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- (71) Applicant: **QL, Huan** [CN/CN]; #302, Unit 2, Ruimin Yaju, Gongzhuling City, Gong Zhuling, Jilin 136100 (CN).
- (72) Inventor: **COHEN, Assaf**; #07-07, 48 Nanyang Crescent, Singapore 637121 (SG).
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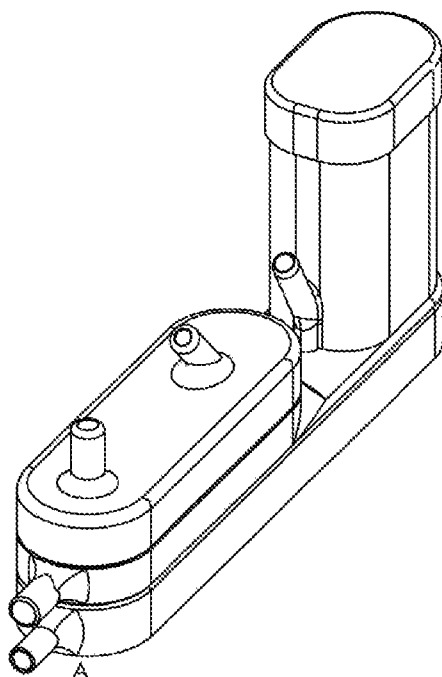


Figure 6A

(57) Abstract: It relates to a field of sensing and diagnostics, in general, and to an analytical device, which performs automatic on-site measurements of toxins, pathogens, heavy metals, explosives and any other analytes of interest.

Method and System for Analyte Sensing

BACKGROUND OF THE INVENTION

Development of rapid, portable, sensitive, inexpensive and reusable molecular sensors has been of growing importance. Amongst the numerous sensors including, for example, optical, electrochemical and piezoelectric, magnetic-based sensors utilizing magnetic particles have gained popularity over recent years due to their unique properties allowing for efficient target capture and separation, relatively high sensitivity, and signal amplification.

The aforementioned magnetic particles have found wide usage in many sensing applications, such as in-vitro assays, cell sorting and target-specific MR contrast imaging. Easy surface functionalization of magnetic particles makes them attractive for use as labels and probes in biosensing applications. US 4,452,773 relates to colloidal sized particles composed of magnetic iron oxide (Fe_3O_4) coated with a polysaccharide, preferably dextran, or a derivative thereof having pendant functional groups. The particles are used to label and separate cells, cellular membranes and other biological particles and molecules by means of a magnetic field.

US 8,420,055 mentions amine functionalized magnetic nanoparticle compositions and processes for their preparation. These nanoparticles coated with various biomolecules are used in vitro assays, cell sorting applications and target specific MR contrast imaging.

Various mechanisms have been used to report the presence of magnetic particle-based labels and probes. Ferreira H A et al, "*Biodetection using magnetically labelled molecules and arrays of spin valve sensors*", Journal of Applied Physics, Vol. 93, no. 10, 2004, pages 7281-7286, reports an on-chip spin-valve to detect surface binding of magnetic particle labels; WO 2005/010543 and WO 2005/010542 A2 mention the use of a Giant-Magneto-Resistance (GMR) sensor for detection of stray fields generated by magnetized particles. US 2008/0309329 A1 relates to a magneto-resistive sensor for detection of a magnetic field arising from the presence of

magnetic particles. U.S. Pat 2010/0259254 A1 mentions a microfluidic device comprising a magnetic field generator and GMR for detection of stray fields generated by magnetized beads used as labels for biological molecules.

US 20080206104 A1 provides a method, a device and a system for determining the concentration of analytes in a fluid containing polarizable or polarized magnetic labels applied to biomolecular diagnostics.

US 20090170212 A1 relates to methods and (bio)sensor systems based on magnetic particles which are transported laterally under magnetic fields over a sensor surface with analyte specific probes. US 20110050215 A1 mentions a magnetic system for biosensors or a biosystem, wherein magnetic particles, which interact with molecules, are brought into a magnetic field, in order to be influenced via magnetic attraction or repulsion forces.

SUMMARY OF THE INVENTION

One aspect of the present invention is a method for sensing an analyte in a flow system using magnetic beads as sensing species, comprising the following steps:

- a) pumping the flow, which contains an analyte into the system;
- b) filtering off magnetic impurities from the flow;
- c) reacting the analyte with a binding entity attached to the magnetic beads inside a reaction cartridge, thereby releasing the magnetic beads from the cartridge into the flow;
- d) accumulating the magnetic beads in the collecting area; and
- e) signal recording.

Another aspect of the present invention is a device for use in the method of the present invention comprising:

- a) Housing;
- b) Inlets;
- c) Pump pumping the flow into the device;
- d) Tubing carrying the flow inside the device;

- e) Filters;
- f) Valves;
- g) Collecting chamber;
- h) Reaction chamber containing one or more reaction cartridges;
- i) Amplification chamber accumulating and magnetizing the magnetic beads in a magnetic field;
- j) Control unit to regulate the flow;
- k) Magnetic beads collection chamber;
- l) Signal transduction unit amplifying and converting the flow of the magnetic beads into an electrical current; and
- m) Processing unit processing the obtained electrical current for readout.

Various embodiments of the invention may allow various benefits, and may be used in conjunction with various applications. The details of one or more embodiments are set forth in the accompanying figure and the description below. Other features, objects and advantages of the described techniques will be apparent from the description and drawings and from the claims.

BRIEF DISCRIPTION OF THE DRAWINGS

The present invention will be understood and appreciated more fully from the following detailed description taken in conjunction with the appended drawings.

Figure 1 is a schematic diagram demonstrating the method according to one embodiment of the present invention.

Figure 2 is a schematic diagram demonstrating the method according to another embodiment of the present invention, including the secondary amplification (or cascade).

Figure 3 is the block diagram showing the electronics of the device.

Figure 4A is a perspective view of the sensing device (Example 1) of the present invention.

Figure 4B is a side view of the sensing device (Example 1) of the present invention.

Figure 4C is a cross-sectional front view (A-A) of the sensing device (Example 1) of the present invention.

Figure 5A is a perspective view of the sensing device (Example 2) of the present invention.

Figure 5B is a side view of the sensing device (Example 2) of the present invention.

Figure 5C is a cross-sectional front view (A-A) of the sensing device (Example 2) of the present invention.

Figure 6A is a perspective view of the sensing device (Example 3) of the present invention.

Figure 6B is a side view of the sensing device (Example 3) of the present invention.

Figure 6C is a cross-sectional front view (A-A) of the sensing device (Example 3) of the present invention.

Figure 7A is a perspective view of the sensing device (Example 4) of the present invention for simultaneous sensing of multiple analytes

Figure 7B is a front view of the sensing device (Example 4) of the present invention for simultaneous sensing of multiple analytes.

DETAILED DESCRIPTION OF THE INVENTION

In the following description, various aspects of the invention will be described. For purposes of illustration, specific configurations and details are set forth in order to provide a thorough understanding of the invention. However, it will be apparent to one skilled in the art that the invention is not limited to the specific details presented herein. Furthermore, well-known features may be omitted or simplified in order not to obscure the invention.

Amplification of the signal obtained from magnetic beads circulated in a flow system by their accumulation in a magnetic field is in the core of the present invention. Such accumulation can also be achieved through a magnetic attraction, chemical or physical reaction, enzymatic reaction, physical separation of the beads and the like.

