

METHOD FOR DETECTING ANALYTES

FIELD OF THE INVENTION

[0001] The invention relates to a method for detecting one or more target analytes in a sample, and more specifically, the use of magnetisable nanoparticles and a magnetic sensor system. The invention also relates to a device for detecting analytes based on the use of magnetisable nanoparticles.

BACKGROUND

[0002] There are many known methods to detect and quantify analytes in a sample. Such systems require an indirect method to quantify the analyte by detecting and measuring a complex that is bound to the analyte. Typically, such methods rely on a binding or recognition system whereby a visualisation aid is coated or linked to a binding molecule that binds to the analyte in the sample.

[0003] The binding molecule may include antibodies, enzymes or pharmacological agents that are specifically chosen based on their affinity for the target analyte. The molecule that directly binds the analyte may itself be labelled with an enzyme or a fluorophore (in the case of fluorescent labelling).

[0004] Alternatively, the molecule that directly binds the analyte may itself be unlabelled and is instead bound to a further binding agent that is itself labelled with an enzyme or fluorophore. This additional labelling procedure can amplify the signal and reduce background staining. A well-known complex is the avidin-biotin complex and the peroxidase-antiperoxidase technique.

[0005] Techniques for detecting and quantifying analytes in a sample need to be rapid, sensitive, qualitative and/or miniaturisable to fulfil the needs of in vitro diagnostics. Miniaturisation of devices can lead to slow and inefficient mixing of fluids due to an increase in viscous forces.

[0006] Point-of-care testing can reduce the turn-around time for diagnostic testing giving improved workflows and thus potentially aiding improved patient care. Such systems must include sensing technology to detect biomarkers (e.g. protein markers or nucleic acid markers). Magnetisable particles have been used for detecting analytes across manual assays for basic research to high throughput testing.

[0007] Many existing devices for detecting analytes attached to magnetisable particles require complex configurations that are unsuitable or are not easily adapted for miniaturisation in point-of-care testing applications.

[0008] The use of magnetisable particles relies on the functionalisation of the particles with binding molecules (e.g. antibodies with high affinity for the target analyte) to allow binding to the target analyte, followed by fluid exchange steps to achieve isolation and purification. It has been reported that the analyte capture rate scales with the total surface area of the suspended particles and therefore with the particle concentration. However, the use of a very high concentration of particles has disadvantages for downstream processes in an integrated multi-step lab-on-chip assay since high particle concentrations generally increase non-specific particle–particle and particle–surface interactions, enhance field-induced particle aggregation, cause steric hindrance in particle concentration steps, obstruct chemical reactions on the particles, and sterically hinder reactions between the particles and a biosensing surface.

[0009] Target analytes may be present in low concentrations within a sample, that also contains a high concentration of background material, such as blood or saliva. In such complex matrices, non-specific adhesion of non-targeted molecules to the magnetisable particles can reduce the effectiveness of the assay.

[0010] The process of magnetic particle-based capture of a target analyte consists of an encounter between the two components (the target analyte and the magnetic particle) and may rely upon the two components aligning their outer surfaces in a very specific manner relative to each other. Thus, the association rate of the two components may be limited by diffusion and by the geometric constraints of the two component's binding sites, and may also be reduced by the final chemical reaction.

[0011] Analytes can be captured in a flowing fluid or a static fluid. Without flow, a method that relies upon surface immobilised antibodies is limited by diffusion and can have a reduced binding rate.

[0012] After the capture of the target analyte by the magnetic particle, additional processing is required for detection. If used as a carrier only, the magnetisable particle is typically bound to identification molecules such as luminescent labels or fluorescent molecules. For accurate detection, it is important that only bound analytes are labelled, and that only bound labels are detected. This requires several washing or separation steps.

[0013] The magnetisable particles may also be used as a label to indicate binding of the target analyte at a sensing surface. Agglutination assays exploit a process wherein aggregates of particles are formed when specific analytes are present in the sample fluid. The degree of aggregation is a measure for the concentration of analytes within the fluid. The agglutination assays are demanding on the reagents because the assays are performed in one step without separation or stringency.

[0014] In magnetic agglutination assays, the formation of particle clusters is accelerated by bringing particles together under the influence of a magnetic field. An issue with such methodologies is that when the analyte concentration is much smaller than the magnetisable particle concentration, a small number of particle aggregates are formed, governed by Poisson statistics. Application of a magnetic field may be enhanced by applying a magnetic field during incubation. However, the magnetic field may also increase the non-specific binding between the particles. Non-specific binding (i.e. the bond is not mediated by the target analyte), results in a false positive signal. Non-specific binding can originate from several types of interactions such as van der Waals interactions, electrostatic interactions, and hydrophobic interactions causing background levels as well as statistical variations of the results, which therefore affect the limit of quantification and the precision of the method.

[0015] The use of magnetisable particles means that additional forces can be applied to the particles, for example, to separate bound from unbound particles.

[0016] An evaluation of the analytical performance of a detection methodology is based on the limit of quantification (LoQ) i.e. the lowest biomarker concentration that can be quantified with a given required precision.

[0017] Optimizing magnetisable particles for specific applications and selecting appropriate detection methods remain challenging for the magnetic nanotechnology community due to the increasing demands of detection sensitivity, molecular specificity, and application complexity.

[0018] The use of GMR in immunoassays has been used in a sandwich-type approach (such as an ELISA), where the molecular target is immobilised on the sensor surface with the addition of tagged magnetic probes (see Koh and Josephson "Magnetic nanoparticle sensors" *Sensors* 2009: 9; 8130-45 and Yao and Xu "Detection of magnetic nanomaterials in molecular imaging and diagnosis applications" *Nanotechnol. Rev* 2014: 3;247-268).

[0019] Some techniques use superconducting quantum interference device (SQUID) to detect and measure Néel relaxation (misalignment of magnetic dipole) in magnetically labelled bacteria. In such techniques, a magnetic field is pulsed to cause magnetic dipole alignment and the subsequent dipole misalignment is detected.

[0020] It is an object of the present invention to address one or more of the abovementioned issues, and/or to provide a method for detecting an analyte in a sample and/or to at least provide the public with a useful choice.

SUMMARY

[0021] According to a first aspect, the present disclosure may broadly provide a method for detecting a target analyte in a sample, the method comprising:

- providing a quantity of magnetisable particles having a reference magnetic signal that is known or measured before or after the sample is added, the particles being coated with binding molecules complementary to the target analyte,
- bringing the sample comprising the target analyte into contact with the magnetisable particles resulting in bound and unbound binder complexes,
- applying a magnetic field to the sample for a period of time,
- obtaining a magnetic signal, the magnetic signal being the magnetic signal of the bound and unbound binder complexes in the presence of the magnetic field,
- removing the magnetic field for a period of time, and
- comparing the reference magnetic signal and the magnetic signal, wherein a difference between the reference magnetic signal and the magnetic signal correlates to the presence and/or quantity of the target analyte in the sample.

[0022] The reference magnetic signal may be determined by measuring the magnetic signal of the quantity of magnetisable particles in the absence of the target analyte.

[0023] The reference magnetic signal may be measured at any point between steps of bringing the sample comprising the target analyte into contact with the magnetisable particles, applying a magnetic field to the sample for a period of time, obtaining a magnetic signal, and removing the magnetic field for a period of time.

[0024] The magnetic field may be generated using an electromagnet.

[0025] The magnetic field may be generated using a permanent magnet.

[0026] The magnetic signal may be obtained after an electromagnetic coil of the electromagnet reaches saturation.

[0027] The target analyte in the sample may be determined by correlating the change in the measured magnetic signal relative to the reference/predetermined magnetic signal.

[0028] The method may further comprise: generating a reference data set based on known analyte quantity values, comparing the value obtained from the difference between

the reference magnetic signal and the magnetic signal with the reference data set to determine the quantity of analytes in the sample.

[0029] The magnetic field may be applied for a predetermined time.

[0030] The magnetic field may be applied for about 0.1, 0.25, 0.5, 1, 2, 3, 4 or 5 seconds, and any range therebetween.

[0031] The magnetic field may be removed for about 0.1, 0.25, 0.5, 1, 2, 3, 4 or 5 seconds, and any range therebetween.

[0032] The magnetic field may be removed for a predetermined time.

[0033] The magnetic field may be removed for about 3 to about 7 seconds.

[0034] The magnetic field may be applied and removed for a substantial equal amount of time.

[0035] The magnetic field may be applied and removed for about 1 second.

[0036] The steps of applying a magnetic field to the sample for a period of time, obtaining a magnetic signal, and removing the magnetic field for a period of time may be repeated for two or more times.

[0037] The magnetisable particles may be superparamagnetic nanoparticles.

[0038] The magnetisable particles may be ferromagnetic nanoparticles.

[0039] The superparamagnetic nanoparticles may have an average particle size of about 20 nm to about 40 nm.

[0040] The superparamagnetic nanoparticles may have an average particle size of about 30 nm.

[0041] The magnetisable particles may have an average particle size of about 5 to about or 5000 nm.

[0042] The method may further comprise magnetically shielding the sample from ambient magnetic field.

[0043] The method may further comprise detecting the ambient magnetic field, and adjusting the reference magnetic signal and the magnetic signals based on the ambient magnetic field.

[0044] The magnetic signal may be measured with a sampling rate of at least about 10,000 samples per second.

[0045] The sample may be incubated to a temperature of about 20°C.

[0046] The magnetic signal may be magnetic field strength.

[0047] The method may further comprise:

- applying a second magnetic field to the magnetisable particles, the second magnetic field may be positioned above or below the sample,
- measuring the magnetic signal of the bound and unbound binder complexes in the presence of the magnetic field and the second magnetic field.

[0048] The method may be performed using a sample testing device comprising:

- a sample well or sample reservoir,
- one or more magnets located on one of an upper side or lower side of the sample well or sample reservoir,
- one or more permanent magnets located on the other of the upper side or lower side of the sample well or sample reservoir, and
- a magnetic field sensor for measuring changes over time in the magnetic signal in the sample well or sample reservoir.

[0049] The magnetisable particles may be functionalised with molecules that specifically bind to the target analyte.

[0050] The sample and magnetisable particles may be processed by a microfluidic device.

[0051] The microfluidic device may facilitate binding between the magnetisable particles and analyte.

[0052] One or more electromagnets may generate a magnetic field that changes over time.

[0053] The one or more electromagnets may generate a continuity of magnitudes.

[0054] The one or more electromagnets may alternate the magnetic field between on and off.

[0055] The magnetic field sensor may measure the changes in magnetic field strength generated by the magnetisable particles over time.

[0056] The signal output from the magnetic field sensor may be boosted by a signal amplifier.

[0057] The signal output from the magnetic field sensor may be a voltage reading that is proportional to the magnetic signal measured by the magnetic field sensor.

[0058] The amplified signal may be converted from a voltage reading into digital bitstream and recorded and/or analysed by a computer.

[0059] The conversion may be performed by Analog to Digital Converter.

[0060] The method may:

- generate sufficient magnetic signal within 15 seconds to detect and/or quantitate the target analyte in the sample, or
- have a limit of detection (LOD) of at least about 0.05 pg/mL, or
- have a limit of quantification (LOQ) of at least about 0.1 pg/mL, or
- one or more of above.

[0061] The second magnetic field may be applied from an opposite side to the magnetic field.

[0062] The second magnetic field may be provided using a permanent magnet.

[0063] The second magnetic field may be provided using an electromagnet.

[0064] The electromagnet may be positioned closer to the sample than the permanent magnet.

[0065] The electromagnet may be calibrated to have a magnetic density at least twice to that of the permanent magnet.

[0066] The magnetic signal may be processed using Fast Fourier Transform (FFT).

[0067] The magnetic signal may be pre-processed by truncating and/or concatenating the magnetic signal in one or more dimensions before processing using FFT.

[0068] The magnetic signal may be windowed before FFT processing.

[0069] According to another aspect, the present disclosure may broadly provide a device for detecting an analyte in a sample, comprising:

- a sample well that is separate to, or integrated into a microfluidic device,
- a magnet for generating a magnetic field,
- a magnetic field sensor configured to measure the magnetic signal of magnetisable particles in the sample well in the presence of the magnetic field, the magnetic signal detected from the overall magnetic response that is proportional to the size of the aggregates.

[0070] The term “comprising” as used in this specification means “consisting at least in part of”. When interpreting statements in this specification which include that term, the features, prefaced by that term in each statement, all need to be present but other features can also be present. Related terms such as “comprise” and “comprised” are to be interpreted in the same manner.

[0071] It is intended that reference to a range of numbers disclosed herein (for example, 1 to 10) also incorporates reference to all rational numbers within that range (for example, 1, 1.1, 2, 3, 3.9, 4, 5, 6, 6.5, 7, 8, 9 and 10) and also any range of rational numbers within that range (for example, 2 to 8, 1.5 to 5.5 and 3.1 to 4.7).

[0072] This invention may also be said broadly to consist in the parts, elements and features referred to or indicated in the specification of the application, individually or collectively, and any or all combinations of any two or more of said parts, elements or features, and where specific integers are mentioned herein which have known equivalents in the art to which this invention relates, such known equivalents are deemed to be incorporated herein as if individually set forth.)

[0073] To those skilled in the art to which the invention relates, many changes in construction and widely differing embodiments and applications of the invention will suggest themselves without departing from the scope of the invention as defined in the appended claims. The disclosures and the descriptions herein are purely illustrative and are not intended to be in any sense limiting.

BRIEF DESCRIPTION OF THE FIGURES

[0074] The invention will now be described by way of example only and with reference to the drawings in which:

[0075] Figure 1 is the magnetic field sensor signal output over N cycles (time) during the sample read stage according to an embodiment of the present disclosure.

[0076] Figure 1a illustrates the magnet-on phase at the start of a read cycle and represents a profile of average values obtained from the magnetic field sensor over time according to an embodiment of the disclosure

[0077] Figure 1b illustrates the magnet-off phase which represents a profile of average values from the magnetic field sensor over time according to an embodiment of the present disclosure

[0078] Figure 2 is a sample introduction device/microfluidic chip according to an embodiment of the present disclosure.

DETAILED DESCRIPTION

[0079] The present disclosure may broadly provide a method of detecting and/or quantitating a target analyte in a sample.

[0080] The described method for detecting a target analyte in a sample, may broadly comprise the steps of:

- providing a quantity of magnetisable particles having a reference magnetic signal that is known or measured before or after the sample is added, the particles being coated with binding molecules complementary to the target analyte,
- bringing the sample comprising the target analyte into contact with the magnetisable particles resulting in bound and unbound binder complexes,
- applying a magnetic field to the sample for a period of time,
- obtaining a magnetic signal, the magnetic signal being the magnetic signal of the bound and unbound binder complexes in the presence of the magnetic field,
- removing the magnetic field for a period of time, and
- comparing the reference magnetic signal and the magnetic signals, wherein a difference between the reference magnetic signal and the magnetic signal correlates to the presence and/or quantity of the target analyte in the sample.

[0081] The described method is based on the concept of measuring the changes in the magnetic signal generated by the magnetisable particles due to Brownian rotation or diffusion that allows quantification of the amount of magnetisable particles-analyte complex

(bound binder complex), which then allows the amount of analyte to be determined in the sample.

[0082] Brownian movement (i.e. rotational or translational) of particles may be affected by a number of factors including, but not limited to, temperature, viscosity of the suspending medium, size of the particles, and surface features of the particles. For example, surface features such as analytes bound to magnetisable particles changes the hydrodynamic characteristic of the chemical conjugates formed.

[0083] As concentration of the analyte changes, the magnetisable particle conjugation/aggregation changes due to the chemical bonding between analyte and binder, binder and binder, and particle and particle resulting in particles of a spectrum of hydrodynamic sizes across the different analyte concentrations.

[0084] For example, when a relatively large concentration of analyte is present in a sample, binders on the magnetisable particles become more saturated with analytes reducing the potential for inter-binder and/or inter-particle interactions (e.g. non-specific binding) and resulting in formation of smaller aggregates.

[0085] Conversely, when a relatively small concentration of analyte is present in a sample, binders on the magnetisable particles are less saturated with analytes increasing the potential for inter-binder and/or inter-particle interactions such that larger aggregates may form.

[0086] Brownian movement is inversely related to the hydrodynamic size of the magnetisable particles and analyte complexes. As such, changes in the amount of analytes changes the hydrodynamic size of the magnetisable particles which affects the effective translational or rotational Brownian motion under an external magnetic field. The change in Brownian motion may be detectable and measurable as an overall magnetic response that is proportional to the size of the aggregates.

[0087] Large aggregations of magnetisable particles may result in densely packed particles that increase dipole-dipole interaction (or dipole coupling). Increased dipole coupling results in a larger magnetic signal.

[0088] The difference in the aggregate formation and resulting magnetic signal may be used to determine the concentration of analyte in a sample.

[0089] Without wishing to be bound by theory, in the magneto-equilibrium state, the magnetisable particles attract each other in a first dimension but repel each other in another dimension which results in the formations of micro-assemblies. Factors such as size of the

magnetisable particles, the amount of target analyte, size of the target analyte may have may affect the distance between micro-assemblies. For example, a greater amount of analyte may result in an effectively larger magnetisable particle-analyte complex. As a result, the larger bound complexes may be kept further apart while having the same attractive forces affecting the formation of micro-assemblies which is detectable as a change in the signal generated by the magnetisable particles.

[0090] The amount of analyte in a sample is determined based on the change in the signal generated by the magnetisable particles as detected by the sensor. The sensor detects the change based on the aggregation of particles.

[0091] The described method may comprise multiple stages.

[0092] The first stage may be a pre-sample stage. The pre-sample stage may comprise providing a quantity of magnetisable particles to a sample device such as a microfluidic device.

[0093] The magnetisable particles may be functionalised with binders that bind the specific target analytes.

[0094] The quantity of magnetisable particles may have a reference signal that is known or predetermined. For example, based on a known number of beads present in the device. The reference signal may be any measurable signal produced by the magnetisable particles in the absence of target analytes. For example, the signal may comprise magnetic or electrical signals.

[0095] The signal generated by the magnetisable particles may be inherent or induced. For example, the signal generated can be inherent to its atomic construct, or can be induced by a magnetic field such as an external magnetic field.

[0096] Alternatively or additionally, the method may comprise a reference calibration step comprising measuring the total signal generated by the magnetisable particles in the absence of analytes. In a reference calibration step, the signal generated by the magnetisable particles is measured using suitable signal sensors after the quantity of magnetisable particles is added to the sample device but before the sample is added.

[0097] In some embodiments, the reference calibration step may be performed contemporaneously with the sample read stage in the proceeding paragraphs. For example, this may be performed using a multiplex system where one channel or well is used for reference calibration and other channels used for sample reading.

[0098] The reference signal provides a base comparison for the subsequent sample reading. The reference calibration step may take 1, 2, 3, 4 or 5 seconds, and suitable ranges may be selected from between any of these values, (for example, about 1 to about 5, about 1 to about 4, about 2 to about 5, about 2 to about 3 or about 3 to about 5 seconds).

[0099] A second stage may comprise introducing a sample to be analysed to the magnetisable particles.

[0100] At this stage, the sample is brought into contact with the magnetisable particles such that any analytes present in the sample may be bound by the binders on the magnetisable particles to form a complex with the particles. This stage results in bound and unbound binder complexes.

[0101] Alternatively, the sample to be analysed maybe incubated with functionalised magnetisable nanoparticles prior to introduction a microfluidic device.

[0102] This stage may include sample mixing and analyte-to-binder complexing (i.e. where the functionalised magnetisable particles bind to the analyte). This stage may take around 3, 4, 5, 6, 7 or 8 minutes, and suitable ranges may be selected from between any of these values, (for example, about 3 to about 8, about 3 to about 7, about 3 to about 5, about 4 to about 8, about 4 to about 6 or about 5 to about 8 minutes).

[0103] A third stage may be the sample read stage.

