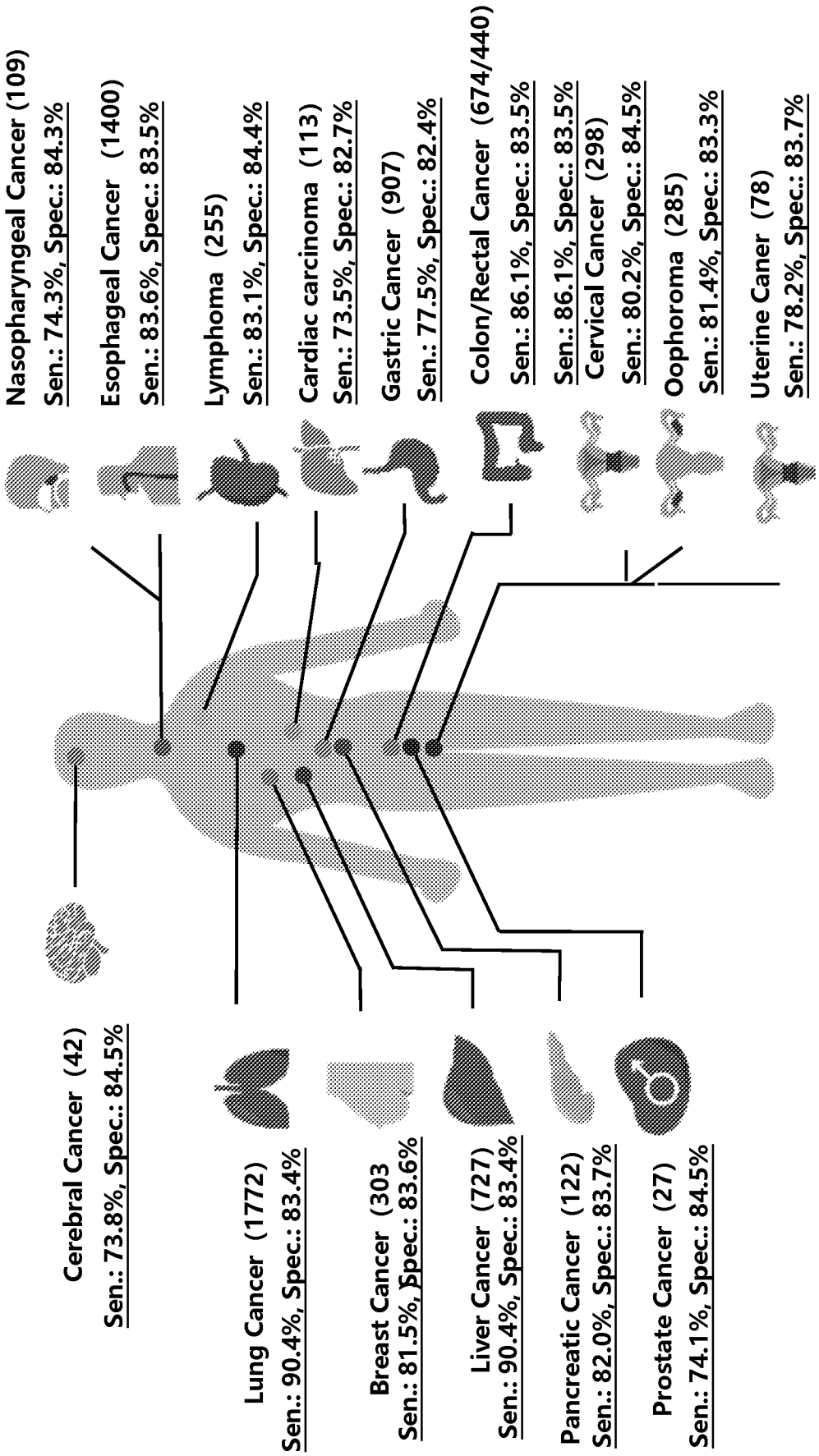

Nasopharyngeal Cancer (109)
Sen.: 74.3%, Spec.: 84.3%
Esophageal Cancer (1400)
Sen.: 83.6%, Spec.: 83.5%
Lymphoma (255)
Sen.: 83.1%, Spec.: 84.4%
Cardiac carcinoma (113)
Sen.: 73.5%, Spec.: 82.7%
Gastric Cancer (907)
Sen.: 77.5%, Spec.: 82.4%
Colon/Rectal Cancer (674/440)
Sen.: 86.1%, Spec.: 83.5%
Sen.: 86.1%, Spec.: 83.5%
Cervical Cancer (298)
Sen.: 80.2%, Spec.: 84.5%
Oophoroma (285)
Sen.: 81.4%, Spec.: 83.3%
Uterine Caner (78)
Sen.: 78.2%, Spec.: 83.7%
Cerebral Cancer (42)
Sen.: 73.8%, Spec.: 84.5%
Lung Cancer (1772)
Sen.: 90.4%, Spec.: 83.4%
Breast Cancer (303
Sen.: 81.5%, Spec.: 83.6%
Liver Cancer (727)
Sen.: 90.4%, Spec.: 83.4%
Pancreatic Cancer (122)
Sen.: 82.0%, Spec.: 83.7%
Prostate Cancer (27)
Sen.: 74.1%, Spec.: 84.5%

Fig. 56

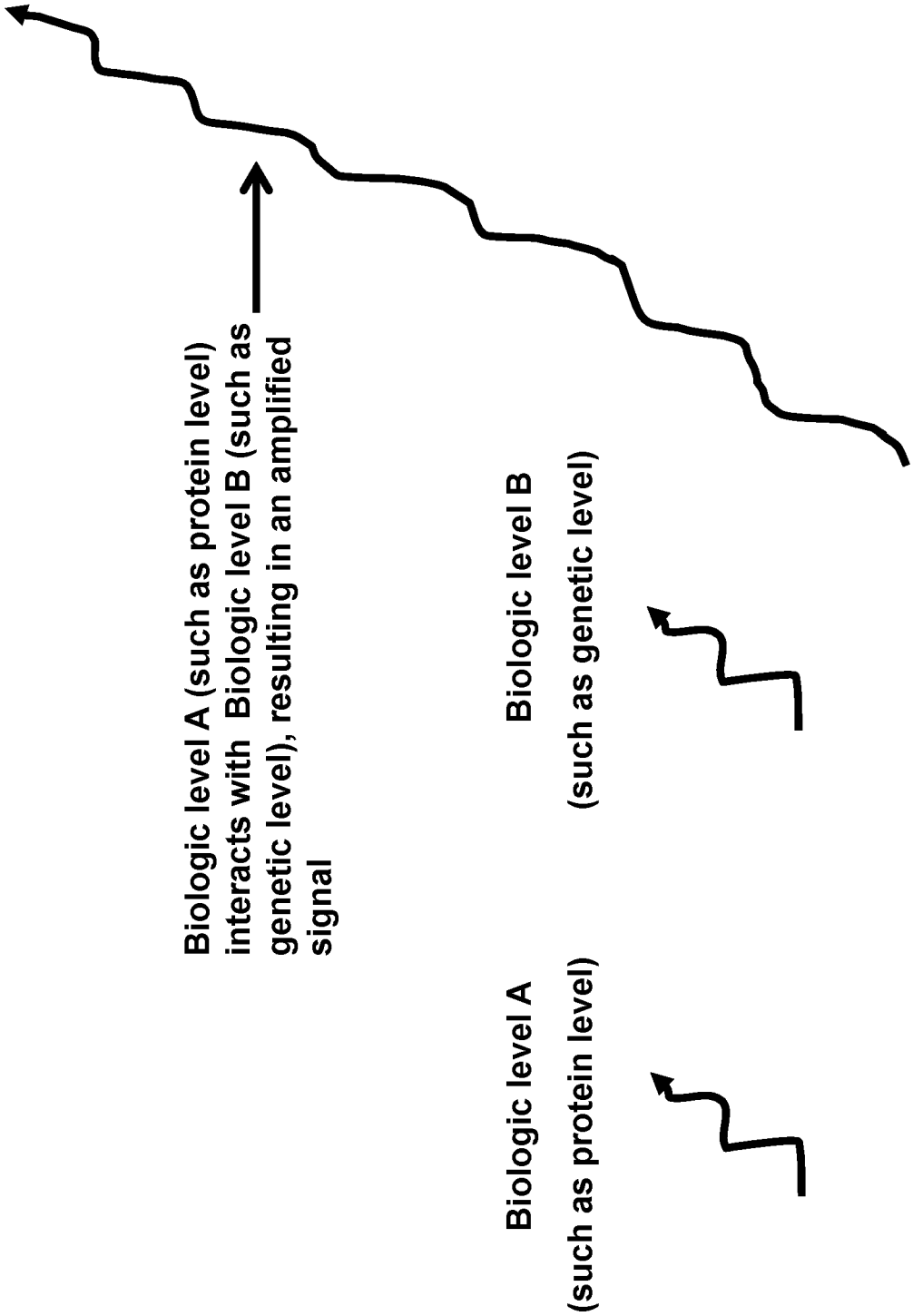

Biologic level A (such as protein level) interacts with Biologic level B (such as genetic level), resulting in an amplified signal
Biologic level B (such as genetic level)
Biologic level A (such as protein level)

