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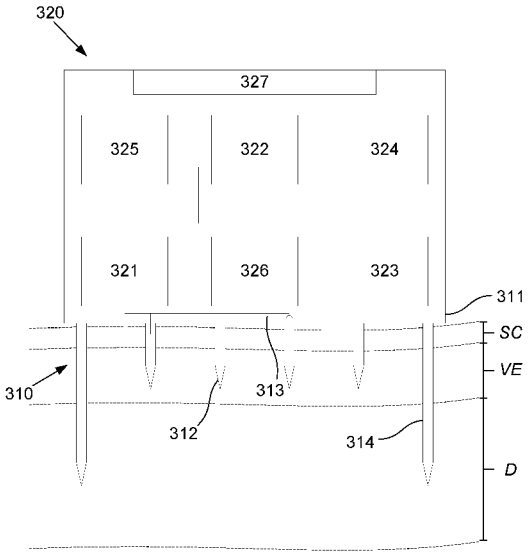

Fig. 3A

(57) Abstract: Disclosed is a system and method for performing measurements on a biological subject, and in one particular example, to performing measurements of analytes in a biological subject by breaching a functional barrier of the subject using microstructures, wherein the one or more microstructures include an aptamer for binding one or more analytes.

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ANALYTE MEASUREMENT SYSTEM

[0001] This application claims priority to Australian Provisional Patent Application No. 2019903696 entitled "Analyte Measurement System" filed on 1 October 2019, the entire content of which is hereby incorporated herein by reference.

Background of the Invention

[0002] The present invention relates to a system and method for performing measurements on a biological subject, and in one particular example, to performing measurements of analytes in a biological subject by breaching a functional barrier of the subject using microstructures.

Description of the Prior Art

[0003] The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that the prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

[0004] Biological markers, such as proteins, antibodies, cells, small chemicals, hormones and nucleic acids, whose presence in excess or deficiency may indicate a diseased state, have been found in blood serum and their levels are routinely measured for research and for clinical diagnosis. Standard tests include antibody analysis for detecting infections, allergic responses, and blood-borne cancer markers (*e.g.* prostate specific antigen analysis for detecting prostate cancer). The biological markers may originate from many organ systems in the body but are extracted from a single compartment, the venous blood.

[0005] However, this is not suitable for all conditions as often blood does not contain key biological markers for diseases originating in solid tissues, and whilst this problem has been partially overcome by taking tissue biopsies, this is time-consuming, painful, risky, costly and can require highly-skilled personnel such as surgeons. In addition, these methods only provide an indication of the level of the biological marker at a single point in time.

[0006] Another serum-rich fluid is the interstitial fluid (ISF) which fills the intercellular spaces in solid tissues and facilitates the passage of nutrients, biomarkers, and excretory products via the blood stream.

[0007] WO2005/072630 describes devices for delivering bioactive materials and other stimuli to living cells, methods of manufacture of the device and various uses of the device, including a number of medical applications. The device comprises a plurality of structures which can penetrate a body surface so as to deliver the bioactive material or stimulus to the required site. The structures are typically solid and the delivery end section of the structure is so dimensioned as to be capable of insertion into targeted cells to deliver the bioactive material or stimulus without appreciable damage to the targeted cells or specific sites therein.

[0008] The use of microneedle versions of such arrays in sampling fluids is also known. However, the techniques focus on the use of micro-fluidic techniques such as capillary or pumping actions to extract fluid, as described for example in US-6,923,764, US-6,052,652, US-6,591,124, US-6,558,361, US-6,908,453, and US2005/0261632, US2006/0264782, US2005/0261632, US2005/0261632, US-6,589,202.

[0009] However, these systems suffer from a number of drawbacks. Firstly, use of capillary or pumping actions can only be achieved using relatively largely structures, which typically pass through the dermis and consequently can end up sampling blood as opposed to interstitial fluid. This can also cause discomfort and irritation to the subject being sampled. Secondly, the requirement for capillary or pumping actions renders the arrays complex, in structure and requiring power sources resulting in arrays that are difficult and expensive to manufacture, liable to infection, making them unsuitable for general use.

[0010] Other *in vitro* diagnostic devices are known, such as the use of arrays that include silicon nanowires, or other complex detection mechanisms, such as direct radio-frequency detection of nucleotide hybridization to perform the detection. Again, the fabrication of such systems is complex and expensive, again making these unsuitable for practical applications.

[0011] US9974471 describes a device and system for measuring and/or monitoring an analyte present on the skin is provided. The system includes a skin-mountable device that may be attached to an external skin surface and a reader device. The skin-mountable device includes

a substrate, a plurality of microneedles, and nanosensors. The microneedles are attached to the substrate such that attachment of the substrate to an external skin surface causes to the microneedles to penetrate into the epidermis, intradermis, or dermis. The nanosensors include a detectable label and are configured to interact with a target analyte present in the interstitial fluid in the epidermis, intradermis, or dermis. The reader device is configured to detect the analyte in interstitial fluid via interaction with the skin-mountable device.

[0012] US20070142885 describes a system and method for revitalizing aging skin using electromagnetic energy that is delivered using a plurality of needles that are capable of penetrating the skin to desired depths. A particular aspect of the invention is the capability to spare zones of tissue from thermal exposure. This sparing of tissue allows new tissue to be regenerated while the heat treatment can shrink the collagen and tighten the underlying structures. Additionally, the system is capable of delivering therapeutically beneficial substances either through the penetrating needles or through channels that have been created by the penetration of the needles.

[0013] US6972013 describes methods for using an electric field to delivery therapeutic or immunizing treatment to a subject by applying non-invasive, user-friendly electrodes to the surface of the skin. Thus, therapeutic or immunizing agents can be delivered into cells of skin for local and systemic treatments or for immunization with optimal gene expression and minimal tissue damage. In particular, therapeutic agents include naked or formulated nucleic acid, polypeptides and chemotherapeutic agents.

[0014] US7285090 describes a monitoring apparatus that includes a sensor device and an I/O device in communication with the sensor device that generates derived data using the data from the sensor device. The derived data cannot be directly detected by the associated sensors. Alternatively, an apparatus that includes a wearable sensor device and an I/O device in communication with the sensor device that includes means for displaying information and a dial for entering information. Alternatively, an apparatus for tracking caloric consumption and caloric expenditure data that includes a sensor device and an I/O device in communication with the sensor device. The sensor device includes a processor programmed to generate data relating to caloric expenditure from sensor data. Alternatively, an apparatus for tracking caloric information for an individual that utilizes a plurality of classification identifiers for classifying

meals consumed by the individual, each of the classification identifiers having a corresponding caloric amount.

[0015] US20110295100 describes methods, systems and/or devices for enhancing conductivity of an electrical signal through a subject's skin using one or more microneedle electrodes are provided. A microneedle electrode may be applied to the subject's skin by placing the microneedle electrode in direct contact with the subject's skin. The microneedles of the microneedle electrode may be inserted into the skin such that the microneedles pierce stratum corneum of the skin up to or through dermis of the skin. An electrical signal passes or is conducted through or across the microneedle electrode and the subject's skin, where impedance of the microneedle electrode is minimal and greatly reduced compared to existing technologies.

[0016] US 2019/0013425 describes a biometric information measuring sensor is provided that includes a base comprising a plurality of bio-marker measuring areas and a plurality of electrodes. Each of the plurality of electrodes is disposed on a respective one of the plurality of bio-marker measuring areas, and each of the plurality of electrodes includes a working electrode and a counter electrode spaced apart from the working electrode. The biometric information measuring sensor also includes a plurality of needles. Each of the needles is disposed on a respective one of the plurality of electrodes. Two or more of the plurality of needles have different lengths.

[0017] WO2009140735 describes an apparatus for use in detecting analytes in a subject, wherein the apparatus includes a number of structures provided on a patch, such that applying the patch to the subject causes at least some of the structures to be inserted into the subject and target one or more analytes and a reagent for detecting the presence or absence of analytes.

Summary of the Present Invention

[0018] In one aspect, there is provided a system for performing measurements on a biological subject, the system including: at least one substrate including one or more microstructures configured to breach a functional barrier of the subject, wherein the one or more microstructures include an aptamer for binding one or more analytes; at least one sensor operatively connected to at least one microstructure, the at least one sensor being configured

to measure response signals from the at least one microstructure; and, one or more electronic processing devices that: determine measured response signals; and, perform an analysis at least in part using the measured response signals to determine at least one indicator at least partially indicative of analyte presence, absence, level or concentration in the subject.

[0019] In one embodiment, the aptamer is a coating on the microstructure.

[0020] In one embodiment, the aptamer selectively bind the one or more analytes.

[0021] In one embodiment, the aptamer undergoes a conformational change upon analyte binding.

[0022] In one embodiment, the aptamer has a first conformation in the absence of analyte binding and a second conformation upon analyte binding.

[0023] In one embodiment, the aptamer comprises a labelling moiety.

[0024] In one embodiment, the labelling moiety is a redox moiety.

[0025] In one embodiment, the redox moiety is selected from the group consisting of methylene blue, ferrocene, vinylferrocene, anthraquinone, nile blue, thionine, anthraquinone-C5, dabcyl, 2,6-dichlorophenal-indophenol, gallocyanine, ROX, pentamethylferrocene, ferrocene-C5, neutral red and horseradish peroxidase.

[0026] In one embodiment, the redox moiety is methylene blue.

[0027] In one embodiment, the labelling moiety is a fluorescent label.

[0028] In one embodiment, the aptamer comprises a moiety for attaching or immobilising the aptamer on the surface of the microstructure.

[0029] In one embodiment, the moiety for attaching or immobilising the aptamer on the surface of the microstructure is a thiol, amine, carboxylic acid, alcohol, carbodiimide, nafion, avidin, biotin or azide.