[0104] During the sample read stage, one or more external magnetic fields may be used to cause and alter the equilibrium of the magnetisable particles (bound and unbound binder complexes) where the transition between the equilibrium states may be measured as a change in signal over time.

[0105] In some embodiments, the external magnetic field may be generated using a magnet. The magnet may be selected from permanent magnets and/or electromagnets.

[0106] In embodiments using an electromagnet, the external magnetic field may be applied and removed by switching the electromagnet on and off. For example, electricity may be supplied or cutoff to the electromagnet coils to activate and deactivate the external magnetic field.

[0107] In embodiments using a permanent magnet, the external magnetic field may be applied and removed by changing the relative positions of the permanent magnet and the sample such that the external magnetic field. For example, the permanent magnet or sample

well may be configured such that the sample moves within or outside an effective range of the permanent magnet depending on the phase of the read cycle.

[0108] In other embodiments, the permanent magnet and sample remain stationary relative to each other but a magnetic shield member may be used to control or redirect the magnetic field generated by the permanent magnet. For example, a movable magnetic shield member positioned between the permanent magnet and sample may be controlled to move such that the magnetic field generated by the permanent magnet is allowed to reach or blocked from reaching the sample.

[0109] In some embodiments, an external magnetic field may be applied and the sample may be read in a number of cycles. In each cycle, the external magnetic field may be activated (applied) and deactivated (removed) for one or more times for a specified time. For example, an external magnetic field may be applied for 2 seconds and removed for 5 seconds.

[0110] Figure 1 illustrates the magnetic field sensor signal output over N cycles (time) during the sample read stage according an embodiment that uses an electromagnet to generate the external magnetic field. In some embodiments, each cycle maybe defined as the time between activations of the external magnetic field. For example, each read cycle starts when the external magnetic field is activated and ends when the external field is re-activated following a period of deactivation.

[0111] The activation of the external magnetic field may be known as the magnet-on phase and the deactivation of the external magnetic field may be known as the magnet-off phase.

[0112] Figure 1a is a zoomed inset of the start of a read cycle and represents a profile of average values obtained from the magnetic field sensor over time according to an embodiment of the disclosure. The magnet-on phase maybe characterised by a number of events. The events described may overlap or occur concurrently.

[0113] At event 2a, the electromagnet is activated at the start of the read cycle and the electromagnetic coil reaches saturation.

[0114] At event 2b, the external magnetic field generated by the electromagnet propagates throughout the sample and the electromagnetic coil achieves equilibrium for input voltage as detected by the magnetic field sensor.

[0115] At event 2c , the propagation of external magnetic field causes bound and unbound magnetisable particles (for example, SPIONs) to spontaneously adopt a magnetic

moment. In particular, the bound and unbound magnetisable particles may adopt an opposite polarity to the net vector to align and optimise harmoniously with nearby magnetisable particles (which are similarly magnetic).

[0116] At event 2d, pre-existing aggregations of bound and unbound magnetisable particles and/or developing through spontaneous magnetic interactions represent rotational and/or translational movement. Magnetic repulsion and attraction results in areas of magnetic equilibrium.

[0117] At event 2e, the magnetisable particle aggregates align with field lines of the magnetic coil and move towards increasing magnetic field gradient (at the source of the external magnetic field).

[0118] The magnet-on phase (i.e. external magnetic field) is applied (activated) for a period of time. The period of time may be a predetermined amount of time.

[0119] The magnet-on phase may be about 0.1, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, 2.75, 3.0, 3.25, 3.5, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, or 12.0 seconds and suitable ranges may be selected from between any of these values, (for example, about 0.25 to about 12, about 0.25 to about 5.0, about 0.25 to about 3.0, about 0.25 to about 2.0, about 0.25 to about 1, about 0.1 to about 12, about 0.1 to about 5.0, about 0.1 to about 3.0, about 0.1 to about 2.0, about 0.1 to about 1, about 0.5 to about 12, about 0.5 to about 5.0, about 0.5 to about 3.0, about 0.5 to about 2.0, about 0.5 to about 1, 0.75 to about 12, about 0.75 to about 5, about 0.75 to about 3.0, about 0.75 to about 2.0, about 0.75 to about 1.0, about 1.0 to about 3.5, about 1.0 to about 3.25, about 1.0 to about 3.0, about 1.0 to about 2.75, about 1.0 to about 2.5, about 1.0 to about 2.25, about 1.0 to about 2.0, about 1.0 to about 1.75, about 1.0 to about 1.5, about 1.0 to about 1.25, about 1.5 to about 3.0, about 1.5 to about 2.5, about 1.5 to about 2.0, about 2.0 to about 2.25, about 2.5, about 2.0 to about 2.75, about 2.0 to about 3.0, about 2.0 to about 3.25, about 2.0 to about 3.5 seconds).

[0120] Preferably, the magnet-on phase is about 1.0 to about 2.0 seconds.

[0121] Without wishing to be bound to theory, minimising the magnet on time may reduce the thermal build up in the electromagnet coils which may affect the accuracy of the readings obtained by sensors..

[0122] Where the magnet on phase may be prolonged, a calibration reference may be used to compensate for any inaccuracies from the sensor readings that may be introduced due to the thermal build up from the electromagnetic coils.

[0123] Figure 1b is a zoomed inset of the magnet-off phase which represents a profile of average values from the magnetic field sensor over time according to an embodiment of the present disclosure.

[0124] The magnet-off phase is a reverse of the magnet-on phase and may be broadly characterised by electromagnetic coil desaturation (event 3a) and a reverse of events 2b to 2e described in relation to the magnet-on phase in the preceding paragraphs.

[0125] The magnet-off phase may be about 0.1, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, 2.75, 3.0, 3.25, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 8.0, 9.0, 10.0, 11.0, or 12.0 seconds and suitable ranges may be selected from between any of these values, (for example, about 0.25 to about 12, about 0.25 to about 5.0, about 0.25 to about 3.0, about 0.25 to about 2.0, about 0.25 to about 1, about 0.1 to about 12, about 0.1 to about 5.0, about 0.1 to about 3.0, about 0.1 to about 2.0, about 0.1 to about 1, about 0.5 to about 12, about 0.5 to about 5.0, about 0.5 to about 3.0, about 0.5 to about 2.0, about 0.5 to about 1, 0.75 to about 12, about 0.75 to about 5, about 0.75 to about 3.0, about 0.75 to about 2.0, about 0.75 to about 1.0, about 3.0 to about 7.0, about 3.0 to about 6.0, about 3.0 to about 5.0, about 3.0 to about 4.0, about 4.0 to about 7.0, about 4.0 to about 6.0, or about 4.0 to about 5.0 seconds).

[0126] In some embodiments, the magnet-on phase and the magnet-off phase may be substantially equal. For example, the magnet-on phase and magnet-off phase time may be substantially equal within about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 20, about 25, about 30, about 35, about 40, about 45, or about 50 % of each other.

[0127] Where the magnet-on phase and magnet-off phase is not equal, as discussed above in some embodiments the device may comprise an onboard reference standard. For example, an onboard standard curve generator that reads known amounts of analytes to create a standard curve. Thus, any deviation from the expected results from the measured results can be used to generate a multiplier that can be applied to the sample results to account for any drift in readings.

[0128] In some embodiments, the sample read stage may comprise two or more sample read cycles. For example, sample read stage may comprise two, three, four, five, six, seven, eight, nine or ten cycles.

[0129] The sample read stage may be around 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, or 70 seconds, and suitable ranges may be selected from between any of these values, (for example, about 5 to about 70, about 5 to about 60, about 5 to about 50, about 5

to about 40, about 5 to about 30, about 5 to about 20, about 5 to about 10, about 10 to about 70, about 10 to about 60, about 10 to about 50, about 10 to about 40, about 10 to about 30, about 10 to about 20, about 20 to about 70, about 20 to about 50, about 20 to 30 seconds).

[0130] In some embodiments, the external magnetic field may comprise a first magnetic field and a second magnetic field.

[0131] The first and second magnetic fields may be generated by magnets located on opposite sides relative to the sample.

[0132] In some embodiments, the first magnetic field may be generated below the sample and the second magnetic field may be generated above the sample. In this embodiment, the first magnetic field may attract the magnetisable particles downwards while the second magnetic field may attract the magnetisable particles upwards.

[0133] In some embodiments, the first and second magnetic fields may have the same or different polarities.

[0134] The first magnetic field may be a permanent magnetic field where the magnetic field is applied continuously at a constant magnitude for the duration of the sample read stage.

[0135] The second magnetic field may be a non-permanent magnetic field where the magnetic field is only applied during the read state such that both the permanent and non-permanent magnetic fields are active during the read state.

[0136] When more than one external field is used, the signal generated by the magnetisable particles (bound and unbound binder complexes) is measured by the signal sensor in the read state when the magnetisable particles are transitioning between equilibrium states when both magnetic fields are active.

[0137] The signal generated by the magnetisable particles may be measured for a portion of or the duration of the read state.

[0138] The permanent magnetic field may be weaker relative to the non-permanent magnetic field.

[0139] The permanent magnetic field may be generated at a position distal to the sample while the non-permanent magnetic field may be generated at a position proximal to the sample.

[0140] The strength of the non-permanent magnetic field may be modulated.

[0141] Without wishing to be bound by theory, the modulation of this magnetic field (i.e. the bias field) has the primary function of aligning the magnetisable particles to the sensor to achieve the highest sensitivity of detection during detection. For ferromagnetic particles, given they have their own permanent magnetic field, where the bias field is switched off resulting in misalignment of the magnetic particles. For paramagnetic (or superparamagnetic) particles, as their magnetic field has to be induced by an external field, the bias field serves the additional function of inducing such a field.

[0142] The non-permanent magnetic field may be modulated in order to support different magnetisable particles since different particles (whether by chemical composition or physical size) may require different bias field strengths and configurations.

[0143] The magnetic fields may be generated by one or more magnetic field generators.

[0144] The permanent magnetic field may be generated using one or more permanent magnets. Alternatively, one or more electromagnets may be configured to apply a continuous and constant magnetic field during the sample read stage.

[0145] The non-permanent magnetic field may be generated using one or more electromagnets.

[0146] In some embodiments, the electromagnet may be configured to have a magnetic density at least twice to that of the permanent magnet.

[0147] The non-permanent magnetic field may be applied for a duration of about 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, 2.75, 3.0, 3.25, or 3.5 seconds and suitable ranges may be selected from between any of these values, (for example, about 1.0 to about 3.5, about 1.0 to about 3.25, about 1.0 to about 3.0, about 1.0 to about 2.75, about 1.0 to about 2.5, about 1.0 to about 2.25, about 1.0 to about 2.0, about 1.0 to about 1.75, about 1.0 to about 1.5, about 1.0 to about 1.25, about 1.5 to about 3.0, about 1.5 to about 2.5, about 1.5 to about 2.0, about 2.0 to about 2.25, about 2.5, about 2.0 to about 2.75, about 2.0 to about 3.0, about 2.0 to about 3.25, about 2.0 to about 3.5 seconds).

[0148] In some embodiments, each sample read cycle may comprise a first state in which the first magnetic field is active and second magnetic field is inactive, a second state in which the first magnetic field is active and second magnetic field is active, and a third state in which the first magnetic field is active and second magnetic field is inactive.

[0149] A fourth stage may be the data analysis stage.

[0150] The method may comprise processing the raw data output from the magnetic field sensor and analysing the processed data to quantify the amount of target analyte in the sample. Raw data processing may be carried out using a combination of hardware and software implementations described in detail elsewhere in the specification.

[0151] The signals detected and measured during the read stage may be recorded as data and analysed. The magnetic field sensor output may be recorded for the duration of the sample read stage.

[0152] The amount of analyte in a sample may be determined based on the change in the magnetic response of the chemically-bound magnetic nanoparticle and analyte complexes detected by a magnetic field sensor.

[0153] In some embodiments, data obtained is analysed to identify portions of the dataset corresponding to the events 2a-2f and 3a-3b illustrated in figures 1a and 1b as described in paragraphs [103] to [110].

[0154] In some embodiments, detection and quantitation is derived using data obtained when the external magnetic field is applied or activated. For example, data used for processing and analysis may be derived from events 2b to 2f during magnet-on after the electromagnetic coil reaches saturation.

[0155] In embodiments using a permanent magnet, data used for processing and analysis may be derived from events 2b to 2f during magnet-on immediately after the magnetic field from the permanent magnet is applied.

[0156] An evaluation of the analytical performance of a detection methodology is often done by measuring dose–response curves from which the limit of detection (LoD) can be derived. The LoD is the lowest quantity of a substance such as a biomarker that can be detected for a chosen confidence level. The chosen assay (biomarker, biomaterials, sample matrix, incubation times, etc.) may have a strong influence on the LoD. Also used is the limit of quantification (LoQ) that is the lowest biomarker concentration that can be quantified with a given required precision. The LoQ is close to the LoD if a dose–response curve has a good sensitivity, i.e. if the signal changes strongly as a function of the target concentration.

[0157] The present method may provide for an LoQ of about 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5 or 2.0 pg/mL, and suitable ranges may be selected from between any of these values.

[0158] The present method may provide for an LoD of about 0.1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2.0 pg/mL, and suitable ranges may be selected from between any of these values.

[0159] In some embodiments, the ambient magnetic field is measured at one or more stages. For example, the ambient magnetic field is measured at the pre-sample stage, sample introduction stage, sample read stage, and data analysis stage. The measured ambient magnetic field may be used to adjust the reference magnetic field signal and magnetic field signal obtained during the sample read stage.

[0160] In some embodiments, the sample may be magnetically shielded from interference of ambient magnetic fields.

[0161] Discrete Fourier Transform may be used to analyse the spectrum of the signal obtained from the sensors, for example, by separating the time domain signal into the frequency components.

[0162] In some embodiments, Fast Fourier Transform (FFT) algorithm may be used to process the signal data obtained from the sensors. FFT processing may convert the signals obtained from the sensors into individual spectral components including but not limited to frequency and magnitude.

[0163] Data from the sensors may be pre-processed prior to FFT processing. The signal data may be truncated (and concatenated) in one or more dimensions to achieve an optimal data set for FFT processing. For example, the sensor signal output data may be truncated in the time dimension corresponding to events 2a to 2f, and/or event 3a illustrated in figures 1a and 1b.

[0164] In some embodiments, the one or more portions of the sensor data may be truncated in one or more dimensions.

[0165] In some embodiments, the portion of the signal data between coil saturation and desaturation of each cycle is truncated.

[0166] In some embodiments, the truncated signal data may be concatenated. The concatenated data set then be subject to FFT processing.

[0167] In some embodiments, windowing may be applied to the data set. For example, one or more data windows may be concatenated and subject to FFT processing.

[0168] In some embodiments, the signal data for FFT processing may be selected from the intermediate cycles during the sample read stage. For example, the signal data from the third cycle may be selected for FFT processing from a sample read stage comprising five cycles.

[0169] The magnetic properties of nano- and micron-sized magnetic materials differ from those of the corresponding bulk magnetic materials. Typically, magnetisable particles are classified as paramagnetic, ferromagnetic, ferrimagnetic, antiferromagnetic, or superparamagnetic based on their magnetic behaviour in the presence and absence of an applied magnetic field.

[0170] According to an embodiment, superparamagnetic nanoparticles, such as superparamagnetic iron oxide nanoparticles (SPIONs), may be used to accurately detect and measure the change in Brownian motion as an overall magnetic response that is proportional to the size of the aggregates.

[0171] Superparamagnetic nanoparticles exhibit a tendency to align its magnetic moment in the presence of an external magnetic source and lose magnetisation in the absence of the magnetic field.

[0172] Diamagnetic materials exhibit no dipole moment in the absence of a magnetic field, and in the presence of a magnetic field they align against the direction of the magnetic field.

[0173] Paramagnetic particles exhibit random dipole moments in the absence of a magnetic field, and in the presence of a magnetic field they align with the direction of the magnetic field.

[0174] Ferromagnetic materials exhibit aligned dipole moments.

[0175] Ferrimagnetic and antiferromagnetic materials exhibit alternating aligned dipole moments.

[0176] In one embodiment the magnetisable particles are paramagnetic particles. Such particles will become magnetic when subjected to a magnetic field. Once the magnetic field is removed, the particles will begin to lose their magnetic characteristics.

[0177] In an alternate embodiment the magnetisable particles are ferromagnetic particles. That is, they always exhibit magnetic characteristics regardless of whether subjected to a magnetic field.

[0178] Commercially available magnetisable particles include Dynaparticles M-270, Dynaparticles M-280, Dynaparticles MyOne T1, and Dynaparticles MyOne C1 from Thermo Fisher Scientific, μ MACS MicroParticles from Miltenyi Biotec, SPHERO™ Superparamagnetic Particles, SPHERO™ Paramagnetic Particles, and SPHERO™ Ferromagnetic Particles from Spherotech.

[0179] The magnetisable particles may be ferromagnetic particles coated with Streptavidin. For example, Commercially available streptavidin coated superparamagnetic particles include Ocean NanoTech SHS30-01. The ferromagnetic particles coated with Streptavidin may be functionalised with biotinylated "detection" antibodies.

[0180] The magnetisable particles may be formed by ferrites which are themselves formed from iron oxide (such as magnetite and maghemite). Various methods are known for synthesising iron oxide and metal-substituted ferrite magnetisable particles such as co-precipitation, thermal decomposition, and hydrothermal. Co-precipitation processes use stoichiometric amounts of ferrous and ferric salts in an alkaline solution in conjunction with a water-soluble surface coating material, such as polyethylene glycol (PEG), where the coating provides colloidal stability and biocompatibility. The size and properties of the magnetisable particle can be controlled by adjusting the reducing agent concentration, pH, ionic strength, temperature, iron salts source, or the ratio of Fe^{2+} to Fe^{3+} .

[0181] The size and shape of magnetisable particles can be tailored by varying the reaction conditions, such as the type of organic solvent, heating rate, surfactant, and reaction time. This method leads to narrow size distributions of the magnetisable particles in the size range 10 to 100 nm. Fe^{2+} may be substituted by other metals to boost the saturation magnetisation.

[0182] The magnetisable particles may be coated with a hydrophobic coating during the synthesis process. If so, then the method of manufacturing the magnetisable particles may include an additional step of ligand exchange so that the magnetisable particles can be dispersed in water for further uses.

[0183] The magnetisable particles may be manufactured by polyol-hydrothermal reduction which produces water-dispersed magnetisable particles in the size range from tens to several hundred nanometres. The size and surface-functionalisation of the iron oxide magnetisable particles may be optimised by adjusting the solvent system, reducing agent, and type of surfactant used. This process may be used to synthesise FePt magnetisable particles.

[0184] The magnetisable particles may be manufactured by a reverse water-in-oil micelle methodology. This method forms a microemulsion of aqueous nanodroplets of iron precursors that is stabilized by a surfactant in the oil phase with the magnetic nanoparticles obtained by precipitation. Iron oxide nanocrystals may be assembled by combining the microemulsion and silica sol-gel, which may be obtained via co-precipitation into magnetisable particles having a diameter of more than 100 nm.

[0185] Metallic magnetisable particles may be either monometallic (e.g., Fe, Co, or Ni) or bimetallic (e.g., FePt and FeCo). Alloy magnetisable particles may be synthesised by physical methods including vacuum-deposition and gas-phase evaporation. These methods may produce FeCo magnetisable particles with high saturation magnetisation (about 207 emu/g) and may be synthesised via the reduction of Fe³⁺ and Co²⁺ salts.

[0186] The magnetisable particles may comprise a single metallic or metallic oxide core. The magnetisable particles may comprise multiple cores, multilayers of magnetic materials and nonmagnetic materials. The magnetisable particles may comprise a coating of silica or polymer cores with magnetic shells. The nonmagnetic core particles may comprise silica or other polymers.