Fig. 57

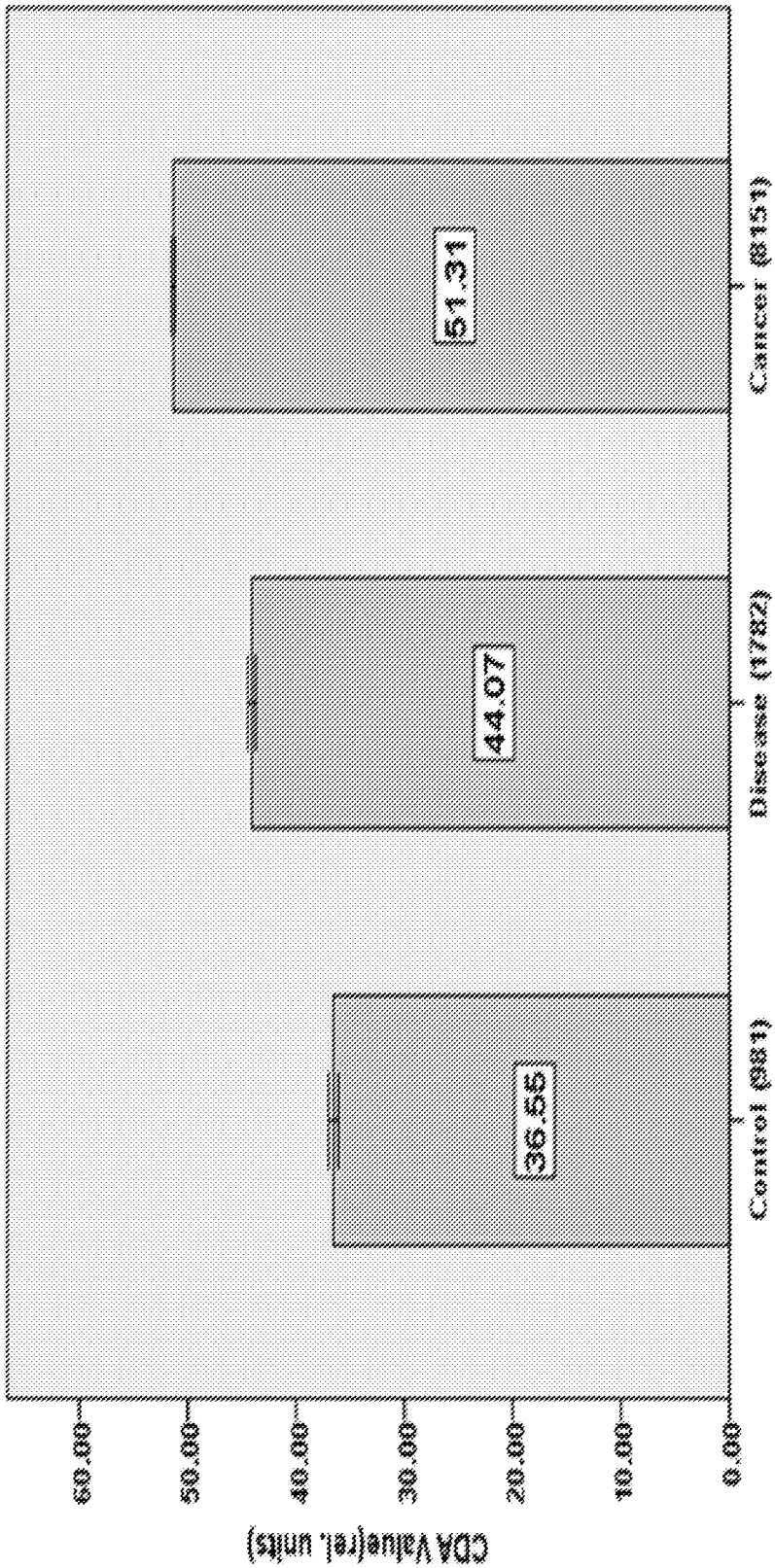

CDA Value(rel. units)
60.00
50.00
40.00
30.00
20.00
10.00
0.00
36.56
44.07
51.31
Control (981)
Disease (1782)
Cancer (8151)
Group

Fig. 58

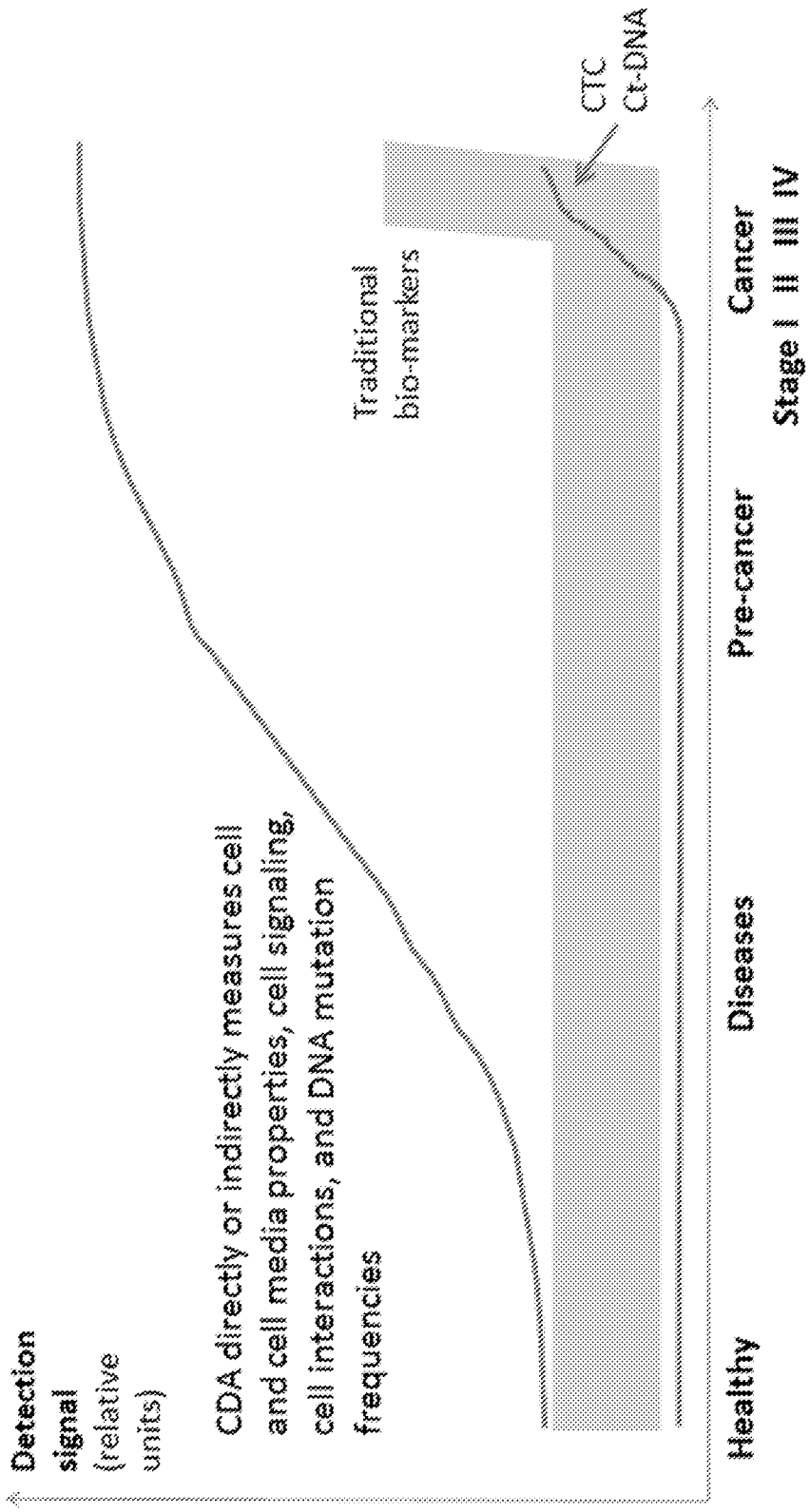

Detection signal (relative units)
CDA directly or indirectly measures cell and cell media properties, cell signaling, cell interactions, and DNA mutation frequencies
Traditional bio-markers
CTC
Ct-DNA
Healthy
Diseases
Pre-cancer
Cancer
Stage I II III IV

Fig. 59

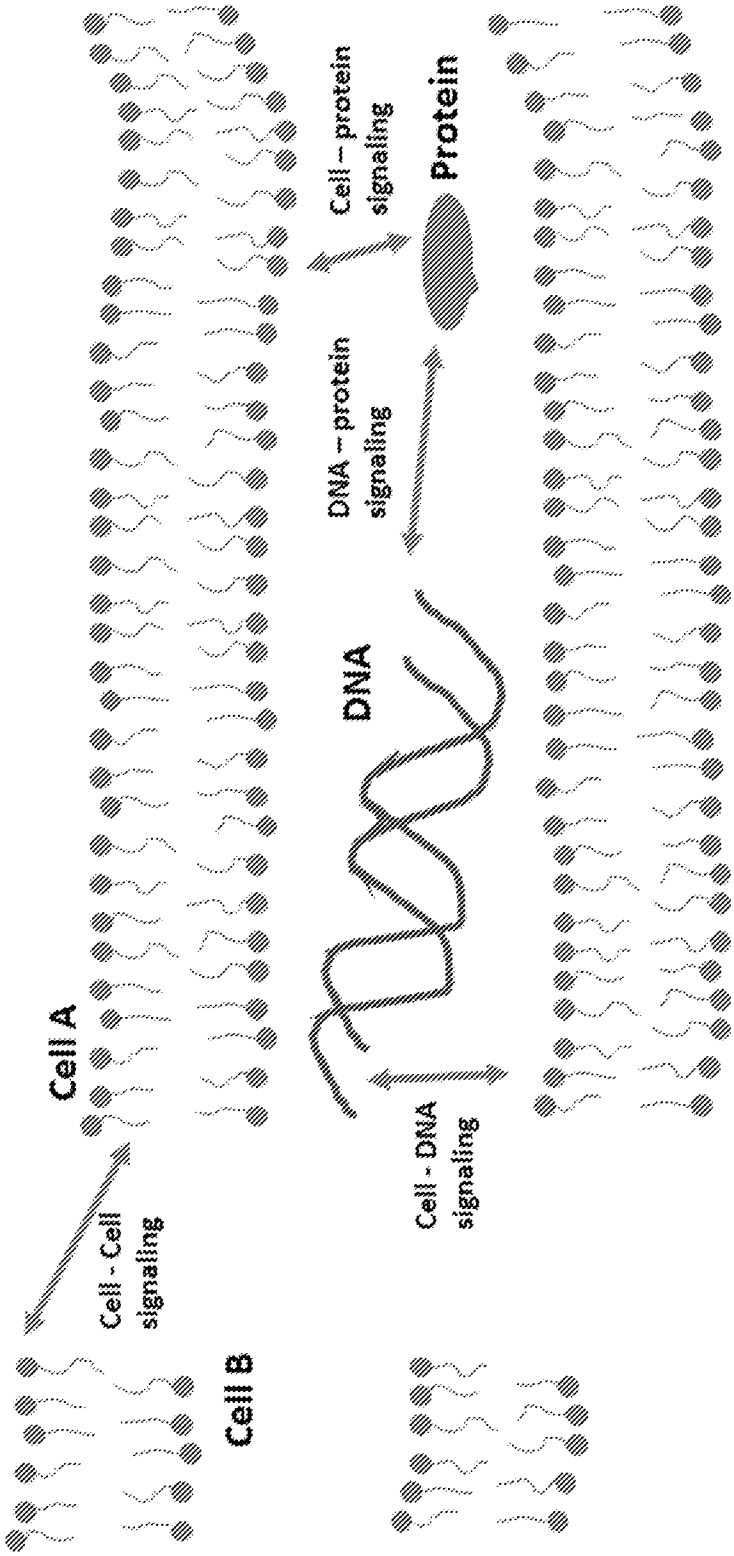

Cell A
Cell - Cell signaling
Cell B
DNA
Cell - DNA signaling
DNA – protein signaling
Cell – protein signaling
Protein