[0030] In one embodiment, the moiety for attaching or immobilising the aptamer on the surface of the microstructure is a thiol.

[0031] In one embodiment, the one or more microstructures are porous.

[0032] In one embodiment, the one or more analytes are selected from the group consisting of a nucleic acid, an antibody or antigen-binding fragment thereof, an allergen, a chemokine, a cytokine, a hormone, a parasite, a bacteria, a virus or virus-like particle, an epigenetic marker, a peptide, a polypeptide, a protein and a small molecule.

[0033] In one embodiment, the one or more analytes is a protein.

[0034] In one embodiment, the protein is troponin or a subunit thereof.

[0035] In one embodiment, the protein is troponin I.

[0036] In one embodiment, the protein is troponin I or a complex thereof.

[0037] In one embodiment, the protein is cardiac troponin I-C complex.

[0038] In one embodiment, the one or more analytes is a cytokine.

[0039] In one embodiment, the cytokine is IL-6.

[0040] In one embodiment, the system is at least partially wearable.

[0041] In one embodiment, the system includes a signal generator operatively connected to at least one microstructure to apply a stimulatory signal.

[0042] In one embodiment, the one or more processing devices are configured to at least one of: control the signal generator to cause a measurement to be performed; and control the signal generator in accordance with measured response signals.

[0043] In one embodiment, response and stimulatory signals include electrical signals, and wherein the substrate includes electrical connections to allow electrical signals to be applied to and/or received from respective microstructures.

[0044] In one embodiment, response and stimulatory signals include optical signals, and wherein the substrate includes optical connections to allow optical signals to be applied to and/or received from respective microstructures.

[0045] In one embodiment, the system includes one or more switches for selectively connecting at least one of at least one sensor and at least one signal generator to one or more of the microstructures.

[0046] In one embodiment, the one or more processing devices are configured to control the switches to at least one of: allow at least one measurement to be performed; and, control which microstructures are used to measure response signals / apply stimulation.

[0047] In one embodiment, at least one of the substrate and the microstructures include at least one of: metal; polymer; and, silicon.

[0048] In one embodiment, the substrate is at least one of: at least partially flexible; configured to conform to an outer surface of the functional barrier; and, configured to conform to a shape of at least part of a subject.

[0049] In one embodiment, the plate microstructures are at least partially tapered and have a substantially rounded rectangular cross sectional shape.

[0050] In one embodiment, the microstructures include anchor microstructures used to anchor the substrate to the subject and wherein the anchor microstructures at least one of: undergo a shape change; undergo a shape change in response to at least one of substances in the subject and applied stimulation; swell; swell in response to at least one of substances in the subject and applied stimulation; include anchoring structures; have a length greater than that of other microstructures; are rougher than other microstructures; have a higher surface friction than other microstructures; are blunter than other microstructures; are fatter than other microstructures; and, enter the dermis.

[0051] In one embodiment, the microstructures are applied to skin of the subject, and wherein at least some of the microstructures at least one of: penetrate the stratum corneum; enter the viable epidermis but not the dermis; and, enter the dermis.

[0052] In one embodiment, at least some of the microstructures have at least one of: a length that is at least one of: less than 2500 μm; less than 1000 μm; less than 750 μm; less than 450 μm; less than 300 μm; less than 250 μm; about 250 μm; about 150 μm; greater than 100

μm; greater than 50 μm; and, greater than 10 μm; a maximum width that is at least one of: less than 2500 μm; less than 1000 μm; less than 750 μm; less than 450 μm; less than 300 μm; less than 250 μm; of a similar order of magnitude to the length; greater than the length; greater than the length; about the same as the length; about 250 μm; about 150 μm; and, greater than 50 μm; and, a maximum thickness that is at least one of: less than the width; significantly less than the width; of a smaller order of magnitude to the length; less than 300 μm; less than 200 μm; less than 50 μm; about 25 μm; and, greater than 10 μm.

[0053] In one embodiment, at least some of the microstructures include at least one of: a shoulder that is configured to abut against the stratum corneum to control a depth of penetration; and, a shaft extending from a shoulder to the tip, the shaft being configured to control a position of the tip in the subject.

[0054] In one embodiment, the microstructures have at least one of: a density that is at least one of: less than 5000 per cm^2; greater than 100 per cm^2; and, about 600 per cm^2; and, a spacing that is at least one of: less than 1 mm; about 0.5 mm; about 0.2 mm; about 0.1 mm; and, more than 10 μm.

[0055] In one embodiment, at least some of microstructures include an electrode.

[0056] In one embodiment, at least one electrode at least one of: extends over a length of a distal portion of the microstructure; extends over a length of a portion of the microstructure spaced from the tip; is positioned proximate a distal end of the microstructure; is positioned proximate a tip of the microstructure; extends over at least 25% of a length of the microstructure; extends over less than 50% of a length of the microstructure; extends over about 60 μm of the microstructure; is configured to be positioned in a viable epidermis of the subject in use; and, has a surface area of at least one of: less than 200,000 μm^2; about 22,500 μm^2; at least 2,000 μm^2.

[0057] In one embodiment, at least some of microstructures include at least part of an active sensor.

[0058] In one embodiment, at least some of the microstructures include an electrically conductive material.

[0059] In one embodiment, at least some of the microstructures include an insulating layer extending over at least one of: part of a surface of the microstructure; a proximal end of the microstructure; at least half of a length of the microstructure; about 90 μm of a proximal end of the microstructure; and, at least part of a tip portion of the microstructure.

[0060] In one embodiment, at least some of the microstructures include plates having a substantially planar face including at least one electrode.

[0061] In one embodiment, at least some of the microstructures are arranged in groups, and wherein at least one of: response signals are measured between microstructures in different group; stimulation is applied between microstructures in different groups; response signals are measured between microstructures in a group; and, stimulation is applied between microstructures in a group.

[0062] In one embodiment, at least one of: there are at least one of: two groups; three groups; and, more than three groups; electrodes of the microstructures within each group are electrically connected; the groups are at least one of: provided on a common substrate; and, provided on different substrates; each group is a pair of microstructures including spaced apart plate microstructures having substantially planar electrodes in opposition; each group includes multiple spaced apart plate microstructures having substantially planar electrodes; and, each group includes multiple pairs of microstructures including spaced apart plate microstructures having substantially planar electrodes in opposition.

[0063] In one embodiment, the groups include: a counter group including a plurality of counter microstructures defining a counter electrode; a reference group including a plurality of reference microstructures defining a reference electrode; and, at least one working group, each working group including a plurality of working microstructures defining a respective working electrode.

[0064] In one embodiment, at least one of: the reference group is smaller than the working and counter groups; the reference group includes fewer microstructures than the working and counter groups; and, the reference group is positioned adjacent each working groups.

[0065] In one embodiment, at least one of: at least some microstructures are angularly offset; at least some microstructures are orthogonally arranged; adjacent microstructures are orthogonally arranged; microstructures are arranged in rows, and microstructures in one row are angularly offset relative to microstructures in other rows; microstructures are arranged in rows, and the microstructures in one row are orthogonally arranged relative to microstructures in other rows; at least some pairs of microstructures are angularly offset; at least some pairs of microstructures are orthogonally arranged; adjacent pairs of microstructures are orthogonally arranged; pairs of microstructures are arranged in rows, and the pairs of microstructures in one row are angularly offset relative to pairs of microstructures in other rows; pairs of microstructures are arranged in rows, and the pairs of microstructures in one row are orthogonally arranged relative to pairs of microstructures in other rows.

[0066] In one embodiment, at least one of: the spacing between the electrodes in each group are at least one of: less than 10 mm; less than 1 mm; about 0.1 mm; and, more than 10 µm; and, a spacing between groups of microstructures is at least one of: less than 50 mm; more than 20 mm; less than 20 mm; less than 10 mm; more than 10 mm; less than 1 mm; more than 1 mm; about 0.5 mm; and, more than 0.2 mm.

[0067] In one embodiment, the one or more microstructures interact with one or more analytes of interest such that a response signal is dependent on a presence, absence, level or concentration of analytes of interest.

[0068] In one embodiment, the analytes interact with a coating on the microstructures to change electrical and/or optical properties of the coating, thereby allowing the analytes to be detected.

[0069] In one embodiment, the microstructures include a material including at least one of: a bioactive material; a reagent for reacting with analytes in the subject; a binding agent for binding with analytes of interest; a material for binding one or more analytes of interest; a probe for selectively targeting analytes of interest; an insulator; a material to reduce biofouling; a material to attract at least one substance to the microstructures; a material to repel or exclude at least one substance from the microstructures; a material to attract at least some analytes to

the microstructures; and, a material to repel or exclude at least some analytes from the microstructures.

[0070] In one embodiment, the substrate includes a plurality of microstructures and wherein different microstructures are at least one of: differentially responsive to analytes; responsive to different analytes; responsive to different combination of analytes; and, responsive to different levels or concentrations of analytes.

[0071] In one embodiment, at least some of the microstructures at least one of: attract at least one substance to the microstructures; repel or excludes at least one substance from the microstructures; attract at least one analyte to the microstructures; and, repel or excludes at least one analyte from the microstructures.

[0072] In one embodiment, at least some of the microstructures are at least partially coated with a coating.

[0073] In one embodiment, at least one of: at least some microstructures are uncoated; at least some microstructures are porous with an internal coating; at least some microstructures are partially coated; different microstructures have different coatings; different parts of microstructures include different coatings; and, at least some microstructures include multiple coatings.

[0074] In one embodiment, stimulation is used to at least one of: release material from the coating on the microstructure; disrupt the coating; dissolve the coating; and, release the coating.

[0075] In one embodiment, at least some of the microstructures are coated with a selectively dissolvable coating.