Reference is now made to **Fig. 1** schematically showing the method according to one embodiment of the present invention. Analyte molecules **104** inside liquid flow **102** or any other carrying media, such as air or gas, passing cartridge **106** are subjected to the reaction with matching binding entity **112** inside the cartridge. Inner walls or surface of cartridge **106** from inside is functionalized with either native analyte **104** or analyte-analogue molecules **109**, which are also capable of reacting with binding entity **112**. Analyte-analogue molecules **109** normally have lower affinity to binding entity **112** than analyte **104**. Different prior-art techniques may be used for surface functionalization of cartridge **106**, followed by immobilization of molecules **104** or **109** using different cross-linkers. For example, surface functional groups may include carboxyl, amine, N-hydroxy succinate (NHS)-activated, thiol, epoxy, hydroxyl, and tosyl. Functional groups can be activated for coupling using, for example, the EDC-coupling chemistry for carboxylates, or glutaraldehyde for amines, in order to attach them to appropriate functional group of molecules **104** or **109**. Alternatively, surface tosyl-, cyanogen bromide, NHS-activated and epoxide groups may be used to attach the molecules directly without cross-linking agents.

In one aspect of the invention, binding entity **112** may be any molecule, macromolecule, biomolecule, material or composite which is capable of specifically recognizing analyte molecules **104**. Some examples of binding entity **112** are aptamers, nucleic acids, oligonucleotides, polymers, imprinted polymers, antibodies, enzymes, proteins, Fabs or phage displays.

In another aspect of the invention, magnetic beads **108** may be any type of magnetic, ferromagnetic, paramagnetic, superparamagnetic, superferromagnetic, particles or nanoparticles or large magnets made of any magnetic material. Magnetic beads have high

surface areas per unit volume, good stability, and enable fast kinetic processes involving solution species compared to bulk solid surfaces. A great advantage of magnetic beads or nanoparticles, as opposed to non-magnetic nanoparticles, is their ease of manipulation with simple, inexpensive magnets. Very efficient isolation of analytes from liquid samples can be achieved inside or outside of the detection system, so that detectors can be versatile and need never be exposed to the complex liquid sample matrix.

novel in this invention is the use of ferromagnetic particles for detection. Unlike prior art techniques which utilizes paramagnetic particles such as iron oxides in order to isolate them and preform a detection using florescent labeling or NMR for example, ferromagnetic particles such as NdFeB particles can undergo temporary or permanent magnetization after exposure to external field. This enable direct quantification of the number of particles with ease and without the necessity of utilizing a secondary process for quantification. Moreover, as they generate no magnetic forces prior their magnetization, the use ferromagnetic particles solves many issues of unwanted coagulation and aggregation raised in prior art use of paramagnetic particles. Additionally ferromagnetic particles may be coated with any material of form that further prevent unwanted aggregation such as materials that will result in mutual repulsion. A specific additional benefit of this is that after magnetization has occurred, these said particles that are natively repulsive to each other will attract and aggregate forming a single entity that is much easier to isolate and quantify by measuring it's weight, magnetic field, light emission intensity, the amount of force it applies when pressing against a surface connected to force measurement device under exposure to large magnetic field that propels the particles to move, etc.

Another novelty that is derived from the use of ferromagnetic particles can arise from using magnetic particles with intrinsic or extrinsic parameters that results in only temporary magnetization. This can allow recycling of the beads, reattaching them in the immunoassay format for another round of sensing. This can be used to develop a multiuse sensor by performing temperature, ionic concentration or pH change cycles to separate the binding entities from the analyte or analogues. At this cycle a magnetic filter, such as electromagnet,

can be used to ensure that the magnetic particles do not leave the system, while the fluid containing unwanted analyte and matter is discarded. After lapse of time that is sufficient to unbond and discard unwanted analyte the binding conditions can be reset to allow binding of the functionalized analyte analogue to the binding entity. After a lapse of time that is sufficient to allow rebinding, the system can be reused as a sensor again.

Magnetic beads **108** used in the present invention may be commercially available or prepared in the lab. According to a particular aspect of the present invention, magnetic beads **108** are ferromagnetic beads, which are the most useful for systems requiring magnetic separation and transport as they become magnetic in an applied magnetic field, but have zero magnetization in the absence of a magnetic field. These beads are often called “superparamagnetic”, while ferromagnetic beads feature permanent magnetism after they are exposed to applied magnetic field.

In yet further aspect of the invention, magnetic beads **108** are paramagnetic beads, which are the most useful for systems requiring magnetic separation and transport as they become magnetic in an applied magnetic field, but have zero magnetization in the absence of a magnetic field. These beads are often called “superparamagnetic”, while ferromagnetic beads feature permanent magnetism after they are exposed to the applied magnetic field. The most common examples of paramagnetic beads have magnetic iron oxide cores and non-magnetic polymer shells featuring surface chemical functionality for attachment of binding entity **112**. The magnetic core may also consist of a collection of paramagnetic nanoparticles embedded in a polymer core. Beads with sizes in the range 100 nm to 100 μ m in diameter are commercially available with variability in size $<\pm 5$ %. Suppliers include Solulink, Invitrogen, Bangs Labs, Merck, and others. Bead size determines sedimentation rate and mobility in a liquid flow. The outer polymer shell serves to add surface functional groups to the bead and protects the metal oxide core magnetic core from external media. The outer shell may also consist of agarose, cellulose, porous glass or silica.

The magnetic beads are also available with surface molecules such as streptavidin, biotin, protein A, protein G, IgG, IgE and IgM. The beads pre-coated with streptavidin may capture the biotin-labeled binding entities. Protein A coated surface may selectively bind to Fc regions of antibodies for orientated immobilization.

Superparamagnetic beads are commercially available with coatings of either organic functional groups to attach biomolecules like antibodies and enzymes, or pre-coated with biomolecules that can bind specific partners.

According to a further aspect of the invention, magnetic beads **108** are magnetic beads with sizes in the range of 100 nm to 1 mm in diameter, having the polymer-embedded iron oxide nanoparticle cores. Such beads with paramagnetic nanoparticles embedded in a polymer core matrix are superparamagnetic, but may feature multidomain magnetic structures with remnant magnetic moment. They show some degree of magnetic clustering in liquids due to induced magnetism in neighboring particles.

In a further aspect of the invention, magnetic beads **108** are coated with binding entity **112**. Since binding entity **112** specifically recognizes molecules **109**, magnetic beads **108** may be attached to the inner walls, matrix or surface of cartridge **106** in the reaction between molecules **109** and binding entity **112**. This is the step of the cartridge **106** preparation.

When analyte **104** passes through cartridge **106**, it binds to binding entity **112**, thereby replacing molecules **109** and releasing magnetic beads **108** from the cartridge into the flow. Magnetic beads are then separated from the flow by an applied magnetic field to be positioned just above magnetometer **110**. When magnetic beads **108** reach magnetometer **110**, signal proportional to the amount of the beads is read out. This amount of beads **108** is in turn proportional to the amount of analyte molecules **104** in the flow and the amount of time the process was taking place and sample flow rate. Magnetometer **110** may be any commercial magnetoresistive, Hall Effect, coil-based, SQUID, or any other type of device, which is capable of sensing the magnetic field or properties.

Reference is now made to **Fig. 2** schematically showing the method of the present invention including a secondary amplification technique (or so called “magnetic cascade”). In one example, one or more magnetic cartridges **116** are introduced in the system for the purpose of signal amplification. In the example, magnetic beads **108** passing through magnetic cartridge **116** after being magnetized by the magnetic field generator, cause the release of additional magnetic beads **118**, which amplify the signal registered by magnetometer **110**. The quantity and the amount of magnetic beads **108** allowed to remain in cartridge **116** is proportional to the amount of magnetic beads **118** that are released.

In addition, left pane or inlay in **Fig. 2** shows the example when magnetic beads **108** are coated with molecules **109**, while binding entity **112** is immobilized on the walls of cartridge **106**. In this case, analyte **104** from the flow will bind to binding entity **112**, thereby releasing magnetic beads **108** in the flow. Amount of the released beads is proportional to the amount of the analyte in the flow. In this case sensitivity may be higher as no free binding sites on the magnetic beads will exist such in the case where the beads are functionalized with binding entities.