Fig. 60

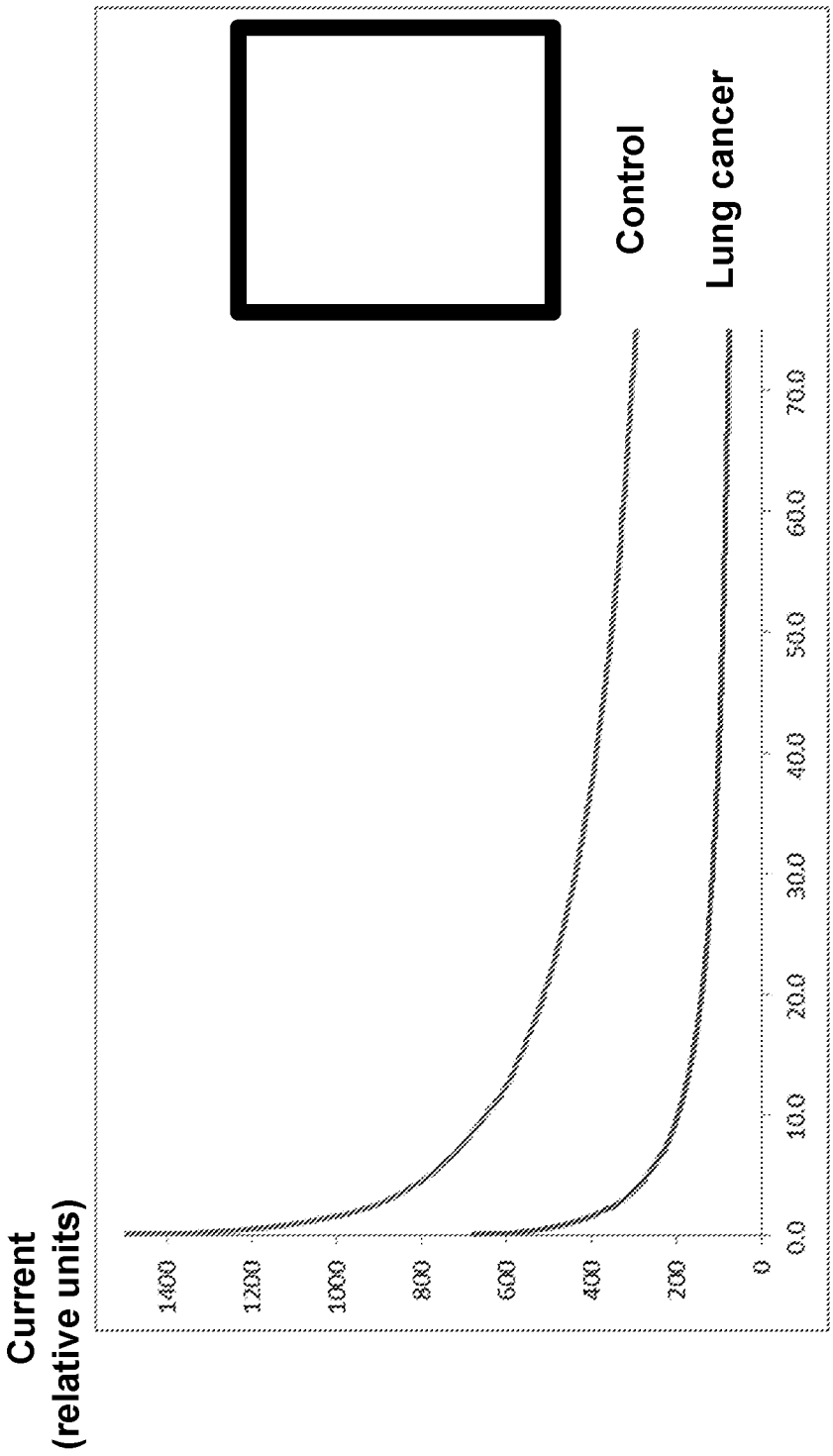

Current
(relative units)
1400
1200
1000
800
600
400
200
0
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
Scanning time
(second)
Control
Lung cancer

Fig. 61

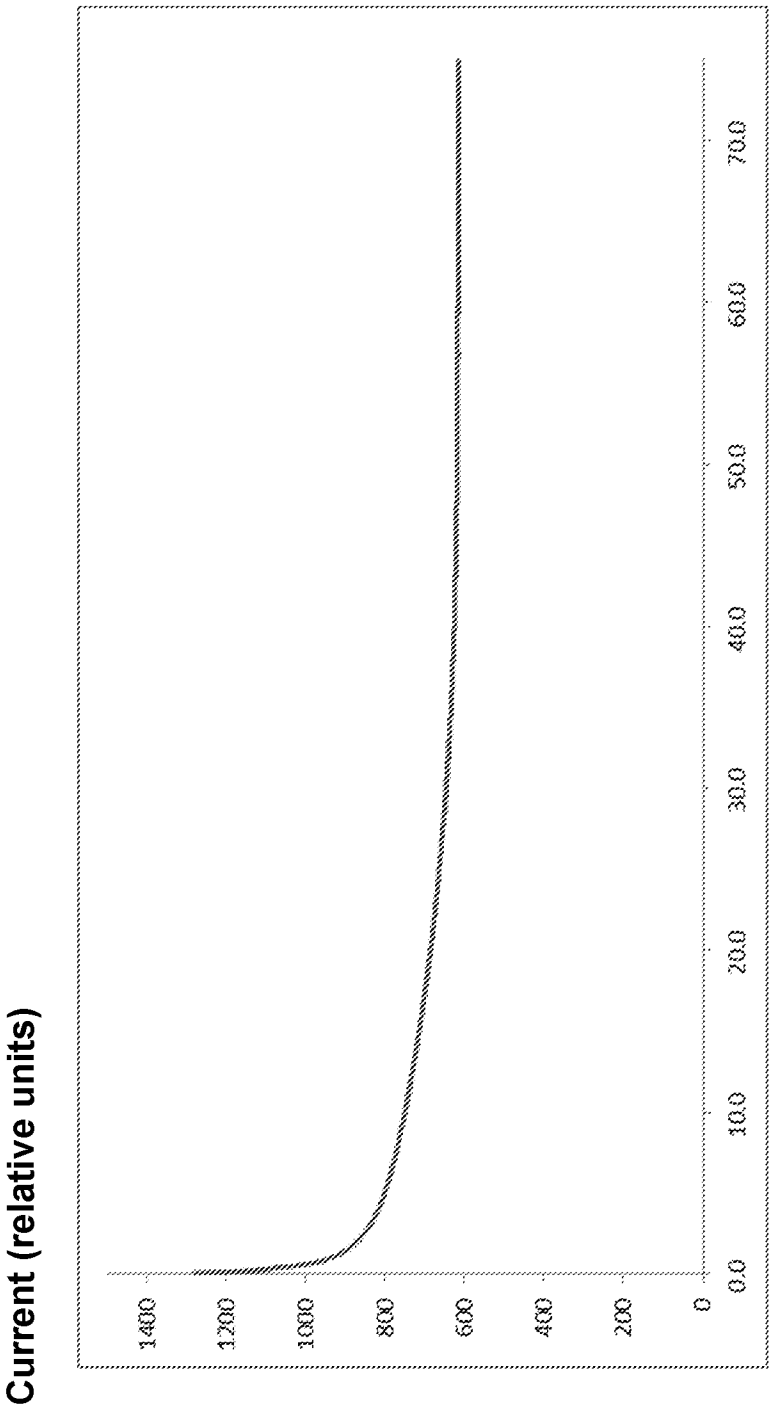

Current (relative units)
1400
1200
1000
800
600
400
200
0
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
Scanning time (second)

Fig. 62

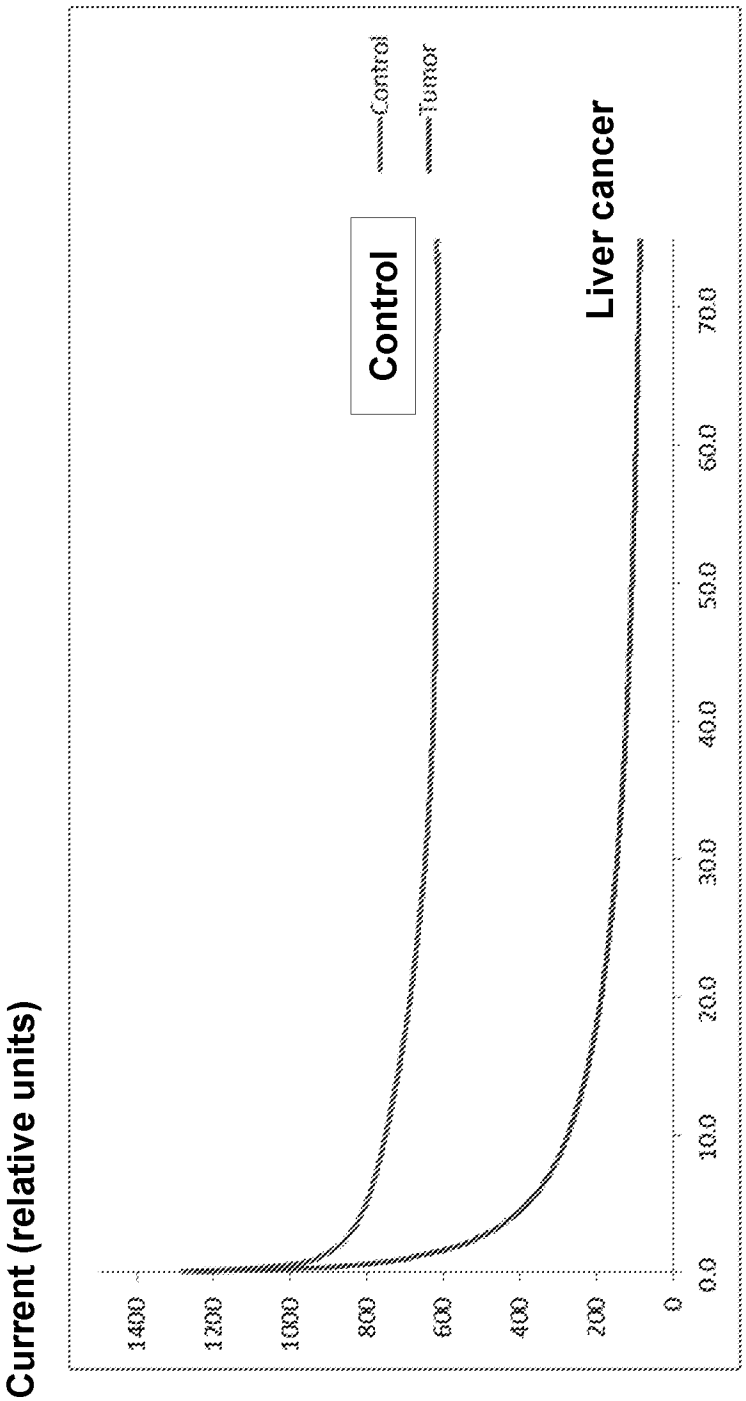

Current (relative units)
1400
1200
1000
800
600
400
200
0
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
Scanning time(s)
Liver cancer
Control
Control
Tumor