[0076] In one embodiment, the coating at least one of: interacts with analytes; undergoes a change in properties upon exposure to analytes; undergoes a shape change to selectively anchor microstructures; modifies surface properties to at least one of: increase hydrophilicity; increase hydrophobicity; and, minimize biofouling; attracts at least one substance to the microstructures; repels or excludes at least one substance from the microstructures; provides a physical structure to at least one of: facilitate penetration of the barrier; strengthen the

microstructures; and, anchor the microstructures in the subject; dissolves to at least one of: expose a microstructure; expose a further coating; and, expose a material; provides stimulation to the subject; contains a material; selectively releases a material; acts as a barrier to preclude at least one substance from the microstructures; and, includes at least one of: polyethylene; polyethylene glycol; polyethylene oxide; zwitterions; peptides; hydrogels; and, self-assembled monolayer.

[0077] In one embodiment, the system includes an actuator configured to apply a force to the substrate to at least one of pierce and penetrate the stratum corneum.

[0078] In one embodiment, the actuator is at least one of: an electromagnetic actuator; a vibratory motor; a piezoelectric actuator; and, a mechanical actuator.

[0079] In one embodiment, the actuator is configured to apply at least one of: a biasing force; a vibratory force; and, a single continuous force.

[0080] In one embodiment, the force at least one of: includes a continuous force that is at least one of: greater than 1 N; less than 10 N; less than 20 N; and, about 2.5 to 5 N; and, includes a vibratory force that is at least one of: at least 1 mN; about 200 mN; and, less than 1000 mN; and, is applied at a frequency that is at least one of: at least 10 Hz; about 100 to 200 Hz; and, less than 1 kHz.

[0081] In one embodiment, at least one of a force and frequency are at least one of: varying; varying depending on at least one of: a time of application; a depth of penetration; a degree of penetration; and, an insertion resistance; and, increasing with an increasing depth of penetration; decreasing with an increasing depth of penetration; increasing until a point of penetration; and decreasing after a point of penetration.

[0082] In one embodiment, the one or more electronic processing devices control the actuator.

[0083] In one embodiment, the system includes a housing containing the at least one sensor and at least one electronic processing device.

[0084] In one embodiment, the housing selectively couples to the substrate.

[0085] In one embodiment, the housing couples to the substrate using at least one of: electromagnetic coupling; mechanical coupling; adhesive coupling; and, magnetic coupling.

[0086] In one embodiment, at least one of the housing and substrate are at least one of: secured to the subject; secured to the subject using anchor microstructures; secured to the subject using an adhesive patch; and, secured to the subject using a strap.

[0087] In one embodiment, the housing includes housing connectors that operatively connect to substrate connectors on the substrate to communicate signals with the microstructures.

[0088] In one embodiment, the system is configured to perform repeated measurements over a time period and wherein the microstructures are configured to remain in the subject during the time period.

[0089] In one embodiment, the time period is at least one of: at least one minute; at least one hour; at least one day; and, at least one week.

[0090] In one embodiment, the system is configured to perform repeated measurements with a frequency that is at least one of: substantially continuously; every second; every minute; every 5 to 10 minutes; hourly; daily; and, weekly.

[0091] In one embodiment, the one or more electronic processing devices analyse measured response signals to determine at least one indicator at least partially indicative of a physiological status associated with the subject.

[0092] In one embodiment, the one or more electronic processing devices: analyse measured response signals to determine at least one metric; and, use the at least one metric to determine at least one indicator, the at least one indicator being at least partially indicative of a physiological status associated with the subject.

[0093] In one embodiment, the one or more electronic devices apply the at least one metric to at least one computational model to determine the indicator, the at least one computational model embodying a relationship between a health status and the at least one metric.

[0094] In one embodiment, the at least one computational model is obtained by applying machine learning to reference metrics derived from subject data measured for one or more reference subjects.

[0095] In one embodiment, the one or more electronic devices are configured to determine an indicator by performing at least one of: pattern matching; a longitudinal analysis; and comparison to a threshold.

[0096] In one embodiment, the one or more processing devices are configured to determine a physiological status indicative of at least one of: a presence, absence or degree of a medical condition; a prognosis associated with a medical condition; a presence, absence, level or concentration of a biomarker; a presence, absence, level or concentration of an analyte; fluid levels in the subject; blood oxygenation; and, bioelectric activity.

[0097] In one embodiment, the one or more electronic devices are configured to generate an output at least one of: including a notification; including an alert; indicative of an indicator; derived from an indicator; and, including a recommendation based on an indicator.

[0098] In one embodiment, the system includes a transmitter that transmits at least one of: subject data derived from the measured response signals; at least one metric derived from measured response signals; an indication of measured response signals; and, at least one metric derived from the subject data.

[0099] In one embodiment, the one or more electronic processing devices: generate subject data indicative of the measured response signals; and, at least one of: at least partially process measured response signals; at least partially process the subject data; at least partially analyse the subject data; and, store an indication of the subject data.

[0100] In one embodiment, the system includes a monitoring device and a patch including the substrate and microstructures.

[0101] In one embodiment, the monitoring device is at least one of: inductively coupled to the patch; attached to the patch; and brought into contact with the patch when a reading is to be performed.

[0102] In one embodiment, the monitoring device is configured to at least one of: cause a measurement to be performed; at least partially analyse measurements; control stimulation applied to at least one microstructure; generate an output; provide an output indicative of the indicator; provide a recommendation based on the indicator; and, cause an action to be performed.

[0103] In one embodiment, the system includes: a wearable monitoring device that performs the measurements; and, a processing system that: receives subject data derived from the measured response signals; and, analyses the subject data to generate at least one indicator, the at least one indicator being at least partially indicative of a health status associated with the subject.

[0104] In one embodiment, the system includes a client device that: receives measurement data from the wearable monitoring device; generates subject data using the measurement data; transfer the subject data to the processing system; receive an indicator from the processing system; and, displays a representation of the indicator.

[0105] In one embodiment, the system includes: a substrate coil positioned on the substrate and operatively coupled to one or more microstructure electrodes; and, an excitation and receiving coil positioned in proximity to the substrate coil such that alteration of a drive signal applied to the excitation and receiving coil acts as a response signal.

[0106] In one embodiment, one or more microstructure electrodes interact with one or more analytes of interest such that the response signal is dependent on a presence, absence, level or concentration of analytes of interest.

[0107] In one embodiment, the system includes: a first substrate coil positioned on a substrate and operatively coupled to one or more first microstructure electrodes; a second substrate coil positioned on a substrate and operatively coupled to one or more second microstructure electrodes, the second microstructure electrodes being configured to interact with analytes of interest; and, at least one excitation and receiving coil positioned in proximity to at least one of the first and second substrate coils such that alteration of a drive signal applied to the at least one excitation and receiving coil acts as a response signal, and wherein the one

or more electronic processing devices use the first and second response signals to a presence, absence, level or concentration of analytes of interest.

[0108] In one embodiment, first and second excitation and receiving coils are positioned in proximity to respective ones of the first and second substrate coils such that alteration of a drive signal applied to each excitation and receiving coil acts as a respective response signal.

[0109] In another aspect, there is provided a system for performing measurements on a biological subject, the system including: at least one sensor configured to be operatively connected to one or more microstructures configured to breach a functional barrier of the subject in use, the at least one sensor being configured to measure response signals from the at least one microstructure, wherein the one or more microstructures include an aptamer for binding one or more analytes; and, one or more electronic processing devices that: determine measured response signals; and, at least one of: perform an analysis at least in part using the measured response signals; and, store data at least partially indicative of the measured response signals.

[0110] In a further aspect, there is provided a method for performing measurements on a biological subject, the method including: using at least one substrate including one or more microstructures to breach a functional barrier of the subject, wherein the one or more microstructures include an aptamer for binding one or more analytes; using at least one sensor operatively connected to at least one microstructure to measure response signals from the at least one microstructure; and, in one or more electronic processing devices: determining measured response signals; and, at least one of: performing an analysis at least in part using the measured response signals; and, storing data at least partially indicative of the measured response signals.

[0111] It will be appreciated that the broad forms of the invention and their respective features can be used in conjunction and/or independently, and reference to separate broad forms is not intended to be limiting. Furthermore, it will be appreciated that features of the method can be performed using the system or apparatus and that features of the system or apparatus can be implemented using the method.

Brief Description of the Drawings

[0112] Various examples and embodiments of the present invention will now be described with reference to the accompanying drawings, in which:

[0113] Figure 1 is a schematic diagram of an example of a system for performing measurements on a biological subject;

[0114] Figure 2 is a flow chart of an example of a process for performing measurements on a biological subject;

[0115] Figure 3A is a schematic side view of a further example of a system for performing measurements on a biological subject;

[0116] Figure 3B is a schematic underside view of an example of a patch for the system of Figure 3A;

[0117] Figure 3C is a schematic plan view of the patch of Figure 3B;

[0118] Figure 3D is a schematic underside view of an alternative example of a patch for the system of Figure 3A;

[0119] Figure 3E is a schematic side view of the patch of Figure 3D;

[0120] Figure 3F is a schematic side view of an example of a housing arrangement for the system of Figure 3A;

[0121] Figure 3G is a schematic plan view of the housing arrangement of Figure 3F;

[0122] Figure 3H is a schematic side view of an example of a flexible segmented substrate arrangement;

[0123] Figure 3I is a schematic side view of a further example of a flexible segmented substrate arrangement;

[0124] Figure 3J is a schematic side view of a further example of a flexible segmented substrate arrangement;

[0125] Figure 3K is a schematic side view of a further example of a flexible segmented substrate arrangement;

[0126] Figure 3L is a schematic side view of an example actuator arrangement;

[0127] Figure 3M is a schematic side view of a further example actuator arrangement;

[0128] Figure 3N is a schematic underside view of an alternative example of a patch for the system of Figure 3A;

[0129] Figure 3O is a schematic plan view of the patch of Figure 3N;

[0130] Figure 4A is a schematic side view of a first example of a microstructure configuration;