In yet another example, the additional magnetic beads **118** can reside in any suitable place in the same cartridge where the reaction takes place, such as the bottom just before the outlet, where the magnetic beads released by the analyte molecule can trigger the release of more magnetic beads through various reactions such as physical, chemical or enzymatic reactions. The beads can also reside mixed with the assay beads for easier, immediate access and interaction while the bead are still on the surface and not yet suspended in the flow which may make the chances of them interacting with the secondary beads lower. In this form cascade will be initiated immediately upon primary beads release. In a specific case of the enzymatic reaction, the released beads are functionalized with an enzyme that can degrade a linker of parking magnetic beads causing their controlled release. Cascading beads may or may not include enzymatic element of their own that may further amplify the cascade in an exponential rather than linear manner. In another example, primary beads that are released in the displacement assay are magnetized and let to flow in channels or microchannels with

secondary beads residing within. The presence of magnetized beads will cause the controlled released of these beads depending on the exposure time and the amount of primary beads. In another example, both primary and secondary beads can be let to flow again into the same channels, microchannels or chambers, or into another third and fourth chambers to release more and more beads in the magnetic cascade.

One of the advantages of the magnetic cascade system is in that the additional magnetic beads are not dependent on the equilibrium and do not have any risk of being randomly displaced. Thus, the beads of any size can be used in the system to optimize the amplification of the signal. Having very large beads in the range that can even be in thousands of microns can have a huge advantage as it allows for a very easily detection using noncomplex means. In here lies a hidden novelty as today, beads no larger than approximately 3 microns are used in immunoassays. The field of bio sensing using larger beads have never been suggested before as large beads held no advantage. However, as we're directly quantifying the beads without using a secondary label, this have huge advantage the magnetic signal from each bead is proportional to the power of three of the volume. The larger the beads are, the signal is exponentially amplified. Moreover, the use of ferromagnetic beads such as NdFeB or Samarium Cobalt and any other ferromagnetic alloys means that they already natively possesses the ability to generate much higher magnetic field than that generated by paramagnetic particles.

Reference is now made to **Fig. 3** which shows a block diagram of an exemplary system consisting of a central core that may be a microcontroller responsible for coordinating the function of an electromagnet used for trapping and magnetizing the magnetic pollutants and released magnetic particles, and can also receive and manipulate data from other sensors measuring magnetic field (counting released beads), pH, ionic concentration and temperature of the sample (calibrating to the changing binding coefficients). It also handles data processing and automated conversion based on stored calibration table and outputs data to a LCD display or transmitted to external computer or mobile devices via USB, Wi-Fi, GSM for further processing, user alerting or storage. An external energy harvester, such as solar panel, turbine and vortex induced vibration that harvest energy from the surrounding flow may be used to

produce a completely autonomous system that can function for long times in remote areas. The use of multiple cartridges that are hibernated (by freezing for example) to increase their lifespan can allow even longer lapse of time without human intervention. Only one cartridge is used until it expires whereupon another cartridge is being automatically commissioned. The system may also be adjusted to perform auto calibration by deliberately exposing itself to known amounts of analyte or in many similar way at needed times. To prevent false positives and false negatives, the system can include multiple cartridges that complement each other's signals. Another optional way to allow better sensitivity is to ensure that the media conditions are strictly monitored. Anti-bacterial, omniphobic coating is applied to the internals of the device by covalent binding using adhesives to bind superhydrophobic NPs for example. This both to save energy required to drive the fluid, especially in microchannels, and also prevent microorganisms' growth. Preservatives can periodically or constantly secrete antibiofouling agents into the system and the system's pH, flow rate, ionic concentration, internal pressure and temperature can be strictly controlled to allow more accurate quantification of the analyte.

Another novelty of this detection scheme is the fact that the use of magnetic beads allows for time-dependent signal sensitivity. Unlike all prior art techniques which deal with finite, usually very small samples, this new method allows for continuous sample input. The continuous sample interacting with the binding entity results in beads displacement overtime that is proportional to the amount of analyte in the sample. Unlike prior art techniques which require ultra-sensitive transduction systems in order to quantify very small amount of analyte, this time-dependent process allows for signal amplification overtime by collection of more and more beads until the signal levels are large enough for even crude transduction units to read it without error. The signal read can be correlated along with the sample flow rate data and the amount of time the process was allowed to take place, to the original amount of analyte in the sample being tested. Thus, this method of sensing allows for unprecedented accuracy without the necessity of a complex transduction unit by collecting beads and amplifying the signal overtime.

Below are the exemplary devices for use in the method of the present invention.

EXAMPLES

Reference is now made to **Figs. 4A-C** showing the first exemplary sensing device according to one embodiment of the present invention. The device is placed in housing **420**. Flow enters the device through the inlet, located at pump **406**. The inlet may contain filters for solid particles present in any sample effluent and may also contain a solids shredder and water injection and mixing channel before the filter for cases where the sample being diagnosed is a solid sample, for example. A condenser or a device such as gas-to-fluid flow exchange coulomb or otherwise may be introduced if the sample being diagnosed is in gaseous form.

The flow passing through tubing **408** reaches the first valve **410** mounted just after the flow passes above the magnetic field generator **412**. The magnetic field generator is kept turned-on until the signal readout moment, or in another case the magnetic field generator can work in a waveform signal which oscillates from plus to minus and the signal is taken when the field strength is zero, thereby separating any residual magnetic material from the flow that was able to elude the initial filters. The separated magnetic material is collected, and can be further washed away from the device through outlet **418**.

Sample continues to flow through tubing **408** and enters immunoassay cartridge **402**, where the reaction takes place (as explained above). As a result of the reaction, the magnetic beads are released into the flow, leave cartridge **402** via outlets **404**, pass through tubing **408** (where the tubing cross section may become wider in order to slow down the beads) and collected in the chamber **416**, which is designed to control the flow, in order to maximize the collection by magnetic field generator, reaching magnetic field generator **412** where they are separated from the flow as they're being trapped and magnetized by the magnetic field. After that, magnetic field generator **412** is turned off, and the signal from the magnetic beads is registered with magnetometer **414**. The signal is proportional to the amount of the magnetic beads released, and in turn is proportional to the concentration of the analyte in the sample. The longer the time the beads are collected, the higher the signal and the latter can be interpolated back to indicate the original concentration of analyte in the flow. This time-dependent signal

amplification is controlled by the user which is able to take the measurement at any time depending on the level of sensitivity the user requires. One of the novel aspects of the present invention is that there is no sensor today that would allow an infinite signal amplification such as the one described above. Normal amplification today is done using a secondary amplification reaction, however the use of the micro magnets in the present invention enables this unique way of single-step signal amplification, which allows the use of very cheap and simple components for detection such as magnetometer or even scales.

When using the magnetometer **414**, multiple ways can be used to reduce residual and ambient magnetic noise. One example is to use waveform magnetic field generated by the magnetic field generator (from $+x$ T to $-x$ T), in this way the signal can be taken when the magnetic field is zero, to reduce noise due to residual magnetization. Nevertheless, there are endless ways to reduce noise in this invention and the latter does not mean to confine the invention but an example only.

In yet another example, multiple magnetometers can be installed in the surroundings and measure the signal together with the magnetometer **414** to subtract the residual noise from the actual signal produced by the magnetized beads.

In yet another example, magnetic shielding can be used in conjunction to any other method, to attenuate ambient fields. In an example derived from this case, a permanent magnet can also be used as the magnetic field generator to reduce power consumption. This magnetic field can be attenuated prior to signal measurement by moving the magnet away or alternatively moving a magnetic shield, such as layers of pyrolytic carbon and Mu-metal or any other, in between the magnet and the magnetometer.

Reference is now made to **Figs. 5A-C** showing the second exemplary sensing device according to another embodiment of the present invention. When the system is in use, sample is allowed to flow into upper chamber **502** through inlet **510** which is designed to slow down the flow. Magnetic field generator **520** that is kept-on during normal operation is used to separate

magnetic pollutants that potentially exist in the incoming sample and may disturb accurate measurements later-on.