[0187] The magnetisable particles may comprise a dielectric silica core coated with a magnetic shell. The magnetic shell may be formed from Co, FePt, or Fe₃O₄. The shell may also comprise a stabiliser such as silica shell or polyelectrolyte layer. The magnetisable particles may be mesoporous magnetisable particles.

[0188] The coating on the magnetisable particle may define the interactions between the magnetisable particles and biological molecules (such as analytes) and their biocompatibility. The coating can be used to define the surface charge, which together with the coating may alter the hydrodynamic size of the magnetic particle. The hydrodynamic size of the magnetisable particle may alter the functionality of the magnetic particle.

[0189] The magnetisable particles may be coated with specific coatings that provide forces of electrostatic and steric repulsion. Such coatings may assist stabilisation of the magnetisable particles which may prevent agglomeration or precipitation of the magnetisable particles.

[0190] The magnetisable particles may comprise of a coating formed from inorganic materials. Such magnetisable particles may be formed with a core-shell structure. For example, a magnetisable particle coated by biocompatible silica or gold (e.g. alloy magnetic nanoparticles, FeCo and CoPt coated with silica). The shell may provide a platform to modify the magnetisable particles with ligands (e.g. thiols). Other inorganic coating materials may

include titanate or silver. For example, silver-coated iron oxide magnetisable particles may be synthesised and integrated with carbon paste.

[0191] The shell may be formed from silica. A benefit of coating with silica is the ability of the silica-coated magnetisable particles to bind covalently with versatile functional molecules and surface-reactive groups. The silica shell may be manufactured, for example, by the Stober method using sol-gel principles or the Philipse method or a combination thereof. The core of the magnetisable particle may be coated with tetraethoxysilane (TEOS), for example, by hydrolysis of TEOS under basic conditions which condenses and polymerises TEOS into a silica shell on the surface of the magnetic core. A cobalt magnetisable particle may be coated using a modified Stober method that combines 3-aminopropyl)trimethoxysilane and TEOS.

[0192] The Philipse method forms a silica shell of sodium silicate on the magnetic core. A second layer of silica may be deposited by the Stober method. The reverse microemulsion method may be used to coat with silica. This method may be used with surfactants. The surfactant may be selected from Igeal CO-520 to provide a silica shell thicknesses of about 5 to about 20 nm. Preferably the reagents for manufacturing silica shells is selected from amino-terminated silanes or alkene-terminated silanes. Preferably the amino-terminated silanes is (3-aminopropyl)trimethoxysilane (APTMS). Preferably the alkene-terminated silanes is 3-methacryloxypropyl)trimethoxysilane.

[0193] The magnetisable particles may be coated with gold. Gold-coated iron oxide nanoparticles may be synthesised by any one of chemical methods, reversed microemulsion, and laser-promoted methods. Gold-coated magnetisable particles may be synthesised by directly coating gold on the magnetisable particle core. Alternately, the gold-coated magnetisable particle may be synthesised by using silica as an intermediate layer for the gold coating. Preferably reduction is used method to deposit gold shells on the magnetisable particles.

[0194] Metal oxide or silica-coated magnetic cores may first be functionalized with 3-aminopropyl)trimethoxysilane prior to the electrostatically attachment of about 2 to about 3 nm gold nanocrystal seeds (from chloroauric acid) to the surface followed by the addition of a reducing agent to form the gold shell. Preferably the reducing agent is a mild reducing agent selected from sodium citrate or tetrakis(hydroxymethyl)phosphonium chloride. In some embodiments the gold shell is formed from reduction of gold(III) acetate ($\text{Au}(\text{OOCCH}_3)_3$). In some embodiments the gold shells are formed on metallic magnetic cores (e.g. nickel and iron) by reverse micelles.

[0195] The magnetisable particles may be functionalised with organic ligands. This may be performed in-situ (i.e. functional ligands provided on the magnetisable particle during the synthesis step), or post-synthesis. The magnetisable particles may be functionalised with terminal hydroxyl groups (-OH), amino groups (-NH₂), and carboxyl groups (-COOH). This may be achieved by varying the surfactant (e.g., dextran, chitosan, or poly(acrylic acid)) used in the hydrothermal synthesis.

[0196] The functionalisation of the magnetisable particle post-synthesis may allow for the functionalisation of customised ligands on any magnetisable particle surface. Post-synthesis functionalisation may be carried out by ligand addition and ligand exchange. Ligand addition comprises the adsorption of amphiphilic molecules (that contain both a hydrophobic segment and a hydrophilic component) to form a double-layer structure. Ligand-exchange replaces the original surfactants (or ligands) with new functional ligands. Preferably the new ligands contain a functional group that is capable of binding on the magnetisable particle surface via either strong chemical bonding or electrostatic attraction. In some embodiments the magnetisable particle also includes a functional groups for stabilisation in water and/or bio-functionalisation.

[0197] The magnetisable particles may be coated with ligands that enhance ionic stability. The functional groups may be selected from carboxylates, phosphates, and catechol (e.g. dopamine). The ligand may be a siloxane group for coating of surfaces enriched in hydroxyl groups (e.g. metal oxide magnetic particle or silica-coated magnetic particles). The ligand may be a small silane ligand that links the magnetisable particle and various functional ligands (e.g. amines, carboxylates, thiols, and epoxides. The silane ligand may be selected from N-(trimethoxysilylpropyl)ethylene diaminetriacetic acid and (triethoxysilylpropyl)succinic anhydride to provide a carboxylate-terminated magnetic particles. The functional groups may be selected from phosphonic acid and catechol (to provide hydrophilic tail groups). The functional groups may be selected from amino-terminated phosphonic acids. Functional groups may be selected from 3-(trihydroxysilyl)propyl methylphosphonate for dispersion in aqueous solution. The ligand may be selected from dihydroxyhydrocinnamic acid, citric acid, or thiomalic acid for magnetisable particles for dispersion in water.

[0198] In some embodiments the magnetisable particle is functionalised with polymeric Ligands. The polymer may be selected from natural polymers (e.g. starch, dextran or chitosan), PEG, polyacrylic acid (PAA), poly(methacrylic acid) (PMAA), poly(N,N-methylene-bisacrylamide) (PMBBAm), and poly(N,N'-methylenebisacrylamide-co-glycidyl methacrylate) (PMG).

[0199] The functional group on the magnetisable particle surface serves as a linker to bind with a complementary biomolecules. The biomolecules may be a small biomolecules. The small biomolecule may be selected from vitamins, peptides, and aptamers. The biomolecule may be a larger biomolecule. The larger biomolecule may be selected from DNA, RNA and proteins.

[0200] In relation to nucleic acid attachment, the nucleic acid may be conjugated by non-chemical methods (e.g. electrostatic interaction) or chemical methods (e.g. covalent bonding). The nucleic acid chain may be modified with functional groups. The functional groups may be selected from thiols or amines, or any combination thereof.

[0201] The conjugation of larger biomolecules may rely on their specific binding interaction with a wide range of substrates and synthetic analogues, such as specific receptor-substrate recognition (i.e. antigen-antibody and biotin-avidin interactions).

[0202] A specific pair of proteins may be used to immobilise species on the magnetic particle. Physical interactions include electrostatic, hydrophilic-hydrophobic, and affinity interactions.

[0203] In some embodiments the biomolecule has a charge opposite to that of the magnetic polymer coating (e.g. polyethylenimine or polyethylenimine). For example, a positively charged magnetisable particle binding with negatively charged DNA.

[0204] The magnetisable particles may utilise the biotin-avidin interaction. The biotin molecules and tetrameric streptavidin have site-specific attraction with low nonspecific binding for controlling the direction of interacted biomolecules, such as the exposure of the Fab region of an antibody toward its antigen.

[0205] The magnetisable particles may bind to biomolecules using covalent conjugation. The covalent conjugation may be selected from homobifunctional/heterobifunctional cross-linkers (amino group), carbodiimide coupling (carboxyl group), maleimide coupling (amino group), direct reaction (epoxide group), maleimide coupling (thiol group), schiff-base condensation (aldehyde group), and click reaction (alkyne/azide group).

[0206] The magnetisable particles may have an average particle size of about 5, 10, 15, 20, 25, 30, 35, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450 or 500 nm, and suitable ranges may be selected from between any of these values, (for example, about 5 to about 500, about 5 to about 400, about 5 to about 250, about 5 to about 100, about 5 to about 50, about 10 to about 500, about 10 to about 450, about 10 to about 300, about 10 to about 150,

about 10 to about 50, about 50 to about 500, about 50 to about 350, about 50 to about 250, about 50 to about 150, about 100 to about 500, about 100 to about 300, about 150 to about 500, about 150 to about 450 or about 200 to about 500 nm).

[0207] The magnetisable particles may have an average particle size of about 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 or 1000 nm, and suitable ranges may be selected from between any of these values, (for example, about 500 to about 1000, about 500 to about 850, about 500 to about 700, about 550 to about 1000, about 550 to about 800, about 600 to about 1000, about 600 to about 900, about 650 to about 1000, about 650 to about 950, about 650 to about 800 or to about 700 to about 1000 nm).

[0208] The magnetisable particles may have an average particle size of about 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500 or 5000 nm, and suitable ranges may be selected from between any of these values, (for example, about 1000 to about 5000, about 1000 to about 4000, about 1500 to about 5000, about 1500 to about 4500, about 1500 to about 3500, about 2000 to about 5000, about 2000 to about 4000, about 2500 to about 5000, about 2500 to about 3500, about 3000 to about 5000 nm).

[0209] The variation in the particle size of the magnetisable particles may be less than 25, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1%, and suitable ranges may be selected from between any of these values.

[0210] In some embodiments, the magnetisable particles may comprise 30 nm superparamagnetic particles.

[0211] In some embodiments, the magnetisable particles may comprise 50 nm superparamagnetic particles.

[0212] In some embodiments, the binders and magnetisable particles (beads) may be provided in specified ratios.

[0213] The binders and magnetisable particles may have a binder-to-magnetisable particle ratio of about 10:1, 9:1, 8:1, 7:1, 6:1 5:1, 4:1, 3:1, 2:1, 1.5:1, 1:1, 0.75:1, 0.5:1, 0.25:1, 1:0.25, 1:0.5, 1:0.75, 1:1.5, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, or 1:10, and suitable ranges may be selected from between any of these values, (for example about 10:1 to about 1:10, about 9:1 to about 1:9, about 8:1 to about 1:8, about 7:1 to about 1:7, about 6:1 to about 1:6, about 5:1 to about 1:5, about 4:1, to about 1:4, about 3:1 to about 1:3, about 2:1 to about 1:2, about 0.75:1 to about 1:0.75).

[0214] The binder-to-magnetisable particle ratio may be a ratio of the number of magnetisable particles to the number of binders. For example, for a binder to bead ratio of 0.75:1, is 0.75ng of binders corresponding to 1 μ g of beads.

[0215] Following on from the example above, the 30nm beads (Ocean Nanotech) may have a molar concentration of 34fmole/ μ l or 0.034 μ M, it may be calculated that the number of beads per μ l (where the concentration of beads is 1 μ g/ μ l) to be approximately 2.047×10^{10} beads (34×10^{-15} times Avogadro's number = $34 \times 10^{-15} \times 6.023 \times 10^{23}$ equals to 2.047×10^{10} beads).

[0216] The molecular weight of the binders may be approximately 150977.24 g/mol. The number of binders for 0.75ng then amounts to 2.99×10^9 binders (The number of moles of binder is $0.75\text{ng} / 150977.24 \text{ g/mol} = 4.97 \times 10^{-15}$ moles. To get the number of binders per 0.75ng, the moles may be multiplied to Avogadro's number = 4.97×10^{-15} moles times 6.023×10^{23} equals to 2.99×10^9 binders).

[0217] The molar ratios is calculated to be 2.047×10^{10} beads/ 2.99×10^9 binders = 6.846. That is, 1 binder per ~7 beads. The surface area of one bead of 30nm diameter is approximately 2827.43nm². Therefore, 1 binder will correspond to $7 \times 2827.43\text{nm}^2 = 19792.01\text{nm}^2$.

[0218] In some embodiments, the binders and magnetisable particle surface may be provided in specified binder-to-surface area ratio.

[0219] The binder-to-surface area ratio may be about 1 to about 2,000, about 2,500, about 3,000, about 3,500, about 4,000, about 4,500, about 5,000, about 5,500, about 6,000, about 6,500, about 7,000, about 7,500, about 8,000, about 8,500, about 9,000, about 9,500, about 10,000, about 10,500, about 11,000, about 11,500, about 12,000, about 12,500, about 13,000, about 13,500, about 14,000, about 14,500, about 15,000, about 15,500, about 16,000, about 16,500, about 17,000, about 17,500, about 18,000, about 18,500, about 19,000, about 19,500, about 20,000, about 20,500, about 21,000, about 21,500, about 22,000, about 22,500, about 23,000, about 23,500, about 24,000, about 24,500, or about 25,000 nm² and suitable ranges may be selected from between any of these values.

[0220] The particles may be tethered or untethered. Tethered particles are tethered to larger secondary particles (macromolecules). Untethered particles may freely diffuse throughout the sample while tethered particles have limited diffusability and may freely diffuse in the sample within the range of the tether. As stated above, the amount of analyte in a sample is determined based on the change in the signal detected by the sensing

module. The sensing module detects the change based on the net movement of the particles.

[0221] The particles may be attached to other objects such as larger secondary particles or molecules. The magnetisable particles may also be attached to surfaces. Attachment to other objects or surfaces allow the magnetisable bead to be positioned at a specific location whilst retaining the ability to undergo Brownian diffusion (within the limits of the attachment or tether) that is detectable and measurable by the apparatus.

[0222] The tethering advantageously allows retains the ability for the particles to undergo Brownian diffusion whilst being localised as a specific location in a larger shared volume, and as such, multiple types of magnetisable particles (types by analyte recognition or other properties) can all be in their discrete locations (e.g. aligned to a specific magnetic sensor) whilst in a shared volume, and this allows for multiplex detection of different target analytes in the one volume.

[0223] Tethering to the non-magnetisable beads or surfaces of a microchannel allows for this multiplex detection as the non-magnetisable bead can act as an 'anchor' to keep the tethered particles in a location via a combination of size, surface chemistry and interaction with its local environment.

[0224] For example, magnetisable particles may be molecularly tethered to a larger non-magnetisable particle such as a latex bead such that the magnetisable particles are localised in a specific area due to the larger non-magnetisable bead but may still freely diffuse within the limit of the tethers. In another example, the magnetisable particles may be molecularly tethered to a surface, such as a surface of the microfluidic device corresponding to sensing zone of the sensing module.

[0225] The non-magnetisable particles may comprise any suitable non-magnetisable particles, including but not limited to, latex beads, polystyrene beads, or other types of polymer beads.

[0226] In some embodiments, non-magnetisable particles such as latex beads with surface chemistries (such as amines and carboxyl groups) can have molecular tethers attached to them (e.g. Polyethylene glycol – PEG) such that one end of the molecular tether is attached to the latex bead (with chemistries compatible with the latex bead surface) and the other end is attached to the magnetisable bead (with chemistries compatible with the magnetic bead surface e.g. Biotin on the tether attaching to Streptavidin on the surface of the magnetic bead), thus forming a tethered connection between the two beads.

[0227] The molecular tether may be about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80 nm in length.

[0228] The method may be carried out using a microfluidic device or system.

[0229] The microfluidics may require some degree of sample preparation. The sample preparation may include cell lysis, washing, centrifugation, separation, filtration, and elution. In some embodiments the sample preparation is prepared off-chip. In an alternative sample preparation is prepared on-chip.

[0230] In some configurations, the sample to be analysed may be added directly to a sample well or microfluidic device without additional processing. The microfluidic system may comprise a fluid. The fluid may be selected from phosphate buffered saline (PBS). The phosphate buffered saline may comprise potassium phosphate dibasic (K_2HPO_4), sodium chloride (NaCl) and disodium phosphate (Na_2HPO_4). The PBS provides the continuous phase which the particles are suspended.

[0231] Microfluidic systems enable faster analysis and reduced response times. Microfluidic systems also offer the ability to automate the preparation of the sample, thereby reducing the risk of contamination and human error. Additionally, microfluidic systems require low sample volumes. Microfluidics may reduce diffusional distances by increasing the surface area to volume ratios, reducing reagent consumption through micro- and nanofabricated channels and chambers, and/or automating all steps of the process.

[0232] In some configurations the microfluidics system includes hard or flexible materials, and may include electronics that may be integrated into the device. The electronics may include wireless communication electronics.

[0233] The microfluidic system may be a flow-through or stationary system. For example, the microfluidic system may comprise magnetic field sensor that is stationary relative to the microfluidic system.

[0234] The microfluidic system may operate passively. For example, the microfluidic system may operate under passive diffusion. That is, the microfluidic system does not require flow generated actively to perform effectively.

[0235] The microfluidic system may include a network of reservoirs, and that may be connected by microfluidics channels. The microfluidics channels may be configured for active metering or passive metering. This may allow for sample fluid to be drawn into the microfluidics channel and passed into a sample chamber.

[0236] Microfluidic systems allows for miniaturisation which allows for lab-on-chip applications. Microfluidic systems may be used as part of the biosensor, for example, including channels for acquiring a biological sample (e.g., saliva and/or Gingival Crevicular Fluid and/or tears and/or sweat, etc.), processing the fluid (e.g., combining with one or more reagents and/or detecting an interaction with a biomolecule, etc.)

[0237] Microfluidic systems may be implemented in the form of microfluidic chips. Microfluidic chips comprise a set of micrometre or millimetre sized channels provided, for example by moulding or etching, onto a material or combination of materials such as glass, silicon, or other types of polymers. The microfluidic channels may be interconnected to form a network of channels. The channels may vary in length from millimetres to centimetres long.

[0238] The microfluidic chips may comprise one or more ports for receiving samples, and/or reagents. For example, the microfluidic chip may comprise sample inlet ports, and reagent ports.

[0239] The microfluidic chips may comprise a plurality of detection areas. The detection areas define portions of the channels in which detection and quantitation of the analyte or biomarkers in a sample occurs. The detection areas of the microfluidic chip correspond to the position of the magnetic sensors of the device such that when a microfluidic chip is placed over the detection surface of the device, each detection area vertically aligns with a corresponding magnetic/other sensor.

[0240] The detection areas may be located at any position along the channels. In some embodiments, the detection areas are located channel juncture points. That is, the detection area is located at the intersection of two or more channels.

[0241] The channel juncture points may comprise a reaction/detection well. The reaction/detection well may comprise a dimension that is larger than the channels.

[0242] The microfluidics may require some degree of sample preparation. The sample preparation may include cell lysis, washing, centrifugation, separation, filtration, and elution. In some embodiments the sample preparation is prepared off-chip. In an alternative sample preparation is prepared on-chip.

[0243] The microfluidic chip may be provided in a 'ready to use' format. For example, the microfluidic chip may be pre-loaded with all the necessary elements and cell separation (such as binder complex and reagents) for performing analyte detection and quantitation.

That is, the 'ready to use' format only requires the addition of a sample to the microfluidic device.

[0244] The reaction/detection wells may be pre-loaded with binder complexes for binding one or more target analytes. The binder complex may be provided within a gel matrix in the reaction/detection wells. For example, each reaction/detection well may comprise hydrogel, agarose gel, or agar containing binder complexes. Binder complexes are described in detail later in the description.

[0245] The binder complexes and/or reagents may be added to the reaction/detection wells before use.

[0246] The microfluidic system may include hard or flexible materials, and may include electronics that may be integrated into the microfluidic chips. The electronics may include wireless communication electronics.

[0247] The microfluidic system may be a flow-through or stationary system. For example, the microfluidic system may comprise magnetic field or other sensors that are stationary relative to the microfluidic system.

[0248] The microfluidic system may operate passively. For example, the microfluidic system may operate under passive diffusion. That is, the microfluidic system does not require flow generated actively to perform effectively.