[0131] Figure 4B is a schematic side view of a second example of a microstructure configuration;

[0132] Figure 4C is a graph illustrating the electric field between closely spaced electrodes;

[0133] Figure 4D is a graph illustrating the electric field between distant spaced electrodes;

[0134] Figure 5A is a schematic side view of an example of a plate microstructure;

[0135] Figure 5B is a schematic front view of the microstructure of Figure 5A;

[0136] Figure 5C is a schematic underside view of an example of a patch including the microstructure of Figure 5A;

[0137] Figure 5D is a schematic perspective topside view of an example of substrate including pairs of blade microstructures of Figures 5A and 5B;

[0138] Figure 5E is a schematic front view of an example of a blade microstructure;

[0139] Figure 5F is a schematic perspective topside view of an example of substrate including blade microstructures;

[0140] Figure 5G is a schematic plan view of an example of a hexagonal grid microstructure array;

[0141] Figure 5H is a schematic plan view of an alternative example of a grid of pairs of microstructures;

[0142] Figure 5I is a schematic plan view of the grid of Figure 5H showing example connections;

[0143] Figure 5J is a schematic perspective view of an example of a grid of pairs of microstructures;

[0144] Figure 5K is an image of an example of a patch including arrays of pairs of angularly offset plate microstructures;

[0145] Figure 5L is a schematic side view of a specific example of a plate microstructure;

[0146] Figure 5M is a schematic perspective view of the plate microstructure of Figure 5I;

[0147] Figure 5N is a schematic side view of an example of a pair of microstructures inserted into a subject for epidermal measurement;

[0148] Figure 5O is a schematic side view of an example of a pair of microstructures inserted into a subject for dermal measurement;

[0149] Figure 5P is a schematic perspective view of a first example of a patch including groups of microstructures acting as reference, counter and working electrodes;

[0150] Figure 5Q is a schematic perspective view of a first example of a patch including groups of pairs of microstructures acting as reference, counter and working electrodes;

[0151] Figure 5R is a schematic perspective view of a second example of a patch including groups of microstructures acting as reference, counter and working electrodes;

[0152] Figure 5S is a schematic perspective view of a second example of a patch including groups of pairs of microstructures acting as reference, counter and working electrodes;

[0153] Figure 5T is a schematic perspective view of a third example of a patch including groups of microstructures acting as reference, counter and working electrodes;

[0154] Figure 5U is a schematic perspective view of a third example of a patch including groups of pairs of microstructures acting as reference, counter and working electrodes;

[0155] Figure 5V is a schematic perspective view of a fourth example of a patch including groups of microstructures acting as reference, counter and working electrodes;

[0156] Figure 5W is a schematic perspective view of a fourth example of a patch including groups of pairs of microstructures acting as reference, counter and working electrodes;

[0157] Figure 6A is a schematic side view of a second example of a microstructure;

[0158] Figure 6B is a schematic front view of the microstructure of Figure 6A;

[0159] Figure 7A is a schematic diagram of a third example of a microstructure;

[0160] Figure 7B is a schematic diagram of a modified version of the microstructure of Figure 7A;

[0161] Figure 8A is a schematic side view of an example of a first step of a microstructure construction technique;

[0162] Figure 8B is a schematic side view of an example of a second step of a microstructure construction technique;

[0163] Figure 8C is a schematic side view of an example of a third step of a microstructure construction technique;

[0164] Figure 8D is a schematic side view of a first example of a microstructure configuration created using the construction technique of Figures 8A to 8C;

[0165] Figure 8E is a schematic side view of a second example of a microstructure configuration created using the construction technique of Figures 8A to 8C;

[0166] Figure 9 is a schematic diagram of an example of a distributed computer architecture;

[0167] Figure 10 is a schematic diagram of an example of a processing system;

[0168] Figure 11 is a schematic diagram of an example of a client device;

[0169] Figures 12A and 12B are a flow chart of an example of a process for performing a measurement on a biological subject;

[0170] Figure 13 is a flow chart of an example of a process for creating a subject record;

[0171] Figures 14A and 14B are a flow chart of a specific example of a process for performing measurements in a biological subject;

[0172] Figure 15A is a schematic perspective topside view of an example of a patch including a substrate incorporating microstructure electrodes and a substrate coil;

[0173] Figure 15B is a schematic diagram of an equivalent circuit representing the electrical response of the patch of Figure 15A;

[0174] Figure 15C is a graph illustrating the response to a drive signal for the patch of Figure 15A;

[0175] Figure 15D is a graph illustrating the resonance response of the patch of Figure 15A;

[0176] Figure 15E is a schematic perspective topside view of an example of a dual patch arrangement;

[0177] Figure 15F is a graph illustrating an example of drive signal attenuation for the dual patch configuration of Figure 15E;

[0178] Figure 15G is a schematic diagram illustrating an example of a drive and measurement circuit for performing measurements using working, reference and counter electrodes;

[0179] Figure 16A is an equivalent circuit for skin based impedance measurements;

[0180] Figure 16B is an equivalent circuit for epidermal based impedance measurements;

[0181] Figure 16C is a schematic diagram comparing skin and microstructure based impedance measurements;

[0182] Figures 17A to 17P are schematic diagrams illustrating steps in an example manufacturing process;

[0183] Figures 18A to 18D are micrograph images of examples of microstructures manufactured using the approach of Figures 17A to 17P;

[0184] Figures 18E to 18G are micrograph images of further example microstructures;

[0185] Figures 19A to 19L are schematic diagrams illustrating steps in an example manufacturing process;

[0186] Figures 20A and 20B are micrograph images of examples of microstructures manufactured using the approach of Figures 19A to 19L;

[0187] Figures 20C and 20D are micrograph images of further examples of microstructures manufactured using the approach of Figures 19A to 19L;

[0188] Figures 20E and 20F are micrograph images of further example microstructures;

[0189] Figures 21A and 21B are micrograph images of examples of partially coated microstructures;

[0190] Figures 21C and 21D are micrograph images of further examples of partially coated microstructures;

[0191] Figure 22A is a schematic diagram illustrating an example of an aptamer configuration;

[0192] Figure 22B is a schematic diagram illustrating an example of an aptamer configuration after interaction with an analyte;

[0193] Figure 23 is a graph illustrating changes in cyclic voltammetry readings following exposure of troponin I aptamer functionalised microstructures to troponin I;

[0194] Figure 24A is a graph illustrating changes in cyclic voltammetry readings following exposure of troponin I aptamer functionalised microstructures to various concentrations of troponin I;

[0195] Figure 24B is a graph illustrating changes in cyclic voltammetry readings following exposure of troponin I aptamer functionalised microstructures to a bovine serum albumin solution;

[0196] Figure 25A is an image of a microstructure patch application site on a human forearm skin immediately post-removal;

[0197] Figure 25B is a Scanning Electron Micrograph of a microstructure after application to human skin;

[0198] Figure 26A is a graph of example qualitative scores of erythema at microstructure patch application sites on human forearm skin from a first study;

[0199] Figure 26B is a graph of example qualitative scores of erythema at microstructure patch application sites on human forearm skin from a second study;

[0200] Figure 27A is a Scanning Electron Micrographs of microstructure prior to application into human forearm skin;

[0201] Figure 27B is a Scanning Electron Micrographs of the microstructure of Figure 27A post application into human forearm skin;

[0202] Figure 27C is a Scanning Electron Micrographs of a microstructure patch post application into human forearm skin;

[0203] Figure 27D is a Scanning Electron Micrographs of microstructure prior to application into human forearm skin;

[0204] Figure 27E is a Scanning Electron Micrographs of the microstructure of Figure 27D post application into human forearm skin;

[0205] Figure 27F is a Scanning Electron Micrographs of a microstructure patch post application into human forearm skin;

[0206] Figure 28A is a graph showing percentage changes in square wave voltammetry readings following exposure of a troponin I aptamer functionalised electrode to increasing concentrations of troponin I (Trop-I) and human serum albumin (HSA);

[0207] Figure 28B is a graph showing square wave voltammetry readings following exposure of a troponin I aptamer functionalised electrode to troponin I (Trop-I);

[0208] Figure 28C is a graph showing square wave voltammetry readings following exposure of a troponin I aptamer functionalised electrode to HSA;

[0209] Figure 29A is a graph showing in square wave voltammetry readings following exposure of a human IL-6 aptamer functionalised electrode to human IL-6;

[0210] Figure 29B is a graph showing percentage changes in square wave voltammetry readings following exposure of a human IL-6 aptamer functionalised electrode to increasing concentrations of human IL-6; and

[0211] Figure 30 is a graph showing percentage changes in square wave voltammetry readings following exposure of a human IL-6 aptamer functionalised electrode to increasing concentrations of human IL-6 (IL-6 response), and troponin I (non-specific response).

Detailed Description of the Preferred Embodiments

Definitions

[0212] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below.

[0213] The articles “a” and “an” are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

[0214] The terms “about” and "approximately" are used herein to refer to conditions (e.g. amounts, levels, concentrations, time, etc.) that vary by as much as 20% (i.e. ±20%), especially by as much as 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% to a specified condition.