Fig. 63

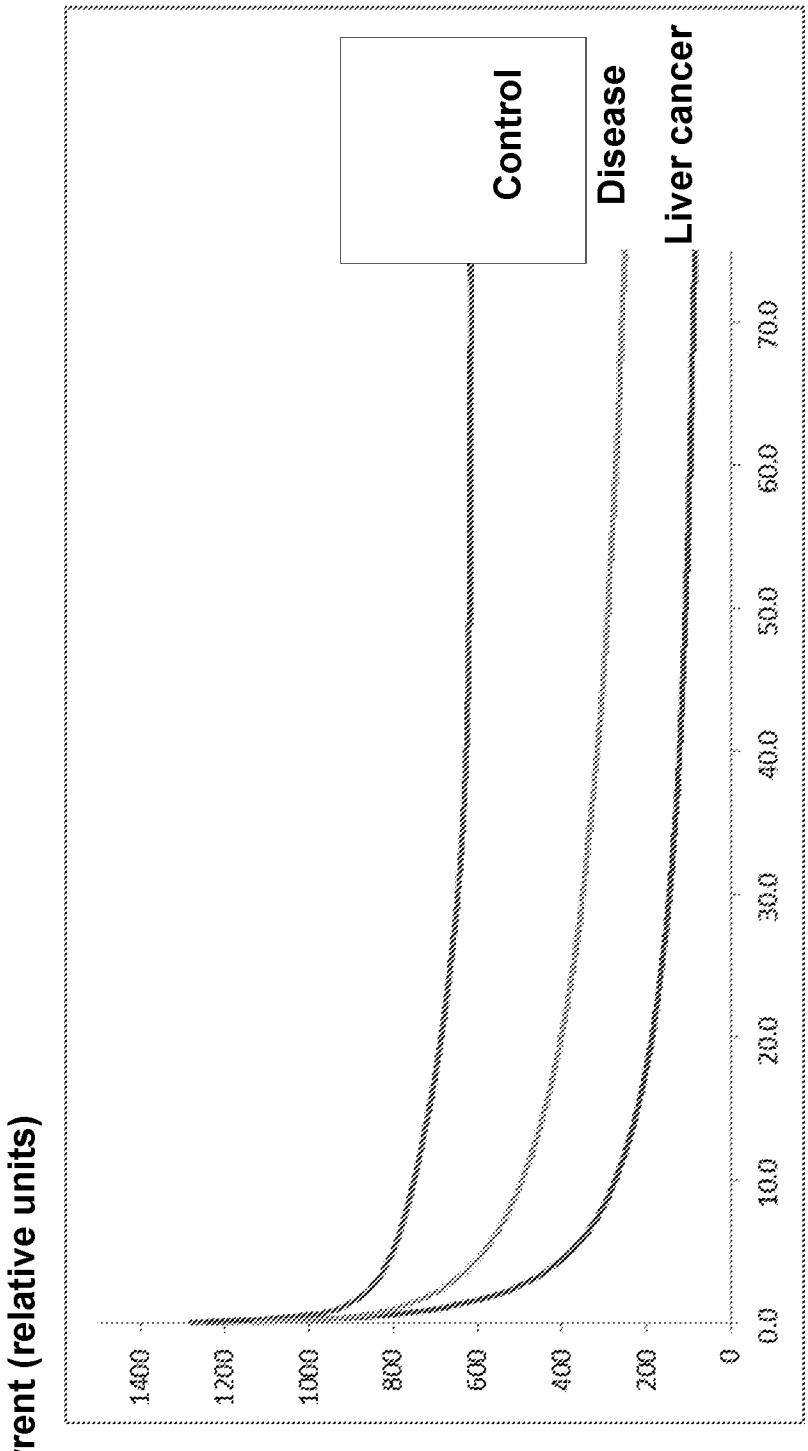

Current (relative units)
1400
1200
1000
800
600
400
200
0
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
Scanning time (second)
Control
Disease
Liver cancer

Fig. 64

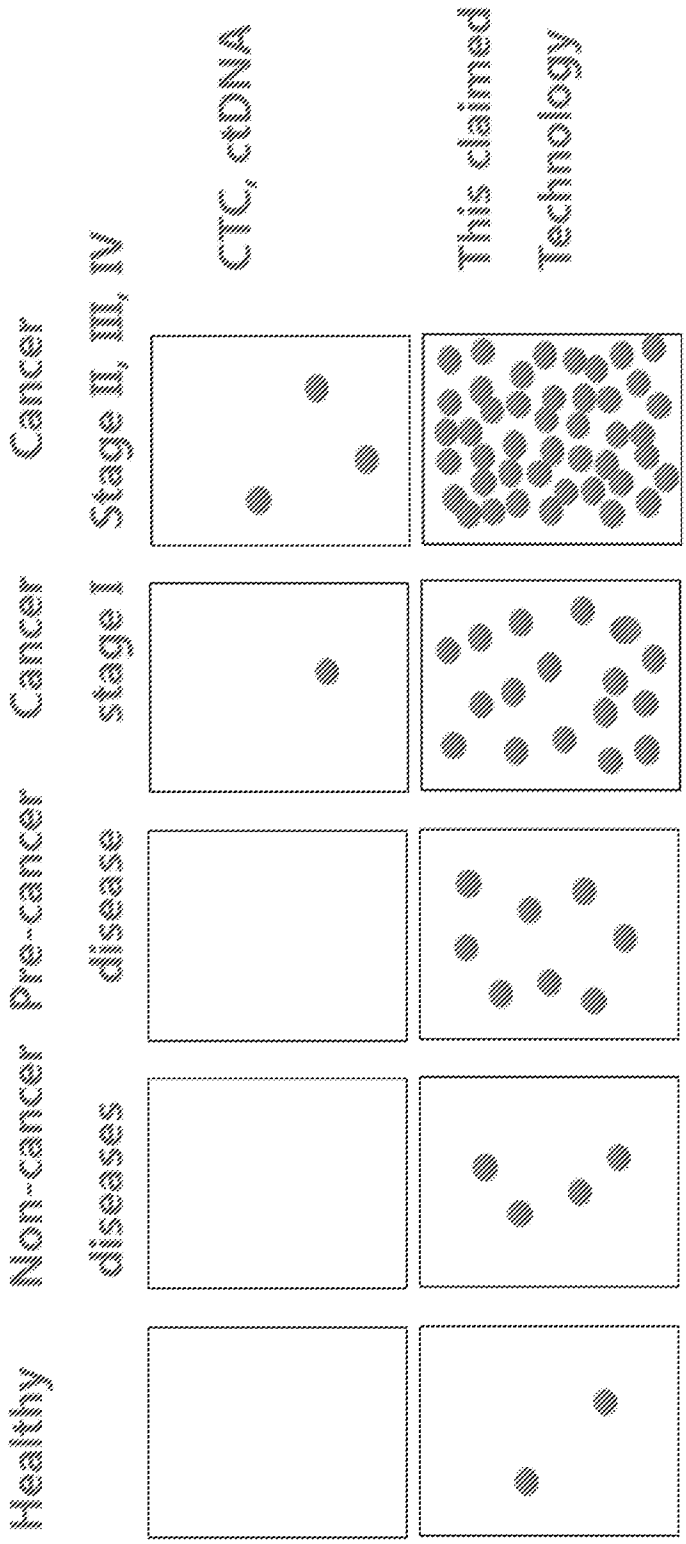
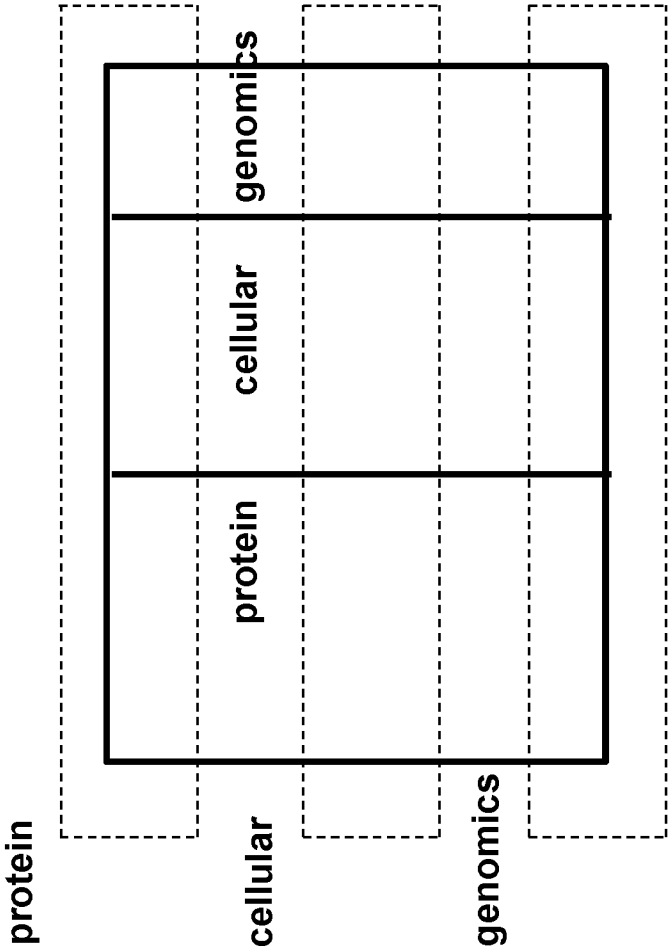

Healthy
Non-cancer diseases
Pre-cancer disease
Cancer stage I
Cancer Stage II, III, IV
CTC, ctDNA
This claimed Technology