[0249] The microfluidic system may include a network of reservoirs, and that may be connected by microfluidics channels. The microfluidics channels may be configured for active metering or passive metering. This may allow for sample fluid to be drawn into the microfluidics channel and passed into a sample chamber.

[0250] The channels may be arranged in a cross-hatch configuration.

[0251] The microfluidic system may include microfluidic channels that are configured to allow access to various sample and/or detection regions on the device at various times. For example, the microfluidics device integrated into or on an aligner may be configured to provide timing via temporal-sampling of a fluid. For example, a microfluidic system can be designed to enable sampling with chronological order and controlled timing. In some variations, the timing of fluid within the microchannel may be timed actively, e.g., by the opening of a channel via release of a valve (e.g. an electromechanical valve, an electromagnetic valve, a pressure valve). Examples of valves controlling fluid in a microfluidic network include piezoelectric, electrokinetics and chemical approaches.

[0252] The channels of the microfluidic chip may comprise wicking structures. The wicking structures may improve the speed in fluid is transported by capillary action. The wicking structure may comprise porous media such as paper based material.

[0253] The microfluidic chip may comprise a plurality of microfluidics channels that are sequentially arranged. The fluid may be drawn into the microfluidics at a metered rate. The timing of access of samples to the channels may be staggered.

[0254] The microfluidics may carry out signal multiplexing. That is the microfluidics may be used to sample and/or measure multiple biomarkers in controlled intervals. For example, the microfluidics may be used to provide access to one or more sample chambers. The microfluidics may include one or more valves that are controlled by control circuitry in the device. The one or more valves may be connected to each other. Thus, the microfluidics may be adapted to perform simultaneous detection of multiple analytes in a common sample body. Additionally or alternatively, the microfluidics may be configured to perform simultaneous multiple detection of multiple samples of the same target.

[0255] The microfluidic channel(s) may have a cross section in the range of about 0.001 to 0.01 mm², 0.01 to 0.1 mm², 0.1 to 0.25 mm², 0.25 to 0.5 mm², 0.1 to 1 mm², 0.5 to 1 mm², 1 to 2 mm², or 2 to 10 mm², and useful ranges may be selected between any of these values.

[0256] In some embodiments the microfluidics receives a predetermined sample volume in the range of about 0.1 to 1 µL, 1 to 5 µL, 5 to 10 µL, 10 to 20 µL, or 20 to 50 µL or more, and useful ranges may be selected between any of these values.

[0257] Shown in Figure 2 is an example of a sample introduction device/microfluidic chip. The microfluidic chip may comprise a plurality of channels arranged to direct the sample from the sample insertion area towards a detection area and functionalised particles for analyte detection.

[0258] The channels may have a cross-sectional dimension as mentioned above, and more preferably of about 0.01 mm² (0.1 mm x 0.1 mm). The channels may have a variable length. For example, the channels may be 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, or 300 mm long, and useful ranges may be selected between any of these values, (for example, from about 1 to 10, 1 to 20, 1 to 50, 1 to 100, 1 to 200, 1 to 300, 10 to 20, 10 to 40, 10 to 60, 10 to 80, 10 to 100, 50 to 100, 50 to 150, 50 to 200, 50 to 250, 50 to 300, 100 to 200, or 100 to 300 mm long).

[0259] The above dimensions of the channels facilitate passive capillary flow.

[0260] When in use, a sample is introduced to the microfluidics device via the sample insertion area. The sample insertion area may comprise an inlet port.

[0261] A filter membrane may be present at the insertion area to separate and allow through the desired components of a sample. For example, to allow plasma from blood to pass into the microfluidic chip, but not cells. The presence of the filter membrane is dependent on the nature of the sample, and whether it comprises components for which it is desirable that they do not pass into the microfluidics chip.

[0262] Plasma-cell separation may result from on or of device configuration.

[0263] Once introduced into the insertion area, the sample will then contact the microfluidic channels and flow through the rest of the channel circuit.

[0264] The microfluidic system may be implemented as a lab-on-chip. The lab on chip may comprise of one or more magnetic sensors in close proximity to the channels. For example, the microfluidic device 1 may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 magnetic sensors arrayed around the microfluidic device.

[0265] The lab-on-chip may comprise two or more magnets, such as permanent magnets or electromagnets for example, arranged in close proximity to the channels that can be activated to draw magnetisable particles through the liquid in the channels to enhance mixing. The mixing may, for example, be carried out for 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 min, and suitable ranges may be selected from between any of these values. The timing of mixing may depend on assay requirements such as sample volume, viscosity, composition and detection ranges of target analyte.

[0266] To effect mixing, the magnets (e.g. electromagnets) may be arranged at substantially opposed ends of a channel, or of the microfluidic device. For example, magnets may be controlled or switched such that they push/pull the magnetisable particles towards one end of a well/channel or the microfluidic device, and then the effect reversed to pull the magnetisable particles towards another end of the well/channel or the microfluidic device. This cycle may be repeated multiple times until the desired level of mixing has been achieved.

[0267] According to an embodiment, the described method may be performed using a device for detecting analyte in a sample consisting essentially of:

- a sample well that is separate to, or integrated into a microfluidic device,
- a magnet for generating an external magnetic field,

- a magnetic signal sensor for measuring magnetic signals within the sample well, and wherein the magnetic signal sensor is adapted to measure the magnetic signals while the external magnetic field is active.

[0268] According to another embodiment, the described method may be performed using a device for detecting analyte in a sample consisting essentially of:

- a sample well that is separate to, or integrated into a microfluidic device,
- a first magnet positioned below the sample well, the first magnet adapted to apply a magnetic field continuously,
- a second magnet positioned above the sample well, the second magnet adapted to apply a magnetic field non-continuously,
- a magnetic signal sensor for measuring magnetic signals within the sample well, and wherein the magnetic signal sensor is adapted to measure the magnetic signals when both the continuous and non-continuous magnetic fields are active.

[0269] The magnetic field generator may comprise magnets.

[0270] The magnetic field generators may generate a magnetic field in a direction perpendicular to the sensor. For example, the magnetic field generator may generate a magnetic field from above and/or below the magnetic field sensors such that the magnetic field is perpendicular to the body of the magnetic field sensors.

[0271] The magnetic field generator may generate a magnetic field in a direction parallel to the sensor. For example, the magnetic field generator may generate a magnetic field from the side of the magnetic field sensors such that the magnetic field is parallel to the body of the magnetic field sensors.

[0272] The device may comprise a combination of magnetic field generators that respectively generate magnetic field in perpendicular and parallel directions relative to the sensors.

[0273] The magnetic field generator may be configured to generate a magnetic field from below and/or above the sample.

[0274] The magnets may be electromagnets. The electromagnets may exert a field strength of about 0.5, 1, 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 Gauss, and suitable ranges may be selected from between any of these values.

[0275] In some embodiments, the magnets may comprise a combination of a permanent and an electromagnet magnet. For example, the first magnet may be a permanent magnet that exerts a continuous magnetic field, and the second magnet may be an electromagnet that applies a non-continuous magnetic field.

[0276] The permanent magnet may comprise any suitable permanent magnets. In some embodiments, the permanent magnet may be selected from one or more of ceramic, samarium-cobalt (SmCo), aluminum-nickel-cobalt (AlNiCo), neodymium iron boron magnets.

[0277] The magnets may exert a magnet field strength of about 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 5, 10, 50 or 100 Gauss, and suitable ranges may be selected from between any of these values. The sample data is then acquired as described.

[0278] The magnetisable particles are sensed by a magnetic sensor.

[0279] The magnetic sensor may be selected from spintronic sensors, atomic magnetometers (AMs), nuclear magnetic resonance (NMR) systems, fluxgate sensors, Faraday induction coil sensors, diamond magnetometers, and domain walls-based sensors.

[0280] The volumetric-based sensors, such as planar hall effect (PHE) sensors provide simple and rapid sample preparation and detection. Surface-based sensors, such as giant magnetoresistance (GMR) offer a lower detection limit (single particle) due to the short distance between the magnetisable particles and the sensor. However, these techniques typically require laborious sample and/or substrate preparation. Optimising magnetisable particles for specific applications and selecting appropriate detection methods remain challenging for the magnetic nanotechnology community due to the increasing demands of detection sensitivity, molecular specificity, and application complexity. The spintronic sensors may be selected from giant magnetoresistance (GMR), tunnel magnetoresistance (TMR), anisotropic magnetoresistance (AMR), and planar Hall effect (PHE) sensors.

[0281] The GMR effect was discovered in the 1980s and has traditionally been used in data recording. The spin valve provides higher sensitivity with a micron-sized design. A spin-valve GMR sensor consists of an artificial magnetic structure with alternating ferromagnetic and nonmagnetic layers. The magneto resistance effect is caused by the spin-orbital coupling between conduction electrons crossing the different layers. The variation in magnetoresistance provides quantitative analysis by this spin-dependent sensor. GMR sensors may be used to detect DNA-DNA or protein (antibody)-DNA interactions. The dimensions of the sensor array may be adjusted for the detection of individual magnetisable particles. GMR sensors may be used in combination with antiferromagnetic particles.

[0282] The planar Hall effect is an exchange-biased permalloy planar sensor based on the anisotropic magnetoresistance effect of ferromagnetic materials. The PHE sensor may be a spin-valve PHE or PHE bridge sensor. The PHE sensor may be able to carry out single-particle sensing.

[0283] As will be appreciated by a skilled addressee in the field of endeavour, Brownian motion or Brownian diffusion may mean that the particles may move in any direction, including towards the magnetic field sensor or electric field sensor. The magnetic signal detected by the magnetic field sensor is based on the net movement of the bound and unbound magnetisable particles. The electric signal detected by the electric field sensor is based on changes in impedance as the particles move through the continuous phase (e.g. the PBS).

[0284] When the bound and unbound particles are positioned in proximity to the magnetic or electric field sensor, the bound and unbound particles may locate at, or close to, the surface of wall of the sample well or sample reservoir until released. Once released from their proximity to the magnetic or electric field sensor, the particles may move, translationally or rotationally. Given their proximity to the surface of the sample well or sample reservoir immediately prior to release from the bias system, the bound and unbound magnetisable particles may typically first tend to move with an approximate 180° freedom of movement relative to the surface of the sample well or sample reservoir.

[0285] The magnetic field may be generated and positioned in such a way as to maximise its effect on the magnetisable particles but minimise its effect on the magnetic field sensor. The magnetic field generator may be generated and/or positioned in close proximity to the magnetic field sensor. In some embodiments, the magnetic field generator is positioned above, below or beside the magnetic field sensor. In some embodiments, the magnetic field generator may be positioned on the same plane vertical or horizontal plane as the magnetic field sensor.

[0286] The magnetic field may be reduced gradually.

[0287] The magnetic field may be removed instantly.

[0288] The magnetic field may be variable in shape.

[0289] As the magnetic field applied to the sample is reduced and/or removed, the bound and unbound binder complexes are released from the magnetic field and may freely diffuse away (translational movement) from their proximity to the magnetic field sensor. The

binder complex may also rotate relative to the magnetic field sensor (rotational movement) as the magnetic field applied to the sample is reduced and/or removed.

[0290] The magnetic field sensor may be an on-chip magnetometer. The magnetic field sensor may have a sensitivity of at least 1 mV/V/gauss. In some embodiments, the magnetic field sensor may detect and/or measure a magnetic field of at least about 10 mGauss, 1 mGauss, 100 μ Gauss, or 10 μ Gauss.

[0291] The magnetic field sensor may comprise multiple axis, for example one, two or three-axis.

[0292] The magnetic field sensor may be a Honeywell HMC 1021S magnetometer. In another embodiment, the magnetic field sensor may be a Honeywell HMC1041Z magnetic sensor. In other embodiments, the magnetic field sensor may be selected from the group comprising Honeywell HMC 1001, HMC 1002, HMC 1022, HMC 1051, HMC 1052, HMC 1053, or HMC 2003 magnetometers.

[0293] The magnetic field sensor may comprise a bespoke magnetic field sensor having custom components.

[0294] In order to achieve a high level of accuracy and sensitivity, the magnetic sensors of the apparatus may comprise a high sampling rate. The magnetic sensors may sample at a sampling rate of about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, or 250 kHz, and suitable ranges may be selected from between any of these values, (for example, about 10 to about 250, about 10 to about 200, about 10 to about 150, about 10 to about 100, about 100 to about 250, about 100 to about 200, about 100 to about 150 kHz.)

[0295] The ADC sampling rate of the magnetic sensors may have a sampling rate of about 100kHz to about 200kHz.

[0296] The magnetic sensors may have a sampling rate of about 150 kHz per channel.

[0297] Multiple magnetic sensors may be provided on the detection surface to simultaneously measure the change in magnetic field. For example, the detection surface may comprise two, three, four, five, six, seven, eight, nine, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50 magnetic sensors.

[0298] The magnetic field sensors may be provided in a relatively small area in the device. For example, 24 magnetic field sensors may be provided to an area of about 13 mm x 19 mm. Such a configuration enables faster sample-to-data times, due the shorter

microfluidic channels that are used with this magnetic field sensor configuration. This configuration further enables a smaller and more portable apparatus.

[0299] The device may comprise about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 magnetic field sensors per cm² of the printed circuit board, and useful ranges may be selected between any of these values, (for example, about 5 to about 15, about 5 to about 13, about 5 to about 10, about 6 to about 15, about 6 to about 12, about 6 to about 9, about 7 to about 15, about 7 to about 14, about 7 to about 13, about 7 to about 10, about 8 to about 15, about 8 to about 14, about 8 to about 11, about 9 to about 15, about 9 to about 13 or about 10 to about 15 sensors per cm² of the printed circuit board).

[0300] In some embodiments, multiple magnetic field sensors may be used simultaneously to measure the change in magnetic field. For example, 50, 60, 70, 80, 90, 100, 110 or 120 magnetic field sensors for small portable applications and in situ laboratory or clinical applications, and useful ranges may be selected between any of these values, (for example about 50 to about 120, about 50 to about 100, about 50 to about 90, about 50 to about 80, about 60 to about 120, about 60 to about 110, about 60 to about 90, about 70 to about 110, about 70 to about 90, about 80 to about 120 or about 80 to about 110 magnetic field sensors).

[0301] In some embodiments, multiple magnetic field sensors may be used simultaneously to measure the change in magnetic field. For example, 1000, 1250, 1500, 1750, 2000, 2250, 2500, 2750 or 3000 magnetic field sensors for laboratory or clinical, research or industrial applications.

[0302] Data acquisition by the sensor may be synchronised with the microfluidic device. This may allow data from the detected sensor to be characterised between sample data or environmental or ambient data. For example, detection by the magnetic sensor of a signal absent sample injection into the microfluidic device would characterise that data as environmental or ambient data. Characterisation of the data as environmental or ambient data may assist to establish background and may also assist preparing calibration data.

[0303] Where the magnetic sensor detects a signal following injection of the sample into a microfluidic device, which coincides with the positioning of the magnetisable particles into close proximity with the magnetic sensor, such data can be characterised as sample data.

[0304] System utilization of such sensing can deliver embodiments in which microfluidic quality control measurement can take place confirming sample displacement and sensing times.

[0305] Data acquisition from the sensor may be continuous. That is, the magnetic sensor continuously transmit signals and, based on the synchronisation of the data collection with the injection of sample into the microfluidics device, characterises the data as sample data or background data.

[0306] The sensor data may be acquired over a period of time in order to measure changes in the magnetic signal from the magnetisable particles. Actions or events may be inferred from changes in the sensed magnetic signal. The actions or events include may include movement of the magnetisable particles from fluid flow, from external magnetic forces, or from diffusion.

[0307] The target analyte can be any substance or molecule that is complementary to and capable of being bound by a binding molecules provided to the magnetisable particles. For example, the target analyte can be selected from the group comprising of a protein, a peptide, a nucleic acid, lipid or a carbohydrate, biochemical, biological agent, virus, bacteria, etc.

[0308] The target analyte may be a protein or a fragment thereof selected from the group comprising of an antibody, an enzyme, a signalling molecule or a hormone.

[0309] The target analyte may be a nucleic acid selected from the group comprising of DNA, RNA, cDNA, mRNA, or rRNA.

[0310] The method may detect more than one target analyte in a single sample. For example, the method may detect two or more, three or more, four or more, five or more, 10 or more, 15 or more, 20 or more 40 or more or 50 or more target analytes in a single sample.

[0311] The sample to be analysed can be any sample that may contain one or more target analyte(s). For example, the sample may be a clinical, veterinary, environmental, food, forensic or other suitable biological samples.

[0312] The clinical sample may be selected from a bodily fluid. For example, the bodily fluid may be selected from blood, sweat, saliva, urine, sputum, semen, mucous, tears, cerebral spinal fluid, amniotic, gastric juices, gingival crevicular or interstitial fluids.

[0313] The environmental sample may be selected from the group comprising of water, soil or an aerosol.

[0314] A benefit of the present invention may be that the sample preparations are not laborious or difficult to prepare. The sample preparation utilises established biochemistries

for molecular functionalisation and attachment, either on microfluidic surfaces or magnetisable particle surfaces.

[0315] The sample to be analysed may be added directly to a sample well or microfluidic device without additional processing.

[0316] The sample may be subjected to one or more sample processing steps. It will be understood that suitable sample processing steps may depend on the type and/or nature of the sample to be analysed. In some embodiments, sample processing steps may be selected from the group comprising dilution, filtration, or extraction (e.g. liquid-liquid, solid-phase). This may also be achieved through microfluidic featured and designs or the use of centripetal force. For example, whole blood samples may be filtered using cellulose based or other filters to isolate plasma to be analysed.

[0317] The method may comprise combining the sample to be analysed with a preparation containing freely diffusible magnetisable particles that are coated with binding molecules (the binder complex) complementary to the target analyte in a sample well or sample reservoir. Where appropriate, the term 'binder complex' may be used interchangeably to refer to the magnetisable particles that are coated with binding molecules.

[0318] In some embodiments the magnetisable particles may have limited diffusibility. This may occur where the magnetisable particles are cross-linked or derivatised with macromolecules. The macromolecules may be a hydrogel or PEG linker. This may occur when using the device for multiplexing assays for detection of multiple targets or samples in the one sample.

[0319] The present method may improve the rate at which the binding molecules bind target analytes by providing binder complexes that are mobile and freely diffusible in solution. When the sample and binder complex preparation are combined, the binder complexes are freely diffusible and the binding molecules are able to interact with the target analytes throughout the entire sample volume. As both the binder complex and target analytes are freely diffusible and suspended in the sample volume, the average physical distance between a target analyte and a binder complex is likely to be small. As such, the rate of binding may be improved and binding equilibrium may be achieved significantly faster.

[0320] In detection assays such as ELISA, binding molecules such as antibodies are immobilised on macro scale objects such as the surface of a test well. In such a method, the physical distance between a target analyte and an antibody may vary significantly depending

on the position of the analyte in the sample volume. For example, a target analyte near the top of the sample volume may be quite far from the immobilised antibody and will be less likely to be captured and bound. As such, the rate of binding may be limited by the rate at which target analytes diffuses in the sample volume towards the immobilised antibodies.

[0321] The sample and binder complex may be allowed to combine for a suitable amount of time to enable binding molecules to reach binding equilibrium. In some embodiments, the suitable amount of time to enable binding to reach equilibrium may be about one, two, three, four, five, 10, 20, 30, 45, 60, 90, 120, 180, 240, 300 or 360 second(s) and useful ranges may be selected between any of these values, (for example from about 1 to 30, 1 to 60, 1 to 120, 10 to 30, 10 to 60, 10 to 90, 30 to 60, 30 to 90, 30 to 120, 60 to 90, 60 to 120, 60 to 180, 90 to 120, 90 to 180, 90 to 240, 180 to 240, 180 to 300, 180 to 360 seconds).