[0215] As used herein, the term “analyte” refers to a naturally occurring and/or synthetic compound, which is a marker of a condition (e.g., drug abuse), disease state (e.g., infectious diseases), disorder (e.g., neurological disorders), or a normal or pathologic process that occurs in a subject (e.g., drug metabolism), or a compound which can be used to monitor levels of an administered or ingested substance in the subject, such as a medicament (substance that treats, prevents and/or alleviates the symptoms of a disease, disorder or condition, e.g., drug, vaccine etc.), an illicit substance (e.g. illicit drug), a non-illicit substance of abuse (e.g. alcohol or prescription drug taken for non-medical reasons), a poison or toxin (including an environmental contaminant), a chemical warfare agent (e.g. nerve agent, and the like) or a metabolite thereof. The term “analyte” can refer to any substance, including chemical and/or biological agents that can be measured in an analytical procedure, including nucleic acids, proteins, illicit drugs, explosives, toxins, pharmaceuticals, carcinogens, poisons, allergens, and infectious agents, which can be measured in an analytical procedure. The analyte may be a compound found directly in a sample such as biological tissue, including body fluids (e.g. interstitial fluid), from a subject, especially in the dermis and/or epidermis. In particular embodiments, the analyte is a compound found in the interstitial fluid. In some embodiments, the analyte is a compound with a molecular weight in the range of from about 30 Da to about 100 kDa, especially about 50 Da to about 40 kDa. Other suitable analytes are as described herein.

[0216] As used herein, the term "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (or).

[0217] As used herein, the term "aptamer" refers to a single-stranded oligonucleotide (e.g. DNA or RNA) that binds to a specific target molecule, such as an analyte. An aptamer may be

of any size suitable for binding such target molecule, such as from about 10 to about 200 nucleotides in length, especially from about 30 to about 100 nucleotides in length.

[0218] The term "bind" and variations such as "binding" are used herein to refer to an interaction between two substances, such as an analyte and an aptamer. The interaction may be a covalent or non-covalent interaction, particularly a non-covalent interaction.

[0219] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps. Thus, the use of the term "comprising" and the like indicates that the listed integers are required or mandatory, but that other integers are optional and may or may not be present. By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present. By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

[0220] The term "plurality" is used herein to refer to more than one, such as 2 to 1×10^{15} (or any integer therebetween) and upwards, including 2, 10, 100, 1000, 10000, 1×10^{6}, 1×10^{7}, 1×10^{8}, 1×10^{9}, 1×10^{10}, 1×10^{11}, 1×10^{12}, 1×10^{13}, 1×10^{14}, 1×10^{15}, etc. (and all integers therebetween).

[0221] As used herein, the term “predetermined threshold” refers to a value, above or below which indicates the presence, absence or progression of a disease, disorder or condition; the presence or absence of an illicit substance or non-illicit substance of abuse; or the presence or absence of a chemical warfare agent, poison and/or toxin. For example, for the purposes of the present invention, a predetermined threshold may represent the level or concentration of a particular analyte in a corresponding sample from an appropriate control subject, such as a

healthy subject, or in pooled samples from multiple control subjects or medians or averages of multiple control subjects. Thus, a level or concentration above or below the threshold indicates the presence, absence or progression of a disease, disorder or condition; the presence or absence of an illicit substance or non-illicit substance of abuse; or the presence or absence of a chemical warfare agent, poison and/or toxin, as taught herein. In other examples, a predetermined threshold may represent a value larger or smaller than the level or ratio determined for a control subject so as to incorporate a further degree of confidence that a level or ratio above or below the predetermined threshold is indicative of the presence, absence or progression of a disease, disorder or condition; the presence or absence of an illicit substance or non-illicit substance of abuse; or the presence or absence of a chemical warfare agent, poison and/or toxin. Those skilled in the art can readily determine an appropriate predetermined threshold based on analysis of samples from appropriate control subjects.

[0222] The terms "selective" and "selectivity" as used herein refer to aptamers that bind an analyte of interest without displaying substantial binding of one or more other analytes. Accordingly, an aptamer that is selective for an analyte, such as troponin or a subunit or complex thereof, exhibits selectivity of greater than about 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-fold or greater than about 500-fold with respect to binding of one or more other analytes.

[0223] The term “subject” as used herein refers to a vertebrate subject, particularly a mammalian subject, for whom monitoring and/or diagnosis of a disease, disorder or condition is desired. Suitable subjects include, but are not limited to, primates; avians (birds); livestock animals such as sheep, cows, horses, deer, donkeys and pigs; laboratory test animals such as rabbits, mice, rats, guinea pigs and hamsters; companion animals such as cats and dogs; bats; and captive wild animals such as foxes, deer and dingoes. In particular, the subject is a human.

System for Performing Measurements

[0224] An example of a system for performing measurements on a biological subject will now be described with reference to Figure 1.

[0225] In this example, the system includes at least one substrate 111 having one or more microstructures 112. In use, the microstructures are configured to breach a functional barrier

associated with a subject. In the current example, the functional barrier is the stratum corneum *SC,* and the microstructures are configured to breach the stratum corneum *SC* by penetrating the stratum corneum *SC* and entering at least the viable epidermis *VE*. In one particular example, the microstructures are configured to not penetrate a boundary between the viable epidermis *VE* and the dermis *D*, although this is not essential and structures that penetrate into the dermis could be used as will be described in more detail below.

[0226] Whilst this example is described with respect to breaching of the stratum corneum *SC*, it will be appreciated that this is not essential, and the techniques could equally be applied to other functional barriers. In this regard, a functional barrier will be understood to include any structure, boundary, or feature, whether physical or otherwise, that prevents passage of signals, and/or analytes, such as biomarkers. For example, functional barriers could include one or more layers, a mechanical discontinuity, such as a discrete change in tissue mechanical properties, a tissue discontinuity, a cellular discontinuity, a neural barrier, a sensor barrier, a cellular layer, skin layers, mucosal layers, internal or external barriers, an inner barrier within an organ, an outer barrier of organs other than the skin, epithelial layers or endothelial layers, or the like. Functional barriers could also include other internal layers or boundaries, including optical barriers such as a melanin layer, electrical barriers, molecular weight barriers that prevent passage of a biomarkers with certain molecular weights, a basal layer boundary between the viable epidermis and dermis, or the like.

[0227] The nature of the microstructure will vary depending upon the preferred implementation. In one example, the microstructures could include needles, but this is not essential and more typically structures, such as plates, blades, or the like, are used, as will be described in more detail below.

[0228] The substrate and microstructures could be manufactured from any suitable material, and the material used may depend on the intended application, for example depending on whether there is a requirement for the structures to be optically and/or electrically conductive, or the like. The substrate can form part of a patch 110, which can be applied to a subject, although other arrangements could be used for example, having the substrate form part of a housing containing other components.

[0229] In one example at least one sensor 121 is provided, which is operatively connected to at least one microstructure 112, thereby allowing response signals to be measured from respective microstructures 112. In this regard, the term response signal will be understood to encompass signals that are intrinsic within the subject, such ECG (Electrocardiograph) signals, or the like, or signals that are induced as a result of the application of stimulation, such as bioimpedance signals, or the like.

[0230] The nature of the sensor will vary depending on the preferred implementation and the nature of the sensing being performed. For example, the sensing could include sensing electrical signals, in which case the sensor could be a voltage or current sensor, or the like. Alternatively, optical signals could be sensed, in which case the sensor could be an optical sensor, such as a photodiode, CCD (Charge Coupled Device) array, or similar, whilst temperature signals could be sensed using a thermistor or the like.

[0231] The manner in which the sensor 121 is connected to the microstructure(s) 112 will also vary depending on the preferred implementation. In one example, this is achieved using connections between the microstructure(s) 112 and the sensor, with the nature of the connections varying depending upon the signals being sensed, so that the connections could include electrically conductive elements to conduct electrical signals, a wave guide, optical fibre or other conductor to conduct electromagnetic signals, or thermal conductor to conduct thermals signals. Connections could also include wireless connections, allowing the sensor to be located remotely. Ionic connections could also be used. Furthermore, connections could be provided as discrete elements, although in other examples, the substrate provides the connection, for example, if the substrate is made from a conductive plate which is then electrically connected to all of the microstructures. As a further alternative, the sensor could be embedded within or formed from part of the microstructure, in which connections may not be required.

[0232] The sensor 121 can be operatively connected to all of the microstructures 112, with connections being collective and/or independent. For example, one or more sensors could be connected to different microstructures to allow different measured response signals to be measured from different groups of microstructures 112, for example to define reference,

counter and one or more working electrodes, as will be described in more detail below. However, this is not essential, and any suitable arrangement could be used.

[0233] In addition to providing sensing, in some examples, the microstructures 112 could additionally and/or alternatively be configured to provide stimulation. For example, microstructures could be coupled to a signal generator that generates a stimulatory signal, as will be described in more detail below. Such stimulation could again include electrical stimulation, using a voltage or current source, optical stimulation, using a visible or non-visible radiation source, such as an LED or laser, thermal stimulation, or the like, and could be delivered via the same microstructures used for measuring response signals, or different microstructures, depending on the preferred implementation. Additionally and/or alternatively, stimulation could be achieved using other techniques, such as through exposure of the subject to the microstructures and materials thereon or therein. For example, coatings can be applied to the microstructures, allowing material to be delivered into the subject beyond the barrier, thereby stimulating a response within the subject.

[0234] These options allow a range of different types of sensing to be performed, including detecting electrical signals within the body, such as ECG signals, plethysmographic signals, electromagnetic signals, or electrical potentials generated by muscles, neural tissue, blood, or the like, detecting photoplethysmographic effects, electromagnetic effects, such as fluorescence, detecting mechanical properties, such as stress or strain, or the like. Sensing could include detecting the body's response to applied electrical signals, for example to measure bioimpedance, bioconductance, or biocapacitance, detecting the presence, absence, level or concentration of analytes, for example by detecting electrical or optical properties, or the like.

[0235] The system further includes one or more electronic processing devices 122, which can form part of a measuring device, and/or could include electronic processing devices forming part of one or more processing systems, such as computer systems, servers, client devices, or the like as will be described in more detail below. In use, the processing devices 122 are adapted to receive signals from the sensor 121 and either store or process the signals. For ease of illustration the remaining description will refer generally to a processing device, but it will be appreciated that multiple processing devices could be used, with processing

distributed between the devices as needed, and that reference to the singular encompasses the plural arrangement and *vice versa*.