Fig. 65



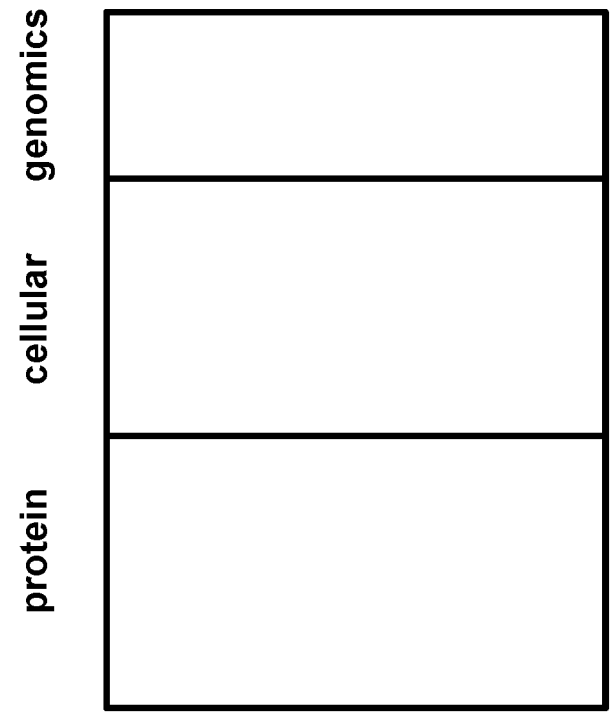


Fig. 66

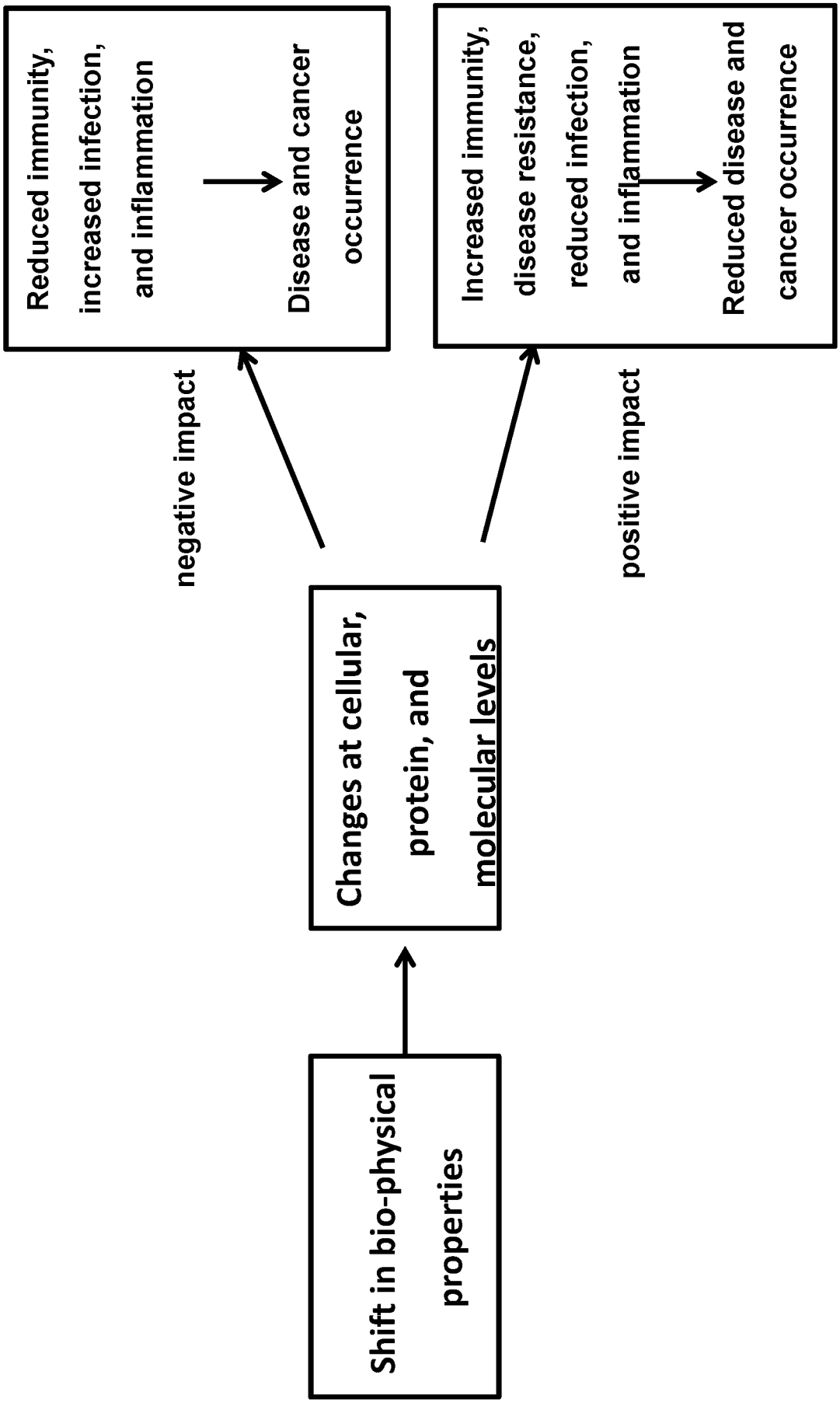

Reduced immunity, increased infection, and inflammation
Disease and cancer occurrence
Increased immunity, disease resistance, reduced infection, and inflammation
Reduced disease and cancer occurrence
negative impact
positive impact
Changes at cellular, protein, and molecular levels
Shift in bio-physical properties

Fig. 67

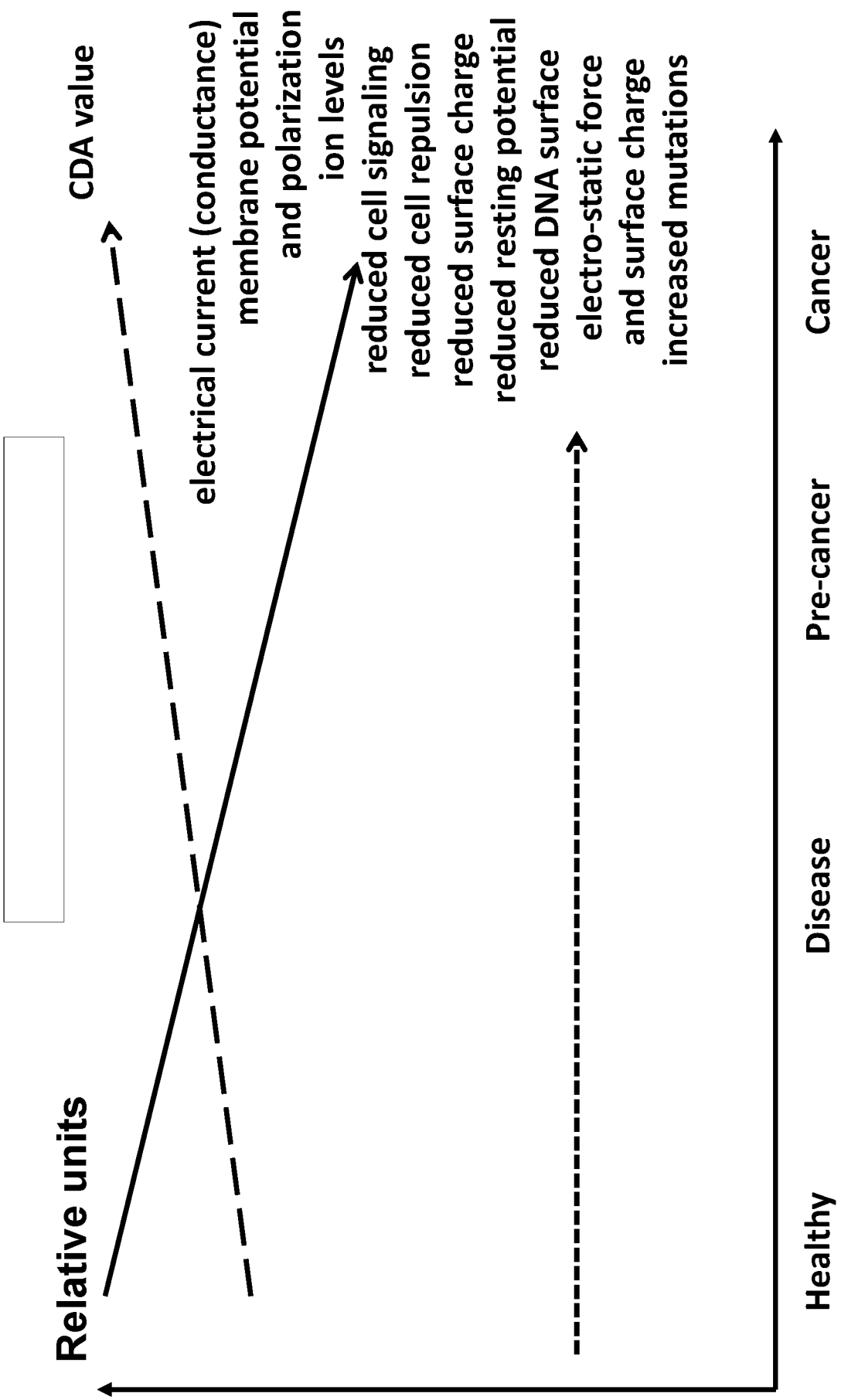

Relative units
CDA value
electrical current (conductance)
membrane potential
and polarization
ion levels
reduced cell signaling
reduced cell repulsion
reduced surface charge
reduced resting potential
reduced DNA surface
electro-static force
and surface charge
increased mutations
Healthy
Disease
Pre-cancer
Cancer