[0322] The magnetic field generator may be used to induce magneto-hydrodynamic mixing of the sample to improve the rate at which binding equilibrium is reached. In such an embodiment, the magnetic field generator is used to induce movement of the binder complexes in the sample volume.

[0323] It will be appreciated that the present method may broadly be used in any application requiring detection and/or quantification of a target analyte. In particular, the method may be used in applications requiring

- rapid determination, or
 - sensitive determination, or
 - quantitative determination , or
 - or any combination of (i) to (iii);
- of the presence of target analytes in samples.

[0324] For example, suitable applications may include clinical, veterinary, environmental, food safety or forensic applications.

[0325] In some embodiments, the clinical application may include diagnostic detection of biomarkers in a sample that may be indicative of a clinical condition. In one example, the method may be used for rapid, sensitive, and quantitative diagnostic detection of specific antibodies in a blood sample which may indicate potential infection by a pathogen. In a further example, the method may be used for diagnostic detection of specific protein biomarkers that are overexpressed in cancers. The diagnostic detection may be performed on samples across different species.

[0326] The clinical condition may be selected from infections, such as infections from bacteria, fungi, viruses (e.g. hepatitis, SARS-CoV-19 and HIV) (e.g. biomarkers such as hepatitis, SARS-CoV-19 and HIV antibodies), parasites (e.g. microbial parasites [e.g. malarial], nematodes, insect parasite).

[0327] The clinical condition may be selected from diseases such as cardiac disease (biomarkers such as BNP), cancer (e.g. solid organ cancers, blood cancers, other cancers), (e.g. biomarkers such as Ca-125 and other tumour markers), neurological diseases (e.g. multiple sclerosis, Alzheimer's disease, Parkinson's disease, Huntington's disease) (e.g. biomarkers such as CNS immunoglobulins), respiratory diseases (e.g. biomarkers such as serum ACE), liver disease (e.g. biomarkers such as liver function tests and albumin), kidney disease (e.g. biomarkers such as creatinine and protein).

[0328] The clinical condition may be selected from organ injury or failure such as brain injury (e.g. biomarkers such as Glial fibrillary acidic protein or GFAP), kidney injury (e.g. biomarkers such as serum creatine), heart damage (e.g. biomarker such as creatine kinase-muscle), lung damage (e.g. biomarkers such as intercellular adhesion molecule-1 or ICAM1), or liver injury (e.g. biomarker such as alkaline phosphatase).

[0329] The clinical condition may be selected from endocrine disorders such as diabetes (e.g. biomarkers such as insulin, elevated, HbA1C, thyroid dysfunction, thyroid hormone, pituitary disorders (e.g. biomarkers such as ACTH, prolactin, gonadotrophins, thyroid stimulating hormone, growth hormone, antidiuretic hormone), parathyroid disorders (e.g. biomarkers such as, parathyroid hormone), adrenal disorder (e.g. biomarkers such as cortisol, aldosterone, adrenaline, DHEAS), sex hormone imbalance (e.g. biomarkers such as androgens and estrogens), carcinoid tumour (e.g. biomarkers such as 5-HIAA, VIPoma, serum VIP), elevated bone turnover (e.g. biomarkers such as P1NP).

[0330] The clinical condition may be selected from lipid disorders (e.g. biomarkers such as cholesterol and triglycerides)

[0331] The clinical condition may be selected from nutritional disorders (e.g. vitamin deficiencies, malabsorption syndrome, malnutrition, disorders of vitamin metabolism), (e.g. biomarkers such as vitamin levels, iron levels, mineral levels).

[0332] The clinical condition may be selected from inflammation or inflammatory disorders (e.g. biomarkers such as ESR, Crp and other acute phase proteins).

[0333] The clinical condition may be selected from autoimmune diseases (e.g. biomarkers such as specific antibody markers).

[0334] The clinical condition may be selected from allergic disease (e.g. biomarkers such as tryptase).

[0335] The clinical condition may be selected from physical trauma such as electrocution (e.g. biomarkers such as creatinine kinase).

[0336] The clinical condition may be selected from immune deficiency disorders (e.g. common variable immune deficiency), (e.g. biomarkers such as complement, leucocytes and immunoglobulins).

[0337] The clinical condition may be selected from clotting disorders (e.g. thrombophilia)(e.g. biomarkers such as biomarkers such as clotting factors and other markers).

[0338] The clinical condition may be selected from inherited or acquired enzyme disorders, deficiency or excess and other congenital or acquired defects of metabolism (e.g. Bartter syndrome, congenital adrenal hyperplasia), (e.g. biomarkers such as electrolytes, enzyme levels, metabolic products of enzymes).

[0339] The clinical condition may be selected from electrolyte disturbance such as hyperkalaemia and hypernatraemia (e.g. biomarkers such as electrolytes).

[0340] The clinical condition may be selected from drug adverse effects or poisoning (eg. biomarkers such as drug levels and levels of drug metabolites).

[0341] The clinical condition may be selected from adverse effects or poisoning from exposure to chemical to biological weapons or other environmental chemical and biological agents.

[0342] Specific to veterinary medicine, the clinical condition may be selected from renal failure, FIV/AIDS (Feline), cancers, and any biomarker for organ function/failure.

[0343] In some embodiments, the clinical conditions may be conditions in veterinary subjects such as feline, canine, bovine, ovine, equine, porcine, or murine.

[0344] In some embodiments, the environmental application may include detection of pollutants in an environmental sample. The environmental pollutant may be selected from such pollutants as, for example, lead, particulate matter, micro plastic and hormones.

[0345] For example, the method may be used for monitoring and quantifying heavy metals in a water sample.

[0346] In some embodiments, the food safety application may include detection of pathogen in food samples. For example, the method may be used to rapidly and sensitively detect post-pasteurisation contamination in milk by bacterial pathogens.

EXAMPLES

1. Sensitivity of detection

[0347] The purpose of this study was to test the sensitivity of detection.

[0348] Specific amounts of magnetisable particles were added to the microfluidics system for detection. The setup of the system is summarised below.

- Magnetic field generators
 - Electromagnet above the microfluidics system
 - Permanent magnet below the microfluidics system
- Signal sensor
 - Honeywell HMC 2003 three-axis magnetic sensor
 - Analog to digital converter (ADC) connected to an oscilloscope recording data at 10,000 samples per second
- Magnetisable particles
 - Functionalised 30 nm superparamagnetic beads - coated in streptavidin and linked via biotin to anti-Human Serum Albumin binder
- Target analyte
 - Human Serum Albumin
- Number of particles:
 - Sample 1: (21.6 °C): control – 0 pg/mL of particles
 - Sample 2: 0.1 pg/mL of particles
 - Sample 3: 1 pg/mL of particles
 - Sample 4: 10 pg/mL of particles
 - Sample 5: 100 pg/mL of particles
 - Sample 6: 1,000 pg/mL of particles
 - Sample 7: 10,000 pg/mL of particles
- Number of sample read cycles

- Five cycles
- Electromagnet (EM) activation on/off time per cycle
 - EM-On for two seconds
 - EM-Off for five seconds

[0349] After being introduced into the microfluidics system the particles were allowed to reach a first equilibrium (Equilibrium A) under the influence of the permanent magnetic field. The electromagnet was then activated for two seconds to allow the particles to reach a second equilibrium (Equilibrium B). The electromagnet is then deactivated to allow the particles to return to a third equilibrium (Equilibrium C) – see Table 1. The magnetic field sensor measured changes in the magnetic signal generated by the particles transitioning between the different equilibrium states.

[0350] Table 1 shows the results for five sample read cycles for the seven samples.

[0351] Shown in table 1 are magnetic field sensor outputs (in voltage) for 0 pg/ml to 10,000 pg/ml.

Table 1.

	0 pg/ml	0.1 pg/ml	1 pg/ml	10 pg/ml	100 pg/ml	1,000 pg/ml	10,000 pg/ml
Sample Temp. (C)	21.6	21.5	21.0	21.4	21.5	21.4	21.5
Cycle 1							
Equilibrium A	2.7710	2.7863	2.7998	2.7861	2.7897	2.7611	2.7858
Equilibrium B	1.8318	1.8797	1.9078	1.7906	1.8110	1.6744	1.5839
Equilibrium C	2.7766	2.7866	2.7858	2.7783	2.7768	2.7432	2.7457
Cycle 2							
Equilibrium A	2.7766	2.7866	2.7858	2.7783	2.7768	2.7432	2.7457
Equilibrium B	1.8265	1.8767	1.9010	1.7889	1.7997	1.6748	1.5818
Equilibrium C	2.7731	2.7898	2.7915	2.7750	2.7748	2.7485	2.7389
Cycle 3							
Equilibrium A	2.7731	2.7898	2.7915	2.7750	2.7748	2.7485	2.7389
Equilibrium B	1.8242	1.8744	1.8989	1.7898	1.8017	1.6746	1.5869
Equilibrium C	2.7692	2.7872	2.7843	2.7667	2.7827	2.7449	2.7653
Cycle 4							
Equilibrium A	2.7692	2.7872	2.7843	2.7667	2.7827	2.7449	2.7653
Equilibrium B	1.8209	1.8702	1.8925	1.7823	1.7945	1.6722	1.5855
Equilibrium C	2.7743	2.7838	2.7838	2.7820	2.5515	2.4624	2.7559
Cycle 5							
Equilibrium A	2.7743	2.7838	2.7838	2.7820	2.5515	2.4624	2.7559
Equilibrium B	1.8160	1.8704	1.8921	1.7811	1.7902	1.6726	1.5855

Equilibrium C	2.7827	2.7838	2.7877	2.7818	2.7662	2.7870	2.7698
---------------	--------	--------	--------	--------	--------	--------	--------

2. Magnetic Equilibrium Detection (MED)

[0352] The purpose of this example was to demonstrate quantitative detection of the biomarker analyte using the claimed methodology. This method used 30 nm superparamagnetic particles. In this example a binder-to-particle ratio of 0.75:1 was tested to determine whether such a ratio can affect the inter-particle interaction in the absence of an analyte.

Table 2.

Human Albumin protein concentration (pg/ mL)	Sensor Value (Volts – V)
10,000	3.325
100	3.751
10	3.881
1	3.957
0.1	3.988

[0353] The results in Table 2 have an R2-value of 0.95.

[0354] The 1,000 pg/ml analyte sample was excluded due to sample handling error.

2.1 Experiment Description

[0355] The experimental design parameters is set out below.

- Magnetisable particles
 - Nanocs MP25-AV-0.5 (30 nm) Streptavidin Coated Superparamagnetic Particles
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor
 - Honeywell HMC 2003 magnetometer
- Amplifier
 - Honeywell HMC2003 in-built amplifier
- Data Acquisition
 - Siglent SDS1204X-E Oscilloscope

- 10,000 samples per second sampling rate
- Approximately 35 seconds total run time on instrument
- Magnets
 - Top magnet – Copper coil electromagnet with 0.2 mm coil gauge. Connected to 3.23V DC power supply.
 - Five cycles of top magnet actuation with each cycle comprising of: two seconds of magnet on time followed by five seconds of magnet off time
- Apparatus Setup
 - Sample is positioned in-between sensor and magnets such that:
 - Top Component – Top magnet
 - Middle Component – Sample
 - Bottom Component – Sensor
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested
 - 1 μ L of magnetisable particles – Nanocs Superparamagnetic Beads (2 mg/mL)
 - 0.75 ng of anti-albumin antibody – Biotinylated "detection" antibody from DY1455 ELISA kit
 - Different concentration of human albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution.
 - All components mixed and sensed in a test volume of 10 μ L

2.2 Data Processing

[0356] Sensor outputs during the 2 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not considered. An automated tool was created for this data processing and each processed output was manually checked.

[0357] Thus the sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all Five cycles. This was fitted to a line-of-best-fit and R^2 -value derived.

3. Quantitative detection of the Biomarker Analyte

[0358] The purpose of this experiment was to demonstrate quantitative detection of a biomarker analyte. 30 nm superparamagnetic particles were used in this example.

Table 3.

Human Albumin protein concentration (pg/mL)	Sensor Value (Volts – V)
10,000	1.585
1,000	1.674
100	1.800
10	1.787
1	1.899
0.1	1.874

[0359] The results in Table 3 have an R^2 -value of 0.94.

3.1 Experiment Description

[0360] The experimental design parameters is set out below.

- Magnetisable particles
 - Nanocs MP25-AV-0.5 (30 nm) Streptavidin Coated Superparamagnetic Particles
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor
 - Honeywell HMC 2003 magnetometer
- Amplifier
 - Honeywell HMC2003 in-built amplifier
- Data Acquisition
 - Siglent SDS1204X-E Oscilloscope
 - 10,000 samples per second sampling rate
 - Approximately 35 seconds total run time on instrument
- Magnets
 - Top Magnet – Copper coil electromagnet with 0.2 mm coil gauge. Connected to 3.33V DC power supply.

- Bottom Magnet – DC cylindrical electromagnet 25 mm diameter.
Disconnected from power source
- Five cycles of top magnet actuation with each cycle comprising of: two seconds of magnet on time followed by five seconds of magnet off time
- Bottom magnet was not actuated or powered.
- Apparatus Setup
 - Sample is positioned in-between sensor and magnets such that:
 - Top Component – Top Magnet
 - Middle Component – Sample
 - Middle Component – Sensor (situated below the Sample)
 - Bottom Component – Bottom Magnet
 - All components were aligned vertically through the center of each component.
 - For each Biomarker Analyte concentration tested
 - 1 μL of magnetisable particles – Nanocs Superparamagnetic Beads (2 mg/mL)
 - 1 nanogram of anti-Albumin Antibody – Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution.
 - All components mixed and sensed in a test volume of 10 μL

3.2 Data Processing

[0361] Sensor outputs during the 2 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not considered. An automated tool was created for this data processing and each processed output was manually checked.

[0362] Thus the sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all Five cycles and fitted to a line-of-best-fit and R²-value derived.

[0363] The aim of this experiment is to demonstrate quantitative detection using a different Biomarker Analyte with the MED detection methodology and 30 nm superparamagnetic particles.

Table 4.

Feline TNF–alpha protein concentration (pg/ mL)	Sensor Value (Volts – V)
10,000	3.794
1,000	3.798
10	3.801
1	3.800
0.1	3.802

[0364] The results in Table 4 have an R²-value of 0.94.

3.3 Experiment Description

[0365] The experimental design parameters is set out below.

- Magnetisable particles
 - Nanocs MP25-AV-0.5 (30 nm) Streptavidin Coated Superparamagnetic Particles
 - Functionalised with Biotinylated "Detection" antibody from DY2586 ELISA kit (anti-Feline TNF- α)
- Magnetic sensor
 - Honeywell HMC 2003 magnetometer
- Amplifier
 - Honeywell HMC2003 in-built amplifier
- Data Acquisition
 - Siglent SDS1204X-E Oscilloscope
 - 10,000 samples per second sampling rate
 - Approximately 35 seconds total run time on instrument
- Magnets
 - Top magnet – Copper coil electromagnet with 0.2 mm coil gauge. Connected to 0.5V DC power supply.

- Bottom magnet – DC cylindrical electromagnet 25 mm diameter.
Disconnected from power source
- Five cycles of top magnet actuation with each cycle comprising of: two seconds of magnet on time followed by five seconds of magnet off time
- Bottom magnet was not actuated or powered.
- Apparatus Setup
 - Sample is positioned in-between sensor and magnets such that:
 - Top Component – Top magnet
 - Middle Component – Sample
 - Middle Component – Sensor (situated below the sample)
 - Bottom Component – Bottom magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested
 - 1 μL of magnetisable particles – Nanocs Superparamagnetic Beads (2 mg/mL)
 - 1 ng of anti-TNF- α Antibody – Biotinylated "Detection" antibody from DY2586 ELISA kit
 - Different concentration of Feline TNF- α recombinant protein (from DY2584 ELISA kit) generated via serial dilution.
 - All components mixed and sensed in a test volume of 10 μL

3.4 Data Processing

[0366] Sensor outputs during the 2 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not considered. An automated tool was created for this data processing and each processed output was manually checked.

[0367] Thus the sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all Five cycles and fitted to a line-of-best-fit and R^2 -value derived.

4. Experiment 1

[0368] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 30 nm superparamagnetic particles with an even lower Binder-to-Bead ratio (0.5:1).

Table 5.

Human Albumin protein concentration (pg/ mL)	Sensor Value (Volts – V)
0	3.751
1	3.768
10	3.780
100	3.773
1,000	3.785
10,000	3.788

[0369] The results in Table 2 have an R^2 -value of 0.91.

4.1 Experiment Description

[0370] The experimental design parameters is set out below.

- Magnetisable particles
 - Nanocs MP25-AV-0.5 (30 nm) Streptavidin Coated Superparamagnetic Particles
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor
 - Honeywell HMC 2003 magnetometer
- Amplifier
 - Honeywell HMC2003 in-built amplifier
- Data Acquisition
 - Siglent SDS1204X-E Oscilloscope
 - 10,000 samples per second sampling rate
 - Approximately 35 seconds total run time on instrument
- Magnets

- Top magnet – Copper coil electromagnet with 0.2 mm coil gauge. Connected to 3.23V DC power supply.
- Five cycles of top magnet actuation with each cycle comprising of: two seconds of magnet on time followed by five seconds of magnet off time
- Apparatus Setup
 - Sample is positioned in-between sensor and magnets such that:
 - Top Component – Top Magnet
 - Middle Component – Sample
 - Bottom Component – Sensor
 - All components were aligned vertically through the center of each component.
- For each biomarker analyte concentration tested
 - 1 μ L of magnetisable particles – Nanocs Superparamagnetic Beads (2 mg/mL)
 - 0.5 ng of anti-Albumin Antibody – Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution.
 - All components mixed and sensed in a test volume of 10 μ L

4.2 Data Processing

[0371] Sensor outputs during the 2 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not considered. An automated tool was created for this data processing and each processed output was manually checked.

[0372] Thus the sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all Five cycles and fitted to a line-of-best-fit and R^2 -value derived.

5. Experiment 2

[0373] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 30 nanometer

superparamagnetic particles. A Binder-to-Particle ratio (0.75:1) was tested to determine whether such ratio affects the inter-particle interaction in the absence of Analyte.

Table 6.

Human Albumin protein concentration (pg/ml)	0 based Sensor Value (Volts - V)
100	0.9993
25	1.0072
6.25	1.0116
3.125	1.0179

[0374] The results in Table 6 has an R^2 -value of 0.99

5.1 Experiment Description

[0375] The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SHS30-01 (30 nanometer) Streptavidin Coated Superparamagnetic Particles
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - Honeywell HMC 1041z magnetometer
- Amplifier:
 - Texas Instruments Amplifier INA819
- Magnets:
 - Top Magnet – Rectangular Copper Coil Electromagnet (Dimension 22x25mm and 3.5mm thickness) with 0.1mm coil gauge. Connected to 0.63V DC power supply with programmable run through RIGOL DP832.
 - 5 Cycles of Top Magnet actuation with each cycle comprising of: 2 seconds of Magnet on time followed by 5 seconds of Magnet off time
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Top Magnet

- Middle Component – Sample
 - Bottom Component – Sensor
- All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech Superparamagnetic Beads (1 mg/mL)
 - 0.75 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (2-fold dilutions starting from 100pg/ml to 3.125pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (0.5µg beads per sample)

5.2 Data Processing

[0376] Sensor outputs during the 2 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not considered. An automated tool was created for this data processing and each processed output was manually checked.

[0377] The sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all 5 cycles. This was fitted to a line-of-best-fit and R^2 -value derived.

6. Experiment 3

[0378] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 30 nanometer superparamagnetic particles. A Binder-to-Particle ratio (0.75:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 7.

Human Albumin protein concentration (pg/ml)	0pg/ml-Based Sensor Value (Volts - V)
25	1.0107
1.56	1.0040

0.39	0.9995
0.098	0.9988

[0379] The results in Table 7 has an R^2 -value of 0.98.