After predetermined amount of time, the magnetic field generator is turned-off and valve is opened allowing to wash the magnetic components that were previously trapped away from the device through outlet **526**. Otherwise, water flow that may be containing the analyte is pumped through outlet **508** toward inlet **512** of cartridge **504** through tubing (which is not shown in the figure and where ambient parameter measurements may take place). Cartridge **504** is optionally equipped with carefully designed preservative magazine holder **530**, which can optionally release anti-biofouling substance, to increase the lifespan of cartridge **504** (preservative can also be released in any other location in the system such as the main inlet or chamber). The reaction described above takes place on reaction substrate **516** which is designed for maximum diffusion rate and low shear forces and is containing the analyte-analogue molecules complex inside cartridge **504**. As a result of the possible displacement reaction with present analyte, the magnetic beads with the newly bound analyte (or bond analogue in cases when the binding entities are bonded to the matrix) are released, then passed through outlet **518** into lower chamber **524** which is designed to slow down the fluid velocity, and finally collected by magnetic field generator **520** which may be located below the magnetometer and is designed to concentrate the beads directly above the magnetometer for more accurate reading of the sample. Magnetometer **522** can read the signal at any time specified and the signal read will be proportional to the amount of magnetic beads collected which is proportional to the concentration of analyte and the period of time the bead collection was made. As above, the recorded signal is proportional to the amount of the magnetic beads collected from the flow, and in turn is proportional to the concentration of the analyte in the water effluent. Optionally, the device can be equipped with flow control device **514** that further improve the flow patterns and prevent shear forces on the surface of **516**. Device outlet **528** is used to discard the sample effluent from the device.

Reference is now made to **Figs. 6A-C** showing the third exemplary sensing device according to yet another embodiment of the present invention. The main difference of this device compared

to the device shown in **Figs. 4A-C** is in the instillation of one or more flow control devices **602** and **604** installed in the upper and in the lower chambers, respectively. Control device **602** installed in the upper chamber is used to redirect the flow patterns and increase particles retention time (by formation turbulences and forming pseudo chambers) and forcing magnetic pollutants, potentially present in the sample, to stay longer in this filtering chamber and closer to its bottom where the magnetic field is stronger and cavities to trap the particles can also be installed, thus maximizing the collection and filtering effects of the magnetic generator, to ensure the maximum number of the magnetic components polluting the sample are separated before the sample entering reaction cartridge. The top of this filtering chamber, or an additional separate chamber can be equipped with a filter to trap and eject suspended solids that can also be an interfering factor.

In order not to have the filtering unit at all, saving costs when manufacturing smaller devices for example, the magnetic filtration unit can be completely removed and instead blank sample that does not interact with the cartridge can be analyzed by the system to have a reading of the amount of magnetic pollutants natively present in the sample, a value that can be removed later on when measuring for the amount of analyte.

One or more control device **604** installed in the lower chamber is used to redirect the displaced magnetic beads towards the magnetic field generator, to ensure the better collection targeted to be above the magnetometer before the signal readout. Another thing that is new about this specific implementation is that there is only one outlet in the reaction chamber **606**. This results in virtually no flow shear forces near the substrates where magnetic bead are, preventing undesirable magnetic beads displacement due to flow forces. It is important to mention however, that shear forces may be wanted in some implementations where the beads are held by multiple bonds. In these cases shear forces are needed to displace the beads once a number of these bonds was incapacitated due to displacement or other reaction of analyte present in the sample. Other reaction can be enzymatic cleavage for example, of a crosslinker due to activation by a present analyte. Thus it is important to emphasize that displacement, competitive, sandwich or any other immunoassay form that may be implemented in this system

is only an example, the release of beads due to presence of analyte can be done in various way that were previously mentioned prior arts and many ways that will become obvious or be developed specifically in order to the release mechanisms for the new systems described here.

Another implementation of the system and method that is presented here is an “upside down” approach which is having beads flowing around and being observed for example, by binding in sandwiched format or due to surface or chemical modification done due to presence of analyte. This causes the removal of beads from the flow and lower magnetic signal generated upon direct measurements of magnetic field or by a similar process to processes described here. This will also include a filter, either size based or magnetic or affinity based to prevent escape of beads from the circulating flow which is continuously replaced in order to continuously measure new sample.

Reference is now made to **Figs. 7A-b** showing one optional configuration of a sensing apparatus for simultaneous sensing of multiple analytes, which all the example configurations can be in this form. The sample will flow in from the pump **702** through tubings, in one example, sample will enter from **706** and pass several closed loop to prevent losing magnetic particles. In another example.. One or more pump may be used to transfer sample solution from inlet **710** and **706** into chamber **712** which distributes the sample into separate sensing channels. Samples that have flown through **704** are collected in **716** and may be pumped to flow through **708** and magnetic beads can be collected in a secondary chamber **708** for reuse.

The above example is only a narrow, specific possible implementation of the said multi-analyte system. This can be done by any means and for example having all or groups of analytes (detection reagents) in separate detection cartridges to be circulated separately and then transferred one by one to the magnetometer for measurements. Alternatively, the system will include one input inlet that is funneled at each time period to a separate cartridge resulting in a system that takes measurement for each analyte one after the other in repeating cycles. Alternatively, for systems when speed is more crucial than cost, each cartridge may contain

separate separation and/or detection unit for detection of all the analyte or groups of them - simultaneously.

A device, system and method in accordance with some aspects of the invention may be used in many variations and different forms, for example, in conjunction with a device which may be built in a lab-on-chip style using microfluidics and allowing multiple analyte sensing configuration. In addition, the disclosed assay may be combined with any labeling technique, for example fluorescence-based technique. The device itself may contain additional components, for example temperature control unit or refrigerating unit using thermoelectric plates, compressor for the main cartridge, part of the cartridge or reserve cartridge to increase the span between the cartridge replacement and maintenance. The method of the present invention may be applied, for example, for on-site testing of water in water reservoirs or water plant effluents, pharmaceuticals production, food production, even gaseous or solids production by transforming the samples into liquid sample by a variety of possible means. It can also be used for on-site testing of explosives in airports, or on-site testing of food products, point-of-care blood and other medical diagnostics or even personal monitoring for food or liquid safety. This is of a particular interest as a personalized device for detection of allergens, pathogens and toxin does not exist today as it will be impractical to even conceive it due to cost and training needed by the user. However the device described here does not require any user training and may even be left by itself inside a food package or at the refrigerator at home. User can easily take a piece of their sample (food for example) and place it in the device which, within minutes or even seconds can report if it is adequate for use. As the machine is cheap and the detection cartridges can be used for a prolonged periods of time, even for multiple positive detections (as there are still large enough amount of beads will still be present), users can use it without significant cost. Cartridges containing a mixture for detection of many analytes simultaneously can be placed in one unit as the user only care for contaminated/not contaminated answer in many cases. It can also be used as an alternative to lateral strips where quantitation is needed, for example in dengue virus detection or monitoring of pregnancy or veterinary, hormonal levels, blood sugar etc. However, the scope of the present invention is not limited in this regard. For example, some embodiments of the invention may be used in in

“non-complete” form, for example, strips containing reagents with magnetic beads can be placed inside food package at home or at the supermarket or factory distribution or packaging stages. The beads from the displaced strip can travel to another strip to be measured by a device that is external to the food package. Similarly, the device can measure the “absence” of beads of the strip directly. Beads released can also be transferred into another strip inducing color change, electrical signal and the like rather than magnetic reading. The measurement device may be placed permanently near the package or by manually or automatically shifter from package to package. Additional conjunction with flow strip devices modified with magnetic beads of the present invention is an option. Such flow strips may be inserted, for example, into food packages. If the specific analyte is present in the food product, the displaced magnetic beads will be removed from the strip and detected with a magnetometer placed near the food package to magnetize the displaced beads.