[0236] An example of the manner in which this is performed will now be described with reference to Figure 2.

[0237] In particular, in this example, at step 200, the substrate is applied to the subject so that the one or more microstructures breach, and in one example, penetrate the functional barrier. For example, when applied to skin, the microstructures could penetrate the stratum corneum and enter the viable epidermis as shown in Figure 1. This could be achieved manually and/or through the use of an actuator, to help ensure successful penetration.

[0238] At step 210, response signals within the subject are measured, with signals indicative of the measured response signals being provided to the electronic processing device 121. This is typically performed following application of stimulation, although this is not essential and will vary depending on the nature of the sensing being performed.

[0239] The one or more processing devices then either analyse the resulting measurement data at step 220, and/or store the data based on the measurement data at step 230 for subsequent analysis, or could alternatively provide an output based on the measured response signals. For example, the processing device could display an indicator indicative of measured response signals and/or values derived therefrom. Alternatively, the processing device could generate a recommendation for an intervention, trigger an action, such as alerting a clinician, trainer or guardian, or the like.

[0240] The analysis can be performed in any suitable manner, and this will vary depending on nature of the measurements being performed. For example, this could involve examining measured response signal values and using these to calculate an indicator indicative of a health status, including the presence, absence, degree or prognosis of one or more medical conditions, a prognosis associated with a medical condition, a presence, absence, level or concentration of a biomarker, a presence, absence, level or concentration of an analyte, a presence, absence or grade of cancer, fluid levels in the subject, blood oxygenation, a tissue inflammation state, bioelectric activity, such as nerve, brain, muscle or heart activity, or a range of other health states. This could be achieved by monitoring changes in the values over time, and may involve

comparison to values measured for reference subjects having known medical conditions. Additionally, and/or alternatively, the indicator could be indicative of measured parameters associated with the subject, such as measured levels or concentrations of analytes or other biomarkers

[0241] In any event, it will be appreciated that the above described system operates by providing microstructures that are configured to breach a barrier, such as the stratum corneum, allowing these to be used to measure response signals within the subject, and in particular, within the epidermis and/or dermis. These response signals can then be processed and subsequently analysed, allowing a variety of values to be derived, which could be indicative of specific measurements, or one or more aspects of subject health.

[0242] For example, the system can be configured to measure an analyte level or concentration, such as the level or concentration of a specific biomarker. Response signals could also be used to generate a visualization, a spatial mapping in 1, 2 or 3 dimensions, details of mechanical properties, forces, pressures, muscle movement, blood pulse wave, an analyte concentration such as the presence, absence, level or concentration of specific biomarkers, a blood oxygen saturation, a bioimpedance, a biocapacitance, a bioconductance or electrical signals within the body, such as ECG (Electrocardiography) signals.

[0243] In one example, the system can be configured so that measurements are performed at a specific location within the subject, such as within the epidermis only, the dermis only, or the like. This allows targeted analyte detection to be performed with a high level of accuracy, providing higher quality data for more precise measures of analytes. Furthermore, constraining the location in which measurements are performed ensures these are repeatable, allowing for more accurate longitudinal monitoring.

[0244] In contrast to traditional approaches, breaching and/or at least partially penetrating a functional barrier, such as the stratum corneum, allows measurements to be performed from within or under the barrier, and in particular within the epidermis and/or dermis, resulting in a significant improvement in the quality and magnitude of response signals that are detected. In particular, this ensures that the response signals accurately reflect conditions within the human body, and in particular within the epidermis and/or dermis, such as the presence, absence, level

or concentration of biomarkers, the impedance of interstitial fluid, or the like, as opposed to traditional external measurements, which are unduly influenced by the environment outside the barrier, such as the physical properties of the skin surface, such as the skin material properties, presence or absence of hair, sweat, mechanical movement of the applied sensor, or the like.

[0245] For example, this allows accurate measurement of high molecular weight biomarkers to be performed, which would otherwise only pass through the skin poorly. A good example of this, is glucose, which whilst present externally, such as in sweat, is typically only present in low concentrations, and often time delayed, meaning the concentration in sweat does not necessarily reflect current glucose levels within the body. In contrast, by breaching the barrier, in this case the stratum corneum, this allows far more accurate measurements to be performed. It will be appreciated that similar considerations apply to a wide range of different biomarkers or signals, and associated barriers that otherwise prevent accurate measurement of the biomarkers or signals.

[0246] For example, in the case of impedance measurements microstructure electrodes tend to measure different impedances as opposed to standard surface electrodes, which is indicative of the fact that the microstructure electrodes do not measure skin impedance, meaning the measured impedance is more indicative of conditions within the body. As the contribution of the skin surface impedance is significant in magnitude this can result in changes in impedance within the body being masked, meaning skin based measurements are less likely to be able to detect meaningful changes.

[0247] A further issue with skin based impedance measurements is that fields generated tend to pass through the stratum corneum and dermis, and are not constrained to the epidermis. An example of this is shown in Figure 16C.

[0248] In this example, skin based electrodes 1601, result in an electric field 1602 extending into the stratum corneum SC, the viable epidermis VEPiD and dermis D. In contrast, a microstructure patch 1603 result in fields 1604 constrained within the viable epidermis VEPiD.

[0249] An example of resulting equivalent circuits for skin based measurements and epidermal measurements are shown in Figures 16A and 16B, respectively. In this regard, each

equivalent circuit includes three circuits for each layer, representing a contribution of current flow through the tissue in orthogonal directions. Thus, for skin based measurements shown in Figure 16A, the impedance of the stratum corneum is represented by the circuits C_{SC1}, R_{SC1}, C_{SC2}, R_{SC2}, C_{SC3}, R_{SC3}, the epidermis is represented by the circuits C_{VE1}, R_{VE1}, C_{VE2}, R_{VE2}, C_{VE3}, R_{VE3}, and the dermis is represented by the circuits C_{D1}, R_{D1}, C_{D2}, R_{D2}, C_{D3}, R_{D3}. In this example, $R_{SC1}>>R_{VE1}$, $R_{SC2}>>R_{VE2}$ and $R_{SC3}>>R_{VE3}$, meaning that the contribution of the impedance in the epidermal layer is minimal compared to the contribution of the impedance in the stratum corneum, so skin based measurements will be more reflective of the impedance in the stratum corneum.

[0250] In contrast, for epidermal sensing only, shown in Figure 16B, the impedance is represented by the circuits C_{VE1}, R_{VE1}, C_{VE2}, R_{VE2}, C_{VE3}, R_{VE3}, only, and hence epidermal measurements are more reflective of the fluid levels in the epidermis.

[0251] Additionally, in some examples, the microstructures only penetrate the barrier a sufficient distance to allow a measurement to be made. For example, in the case of skin, the microstructures are typically configured to enter the viable epidermis and not enter the dermal layer. This results in a number of improvements over other invasive techniques, including avoiding issues associated with penetration of the dermis, such as pain caused by exposure of nerves, erythema, petechiae, or the like. Avoiding penetrating the dermal boundary also significantly reduces the risk of infection, allowing the microstructures to remain embedded for prolonged periods of time, such as several days, which in turn can be used to perform longitudinal monitoring over a prolonged time periods. However, in some instances, such as when detecting troponin or a subunit or complex thereof, penetration of the dermal barrier may be required.

[0252] It will be appreciated that the ability of the microstructures to remain in-situ is particularly beneficial, as this ensures that measurements are made at the same site within the subject, which reduces inherent variability arising from inaccuracies of replacement of measuring equipment which can arise using traditional techniques. Despite this, it will be appreciated that the system can be used in other manners, for example to perform single time point monitoring or the like.

[0253] In one example, this allows the arrangement to be provided as part of a wearable device, enabling measurements to be performed that are significantly better than existing surface based measurement techniques, for example by providing access to signals or biomarkers that cannot otherwise pass through the barrier, but whilst allowing measurements to be performed whilst the subject is undergoing normal activities and/or over a prolonged period of time. This in turn enables measurements to be captured that are more accurately reflective of the health or other status of the subject. For example, this allows variations in a subject's condition during a course of the day to be measured, and avoids measurements being made under artificial conditions, such as within a clinic, which are not typically indicative of the actual condition of the subject. This also allows monitoring to be performed substantially continuously, which can allow conditions to be detected as they arise, for example, in the case of myocardial infarction, cardiovascular disease, vomiting, diarrhoea, or similar, which can allow more rapid intervention to be sought.

[0254] The above described system can be applied to any part of the body, and hence could be used with a wide range of different functional barriers. For example, the functional barrier could be an internal or external barrier, a skin layer, a mucosal layer, an inner barrier within an organ, an outer barrier of an organ, an epithelial layer, an endothelial layer, a melanin layer, an optical barrier, an electrical barrier, molecular weight barrier, basal layer or the stratum corneum. Thus, the microstructures could be applied to the buccal mucosa, the eye, or another epithelial layer, endothelial layer, or the like. The following examples will focus specifically on application to the skin, with the functional barrier including some or all of the stratum corneum, but it will be appreciated that this is intended to be illustrative and is not intended to be limiting.

[0255] Further variations will become apparent from the following description.

[0256] In one example, the system includes a signal generator operatively connected to at least one microstructure to apply stimulation, typically by applying a stimulatory signal to the microstructure. Again, the manner in which the signal generator is connected will vary depending on the preferred implementation, and this could be achieved via connections, such as wired or wireless connections and/or integrating the signal generator into the substrate and/or

microstructures. Example connection types include mechanical, magnetic, thermal, electrical, electromagnetic, optical, or the like.