6.1 Experiment Description

[0380] The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SHS30-01 (30 nanometer) Streptavidin Coated Superparamagnetic Particles
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - Honeywell HMC 1041z magnetometer
- Amplifier:
 - Texas Instruments Amplifier INA819
- Magnets:
 - Top Magnet - Rectangular Copper Coil Electromagnet (Dimension 22x25mm and 3.5mm thickness) with 0.1mm coil gauge. Connected to 0.63V DC power supply with programmable run through RIGOL DP832.
 - 5 Cycles of Top Magnet actuation with each cycle comprising of: 2 seconds of Magnet on time followed by 5 seconds of Magnet-off time.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Top Magnet
 - Middle Component – Sample
 - Bottom Component – Sensor
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles –Ocean NanoTech Superparamagnetic Beads (1 mg/mL)

- 0.75 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
- Different concentration of Human Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (4-fold dilutions starting from 100pg/ml to 0.098pg/ml).

All components mixed and sensed in a test volume of 5 microlitres (0.5µg beads per sample)

6.2 Data Processing

[0381] Sensor outputs during the 2 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not considered. An automated tool was created for this data processing and each processed output was manually checked.

[0382] The sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all 5 cycles. This was fitted to a line-of-best-fit and R²-value derived.

7. Experiment 4

[0383] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 30 nanometer superparamagnetic particles. A Binder-to-Particle ratio (0.75:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 8.

Human Albumin protein concentration (pg/ml)	0pg/ml-Based Sensor Value (Volts - V)
10000	1.0098
1000	1.0011
100	0.9931
1	0.9909

[0384] The results in Table 8 has an R²-value of 0.99.

7.1 Experiment Description

[0385] The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SHS30-01 (30 nanometer) Streptavidin Coated Superparamagnetic Particles
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - Honeywell HMC 1041z magnetometer
- Amplifier:
 - Texas Instruments Amplifier INA819
- Magnets:
 - Top Magnet - Rectangular Copper Coil Electromagnet (Dimension 22x25mm and 3.5mm thickness) with 0.1mm coil gauge.
 - Bottom Magnet - Rectangular Copper Coil Electromagnet (Dimension 22x25mm and 3.5mm thickness) with 0.1mm coil gauge.
 - Both magnets connected together in an Helmholtz arrangement at 0.63V DC power supply with programmable run through RIGOL DP832.
 - 5 Cycles of Top Magnet actuation with each cycle comprising of: 2 seconds of Magnet on time followed by 5 seconds of Magnet-off time.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Top Magnet
 - Middle Component – Sample
 - Subsequent Component – Sensor
 - Bottom Component – Bottom Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech Superparamagnetic Beads (1 mg/mL)
 - 0.75 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit

- Different concentration of Human Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (10-fold dilutions starting from 10,000pg/ml to 0.1pg/ml).
- All components mixed and sensed in a test volume of 5 microlitres (0.5µg beads per sample)

7.2 Data Processing

[0386] Sensor outputs during the 2 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not considered. An automated tool was created for this data processing and each processed output was manually checked.

[0387] The sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all 5 cycles. This was fitted to a line-of-best-fit and R²-value derived.

8. Experiment 5

[0388] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 30 nanometer superparamagnetic particles. A Binder-to-Particle ratio (0.75:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 9.

Human Albumin protein concentration (pg/ml)	0pg/ml-Based Sensor Value (Volts - V)
100	0.9967
10	0.9919
1	0.9801
0.1	0.9792

[0389] The results in Table 9 has an R²-value of 0.91.

8.1 Experiment Description

[0390] The experimental design parameters is set out below.

- Magnetisable particles:

- Ocean NanoTech SHS30-01 (30 nanometer) Streptavidin Coated Superparamagnetic Particles
- Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - Honeywell HMC 1041z magnetometer
- Amplifier:
 - Texas Instruments Amplifier INA819
- Magnets:
 - Bottom Magnet - Rectangular Copper Coil Electromagnet (Dimension 22x25mm and 3.5mm thickness) with 0.1mm coil gauge. Connected at 0.63V DC power supply with programmable run through RIGOL DP832.
 - 5 Cycles of Top Magnet actuation with each cycle comprising of: 2 seconds of Magnet on time followed by 5 seconds of Magnet-off time.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Bottom Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech Superparamagnetic Beads (1 mg/mL)
 - 0.75 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (10-fold dilutions starting from 10,000pg/ml to 0.1pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (0.5µg beads per sample)

8.2 Data Processing

[0391] Sensor outputs during the 2 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not considered. An automated tool was created for this data processing and each processed output was manually checked.

[0392] The sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all 5 cycles. This was fitted to a line-of-best-fit and R^2 -value derived.

9. Experiment 6

The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 30 nanometer superparamagnetic particles. A Binder-to-Particle ratio (0.75:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 10.

Human Albumin protein concentration (pg/ml)	0pg/ml-Based Sensor Value (Volts - V)
1000	1.0059
100	0.9998
1	0.9999
0.1	0.9976

[0393] The results in Table 10 has an R^2 -value of 0.90.

9.1 Experiment Description

[0394] The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SHS30-01 (30 nanometer) Streptavidin Coated Superparamagnetic Particles
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - Honeywell HMC 1041z magnetometer

- Amplifier:
 - Texas Instruments Amplifier INA819
- Magnets:
 - Bottom Magnet - Rectangular Copper Coil Electromagnet (Dimension 22x25mm and 3.5mm thickness) with 0.1mm coil gauge. Connected at 0.63V DC power supply with programmable run through RIGOL DP832.
 - 5 Cycles of Top Magnet actuation with each cycle comprising of: 2 seconds of Magnet on time followed by 5 seconds of Magnet off time
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Bottom Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles –Ocean NanoTech Superparamagnetic Beads (1 mg/mL)
 - 0.75 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (10-fold dilutions starting from 10,000pg/ml to 0.1pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (2µg beads per sample)

9.2 Data Processing

[0395] Sensor outputs during the 2 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not considered. An automated tool was created for this data processing and each processed output was manually checked.

[0396] Thus the sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all 5 cycles. This was fitted to a line-of-best-fit and R^2 -value derived.

10. Experiment 7

[0397] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 30 nanometer superparamagnetic particles. A Binder-to-Particle ratio (0.75:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 11.

Human Albumin protein concentration (pg/ml)	0pg/ml-Based Sensor Value (Volts - V)
10000	0.6900
1000	0.8376
100	1.0017
10	1.0072

[0398] The results in Table 11 has an R^2 -value of 0.96.

10.1 Experiment Description

[0399] The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SHS30-01 (30 nanometer) Streptavidin Coated Superparamagnetic Particles
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - Honeywell HMC 1041z magnetometer
- Amplifier:
 - Texas Instruments Amplifier INA819
- Magnets:

- Bottom Magnet - Rectangular Copper Coil Electromagnet (Dimension 22x25mm and 3.5mm thickness) with 0.1mm coil gauge. Connected at 0.9V DC power supply with programmable run through RIGOL DP832.
- 5 Cycles of Top Magnet actuation with each cycle comprising of: 2 seconds of Magnet on time followed by 5 seconds of Magnet-off time.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Bottom Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech Superparamagnetic Beads (1 mg/mL)
 - 0.75 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (10-fold dilutions starting from 10,000pg/ml to 0.1pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (2µg beads per sample)

10.2 Data Processing

[0400] Sensor outputs during the 2 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not considered. An automated tool was created for this data processing and each processed output was manually checked.

[0401] Thus the sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all 5 cycles. This was fitted to a line-of-best-fit and R^2 -value derived.

11. Experiment 8

[0402] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 30 nanometer superparamagnetic particles. A Binder-to-Particle ratio (0.75:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 12.

Human Albumin protein concentration (pg/ml)	Sensor Value (Volts - V)
100	4.4706
10	4.3886
1	4.2977
0.1	4.4480

[0403] The results in Table 12 has an R²-value of 1.00.

11.1 Experiment Description

[0404] The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SHS30-01 (30 nanometer) Streptavidin Coated Superparamagnetic Particles
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - Honeywell HMC 1041z magnetometer
- Amplifier:
 - Texas Instruments Amplifier INA819
- Magnets:
 - Bottom Magnet - Rectangular Copper Coil Electromagnet (Dimension 22x25mm and 3.5mm thickness) with 0.1mm coil gauge. Connected at 0.9V DC power supply with programmable run through RIGOL DP832.
 - 5 Cycles of Top Magnet actuation with each cycle comprising of: 2 seconds of Magnet on time followed by 5 seconds of Magnet-off time.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:

- Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Bottom Magnet
- All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech Superparamagnetic Beads (1 mg/mL)
 - 0.75 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (10-fold dilutions starting from 10,000pg/ml to 0.1pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (2µg beads per sample)

11.2 Data Processing

[0405] Sensor outputs during the 2 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not considered. An automated tool was created for this data processing and each processed output was manually checked.

[0406] Thus, the sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all 5 cycles. This was fitted to a line-of-best-fit and R²-value derived.

12. Experiment 9

[0407] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 30 nanometer superparamagnetic particles. A Binder-to-Particle ratio (0.75:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 13.

Human Albumin protein concentration (pg/ml)	0pg/ml-Based Sensor Value (Volts - V)
1000	0.9810

100	0.9801
1	0.9799
0.1	0.9795

The results in Table 13 has an R^2 -value of 0.98.

12.1 Experiment Description

[0408] The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SHS30-01 (30 nanometer) Streptavidin Coated Superparamagnetic Particles
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - Honeywell HMC 1041z magnetometer
- Amplifier:
 - Texas Instruments Amplifier INA819
- Magnets:
 - Bottom Magnet - Rectangular Copper Coil Electromagnet (Dimension 22x25mm and 3.5mm thickness) with 0.1mm coil gauge. Connected at 0.6V DC power supply with programmable run through RIGOL DP832.
 - 5 Cycles of Top Magnet actuation with each cycle comprising of: 2 seconds of Magnet on time followed by 5 seconds of Magnet-off time.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Bottom Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech Superparamagnetic Beads (1 mg/mL)

- 0.75 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
- Different concentration of Human Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (10-fold dilutions starting from 10,000pg/ml to 0.1pg/ml).
- All components mixed and sensed in a test volume of 5 microlitres (2µg beads per sample)

12.2 Data Processing

[0409] Sensor outputs during the 2 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not considered. An automated tool was created for this data processing and each processed output was manually checked.

[0410] Thus the sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all 5 cycles. This was fitted to a line-of-best-fit and R²-value derived.

13. Experiment 10

[0411] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 30 nanometer superparamagnetic particles. A Binder-to-Particle ratio (0.75:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 14.

Human Albumin protein concentration (pg/ml)	0pg/ml-Based Sensor Value (Volts - V)
10000	0.9996
1000	1.0012
100	1.0042
1	1.0068

[0412] The results in Table 14 has an R²-value of 0.99.

13.1 Experiment Description

[0413] The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SHS30-01 (30 nanometer) Streptavidin Coated Superparamagnetic Particles
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - Honeywell HMC 1041z magnetometer
- Amplifier:
 - Texas Instruments Amplifier INA819
- Magnets:
 - Bottom Magnet - Rectangular Copper Coil Electromagnet (Dimension 22x25mm and 3.5mm thickness) with 0.1mm coil gauge. Connected at 0.5V DC power supply with programmable run through RIGOL DP832.
 - 5 Cycles of Top Magnet actuation with each cycle comprising of: 2 seconds of Magnet on time followed by 5 seconds of Magnet-off time.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Bottom Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech Superparamagnetic Beads (1 mg/mL)
 - 0.75 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (10-fold dilutions starting from 10,000pg/ml to 0.1pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (2µg beads per sample)

13.2 Data Processing

[0414] Sensor outputs during the 2 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not considered. An automated tool was created for this data processing and each processed output was manually checked.

[0415] Thus, the sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all 5 cycles. This was fitted to a line-of-best-fit and R^2 -value derived.

14. Experiment 11

[0416] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 30 nanometer superparamagnetic particles. A Binder-to-Particle ratio (0.75:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 15.

Human Albumin protein concentration (pg/ml)	0pg/ml-Based Sensor Value (Volts - V)
10000	1.0088
2500	1.0074
1250	1.0060
625	1.0041
312.5	1.0039

[0417] The results in Table 15 has an R^2 -value of 0.96.

14.1 Experiment Description

[0418] The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SHS30-01 (30 nanometer) Streptavidin Coated Superparamagnetic Particles
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)

- Magnetic sensor:
 - Honeywell HMC 1041z magnetometer
- Amplifier:
 - Texas Instruments Amplifier INA819
- Magnets:
 - Bottom Magnet - Rectangular Copper Coil Electromagnet (Dimension 22x25mm and 3.5mm thickness) with 0.1mm coil gauge. Connected at 0.5V DC power supply with programmable run through RIGOL DP832.
 - 5 Cycles of Top Magnet actuation with each cycle comprising of: 3 seconds of Magnet on time followed by 5 seconds of Magnet-off time.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Bottom Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech Superparamagnetic Beads (1 mg/mL)
 - 0.75 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (2-fold dilutions starting from 10,000pg/ml to 312.5pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (2µg beads per sample)

14.2 Data Processing

[0419] Sensor outputs during the 3 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not

considered. An automated tool was created for this data processing and each processed output was manually checked.

[0420] Thus, the sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all 5 cycles. This was fitted to a line-of-best-fit and R^2 -value derived.

15. Experiment 12

The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 30 nanometer superparamagnetic particles. A Binder-to-Particle ratio (0.75:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 16.

Human Albumin protein concentration (pg/ml)	Sensor Value (Volts - V)
5000	5.0387
2500	5.0366
312.5	5.0357
0	5.0347

[0421] The results in Table 16 has an R^2 -value of 0.99.

15.1 Experiment Description

[0422] The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SHS30-01 (30 nanometer) Streptavidin Coated Superparamagnetic Particles
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - Honeywell HMC 1041z magnetometer
- Amplifier:
 - Texas Instruments Amplifier INA819
- Magnets:

- Bottom Magnet - Rectangular Copper Coil Electromagnet (Dimension 22x25mm and 3.5mm thickness) with 0.1mm coil gauge. Connected at 0.5V DC power supply with programmable run through RIGOL DP832.
- 5 Cycles of Top Magnet actuation with each cycle comprising of: 3 seconds of Magnet on time followed by 5 seconds of Magnet off time
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Bottom Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech Superparamagnetic Beads (1 mg/mL)
 - 0.75 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (2-fold dilutions starting from 10,000pg/ml to 312.5pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (2µg beads per sample)

15.2 Data Processing

[0423] Sensor outputs during the 3 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not considered. An automated tool was created for this data processing and each processed output was manually checked.

[0424] Thus, the sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all 5 cycles. This was fitted to a line-of-best-fit and R^2 value derived.

16. Experiment 13

[0425] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 30 nanometer superparamagnetic particles. A Binder-to-Particle ratio (0.75:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 17.

Human Albumin protein concentration (pg/ml)	Sensor Value (Volts - V)
1250	5.1712
625	5.0424
312.5	5.0106
0	5.0063

[0426] The results in Table 17 has an R^2 -value of 0.91.

16.1 Experiment Description

[0427] The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SHS30-01 (30 nanometer) Streptavidin Coated Superparamagnetic Particles
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - Honeywell HMC 1041z magnetometer
- Amplifier:
 - Texas Instruments Amplifier INA819
- Magnets:
 - Bottom Magnet – Rectangular Copper Coil Electromagnet with 0.2mm coil gauge. Bottom Magnet – Rectangular Copper Coil Electromagnet (Dimensions 36x33.5mm, 5.3mm Thickness) with 0.2mm coil gauge. Connected at 0.3V DC power supply with programmable run through RIGOL DP832.
 - 5 Cycles of Top Magnet actuation with each cycle comprising of: 3 seconds of Magnet on time followed by 5 seconds of Magnet off time

- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Bottom Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech Superparamagnetic Beads (1 mg/mL)
 - 0.75 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (2-fold dilutions starting from 10,000pg/ml to 312.5pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (2µg beads per sample)

16.2 Data Processing

[0428] Sensor outputs during the 3 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not considered. An automated tool was created for this data processing and each processed output was manually checked.

[0429] Thus, the sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all 5 cycles. This was fitted to a line-of-best-fit and R²-value derived.

17. Experiment 14

[0430] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 30 nanometer superparamagnetic particles. A Binder-to-Particle ratio (0.75:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 18.

Human Albumin protein concentration (pg/ml)	Sensor Value (Volts - V)
10	4.9962
1	5.0107
0.1	5.0442
0	5.0553

[0431] The results in Table 18 has an R^2 -value of 0.96.

17.1 Experiment Description

[0432] The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SHS30-01 (30 nanometer) Streptavidin Coated Superparamagnetic Particles
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - Honeywell HMC 1041z magnetometer
- Amplifier:
 - Texas Instruments Amplifier INA819
- Magnets:
 - Bottom Magnet - Rectangular Copper Coil Electromagnet (Dimensions 36x33.5mm, 5.3mm Thickness) with 0.2mm coil gauge. Connected at 0.3V DC power supply with programmable run through RIGOL DP832.
 - 5 Cycles of Top Magnet actuation with each cycle comprising of: 3 seconds of Magnet on time followed by 5 seconds of Magnet off time
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Bottom Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:

- 1 microliter of magnetisable particles – Ocean NanoTech Superparamagnetic Beads (1 mg/mL)
- 0.75 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
- Different concentration of Human Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (10-fold dilutions starting from 10,000pg/ml to 0.1pg/ml).
- All components mixed and sensed in a test volume of 5 microlitres (2µg beads per sample)

17.2 Data Processing

[0433] Sensor outputs during the 3 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not considered. An automated tool was created for this data processing and each processed output was manually checked.

[0434] Thus, the sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all 5 cycles. This was fitted to a line-of-best-fit and R²-value derived.

18. Experiment 15

[0435] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 30 nanometer superparamagnetic particles. A Binder-to-Particle ratio (0.75:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 19.

Human Albumin protein concentration (pg/ml)	Sensor Value (Volts - V)
1000	4.9403
100	4.9808
1	4.9802
0.1	5.0066

The results in Table 19 has an R²-value of 0.92.

18.1 Experiment Description

The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SHS30-01 (30 nanometer) Streptavidin Coated Superparamagnetic Particles
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - Honeywell HMC 1041z magnetometer
- Amplifier:
 - Texas Instruments Amplifier INA819
- Magnets:
 - Bottom Magnet - Rectangular Copper Coil Electromagnet (Dimension 22x25mm and 3.5mm thickness) with 0.1mm coil gauge. Connected at 0.5V DC power supply with programmable run through RIGOL DP832.
 - 5 Cycles of Top Magnet actuation with each cycle comprising of: 3 seconds of Magnet on time followed by 5 seconds of Magnet off time
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Bottom Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech Superparamagnetic Beads (1 mg/mL)
 - 0.75 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (10-fold dilutions starting from 10,000pg/ml to 0.1pg/ml).

- All components mixed and sensed in a test volume of 5 microlitres (2µg beads per sample)

18.2 Data Processing

[0436] Sensor outputs during the 3 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not considered. An automated tool was created for this data processing and each processed output was manually checked.

[0437] Thus, the sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all 5 cycles. This was fitted to a line-of-best-fit and R²-value derived.

19. Experiment 16

[0438] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 50 nanometer superparamagnetic particles. A Binder-to-Particle ratio (0.75:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 20.

Human Albumin protein concentration (pg/ml)	5th/1st Harmonic Magnitude
0	0.47106836
0.1	0.375649893
1	0.296456688
10	0.251193033
100	0.216144327
10000	0.191369032

[0439] The results in Table 20 has an R²-value of 1.00.

[0440] In this and subsequent examples, harmonic corresponds to the magnet actuation cycles. For example, the 5th and 1st harmonics correspond to the fifth and first magnet actuation cycles as described in the experiment description below. In this experiment, the fifth harmonic is divided by the first harmonic.