In another implementation this new magnetic detection technique may be combined with other techniques such as fluorescent to give multidimensional information regarding a sample. For example magnetic beads measured and also contain a specific fluorescent label that indicate the presence of another analyte. In this form, for example, beads for detection of a specific bacteria X can be also functionalized with and linked with more specific strain antibodies that will be color-dependent. Beads can contain two or more sets of binding entities for that are needed for their release giving quantification of two separate elements in a single detection. This can be further expended by utilizing fluorescent markers of various colors to provide information on a matrix of parameters with a single detection step. It can also be further expended as cellular elements for example can be pre-labeled with fluorescent markers that are protein- or trait-specific to provide abundant of information on the detected sample. Fluorescent is not the only secondary label possible, the beads, their linkers and the surface may be functionalized with a variety of different elements that may provide additional sample and more specific and accurate analyte information. Specific membranes and filters may be placed to collect beads with markers and labels that are label-specific. In general this technique may be combine with a variety of other techniques that are available today or may be specifically developed in the future.

While certain features of the invention have been illustrated and described herein, many modifications, substitutions, changes, and equivalents will now occur to those of ordinary skill in the art. It is, therefore, to be understood that the appended claims are intended to cover all such modifications and changes as fall within the true spirit of the invention.

CLAIMS

1. A method for sensing an analyte in a flow system using magnetic beads as sensing species, comprising the following steps:
 - a) pumping the flow, which contains an analyte, into the system;
 - b) filtering off magnetic impurities from the flow;
 - c) reacting the analyte with a binding entity attached to the magnetic beads inside a reaction cartridge, thereby releasing the magnetic beads from the cartridge into the flow;
 - d) accumulating the magnetic beads in the collecting area; and
 - e) signal recording.
2. The method according to claim 1, wherein steps a) to d) are continuously repeated for the predetermined amount of time, thereby amplifying the recorded signal.
3. The method according to claims 1 or 2, wherein the analyte is selected from toxins, viruses, pathogens, explosives or any other ecologically, agriculturally, forensically, toxically, therapeutically or pharmaceutically important molecules.
4. The method according to any one of claims 1-3, wherein the flow is any suitable liquid, gas or air.
5. The method according to claim 4, where liquid is water.
6. The method according to any one of claims 1-5, wherein the magnetic beads are paramagnetic beads, superparamagnetic beads, superferromagnetic beads, or ferromagnetic beads or miniaturized magnets all of which can be either undemagnetized or magnetized.
7. The method according to claim 6, where the magnetic beads are ferromagnetic beads.

8. The method according to claims 6 or 7, wherein the magnetic beads have magnetic metal alloy and non-magnetic polymer shells.
9. The method according to claim 8, wherein the non-magnetic polymer shell is suitable for adding surface functional groups to the beads and protecting the magnetic core from external media.
10. The method according to claim 8, wherein the non-magnetic polymer shell is made of agarose, cellulose, porous glass or silica.
11. The method according to any one of claims 8-10, wherein the non-magnetic polymer shell is suitable for surface chemical attachment of the binding entity.
12. The method according to claim 8, wherein the magnetic core consists of a collection of ferromagnetic particles embedded in a polymer core.
13. The method according to any one of claims 6-12, wherein the magnetic beads have with sizes in the range of 100 nm to 1 mm in diameter.
14. The method according to claim 13, wherein the magnetic beads have with sizes in the range of 100 nm to 3 μ m in diameter.
15. The method according to any one of claims 1-14, wherein the magnetic beads are coated with the binding entity.
16. The method according to claim 1, wherein the inner walls or surface or any matrix of the reaction cartridge are functionalized with the analyte or analyte-analogue molecules capable of reacting with the binding entity.

17. The method according to claim 16, wherein the analyte or analyte-analogue molecules are functionalized on the inner walls or surface of the reaction cartridge using cross-linkers.
18. The method according to claims 16 or 17, wherein the inner walls or surface of the cartridge are pre-activated with functional groups.
19. The method according to claim 18, wherein the functional groups are selected from carboxyl, amine, N-hydroxy succinate (NHS)-activated, thiol, epoxy, hydroxyl and tosyl.
20. The method according to claims 18 or 19, wherein the functional groups are activated for coupling with the binding entity using the EDC-coupling chemistry for carboxylates, or glutaraldehyde for amines.
21. The method according to claims 18 or 19, wherein the functional groups are activated for coupling with the binding entity using surface tosyl-, cyanogen bromide, NHS-activated and epoxide groups.
22. The method according to claim 1, wherein the binding entity is a macromolecule, biomolecule or any other entity capable of specifically recognizing the analyte in the flow.
23. The method according to claim 22, wherein the binding entity is selected from aptamers, nucleic acids, oligonucleotides, polymers, imprinted polymers, antibodies, antibody fragments, enzymes, proteins or phage displays.
24. The method according to any one of claims 1-23, wherein the magnetic beads coated with the binding entity are attached to the walls or surface of the reaction cartridge via affinity interactions with the immobilized analyte or analyte-analogue molecules.
25. The method according to any one of claims 1-24, wherein the analyte in the flow is capable of reacting with the binding entity onto the magnetic beads attached to the reaction cartridge, thereby releasing the magnetic beads from the cartridge into the flow.

26. The method according to any one of claims 1-25, wherein the magnetic beads are coated with the analyte or analyte-analogue molecules, and the walls or surface of the cartridge are coated with the binding entity.
27. The method according to claim 1 further comprising the secondary amplification step or magnetic cascade.
28. The method according to claim 1, wherein the signal is recorded with a magnetometer.
29. The method according to claim 28, wherein the magnetometer is magnetoresistive, coil-based, hall effect, SQUID, which is capable of sensing the magnetic beads.
30. A device for use in a method of claim 1 comprising:
 - a) Housing;
 - b) Inlets;
 - c) Pump pumping the flow into the device;
 - d) Tubing carrying the flow inside the device;
 - e) Filters;
 - f) Valves;
 - g) Antifouling elements;
 - h) Collecting chamber;
 - i) Reaction chamber containing one or more reaction cartridges;
 - j) Amplification chamber accumulating the magnetic beads;
 - k) Control unit to regulate the flow;
 - l) Signal transduction unit amplifying and converting the flow of the magnetic beads into an electrical current; and
 - m) Processing unit processing the obtained electrical current for readout.
31. The device can be spitted into two or more units to be fitted cheaply into food packages

32. The method according to claim 15, wherein the magnetic bead is attached directly onto the cartridge surface and a secondary reaction which happens in the presence of analyte causes cleavage of the bond or dislodging of the beads
33. The use of ferromagnetic particles and micro magnets for sensing applications by displacing them due to the presence of analyte, collecting them and counting them.
34. The method according to claim of 33 whereas the method of collection the particles is by utilizing magnetic field, physical barrier, chemical linkage.
35. The method according to claim of 33 where the mean of counting the beads is by using a magnetic properties sensor, a mass scale, or by applying magnetic field to attract the particles and measure the force they apply.
36. The method according to claim 33 where applying magnetic field to attract magnetic particles in a form that will apply force on a surface that can be measured to quantify the number of particles
37. The method according to claim 33 whereas the magnetic particles or the analyte are also modifies fluorescently to provide further information on the selected analyte.
38. A method of signal amplification that utilizes release of magnetic beads due the presence of entities that are released in the presence of analyte.
39. The method according to claim 38 whereas the beads released due to physical, magnetic, chemical affinity, catalytic or enzymatic reaction.
40. The method according to claim 38 whereas the magnetic particles that are released in the assay are functionalized with an enzyme that can degrade linker of secondary magnetic particles.