Fig. 68

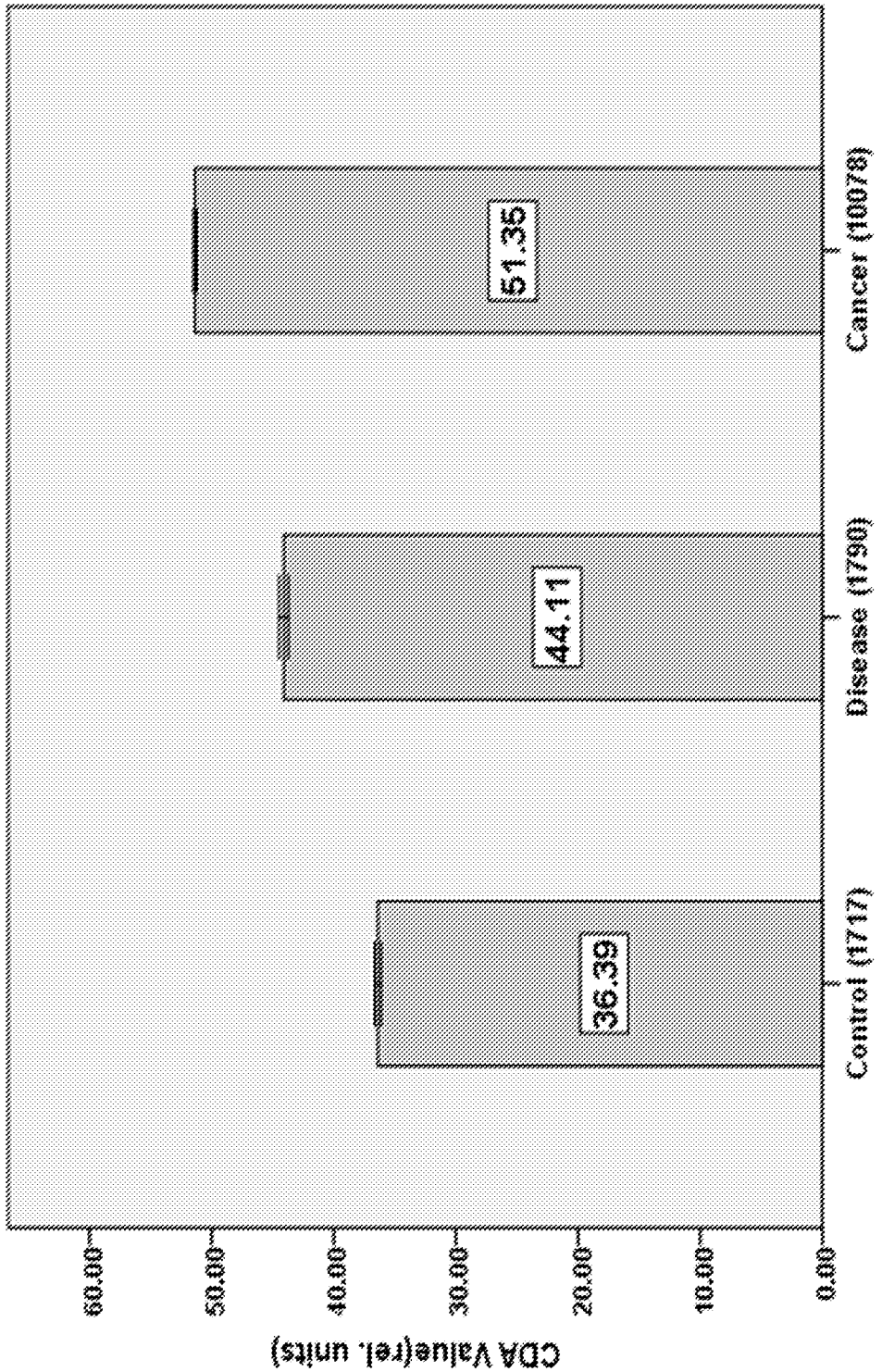

CDA Value(rel. units)
60.00
50.00
40.00
30.00
20.00
10.00
0.00
36.39
44.11
51.35
Control (1717)
Disease (1790)
Cancer (10078)
Group