19.1 Experiment Description

The experimental design parameters is set out below.

- Magnetisable particles:
 - SuperMag Streptavidin Beads, 50nm (Product ID: SV0050)
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - Honeywell HMC 1041z magnetometer
- Amplifier:
 - Texas Instruments Amplifier INA819
- Magnets:
 - Bottom Magnet - Rectangular Copper Coil Electromagnet (Dimensions 36x33.5mm, 5.3mm Thickness) with 0.2mm coil gauge. Connected to DC power supply with programmable run through RIGOL DP832.
 - 5 Cycles of Bottom Magnet actuation with each cycle comprising of: 1 seconds of Magnet on time with step voltage of 0.2V stepping up and down every 1 second.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech SuperMag Streptavidin Beads
 - 0.75 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (10-fold dilutions starting from 10,000pg/ml to 0.1pg/ml).

- All components mixed and sensed in a test volume of 5 microlitres (2µg beads per sample)

19.2 Data Processing

[0441] Sensor outputs including the Magnet On and Off phase along with Magnet powering or depowering were considered as a whole data set. The data was then processed through an automated tool for FFT (Fast Fourier Transform) and each processed output was manually checked.

[0442] Thus, the sensor outputs corresponding to cycles of Magnet On and Off phase were processed using Fast Fourier Transform. The Fast Fourier Transform analysis was utilized to acquire the detected magnitude of the Fundamental Frequency and the magnitudes of the associated harmonics of each concentration. These magnitudes and ratios then being applied to quantitatively determine a correlation co-efficient (R^2 -value) across the indicated concentrations to reflect correlation between the Concentrations and the Harmonics, Magnitudes and Ratios.

20. Experiment 17

[0443] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 50 nanometer superparamagnetic particles. A Binder-to-Particle ratio (0.75:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 20.

Feline TNFa protein concentration (pg/ml)	1st Harmonic Magnitude
1	172.8587188
10	190.9849380
100	219.9066386
1000	238.8395662

[0444] The results in Table 20 has an R^2 -value of 0.99.

20.1 Experiment Description

[0445] The experimental design parameters is set out below.

- Magnetisable particles:

- Ocean NanoTech SuperMag Streptavidin Beads, 50nm (Product ID: SV0050)
 - Functionalised with Biotinylated "Detection" antibody from DY2586 ELISA kit (anti-Feline TNFa)
- Magnetic sensor:
 - Honeywell HMC 1041z magnetometer
- Amplifier:
 - Texas Instruments Amplifier INA819
- Magnets:
 - Bottom Magnet - Rectangular Copper Coil Electromagnet (Dimensions 36x33.5mm, 5.3mm Thickness) with 0.2mm coil gauge. Connected to DC power supply with programmable run through RIGOL DP832.
 - 5 Cycles of Bottom Magnet actuation with each cycle comprising of: 1 seconds of Magnet on time with step voltage of 0.2V stepping up and down every 1 second.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech SuperMag Streptavidin Beads (1mg/ml)
 - 0.75 nanogram of anti-TNFa Antibody - Biotinylated "Detection" antibody from DY2586 ELISA kit
 - Different concentration of Feline TNFa recombinant protein (from DY2586 ELISA kit) generated via serial dilution (10-fold dilutions starting from 10,000pg/ml to 0.1pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (2µg beads per sample)

20.2 Data Processing

[0446] Sensor outputs including the Magnet On and Off phase along with Magnet powering or depowering were considered as a whole data set. The data was then processed through an automated tool for FFT (Fast Fourier Transform) and each processed output was manually checked.

[0447] Thus, the sensor outputs corresponding to cycles of Magnet On and Off phase were processed using Fast Fourier Transform. The Fast Fourier Transform analysis was utilized to acquire the detected magnitude of the Fundamental Frequency and the magnitudes of the associated harmonics of each concentration. These magnitudes and ratios then being applied to quantitatively determine a correlation co-efficient (R^2 -value) across the indicated concentrations to reflect correlation between the Concentrations and the Harmonics, Magnitudes and Ratios.

21. Experiment 18

[0448] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 50 nanometer superparamagnetic particles. A Binder-to-Particle ratio (1.5:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 21.

Human Albumin protein concentration (pg/ml)	7th/3rd Harmonic Magnitude
0	0.44582675
1	0.446124509
10	0.438744048
100	0.412915302
1000	0.392439408
10000	0.369745285
100000	0.344362506

[0449] The results in Table 21 has an R^2 -value of 0.94.

21.1 Experiment Description

[0450] The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SuperMag Streptavidin Beads, 50nm (Product ID: SV0050)

- Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - Honeywell HMC 1041z magnetometer
- Amplifier:
 - Texas Instruments Amplifier INA819
- Magnets:
 - Bottom Magnet - Rectangular Copper Coil Electromagnet (Dimensions 36x33.5mm, 5.3mm Thickness) with 0.2mm coil gauge. Connected to 0.4V DC power supply with programmable run through RIGOL DP832.
 - 30 Cycles of Bottom Magnet actuation with each cycle comprising of: 1 second of Magnet on time and 1 second of Magnet off.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech SuperMag Streptavidin Beads (1mg/ml)
 - 1.5 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Serum Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (10-fold dilutions starting from 10,000pg/ml to 0.1pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (2µg beads per sample)

21.2 Data Processing

[0451] Sensor outputs including the Magnet On and Off phase along with Magnet powering or depowering were considered as a whole data set. The data was then

processed through an automated tool for FFT (Fast Fourier Transform) and each processed output was manually checked.

[0452] Thus, the sensor outputs corresponding to cycles of Magnet On and Off phase were processed using Fast Fourier Transform. The Fast Fourier Transform analysis was utilized to acquire the detected magnitude of the Fundamental Frequency and the magnitudes of the associated harmonics of each concentration. These magnitudes and ratios then being applied to quantitatively determine a correlation co-efficient (R^2 -value) across the indicated concentrations to reflect correlation between the Concentrations and the Harmonics, Magnitudes and Ratios.

22. Experiment 19

[0453] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 50 nanometer superparamagnetic particles. A Binder-to-Particle ratio (1.5:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 22.

Human Albumin protein concentration (pg/ml)	7th/1st Harmonic Magnitude
0	0.082833633
0.1	0.090523707
1	0.097333865
10	0.107331973

[0454] The results in Table 22 has an R^2 -value of 0.99.

22.1 Experiment Description

[0455] The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SuperMag Streptavidin Beads, 50nm (Product ID: SV0050)
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - ST Sensor (LIS2MDL)
- Amplifier:

- ST Sensor in-built amplifier
- Magnets:
 - Bottom Magnet - Oval Copper Coil Electromagnet (Dimensions 43.8x33.5mm, 5.3mm Thickness) with 0.2mm coil gauge. Connected to 0.4V DC power supply with programmable run through RIGOL DP832.
 - 5 Cycles of Bottom Magnet actuation with each cycle comprising of: 2 seconds of Magnet on time and 1 second of Magnet off.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech SuperMag Streptavidin Beads (1mg/ml)
 - 1.5 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Serum Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (10-fold dilutions starting from 100,000pg/ml to 0.1pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (2µg beads per sample)

22.2 Data Processing

[0456] Sensor outputs including the Magnet On and Off phase along with Magnet powering or depowering were considered as a whole data set. The data was then processed through an automated tool for FFT (Fast Fourier Transform) and each processed output was manually checked.

[0457] Thus, the sensor outputs corresponding to cycles of Magnet On and Off phase were processed using Fast Fourier Transform. The Fast Fourier Transform analysis was utilized to acquire the detected magnitude of the Fundamental Frequency and the magnitudes of the associated harmonics of each concentration. These magnitudes and

ratios then being applied to quantitatively determine a correlation co-efficient (R^2 -value) across the indicated concentrations to reflect correlation between the Concentrations and the Harmonics, Magnitudes and Ratios.

23. Experiment 20

[0458] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 50 nanometer superparamagnetic particles. A Binder-to-Particle ratio (1.5:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 23.

Human Albumin protein concentration (pg/ml)	7th/1st Harmonic Magnitude
0	0.174079795
1	0.149582285
10	0.1443692
100	0.139354826
1000	0.133107873
10000	0.138751704
100000	0.13084714

[0459] The results in Table 23 has an R^2 -value of 0.91.

23.1 Experiment Description

[0460] The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SuperMag Streptavidin Beads, 50nm (Product ID: SV0050)
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - ST Sensor (LIS2MDL)
- Amplifier:
 - ST Sensor in-built amplifier
- Magnets:

- Bottom Magnet - Oval Copper Coil Electromagnet (Dimensions 43.8x33.5mm, 5.3mm Thickness) with 0.2mm coil gauge. Connected to 1V DC power supply with programmable run through RIGOL DP832.
- 25 Cycles of Bottom Magnet actuation with each cycle comprising of: 1 second of Magnet on time and 1 second of Magnet off.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech SuperMag Streptavidin Beads (1mg/ml)
 - 1.5 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Serum Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (10-fold dilutions starting from 100,000pg/ml to 0.1pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (2µg beads per sample)

23.2 Data Processing

[0461] Sensor outputs including the Magnet On and Off phase along with Magnet powering or depowering were considered as a whole data set. The data was then processed through an automated tool for FFT (Fast Fourier Transform) and each processed output was manually checked.

[0462] Thus, the sensor outputs corresponding to cycles of Magnet On and Off phase were processed using Fast Fourier Transform. The Fast Fourier Transform analysis was utilized to acquire the detected magnitude of the Fundamental Frequency and the magnitudes of the associated harmonics of each concentration. These magnitudes and ratios then being applied to quantitatively determine a correlation co-efficient (R^2 -value)

across the indicated concentrations to reflect correlation between the Concentrations and the Harmonics, Magnitudes and Ratios.

24. Experiment 21

[0463] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 50 nanometer superparamagnetic particles. A Binder-to-Particle ratio (1.5:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 24.

Human Albumin protein concentration (pg/ml)	1st Harmonic Magnitude
0	3386844.314
0.1	3358218.162
10	3167777.892
100	3099116.6
1000	3086903.761
100000	2824221.833

[0464] The results in Table 24 has an R^2 -value of 0.92.

24.1 Experiment Description

[0465] The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SuperMag Streptavidin Beads, 50nm (Product ID: SV0050)
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - ST Sensor (LIS2MDL)
- Amplifier:
 - ST Sensor in-built amplifier
- Magnets:
 - Top Magnet – Circular Flat Base EM coil (Pancake Coil) Design (Dimension 20.6mm radi, Thickness 1.8mm) coil gauge 0.9mm. Connected to 0.3V DC power supply with programmable run through RIGOL DP832.

- 5 Cycles of Bottom Magnet actuation with each cycle comprising of: 2 seconds of Magnet on time and 1 second of Magnet off.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Magnet
 - Middle Component – Sample
 - Bottom Component – Sensor
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech SuperMag Streptavidin Beads (1mg/ml)
 - 1.5 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Serum Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (10-fold dilutions starting from 100,000pg/ml to 0.1pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (2µg beads per sample)

24.2 Data Processing

[0466] Sensor outputs including the Magnet On and Off phase along with Magnet powering or depowering were considered as a whole data set. The data was then processed through an automated tool for FFT (Fast Fourier Transform) and each processed output was manually checked.

[0467] Thus, the sensor outputs corresponding to cycles of Magnet On and Off phase were processed using Fast Fourier Transform. The Fast Fourier Transform analysis was utilized to acquire the detected magnitude of the Fundamental Frequency and the magnitudes of the associated harmonics of each concentration. These magnitudes and ratios then being applied to quantitatively determine a correlation co-efficient (R^2 -value) across the indicated concentrations to reflect correlation between the Concentrations and the Harmonics, Magnitudes and Ratios.

25. Experiment 22

[0468] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 50 nanometer superparamagnetic particles. A Binder-to-Particle ratio (1.5:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 25.

Human Albumin protein concentration (pg/ml)	1st Harmonic Magnitude
0.1	621.5204
1	729.8993
10	815.047
1000	1028.503
10000	989.3417
100000	1191.741

[0469] The results in Table 25 has an R^2 -value of 0.95.

25.1 Experiment Description

[0470] The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SuperMag Streptavidin Beads, 50nm (Product ID: SV0050)
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - ST Sensor (LIS2MDL)
- Amplifier:
 - ST Sensor in-built amplifier
- Magnets:
 - Bottom Magnet - Oval Copper Coil Electromagnet (Dimensions 43.8x33.5mm, 5.3mm Thickness) with 0.2mm coil gauge. Connected to 1V DC power supply with programmable run through RIGOL DP832. Time controlled run with a SSR switch with 80 duty cycle.

- 10 Cycles of Bottom Magnet actuation with each cycle comprising of: 1 second of Magnet on time and 1 second of Magnet off.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech SuperMag Streptavidin Beads (1mg/ml)
 - 1.5 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Serum Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (10-fold dilutions starting from 100,000pg/ml to 0.1pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (2µg beads per sample)

25.2 Data Processing

[0471] Sensor outputs including the Magnet On and Off phase along with Magnet powering or depowering were considered as a whole data set. The data was then processed through an automated tool for FFT (Fast Fourier Transform) and each processed output was manually checked.

[0472] Thus, the sensor outputs corresponding to cycles of Magnet On and Off phase were processed using Fast Fourier Transform. The Fast Fourier Transform analysis was utilized to acquire the detected magnitude of the Fundamental Frequency and the magnitudes of the associated harmonics of each concentration. These magnitudes and ratios then being applied to quantitatively determine a correlation co-efficient (R^2 -value) across the indicated concentrations to reflect correlation between the Concentrations and the Harmonics, Magnitudes and Ratios.

26. Experiment 23

The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 50 nanometer superparamagnetic particles. A Binder-to-Particle ratio (1.5:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 26.

Human Albumin protein concentration (pg/ml)	5th/1st Harmonic Magnitude
0	0.200179438
1	0.200117
10	0.20001
100	0.199971
1000	0.199979
10000	0.199932
100000	0.199869

[0473] The results in Table 26 has an R^2 -value of 0.93.

26.1 Experiment Description

[0474] The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SuperMag Streptavidin Beads, 50nm (Product ID: SV0050)
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - ST Sensor (LIS2MDL)
- Amplifier:
 - ST Sensor in-built amplifier
- Magnets:
 - Bottom Magnet - Oval Copper Coil Electromagnet (Dimensions 43.8x33.5mm, 5.3mm Thickness) with 0.2mm coil gauge. Connected to 1V DC power supply with programmable run through RIGOL DP832. Time controlled run with a SSR switch with 80 duty cycle.

- 5 Cycles of Bottom Magnet actuation with each cycle comprising of: 1 second of Magnet on time and 1 second of Magnet off.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech SuperMag Streptavidin Beads (1mg/ml)
 - 1.5 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Serum Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (10-fold dilutions starting from 100,000pg/ml to 0.1pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (2µg beads per sample)

26.2 Data Processing

[0475] Sensor outputs including the Magnet On and Off phase along with Magnet powering or depowering were considered as a whole data set. The data was then processed through an automated tool for FFT (Fast Fourier Transform) and each processed output was manually checked.

[0476] Thus, the sensor outputs corresponding to cycles of Magnet On and Off phase were processed using Fast Fourier Transform. The Fast Fourier Transform analysis was utilized to acquire the detected magnitude of the Fundamental Frequency and the magnitudes of the associated harmonics of each concentration. These magnitudes and ratios then being applied to quantitatively determine a correlation co-efficient (R^2 -value) across the indicated concentrations to reflect correlation between the Concentrations and the Harmonics, Magnitudes and Ratios.

27. Experiment 24

The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 2.1µm ferromagnetic particles. A Binder-to-Particle ratio (10:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 27.

Human Albumin protein concentration (pg/ml)	1st Harmonic Magnitude
0	843.9974259
0.1	1150.337503
1	1191.035205
100	1417.11569
1000	1583.855146
100000	2031.19701

[0477] The results in Table 27 has an R^2 -value of 0.95.

27.1 Experiment Description

[0478] The experimental design parameters is set out below.

- Magnetisable particles:
 - SpheroTech 2.1µm ferromagnetic beads (SVFM 20-5) coated with Streptavidin.
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - ST Sensor (LIS2MDL)
- Amplifier:
 - ST Sensor in-built amplifier
- Magnets:
 - Bottom Magnet - Oval Copper Coil Electromagnet (Dimensions 43.8x33.5mm, 5.3mm Thickness) with 0.2mm coil gauge. Connected to 1V DC power supply with programmable run through RIGOL DP832. Time controlled run with a SSR switch with 80 duty cycle.

- 5 Cycles of Bottom Magnet actuation with each cycle comprising of: 1 second of Magnet on time and 1 second of Magnet off.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – SpheroTech 2.1um ferromagnetic beads (1% w/v)
 - 10 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Serum Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (10-fold dilutions starting from 100,000pg/ml to 0.1pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (0.5µg beads per sample)

27.2 Data Processing

[0479] Sensor data relating to the very commencement of the EM-Coil saturation (as evidenced by an increase in the Sensed magnetic field) through to maximum coil saturation (for a given/set power level) was selected with a total measurement period of the EM-On build up into equilibrium of 0.05 seconds. Whilst the entire duration of EM-On equilibrium was of approximately 1.0 seconds total duration per cycle, the next focus was on the mirror image of the time window and data positions aforementioned. That is, now capturing 0.05 seconds commencing prior to the commencement of the EM-Coil desaturation, and through to a return to the EM-Coil desaturation level. In total, an aggregate was collected, concatenated data set of these two data windows (as described above) and reflecting a focus on a total time period of circa 0.10 seconds per EM-Coil power modulation cycle.

[0480] The concatenated data was then processed through an automated computational tool for FFT (Fast Fourier Transform) and each Data-processed output was manually checked.

[0481] Thus, the sensor outputs corresponding to Magnet On periods (commencing from the initiation of the EM-Coil saturating through to equilibrium, and a concatenated period commencing from the last moments of the coil at equilibrium through the initiation of EM-Coil desaturation through to EM-Coil fully desaturation) represent the entirety of the data sets in this data processing design. The Fast Fourier Transform analysis was utilized to acquire the detected magnitude of the Fundamental Frequency and the magnitudes of the associated harmonics of each concentration. These magnitudes and ratios then being applied to quantitatively determine a correlation co-efficient (R^2 -value) across the indicated concentrations to reflect correlation between the Concentrations and the Harmonics, Magnitudes and Ratios.

28. Experiment 25

[0482] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 2.1um ferromagnetic particles. A Binder-to-Particle ratio (10:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 28.

Human Albumin protein concentration (pg/ml)	3rd/1st Harmonic Magnitude
0	0.196748455
1	0.196832
10	0.197239
100	0.197546
1000	0.197925702
100000	0.198433224

[0483] The results in Table 28 has an R^2 -value of 0.97.

28.1 Experiment Description

[0484] The experimental design parameters is set out below.

- Magnetisable particles:
 - SpheroTech 2.1um ferromagnetic beads (SVFM 20-5) coated with Streptavidin.
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:

- ST Sensor (LIS2MDL)
- Amplifier:
 - ST Sensor in-built amplifier
- Magnets:
 - Bottom Magnet - Oval Copper Coil Electromagnet (Dimensions 43.8x33.5mm, 5.3mm Thickness) with 0.2mm coil gauge. Connected to 1V DC power supply with programmable run through RIGOL DP832. Time controlled run with a SSR switch with 80 duty cycle.
 - 5 Cycles of Bottom Magnet actuation with each cycle comprising of: 1 second of Magnet on time and 1 second of Magnet off.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – SpheroTech 2.1um ferromagnetic beads (1% w/v)
 - 10 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Serum Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (10-fold dilutions starting from 100,000pg/ml to 0.1pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (0.5µg beads per sample)

28.2 Data Processing

[0485] Sensor data relating to the very commencement of the EM-Coil saturation (as evidenced by an increase in the Sensed magnetic field) through to maximum coil saturation (for a given/set power level) was selected with a total measurement period of the EM-On build up into equilibrium of 0.05 seconds. Whilst the entire duration of EM-On equilibrium was approximately 1.0 seconds total duration per cycle, the next focus was on the mirror

image of the time window and data positions aforementioned. That is, now capturing 0.05 seconds commencing prior to the commencement of the EM-Coil desaturation, and through to a return to the EM-Coil desaturation level. The concatenated data set of these two data windows (as described above) was used and reflecting a focus on a total time period of circa 0.10 seconds per EM-Coil power modulation of the middle third cycle only.