41. Release of ferromagnetic particles in a sensing assay due to activation on enzyme that break linker or chemical reaction that causes dislodge of the particles that can later be quantified.
42. Using multiple assay cartridge and hibernating the unused cartridges using cold to increase the lifespan of the device.
43. A system to analyse multiple analyte which containing multiple cartridges that operates in sequence or in parallel

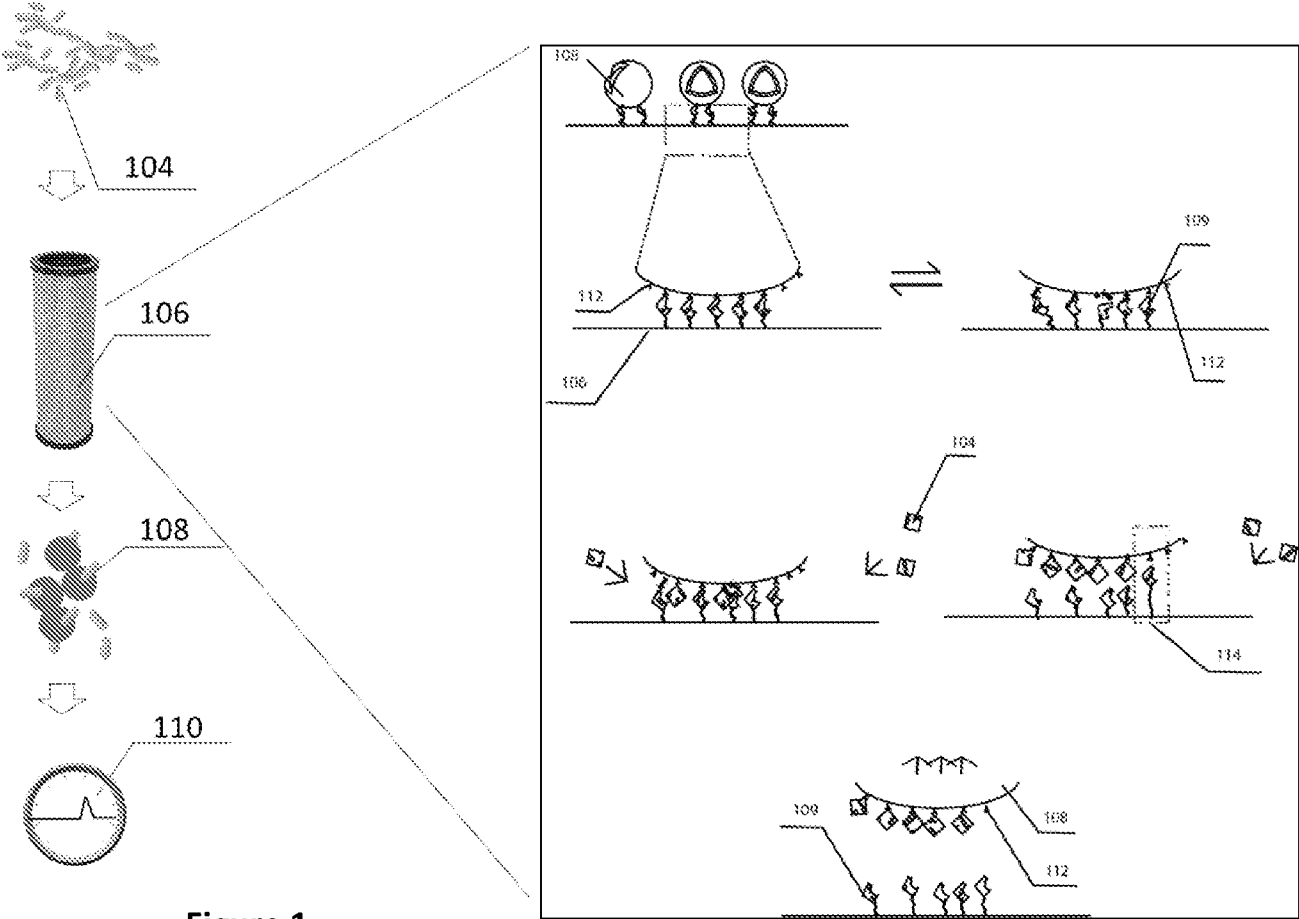


Figure 1

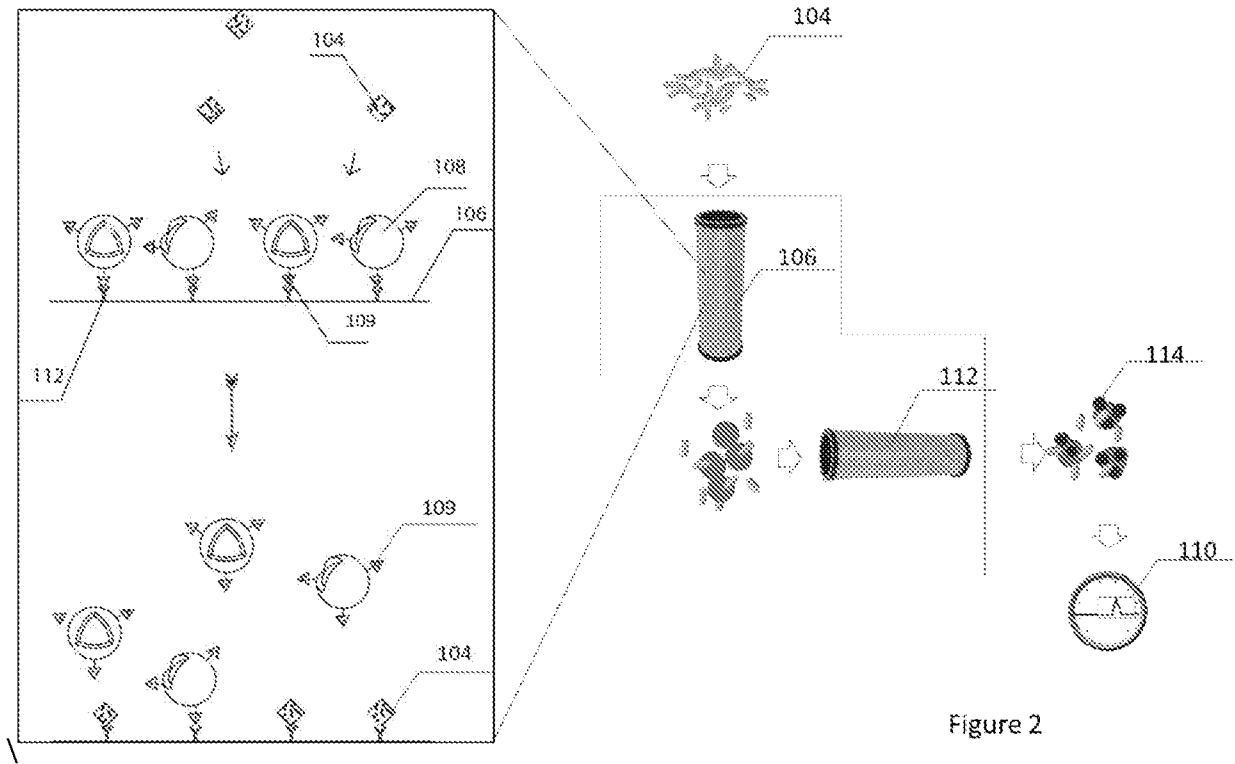


Figure 2

System Block Diagram

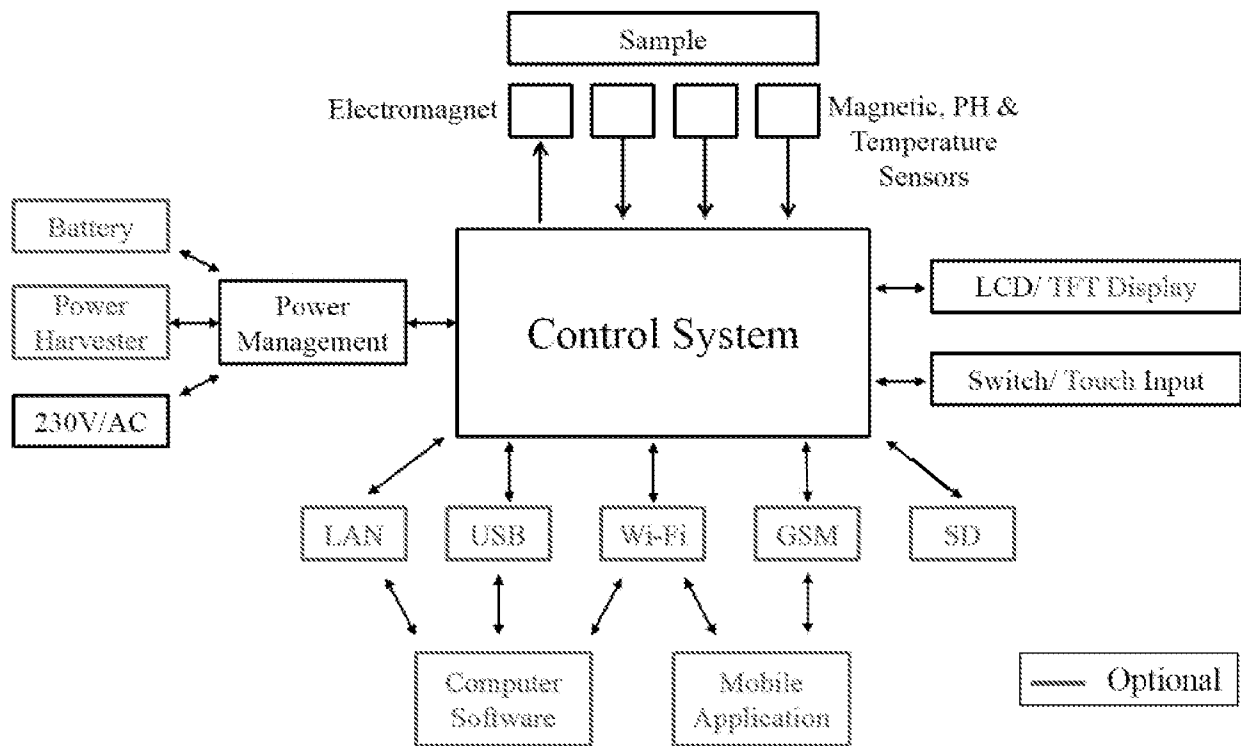


Figure 3

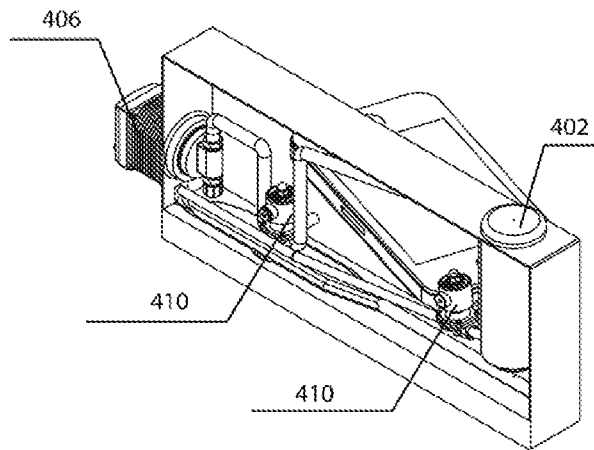


Figure 4A

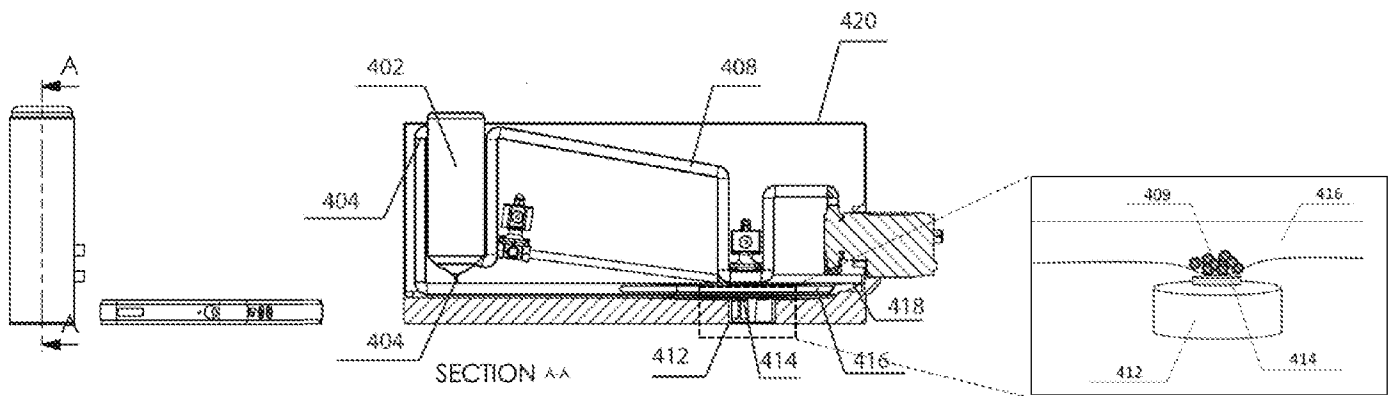


Figure 4B

Figure 4C

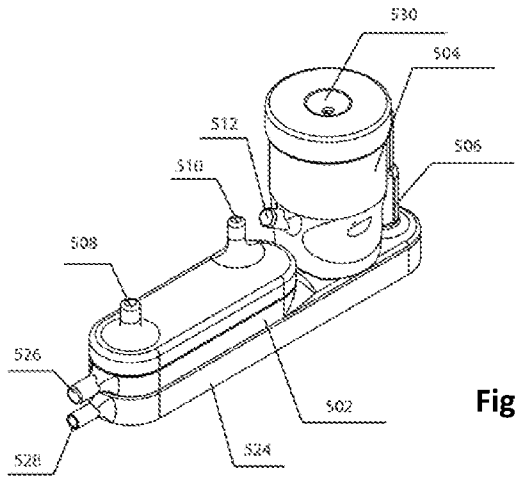


Figure 5A

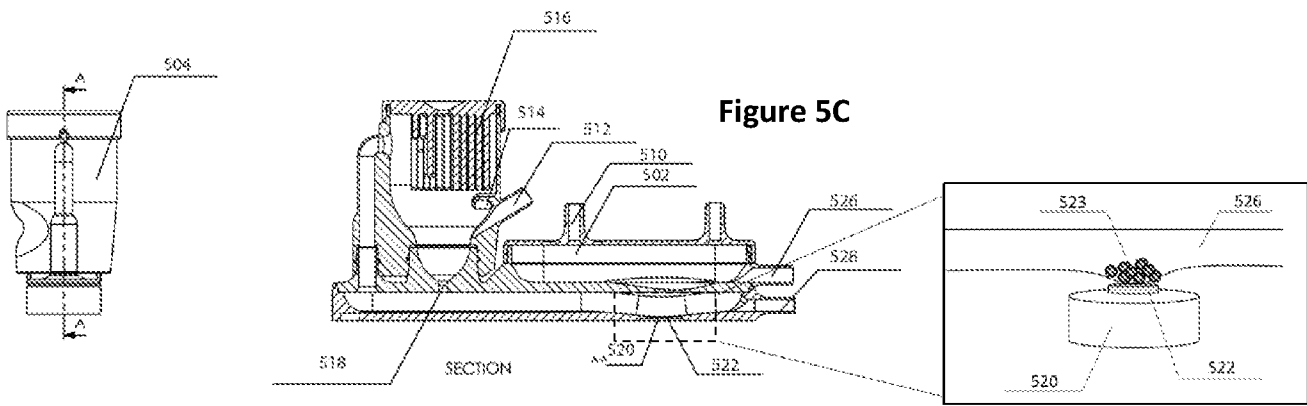


Figure 5C

Figure 5B

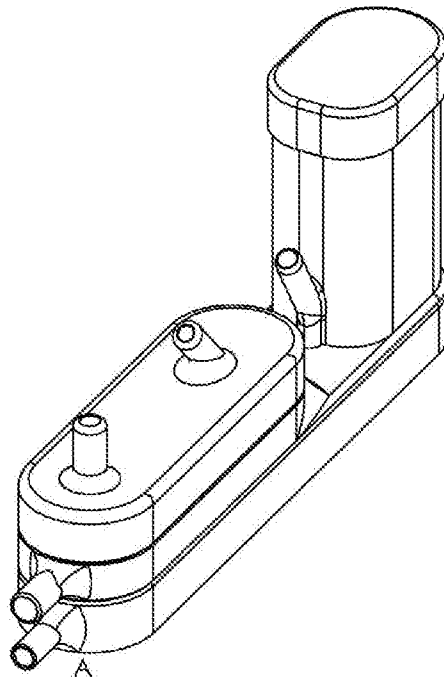


Figure 6A

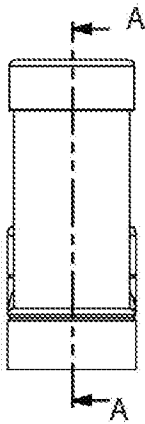


Figure 6B

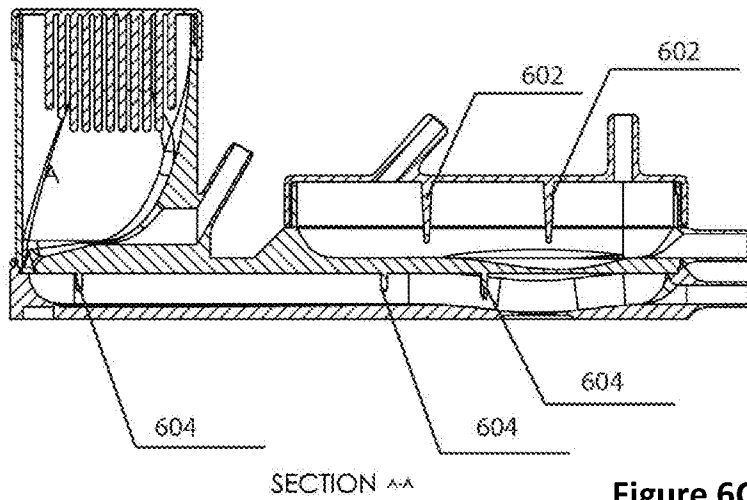


Figure 6C

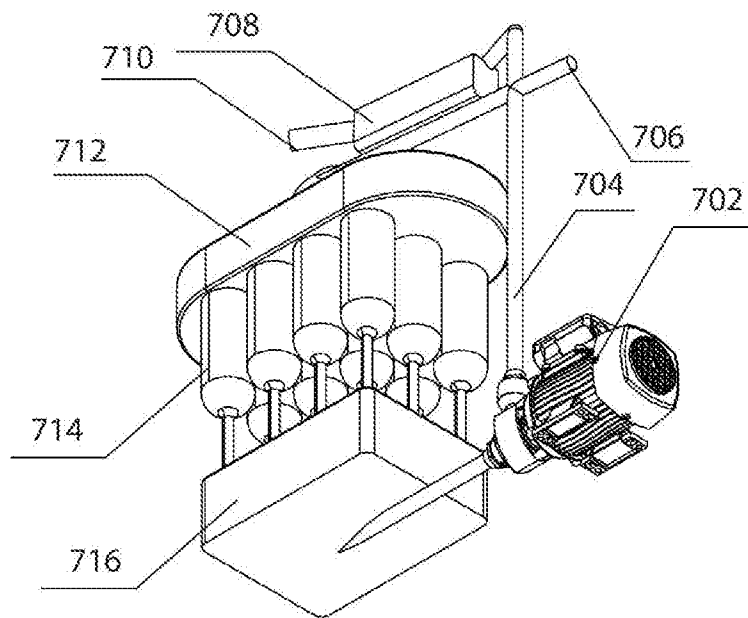


Figure 7A

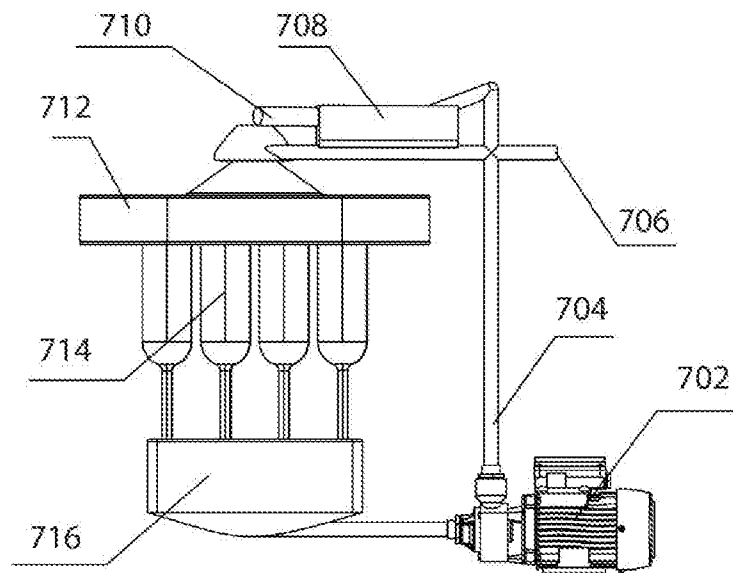


Figure 7B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2015/053482

A. CLASSIFICATION OF SUBJECT MATTER

G01N 27/00(2006.01)i; G01N 33/50(2006.01)i