Fig. 69

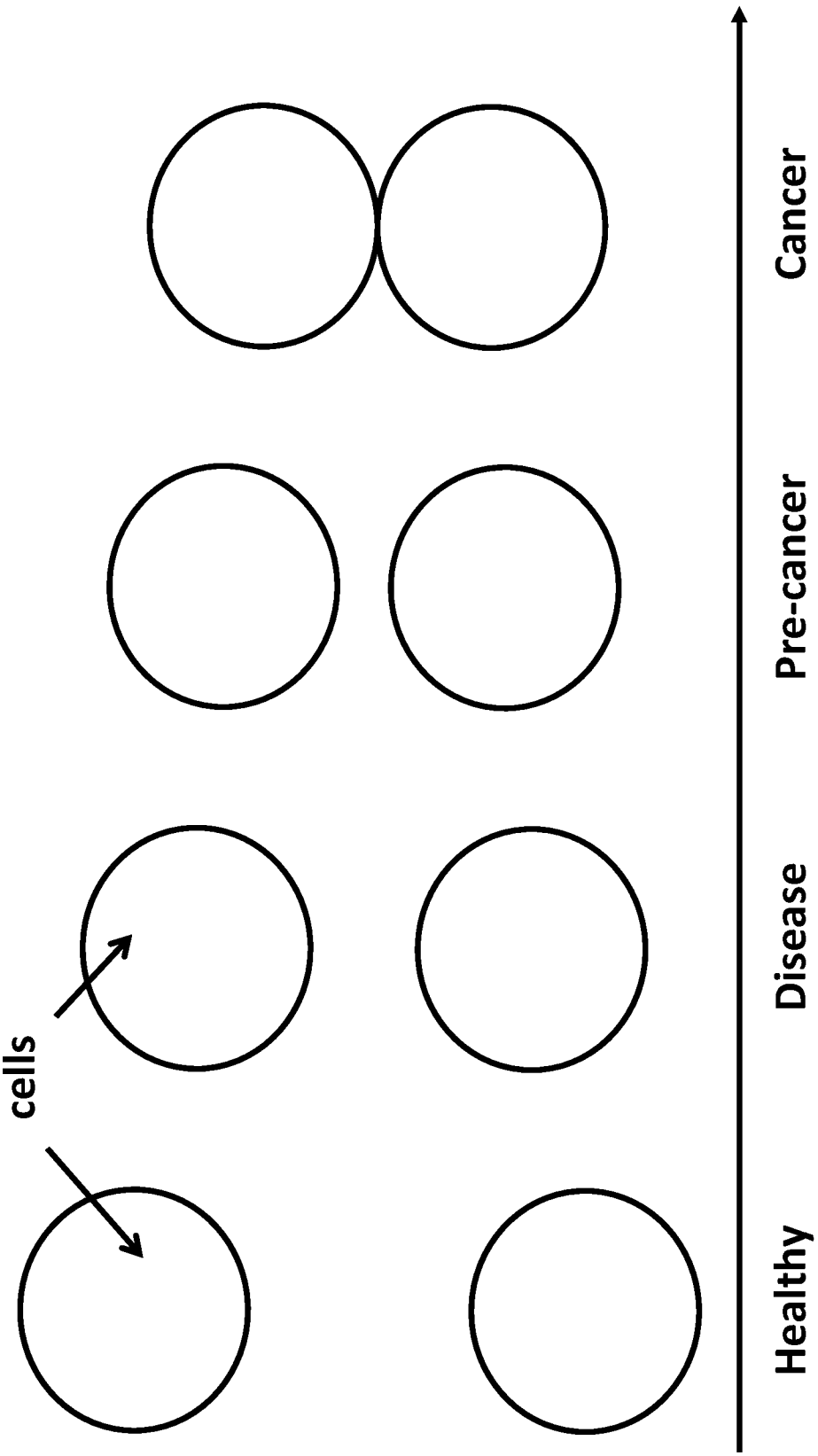


Fig. 70

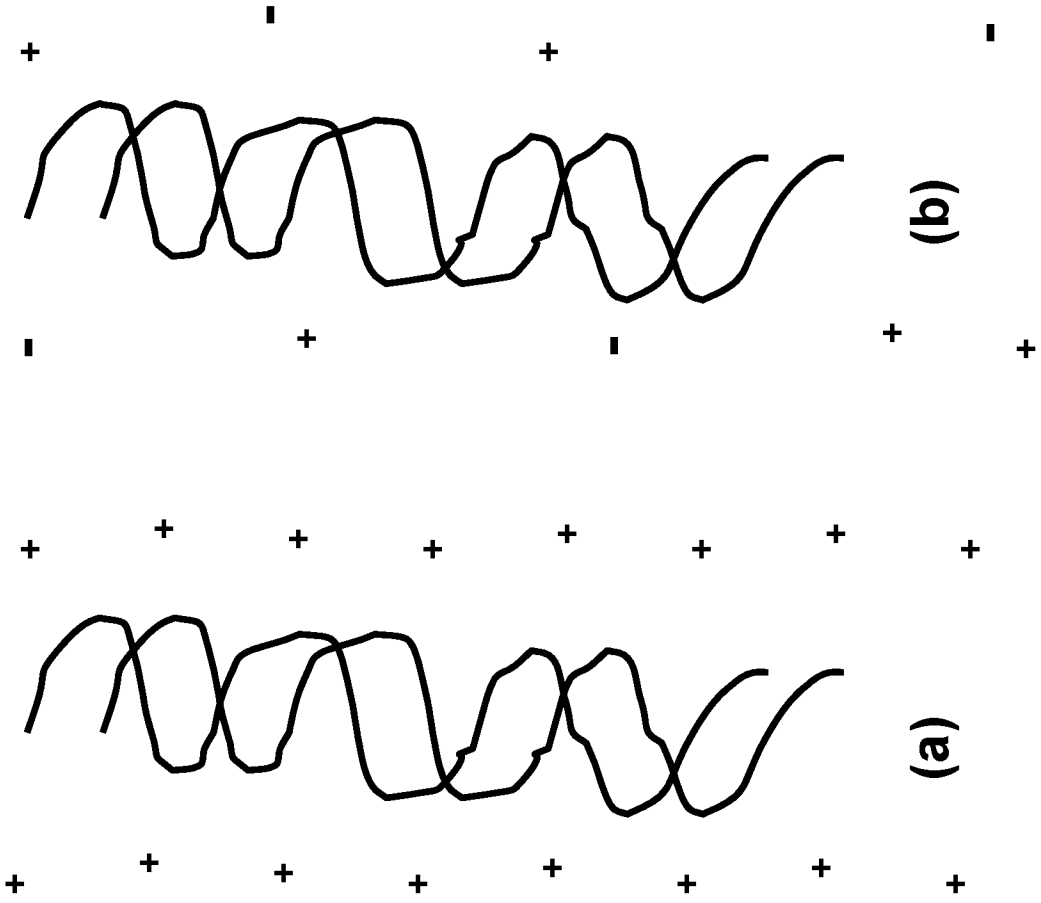


Fig. 71

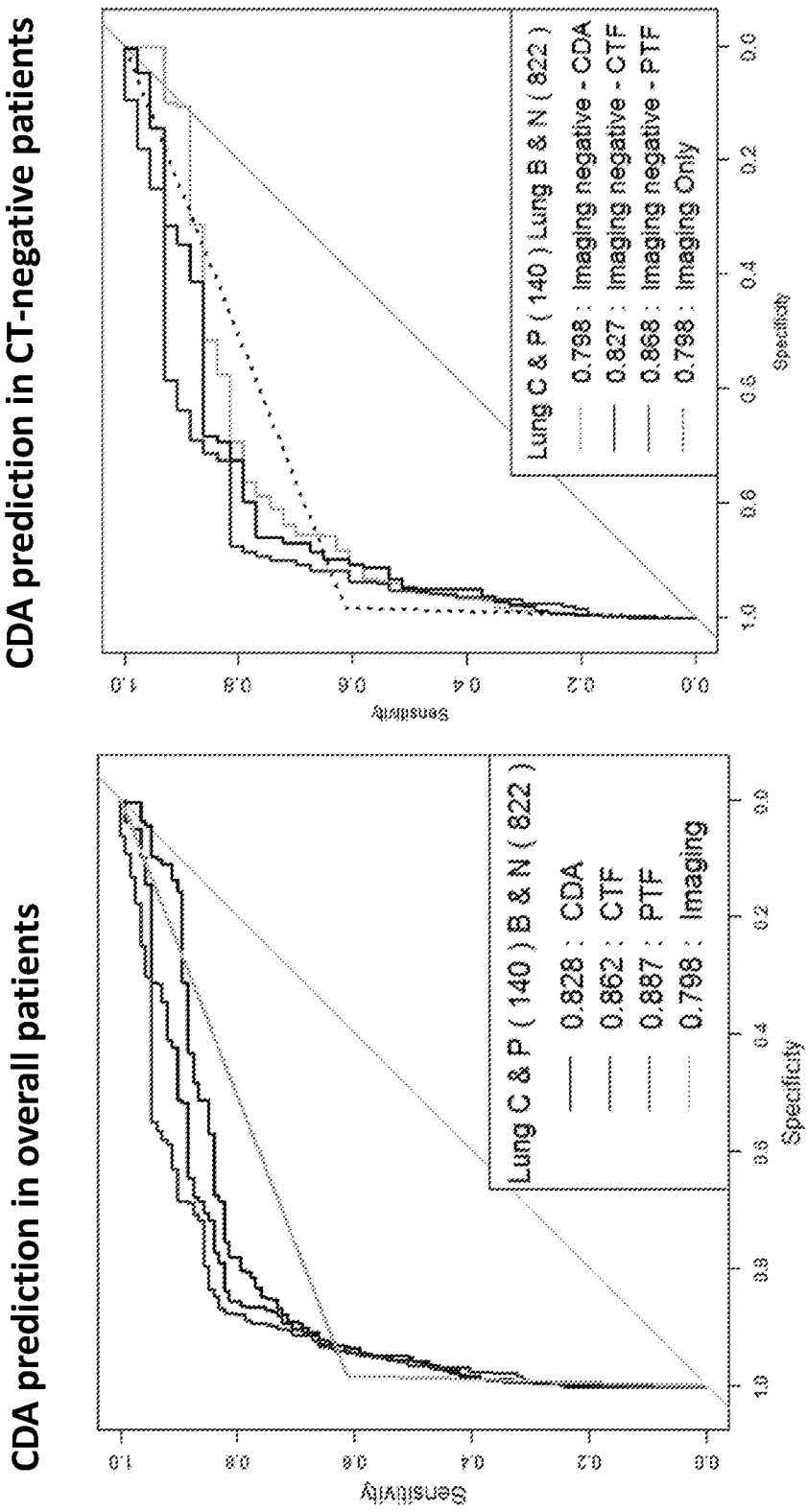

CDA prediction in overall patients
Sensitivity
Specificity
Lung C & P (140) B & N (822)
0.828 : CDA
0.862 : CTF
0.887 : PTF
0.798 : Imaging
C&P : Cancer + precancerous lesion
B&N: benign + normal
CDA prediction in CT-negative patients
Sensitivity
Specificity
Lung C & P (140) Lung B & N (822)
0.738 : Imaging negative - CDA
0.827 : Imaging negative - CTF
0.868 : Imaging negative - PTF
0.798 : Imaging Only
C&P : Cancer + precancerous lesion
B&N: benign + normal

Fig. 72

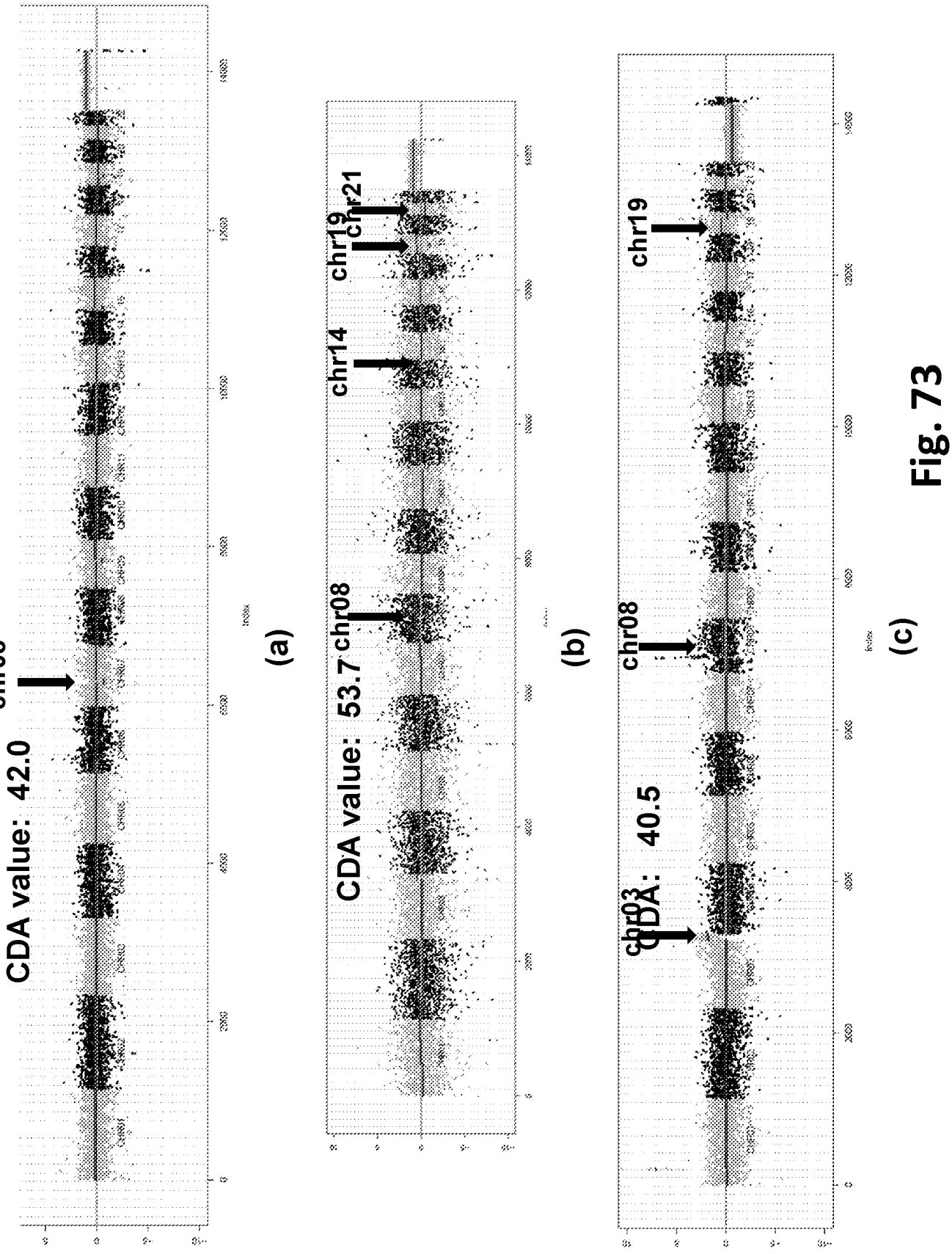

CDA value: 42.0
chr08
(a)
CDA value: 53.7
chr08
chr14
chr19
chr21
(b)
chr08
CDA: 40.5
chr03
chr19
(c)

Fig. 73

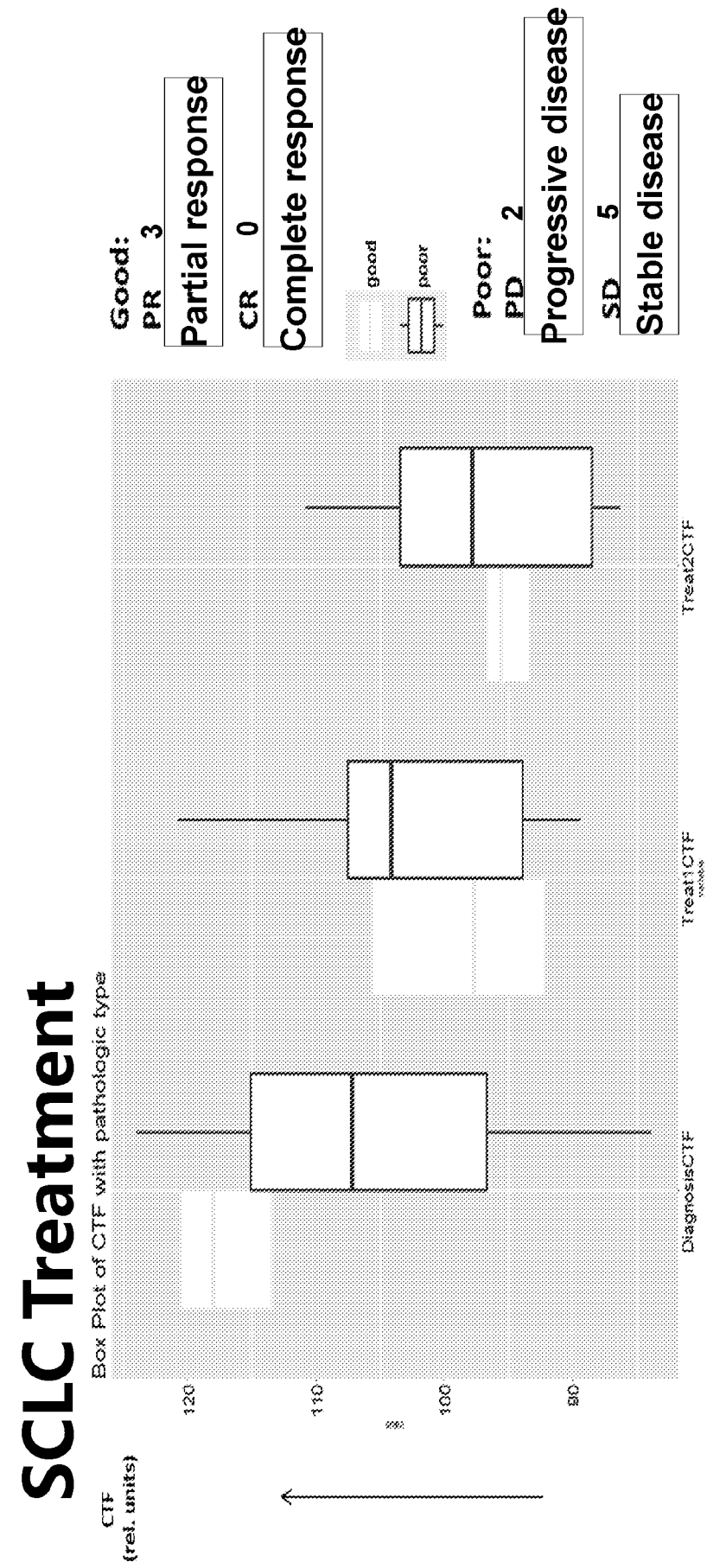

SCLC Treatment
Box Plot of CTF with pathologic type
CTF
(rel. units)
120
110
100
90
DiagnosisCTF
Treat1CTF
Treat2CTF
Good:
PR 3
Partial response
CR 0
Complete response
good
poor
Poor:
PD 2
Progressive disease
SD 5
Stable disease