[0257] The nature of the stimulatory signal and the manner in which this is applied will vary depending upon the preferred implementation and this could include any one or more of biochemical, chemical, mechanical, magnetic, electromagnetic, electrical, optical, thermal, or other signals. The stimulatory signal could be used to allow the response signal to be measured and/or could be used to trigger a biological response, which is then measured. For example this can be used to cause electroporation, to induce local mediators of inflammation, which can in turn release biomarkers, allowing levels or concentrations of these to be measured. In this regard, electroporation, or electropermeabilization, involves applying an electrical field to cells in order to increase the permeability of the cell membrane, allowing chemicals, drugs, or DNA to be introduced into the cell. In another example, stimulation can be used to disrupt a boundary within the subject, for example disrupting a dermal boundary allowing biomarkers within the dermal layer to be detected in the viable epidermis, without requiring penetration of the dermal layer by the microstructures. In a further example, stimulation can be used to trigger additional effects. So for example, an electrical or mechanical signal could be used to disrupt a coating on the microstructures, causing material to be released, which can in turn a chemical or other stimulation.

[0258] Stimulatory signals could also be applied to the microstructures to alter the microstructure form or function. For example, polymer microstructures could be induced to grow or shrink along their length or width with an applied electric field or temperature, whilst microstructures could be configured to move between a retracted flat position and an extended upright position, in order to penetrate and then retract from the skin or other barrier.

[0259] In one example, operation of the signal generator is controlled by the processing device, allowing the processing device to control the signal generator to thereby cause a measurement to be performed, for example by applying an electrical signal to allow an impedance measurement to be performed. Additionally, and/or alternative the processing device could control the signal generator in accordance with measured response signals, for example, allowing stimulation to be applied to the subject and/or microstructures once certain criteria are met. For example, in theranostic applications, a signal applied to microstructures

can be used to release therapeutic materials. In this example, the processing device can monitor response signals and use these to assess when an intervention is required, and then control the signal generator to trigger the release. In one example, such control could be performed in accordance with a dosing regime, for example specifying a dose and timing of delivery of the dose, once it has been determined that therapy is required. In this example, the dosing regimen could be predetermined and stored onboard or could be manually input by a clinician or other individual, as needed.

[0260] As mentioned above, the signal generator and/or sensor can be connected to the microstructures via connections. The nature of the connections will vary depending on the preferred implementation and the nature of the signal. For example, if the signal is an optical or other electromagnetic signal, a waveguide, fibre optic cable, or other electromagnetic conductor can be used. In the case of electrical signals, the connections can be conductive connections, such as wires, or conductive tracks on a substrate, or could be formed by a conductive substrate. Connections could also include wireless connections, such as short-range radio frequency wireless connections, inductive connections, or the like. Connections could also be mechanical, magnetic, thermal, or the like.

[0261] In one example, inductive connections can be used to transmit signals and power, so that for example, inductive coupling could be used to power electronic circuits mounted on the substrate. This could be used to allow basic processing to be performed on board the substrate, such as amplifying and process impedance changes, using a simple integrated circuit or similar, without requiring an in-built power supply on the substrate.

[0262] In one example, the system can include response microstructures used to measure response signals and/or stimulation microstructures used to apply stimulation signals to the subject. Thus, stimulation and response could be measured via different microstructures, in which case the substrate typically incorporates response connections for allowing response signals to be measured and stimulation connections allowing stimulation signals to be applied. In some examples, multiple stimulation and response connections are provided, allowing different measurements to be performed via different connections. For example, different types of measurements could be performed via different microstructures or different parts of given microstructures, to enable multi-modal sensing. Additionally and/or alternatively, the

same type of measurements could be performed at different locations and/or depths, for example to identify localised issues, such as the presence of skin cancers or similar. In other cases, stimulation and measurement could be performed via the same connections, for example when making bipolar impedance measurements.

[0263] Signals could be applied to or measured from individual microstructures and/or to different parts of microstructures, which can be useful to discern features at different locations and/or depths within the body. This can be used for example to perform mapping or tomography, for example to produce images wherein the image contrast or colour is proportional to the levels or concentrations of one or more analytes or the change in a physical property such as bioimpedance. Additionally, and/or alternatively, signals could be applied to or measured from multiple microstructures collectively, which can be used to improve signal quality, or perform measurements, such as bipolar, tetra-polar, or other multi-polar impedance measurements. Additionally and/or alternatively, microstructures might be used for both measuring and stimulation, for example applying a signal to a microstructure and then subsequently measuring a response therefrom.

[0264] In one particular example, sensors and/or signal generators can be connected to microstructures via one or more switching devices, such as multiplexers, allowing signals to be selectively communicated between the sensor or signal generator and different microstructures. The processing device is typically configured to control the switches, allowing a variety of different sensing and stimulation to be achieved under control of the processing device. In one example, this allows at least some electrodes can be used independently of at least some other electrodes. This ability to selectively interrogate different electrodes can provide benefits.

[0265] For example, this allows different electrodes to have different functionality, for example by having different electrodes functionalized with different coatings, and then interrogated or stimulated as needed, so that different measurements can be performed as required. Additionally, and/or alternatively, this allows different measurements to be performed via different microstructures, for example to perform spatial discrimination and hence mapping. For example, interrogating electrodes at different locations on a patch, enables a map of measurements at different locations to be constructed, which can in turn be used to

localise an effect, so as the presence of analytes or specific objects, such as lesions or cancer. Furthermore, this allows stimulation to be delivered to different microstructures. For example, in theranostic embodiments, different therapeutic materials or doses could be associated with different microstructures, so selectively stimulating different microstructures allows a range of different interventions to be performed. In some example, different microstructures could be used for different purposes, so that some microstructures are used for sensing, whilst others are used for delivering stimulation and/or therapy.

[0266] In another example, as described in more detail below, when electrodes are provided as pairs, this allows some pairs of electrodes to be used independently of other pairs. In one particular example, electrodes and/or pairs of electrodes, can be arranged in rows, and this can allows measurements to be performed on a row by row basis, although this is not essential and other groupings could be used.

[0267] The nature of the substrate and/or microstructures will vary depending upon the preferred implementation. For example, substrate and/or microstructures could be made from or contain fabric, woven fabric, electronic fabric, natural fibres, silk, organic materials, natural composite materials, artificial composite materials, ceramics, stainless steel, ceramics, metals, such as stainless steel, titanium or platinum, polymers, such as rigid or semi-rigid plastics, including doped polymers, silicon or other semiconductors, including doped semiconductors, organosilicates, gold, silver, carbon, carbon nano materials, or the like. The substrate and microstructures could be made from similar and/or dissimilar materials, and could be integrally formed, or made separately and bonded together. Microstructures can also be provided on one or more substrates, so for example, signals could be measured or applied between microstructures on separate substrates.

[0268] It will be appreciated that the particular material used will depend on the intended application, so for example different materials will be used if the microstructure needs to be conductive as opposed to insulative. Insulating materials, such as polymers and plastics could be doped so as to provide required conductivity, for example via doping with micro or nano sized metal particles, or conductive composite polymers could be used such as PEDOT:PSS (poly(3,4-ethylenedioxythiophene)polystyrene). If doping is used, this could involve using graphite or graphite derivates, including 2D materials such as graphene and carbon nanotubes,

with these materials also being useable as stand-alone materials or as dopants in blends with polymers or plastics.

[0269] The substrate and microstructures can be manufactured using any suitable technique. For example, in the case of silicon-based structures, this could be performed using etching techniques. Polymer or plastic structures could be manufactured using additive manufacturing, such as 3D printing, or moulding. In one particular example, a mould is filled with a suitable filling material, such as a solution containing a material such as an active compound and/or sugar-based excipient, such as carboxy-methylcellulose (CMC), or one or more polymers, or the like, which is then cured and removed. It will also be appreciated that the filling material may include any required probes, reagents, or the like that are to be contained within the structures, as will be discussed in more detail below. Photosensitive polymers might be used, such as photoresists, including SU8 or polyimides, for direct patterning of electrodes on the substrate or to make microstructures. Successive layers of photosensitive resists, polymers, metals, or the like, can be deposited and/or selectively removed to produce bespoke 3D microstructure geometries.

[0270] In one example, the substrate could be at least partially flexible in order to allow the substrate to conform to the shape of a subject and thereby ensure penetration of the microstructures into the viable epidermis and/or dermis, or other functional barrier. In this example, the substrate could potentially be a textile or fabric, with electrodes and circuitry woven in, or multiple substrates could be mounted on a flexible backing, to provide a segmented substrate arrangement. Alternatively, the substrate could be shaped to conform to a shape of the subject, so that the substrate is rigid but nevertheless ensures penetration of the microstructures.

[0271] In preferred examples, the substrate and microstructures are formed from one or more of metal, polymer or silicon.

[0272] The microstructures could have a range of different shapes and could include ridges, needles, plates, blades, or similar. In this regard, the terms plates and blades are used interchangeably to refer to microstructures having a width that is of a similar order of magnitude in size to the length, but which are significantly thinner. The microstructures can

be tapered to facilitate insertion into the subject, and can have different cross-sectional shapes, for example depending on the intended use. The microstructures typically have a rounded rectangular shape and may include shape changes along a length of the microstructure. For example, microstructures could include a shoulder that is configured to abut against the stratum corneum to control a depth of penetration and/or a shaft extending to the tip, with the shaft being configured to control a position of the tip in the subject and/or provide a surface for an electrode.

[0273] Other example shapes include circular, rectangular, cruciform shapes, square, rounded square, rounded rectangular, ellipsoidal, or the like, which can allow for increased surface area, which is useful when coating microstructures to maximise the coating volume and hence the amount of payload delivered per microstructure, although it will be appreciated that a range of other shapes could be used.

[0274] Microstructures can have a rough or smooth surface, or may include surface features, such as pores, raised portions, serrations, or the like, which can increase surface area and/or assist in penetrating or engaging tissue, to thereby anchor the microstructures within the subject. This can also assist in reducing biofouling, for example by prohibiting the adherence and hence build-up of biofilms. The microstructures might also be hollow or porous and can include an internal structure, such as holes or similar, in which case the cross sectional shape could also be at least partially hollow. In particular embodiments, the microstructures are porous, which may increase the effective surface area of the microstructure. The pores may be of any suitable size to allow an analyte of interest to enter the pores, but exclude one or more other analytes or substances, and thus, will depend on the size of the analyte of interest. In some embodiments, the pores may be less than about 10 μm in diameter, preferably less than about 1 μm in diameter.