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)

G01N 27/; G01N 33/

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

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

WPI, EPODOC, CNPAT, CNTXT, EPTXT, USTXT, WOTXT, CNKI, WANFANG, EBI, NCBI, GOOGLE, BAIDU, ISI Web of Knowledge, PUBMED:QI, Huan, COHEN,Assaf, COHEN A, HUAN Q, sensing, flow, magnetic, bead+, pump+, analyte, filter+, impurit+, cartridge, release+, sensor, magnetic based sensor+, magnetic particles, biosensor, paramagnetic, ferromagnetic, non-magnetic polymer shells, agarose, cellulose, porous glass, silica, functional groups, carboxyl, amine, N-hydroxy succinate -activated, NHS, thiol, epoxy, hydroxyl, tosyl, EDC-coupling chemistry, carboxylates, glutaraldehyde, cyanogen bromide, NHS-activated, epoxide, device, Housing, Inlets, Pump, Tubing, Filters, Valves, Antifouling elements, Collecting chamber, Reaction chamber, reaction cartridges, Amplification chamber, Control unit, Signal transduction unit, magnetometer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2006134546 A2 (KONINKLIJKE PHILIPS ELECTRONICS N.V.) 21 December 2006 (2006-12-21) the whole document	1-43
A	WO 2008072149 A2 (KONINKLIJKE PHILIPS ELECTRONICS N.V.) 19 June 2008 (2008-06-19) the whole document	1-43
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A	US 4452773 A (CANADIAN PATENTS DEV.) 05 June 1984 (1984-06-05) the whole document	1-43



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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 “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

09 August 2015

Date of mailing of the international search report

15 October 2015

Name and mailing address of the ISA/CN

STATE INTELLECTUAL PROPERTY OFFICE OF THE
P.R.CHINA
6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing
100088, China

Facsimile No. (86-10)62019451

Authorized officer

LI, Juanjuan

Telephone No. (86-10)010-82246978

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2015/053482**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	WO 2011086486 A1 (COMMISSARIAT A L'ENERGIE ATOMIQUE ET AUX ENERGIES ALTERNATIVES ET AL.) 21 July 2011 (2011-07-21) the whole document	1-43
A	WO 2012073182 A1 (KONINKLIJKE PHILIPS ELECTRONICS N.V.) 07 June 2012 (2012- 06-07) the whole document	1-43
A	WO 2007089564 A2 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 09 August 2007 (2007-08-09) the whole document	1-43

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/IB2015/053482

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