Fig. 74

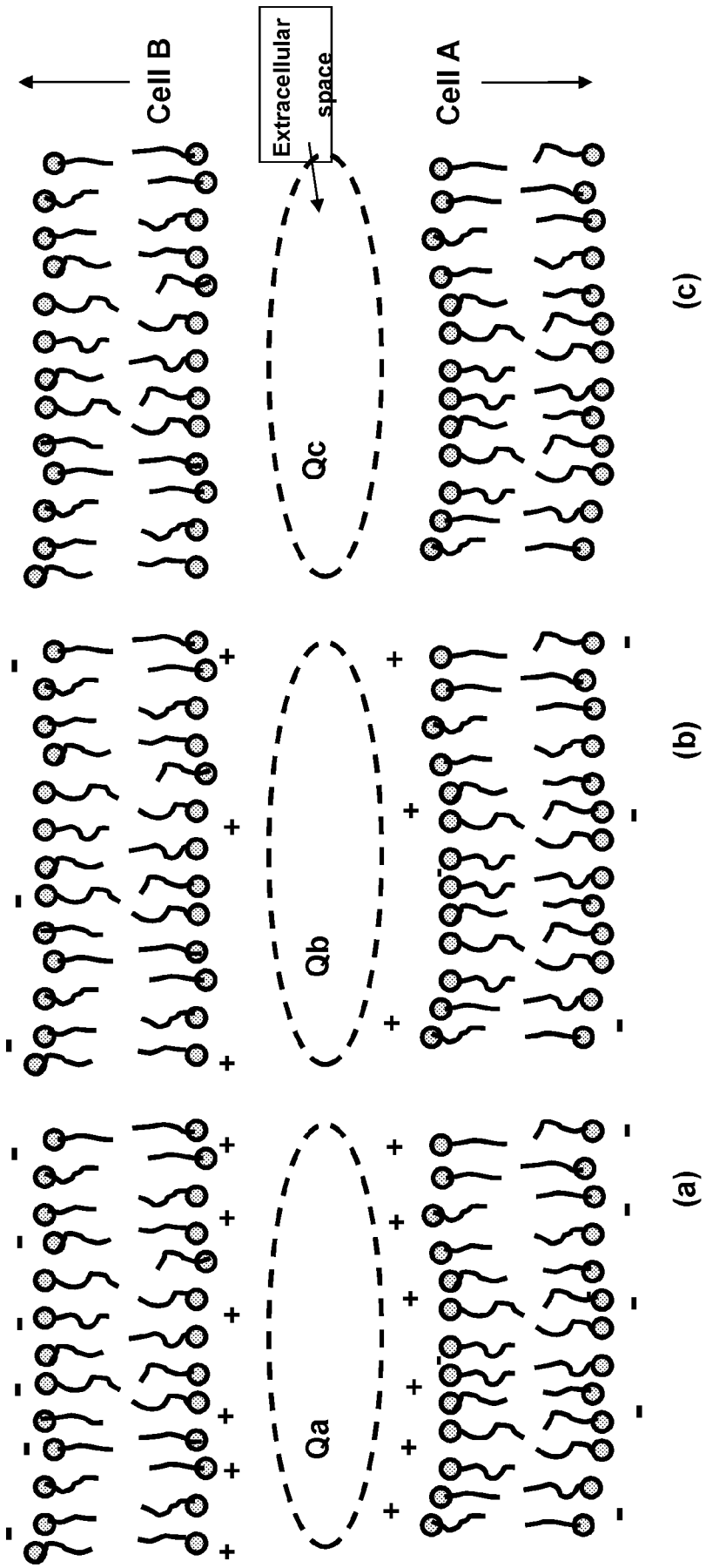


Fig. 75

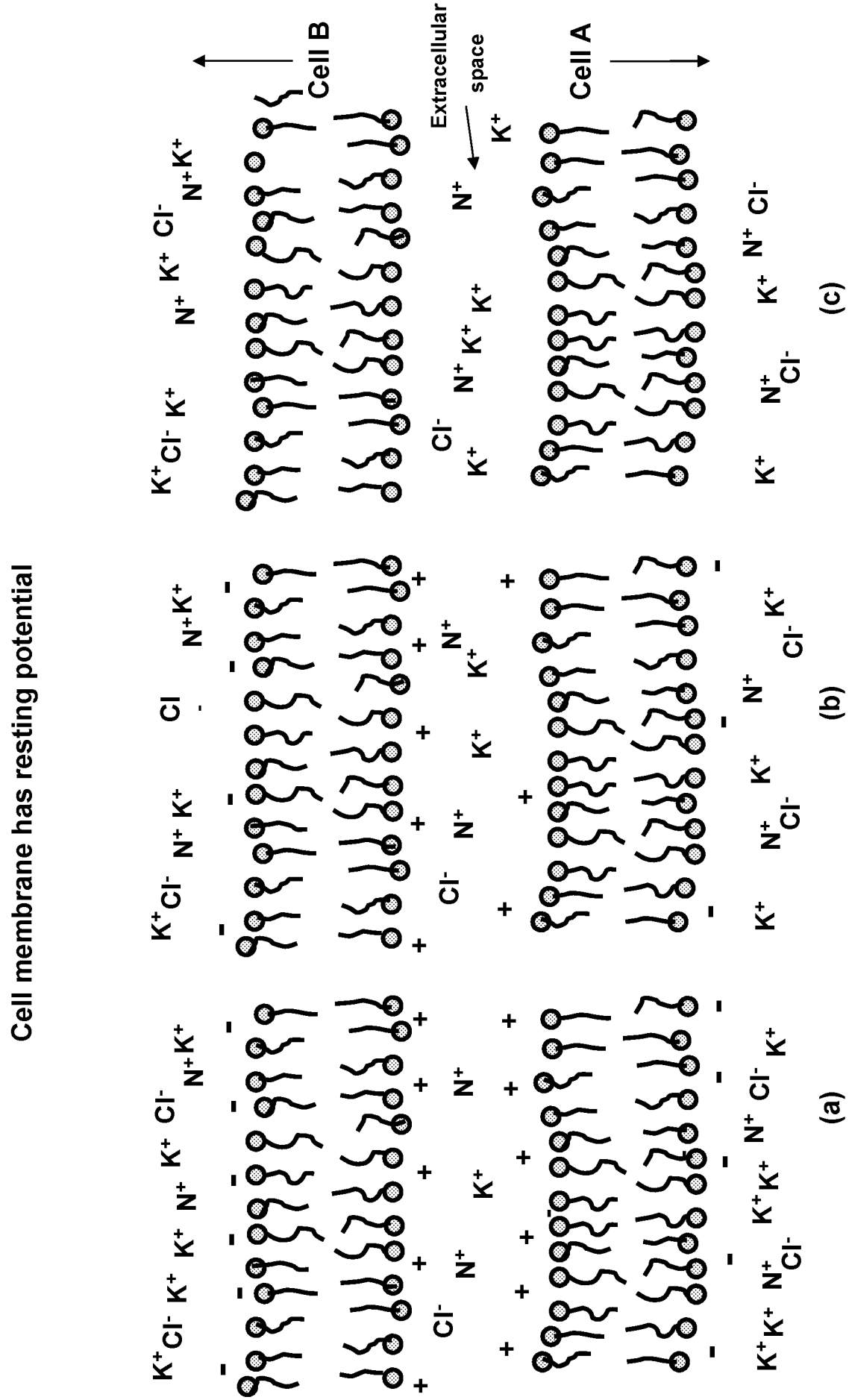

Cell membrane has resting potential
Cell B
Extracellular space
Cell A
(a)
(b)
(c)

Fig. 76

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/28785

A. CLASSIFICATION OF SUBJECT MATTER

IPC - B01L 3/00; C12Q 1/68; G01N 27/26, 29/24, 33/50 (2019.01)

CPC - B01L 3/5027; C12Q 1/68; G01N 27/26, 29/24, 29/2437, 33/50, 33/5005, 33/5091

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014/0342441 A1 (YU, CC et al.) 20 November 2014; paragraphs [0006], [0009], [0045], [0179], [0249], [0287], [0322], [0332]	1-2, 3/1-2, 4/1-2, 5/4/1-2, 6/5/4/1-2, 26-27, 34, 39, 41-49, 112-113, 114/112-113
X	US 2016/0010155 A1 (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK) 14 January 2016; paragraphs [0008], [0009], [0035], [0102]	68-69, 70/68-69, 71/70/68-69
X	US 8,442,629 B2 (SUZUKI, K et al.) 14 May 2013; 2nd column, lines 18-32; 3rd column, lines 29-53; 5th column, lines 32-42	75-76, 77/75-76
X	US 2017/0027485 A1 (ANPAC BIO-MEDICAL SCIENCE CO, LTD) 2 February 2017; entire document	1-2, 49, 112
P, X	US 2018/0259501 A1 (YU, CC) 13 September 2018; entire document	1-2, 49, 112

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 August 2019 (12.08.2019)	27 AUG 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/28785

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7-25, 28-33, 35-38, 40, 50-67, 72-74, 78-111, and 115-126
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.