[0486] The concatenated data was then processed through an automated computational tool for FFT (Fast Fourier Transform) and each Data-processed output was manually checked.

[0487] Thus, the sensor outputs corresponding to third cycle Magnet On periods (commencing from the initiation of the EM-Coil saturating through to equilibrium, and a concatenated period commencing from the last moments of the coil at equilibrium through the initiation of EM-Coil desaturation through to EM-Coil fully desaturation) represent the entirety of the data sets in this data processing design. The Fast Fourier Transform analysis was utilized to acquire the detected magnitude of the Fundamental Frequency and the magnitudes of the associated harmonics of each concentration. These magnitudes and ratios then being applied to quantitatively determine a correlation co-efficient (R^2 -value) across the indicated concentrations to reflect correlation between the Concentrations and the Harmonics, Magnitudes and Ratios.

29. Experiment 26

[0488] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 2.1 μ m ferromagnetic particles. A Binder-to-Particle ratio (10:1) was tested to determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 29.

Human Albumin protein concentration (pg/ml)	3rd/1st Harmonic Magnitude
0	0.197012322
1	0.197546
10	0.197658
1000	0.198171358
10000	0.198431864

[0489] The results in Table 29 has an R^2 -value of 0.97.

29.1 Experiment Description

[0490] The experimental design parameters is set out below.

- Magnetisable particles:
 - SpheroTech 2.1um ferromagnetic beads (SVFM 20-5) coated with Streptavidin.
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - ST Sensor (LIS2MDL)
- Amplifier:
 - ST Sensor in-built amplifier
- Magnets:
 - Bottom Magnet - Oval Copper Coil Electromagnet (Dimensions 43.8x33.5mm, 5.3mm Thickness) with 0.2mm coil gauge. Connected to 1V DC power supply with programmable run through RIGOL DP832. Time controlled run with a SSR switch with 80 duty cycle.
 - 5 Cycles of Bottom Magnet actuation with each cycle comprising of: 1 second of Magnet on time and 1 second of Magnet off.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – SpheroTech 2.1um ferromagnetic beads (1% w/v)
 - 10 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit

- Different concentration of Human Serum Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (10-fold dilutions starting from 100,000pg/ml to 0.1pg/ml).
- All components mixed and sensed in a test volume of 5 microlitres (0.5µg beads per sample)

29.2 Data Processing

[0491] Sensor data relating to the very commencement of the EM-Coil saturation (as evidenced by an increase in the Sensed magnetic field) through to maximum coil saturation (for a given/set power level) was selected with a total measurement period of the EM-On build up into equilibrium of 0.05 seconds. Whilst the entire duration of EM-On equilibrium was approximately 1.0 seconds total duration per cycle, the next focus was on the mirror image of the time window and data positions aforementioned. That is, now capturing 0.05 seconds commencing prior to the commencement of the EM-Coil desaturation, and through to a return to the EM-Coil desaturation level. The concatenated data set of these two data windows (as described above) was used and reflecting a focus on a total time period of circa 0.10 seconds per EM-Coil power modulation of the middle third cycle only.

[0492] The concatenated data was then processed through an automated computational tool for FFT (Fast Fourier Transform) and each Data-processed output was manually checked.

[0493] Thus, the sensor outputs corresponding to third cycle Magnet On periods (commencing from the initiation of the EM-Coil saturating through to equilibrium, and a concatenated period commencing from the last moments of the coil at equilibrium through the initiation of EM-Coil desaturation through to EM-Coil fully desaturation) represent the entirety of the data sets in this data processing design. The Fast Fourier Transform analysis was utilized to acquire the detected magnitude of the Fundamental Frequency and the magnitudes of the associated harmonics of each concentration. These magnitudes and ratios then being applied to quantitatively determine a correlation co-efficient (R^2 -value) across the indicated concentrations to reflect correlation between the Concentrations and the Harmonics, Magnitudes and Ratios.

30. Experiment 27

[0494] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 30 nanometer superparamagnetic particles. A different Binder-to-Particle ratio (0.75:1) was tested to

determine whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 30.

Human Albumin protein concentration (pg/ml)	Sensor Value (Volts - V)
25	5.53390
1.56	5.52464
0.39	5.55030
0.098	5.61762

[0495] The results in Table 30 has an R^2 -value of 0.88.

30.1 Experiment Description

[0496] The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SHS30-01 (30 nanometer) Streptavidin Coated Superparamagnetic Particles
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - Honeywell HMC 1041z magnetometer
- Amplifier:
 - Texas Instruments Amplifier INA819
- Magnets:
 - Top Magnet - Rectangular Copper Coil Electromagnet (Dimension 22x25mm and 3.5mm thickness) with 0.1mm coil gauge. Connected to 0.63V DC power supply with programmable run through RIGOL DP832.
 - 5 Cycles of Top Magnet actuation with each cycle comprising of: 2 seconds of Magnet on time followed by 5 seconds of Magnet off-time.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Top Magnet

- Middle Component – Sample
- Bottom Component – Sensor
- All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech Superparamagnetic Beads (1 mg/mL)
 - 0.75 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (4-fold dilutions starting from 100pg/ml to 0.098pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (0.5µg beads per sample)

30.2 Data Processing

[0497] Sensor outputs during the 2 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not considered. An automated tool was created for this data processing and each processed output was manually checked.

[0498] The sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all 5 cycles. This was fitted to a line-of-best-fit and R^2 -value derived.

31. Experiment 27a

[0499] The aim of this experiment is to observe the difference in R^2 value when FFT is used to process the sensor value outputs obtained in experiment 27.

Table 31.

Human Albumin protein concentration (pg/ml)	5th / 1st Harmonic Magnitude
0.39	0.042917
1.56	0.043164
6.25	0.043687
25	0.044313

[0500] The results in Table 31 has an R^2 -value of 0.97.

[0501] An increase in the R^2 value is observed when the when FFT is used to process the sensor value outputs obtained in experiment 27.

31.1 Data Processing

[0502] Sensor outputs including the Magnet On and Off phase along with Magnet powering or depowering were considered as a whole data set. The data was then processed through an automated tool for FFT (Fast Fourier Transform) and each processed output was manually checked.

[0503] Thus, the sensor outputs corresponding to cycles of Magnet On and Off phase were processed using Fast Fourier Transform. The Fast Fourier Transform analysis was utilized to acquire the detected magnitude of the Fundamental Frequency and the magnitudes of the associated harmonics of each concentration. These magnitudes and ratios then being applied to quantitatively determine a correlation co-efficient (R^2 -value) across the indicated concentrations to reflect correlation between the Concentrations and the Harmonics, Magnitudes and Ratios.

32. Experiment 28

[0504] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 30 nanometer superparamagnetic particles. We tested for a different Binder-to-Particle ratio (0.75:1) to see whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 32.

Human Albumin protein concentration (pg/ml)	Sensor Value (Volts - V)
1000	6.01972
100	6.01411
10	5.65961
1	5.53346

[0505] The results in Table 32 has an R^2 -value of 0.89.

32.1 Experiment Description

[0506] The experimental design parameters is set out below.

- Magnetisable particles:

- Ocean NanoTech SHS30-01 (30 nanometer) Streptavidin Coated Superparamagnetic Particles
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - Honeywell HMC 1041z magnetometer
- Amplifier:
 - Texas Instruments Amplifier INA819
- Magnets:
 - Bottom Magnet - Rectangular Copper Coil Electromagnet (Dimension 22x25mm and 3.5mm thickness) with 0.1mm coil gauge. Connected at 1V DC power supply with programmable run through RIGOL DP832.
 - 5 Cycles of Top Magnet actuation with each cycle comprising of: 2 seconds of Magnet on time followed by 5 seconds of Magnet-off time.
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Bottom Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles –Ocean NanoTech Superparamagnetic Beads (1 mg/mL)
 - 0.75 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (10-fold dilutions starting from 10,000pg/ml to 0.1pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (2µg beads per sample)

32.2 Data Processing

[0507] Sensor outputs during the 2 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not considered. An automated tool was created for this data processing and each processed output was manually checked.

[0508] The sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all 5 cycles. This was fitted to a line-of-best-fit and R²-value derived.

33. Experiment 28a

[0509] The aim of this experiment is to observe the difference in R² value when FFT is used to process the sensor value outputs obtained in experiment 28.

Table 33.

Human Albumin protein concentration (pg/ml)	7th /1st Harmonic Magnitude
0	0.017334834
1	0.022753
10	0.022303
100	0.027039
1000	0.02861
10000	0.031829

[0510] The results in Table 33 has an R²-value of 0.95.

[0511] An increase in the R² value is observed when the when FFT is used to process the sensor value outputs obtained in experiment 28.

33.1 Data Processing

[0512] Sensor outputs including the Magnet On and Off phase along with Magnet powering or depowering were considered as a whole data set. The data was then processed through an automated tool for FFT (Fast Fourier Transform) and each processed output was manually checked.

[0513] Thus, the sensor outputs corresponding to cycles of Magnet On and Off phase were processed using Fast Fourier Transform. The Fast Fourier Transform analysis was

utilized to acquire the detected magnitude of the Fundamental Frequency and the magnitudes of the associated harmonics of each concentration. These magnitudes and ratios then being applied to quantitatively determine a correlation co-efficient (R²-value) across the indicated concentrations to reflect correlation between the Concentrations and the Harmonics, Magnitudes and Ratios.

34. Experiment 29

[0514] The aim of this experiment is to demonstrate quantitative detection of the Biomarker Analyte using the MED detection methodology and 30 nanometer superparamagnetic particles. We tested for a different Binder-to-Particle ratio (0.75:1) to see whether such ratio can affect the inter-particle interaction in the absence of Analyte.

Table 34.

Human Albumin protein concentration (pg/ml)	Sensor Value (Volts - V)
10000	5.0010
5000	5.0194
2500	5.0173
1250	5.0135
625	5.0455
312.5	5.0386
0	5.1317

[0515] The results in Table 34 has an R²-value of 0.84.

34.1 Experiment Description

[0516] The experimental design parameters is set out below.

- Magnetisable particles:
 - Ocean NanoTech SHS30-01 (30 nanometer) Streptavidin Coated Superparamagnetic Particles
 - Functionalised with Biotinylated "Detection" antibody from DY1455 ELISA kit (anti-Human Serum Albumin)
- Magnetic sensor:
 - Honeywell HMC 1041z magnetometer
- Amplifier:

- Texas Instruments Amplifier INA819
- Magnets:
 - Bottom Magnet - Rectangular Copper Coil Electromagnet (Dimension 22x25mm and 3.5mm thickness) with 0.1mm coil gauge. Connected at 0.5V DC power supply with programmable run through RIGOL DP832.
 - 5 Cycles of Top Magnet actuation with each cycle comprising of: 2 seconds of Magnet on time followed by 5 seconds of Magnet off time
- Apparatus Setup:
 - Sample is positioned in-between Sensor and Magnets such that:
 - Top Component – Sample
 - Middle Component – Sensor
 - Bottom Component – Bottom Magnet
 - All components were aligned vertically through the center of each component.
- For each Biomarker Analyte concentration tested:
 - 1 microliter of magnetisable particles – Ocean NanoTech Superparamagnetic Beads (1 mg/mL)
 - 0.75 nanogram of anti-Albumin Antibody - Biotinylated "Detection" antibody from DY1455 ELISA kit
 - Different concentration of Human Albumin recombinant protein (from DY1455 ELISA kit) generated via serial dilution (2-fold dilutions starting from 10,000pg/ml to 312.5pg/ml).
 - All components mixed and sensed in a test volume of 5 microlitres (2µg beads per sample)

34.2 Data Processing

[0517] Sensor outputs during the 2 seconds of Magnet On phase of each cycle, was processed to exclude data points which correspond with the actuation of the Magnet (either turning On or Off) so that effects due to powering or depowering of the Magnet were not considered. An automated tool was created for this data processing and each processed output was manually checked.

[0518] Thus, the sensor outputs corresponding to Magnet On phase were averaged for each cycle, and then averaged across all 5 cycles. This was fitted to a line-of-best-fit and R2-value derived.

35. Experiment 29

[0519] The aim of this experiment is to observe the difference in R^2 value when FFT is used to process the sensor value outputs obtained in experiment 29.

Table 35.

Human Albumin protein concentration (pg/ml)	1st Harmonic Magnitude
312.5	78.80826085
625	79.0195981
1250	79.47621873
2500	80.38422115
500	80.8915852
10000	82.25004617

[0520] The results in Table 35 has an R^2 -value of 0.94.

[0521] An increase in the R^2 value is observed when the when FFT is used to process the sensor value outputs obtained in experiment 29.

35.1 Data Processing

[0522] Sensor outputs including the Magnet On and Off phase along with Magnet powering or depowering were considered as a whole data set. The data was then processed through an automated tool for FFT (Fast Fourier Transform) and each processed output was manually checked.

[0523] Thus, the sensor outputs corresponding to cycles of Magnet On and Off phase were processed using Fast Fourier Transform. The Fast Fourier Transform analysis was utilized to acquire the detected magnitude of the Fundamental Frequency and the magnitudes of the associated harmonics of each concentration. These magnitudes and ratios then being applied to quantitatively determine a correlation co-efficient (R^2 -value) across the indicated concentrations to reflect correlation between the Concentrations and the Harmonics, Magnitudes and Ratios.

WE CLAIM:

1. A method for detecting a target analyte in a sample, the method comprising:
 - a) providing a quantity of magnetisable particles having a reference magnetic signal that is known or measured before or after the sample is added, the particles being coated with binding molecules complementary to the target analyte,
 - b) bringing the sample comprising the target analyte into contact with the magnetisable particles resulting in bound and unbound binder complexes,
 - c) applying a magnetic field to the sample for a period of time,
 - d) obtaining a magnetic signal, the magnetic signal being the magnetic signal of the bound and unbound binder complexes in the presence of the magnetic field,
 - e) removing the magnetic field for a period of time, and
 - f) comparing the reference magnetic signal and the magnetic signal, wherein a difference between the reference magnetic signal and the magnetic signal correlates to the presence and/or quantity of the target analyte in the sample.
2. The method of claim 1, wherein the reference magnetic signal is determined by measuring the magnetic signal of the quantity of magnetisable particles in the absence of the target analyte.
3. The method of claim 2, wherein the reference magnetic signal is measured at any point between steps b) to e).
4. The method of any one of claims 1 to 3, wherein the magnetic field is generated using an electromagnet.
5. The method of any one of claims 1 to 4, wherein the magnetic signal is obtained after an electromagnetic coil of the electromagnet reaches saturation.
6. The method of any one of claims 1 to 3, wherein the magnetic field is generated using a permanent magnet.
7. The method of any one of claims 1 to 6, wherein quantitation of the target analyte in the sample is determined by correlating the change in the measured magnetic signal relative to the reference/predetermined magnetic signal.
8. The method of any one of claims 1 to 7, further comprising:

generating a reference data set based on known analyte quantity values,

comparing the value obtained from the difference between the reference magnetic signal and the magnetic signal with the reference data set to determine the quantity of analytes in the sample.

9. The method of any one of claims 1 to 8, wherein the magnetic field is applied for a predetermined time.
10. The method of claim 9, wherein the magnetic field is applied for about 0.1 to about 5 seconds.
11. The method of any one of claims 1 to 10, wherein the magnetic field is removed for a predetermined time.
12. The method of claim 11, wherein the magnetic field is removed for about 0.1 to about 5 seconds.
13. The method of any one of claims 1 to 12, wherein the magnetic field is applied and removed for a substantial equal amount of time.
14. The method of claim 13, wherein the magnetic field is applied and removed for about 1 second.
15. The method of any one of claims 1 to 14, wherein steps c) to e) are repeated for two or more times.
16. The method of any one of claims 1 to 15, wherein the magnetisable particles are superparamagnetic or ferromagnetic nanoparticles.
17. The method of any one of claims 1 to 16, wherein the magnetisable particles have an average particle size of about 20 nm to about 60 nm.
18. The method of any one of claims 1 to 17, wherein the magnetisable particles have an average particle size of about 30 nm.
19. The method of any one of claims 1 to 18, further comprising magnetically shielding the sample from ambient magnetic field.
20. The method of any one of claims 1 to 19, further comprising measuring the ambient magnetic field, and adjusting the reference magnetic signal and the magnetic signals based on the ambient magnetic field.

21. The method of any one of claims 1 to 20, wherein the magnetic signal is measured with a sampling rate of at least about 10,000 samples per second.
22. The method of any one of claims 1 to 21, wherein the sample is incubated to a temperature of about 20°C.
23. The method of any one of claims 1 to 22, wherein the magnetic signal is magnetic field strength.
24. The method of any one of claims 1 to 23, is performed using a sample testing device comprising:
 - a sample well or sample reservoir,
 - a magnet located on one of an upper side or lower side of the sample well or sample reservoir, and
 - a magnetic field sensor for measuring changes over time in the magnetic signal in the sample well or sample reservoir.
25. The method of any one of claims 1 to 24, wherein the magnetisable particles are functionalised with molecules that specifically bind to the target analyte.
26. The method of any one of claims 1 to 25, wherein the sample and magnetisable particles are processed by a microfluidic device.
27. The method of any one of claims 1 to 26, wherein the microfluidic device facilitates binding between the magnetisable particles and analyte.
28. The method of any one of claims 1 to 27, wherein one or more magnets generates a magnetic field that changes over time.
29. The method of any one of claims 1 to 28, wherein the one or more magnets can generate a continuity of magnitudes.
30. The method of any one of claims 1 to 29, wherein the one or more magnets can alternate the magnetic field between on and off.
31. The method of claim 24, wherein the magnetic field sensor measures the changes in magnetic field strength generated by the magnetisable particles over time.
32. The method of any one of claims 24 to 31, wherein the signal output from the magnetic field sensor is boosted by a signal amplifier.

33. The method of any one of claims 24 to 32, wherein the signal output from the magnetic field sensor is a voltage reading that is proportional to the magnetic signal measured by the magnetic field sensor.
34. The method of claim 32, wherein the amplified signal is converted from a voltage reading into digital bitstream and recorded and/or analysed by a computer.
35. The method of claim 34, wherein the conversion is performed by Analog to Digital Converter.
36. The method of any one of claims 1 to 35, wherein the method:
- generates sufficient magnetic signal within 15 seconds to detect and/or quantitate the target analyte in the sample, or
 - has a limit of detection (LOD) of at least about 0.05 pg/mL, or
 - has a limit of quantification (LOQ) of at least about 0.1 pg/mL, or
 - one or more of (a) to (c).
37. The method of any one of claims 1 to 36, wherein the magnetic signal is processed using Fast Fourier Transform (FFT).
38. The method of claim 37, wherein the magnetic signal is pre-processed by truncating and/or concatenating the magnetic signal in one or more dimensions before processing using FFT.
39. The method of claim 37 or claim 38, wherein the magnetic signal is windowed before FFT processing.
40. A device for detecting an analyte in a sample, comprising:
- a sample well that is separate to, or integrated into a microfluidic device,
 - a magnet for generating a magnetic field,
 - a magnetic field sensor configured to measure the magnetic signal of magnetisable particles in the sample well in the presence of the magnetic field, the magnetic signal detected from the overall magnetic response that is proportional to the size of the aggregates.