[0275] In one example, the microstructures have a rounded rectangular shape when viewed in cross section through a plane extending laterally through the microstructures and parallel to but offset from the substrate. The microstructures may include shape changes along a length of the microstructure. For example, microstructures could include a shoulder that is configured to abut against the stratum corneum to control a depth of penetration and/or a shaft extending

to the tip, with the shaft being configured to control a position of the tip in the subject and/or provide a surface for an electrode.

[0276] Different microstructures could be provided on a common substrate, for example providing different shapes of microstructure to achieve different functions. In one example, this could include performing different types of measurement. In other examples, microstructures could be provided on different substrates, for example, allowing sensing to be performed via microstructures on one patch and delivery of therapy to be performed via microstructures on a different patch. In this example, this could allow a therapy patch to be replaced once exhausted, whilst a sensing patch could remain in situ. Additionally, measurements could be performed between patches, for example, performing whole of body impedance measurements between patches provided at different locations on a subject.

[0277] Additionally and/or alternatively anchor microstructures could be provided, which can be used to anchor the substrate to the subject. In this regard, anchor microstructures would typically have a greater length than that of the microstructures, which can help retain the substrate in position on the subject and ensure that the substrate does not move during the measurements or is not being inadvertently removed. Anchor microstructures can include anchoring structures, such as raised portions, which can assist with engaging the tissue, and these could be formed by a shape of the microstructure and/or a shape of a coating. Additionally, the coating could include a hydrogel or other similar material, which expands upon expose to moisture within the subject, thereby further facilitating engagement with the subject. Similarly the microstructure could undergo a shape change, such as swelling either in response to exposure to substances, such as water or moisture within the subject, or in response to an applied stimulation. When applied to skin, the anchor microstructures can enter the dermis, and hence are longer than other microstructures, to help retain the substrate in place, although it will be appreciated that this is not essential and will depend upon the preferred implementation. In other examples the anchor microstructures are rougher than other microstructures, have a higher surface friction than other microstructures, are blunter than other microstructures or are fatter than other microstructures.

[0278] In a further example, at least part of the substrate could be coated with an adhesive coating in order to allow the substrate and hence patch, to adhere to the subject.

[0279] As previously mentioned, when applied to skin, the microstructures typically enter the viable epidermis and in one example, do not enter the dermis, although in other examples, may enter the dermis. But this is not essential, and for some applications, it may be necessary for the microstructures to enter the dermis, for example projecting shortly through the viable epidermis/dermis boundary or entering into the dermis a significant distance, largely depending on the nature of the sensing being performed. In one example, for skin, the microstructures have a length that is at least one of less than 2500 μm, less than 1000 μm, less than 750 μm, less than 600 μm, less than 500 μm, less than 400 μm, less than 300 μm, less than 250 μm, greater than 100 μm, greater than 50 μm and greater than 10 μm, but it will be appreciated that other lengths could be used. More generally, when applied to a functional barrier, the microstructures typically have a length greater than the thickness of the functional barrier, at least 10% greater than the thickness of the functional barrier, at least 20% greater than the thickness of the functional barrier, at least 50% greater than the thickness of the functional barrier, at least 75% greater than the thickness of the functional barrier and at least 100% greater than the thickness of the functional barrier.

[0280] In another example, the microstructures have a length that is no more than 2000% greater than the thickness of the functional barrier, no more than 1000% greater than the thickness of the functional barrier, no more than 500% greater than the thickness of the functional barrier, no more than 100% greater than the thickness of the functional barrier, no more than 75% greater than the thickness of the functional barrier or no more than 50% greater than the thickness of the functional barrier. This can avoid deep penetration of underlying layers within the body, which can in turn be undesirable, and it will be appreciated that the length of the microstructures used will vary depending on the intended use, and in particular the nature of the barrier to be breached, and/or signals to be applied or measured. The length of the microstructures can also be uneven, for example, allowing a blade to be taller at one end than another, which can facilitate penetration of the subject or functional barrier.

[0281] Similarly, the microstructures can have different widths depending on the preferred implementation. Typically, the widths are at least one of less than 25% of the length, less than 20% of the length, less than 15% of the length, less than 10% of the length, or less than 5% of the length. Thus, for example, when applied to the skin, the microstructures could have a width

of less than 50 μm, less than 40 μm, less than 30 μm, less than 20 μm or less than 10 μm. However, alternatively, the microstructures could include blades, and could be wider than the length of the microstructures. In some example, the microstructures could have a width of less than 50000 μm, less than 40000 μm, less than 30000 μm, less than 20000 μm, less than 10000 μm, less than 5000 μm, less than 2500 μm, less than 1000 μm, less than 500 μm or less than 100 μm. In blade examples, it is also feasible to use microstructures having a width substantially up to the width of the substrate.

[0282] In general the thickness of the microstructures is significantly lower in order to facilitate penetration and is typically less than 1000 μm, less than 500 μm, less than 200 μm, less than 100 μm, less than 50 μm, less than 20 μm, less than 10 μm, at least 1 μm, at least 0.5 μm or at least 0.1 μm. In general the thickness of the microstructure is governed by mechanical requirements, and in particular the need to ensure the microstructure does not break, fracture or deform upon penetration. However, this issue can be mitigated through the use of a coating that adds additional mechanical strength to the microstructures.

[0283] In one specific example, for epidermal sensing, the microstructures have a length that is less than 300 μm, greater than 50 μm, greater than 100 μm and about 150 μm, and, a width that is greater than or about equal to a length of the microstructure, and is typically less than 300 μm, greater than 50 μm and about 150 μm. In another example, for dermal sensing, the microstructures have a length that is less than 450 μm, greater than 100 μm, and about 250 μm, and, a width that is greater than or about equal to a length of the microstructure, and at least of a similar order of magnitude to the length, and is typically less than 450 μm, greater than 100 μm, and about 250 μm. In other examples, longer microstructures could be used, so for example for hyperdermal sensing, the microstructures would be of a greater length. The microstructures typically have a thickness that is less than the width, significantly less than the width and of an order of magnitude smaller than the width. In one example, the thickness is less than 50 μm, greater than 10 μm, and about 25 μm, whilst the microstructure typically includes a flared base for additional strength, and hence includes a base thickness proximate the substrate that is about three times the thickness, and typically is less than 150 μm, greater than 30 μm and about 75 μm. The microstructures typically have a tip has a length that is less than 50% of a length of the microstructure, at least 10% of a length of the microstructure and

more typically about 30% of a length of the microstructure. The tip further has a sharpness that is at least 0.1 μm, less than 5 μm and typically about 1 μm.

[0284] In one example, the microstructures have a relatively low density, such as less than 10000 per cm^2, such as less than 1000 per cm^2, less than 500 per cm^2, less than 100 per cm^2, less than 10 per cm^2 or even less than 5 per cm^2. The use of a relatively low density facilitates penetration of the microstructures through the stratum corneum and in particular avoids the issues associated with penetration of the skin by high density arrays, which in turn can lead to the need for high powered actuators in order for the arrays to be correctly applied. However, this is not essential, and higher density microstructure arrangements could be used, including less than 50,000 microstructures per cm^2, less than 30,000 microstructures per cm^2, or the like. As a result, the microstructures typically have a spacing that is less than 20 mm, less than 10 mm, less than 1 mm, less than 0.1 mm or less than 10 μm. It should be noted that in some circumstances, microstructures are arranged in pairs, with the microstructures in each pair having a small spacing, such as less than 10 μm, whilst the pairs have a great spacing, such as more than 1 mm, in order to ensure a low overall density is maintained. However, it will be appreciated that this is not essential, and higher densities could be used in some circumstances.

[0285] In one specific example, the microstructures have a density that is less than 5000 per cm^2, greater than 100 per cm^2, and about 600 per cm^2, leading to a spacing of less than 1 mm, more than 10 μm, and about 0.5 mm, 0.2 mm or 0.1 mm.

[0286] In one example, when optical sensing is performed, the connections in the substrate include waveguides, or other electromagnetically conductive paths, such as optical fibres, which extend through the microstructures to one or more ports in the microstructure, to allow electromagnetic radiation to be emitted from or received via the ports. In one example, this is achieved by having the microstructure made from, or contain, polymer, or another similar material, which is at least partially transparent to the frequency of electromagnetic radiation being applied or received, which could include visible radiation, ultra-violet radiation, infra-red radiation, or the like, depending on the preferred application.

[0287] In one example, an at least partially electromagnetically transparent core can be surrounded by an outer electromagnetically opaque layer, with ports extending through the

opaque layer, to allow electromagnetic radiation to be emitted or received via the ports. In this example, it will be appreciated that appropriate positioning of the ports, allows radiation to be delivered or received in a targeted manner, for example allowing this to be directed into a particular depth within the viable epidermis, or elsewhere. In one example, the transparent core could be made from a waveguide, such as a fibre optic cable, or part thereof. For example, the outer layer and/or reflective layer could be removed, allowing the transparent core of the microstructure to be made of the fibre optic core. In a further example, the microstructures include electromagnetically reflective layers to allow electromagnetic radiation to be conducted to and from designated ports.

[0288] Similar arrangements could be provided for electrical signalling, with the microstructures including an electrically conductive core material and optionally including an electrically insulating layer including ports to allow electrical signals to be emitted from or received by the ports, again with ports optionally being at different depths, to allow electrical signals to be measured at different locations and/or depths.

[0289] Thus, the microstructure could include an electrically conductive material covered by a non-conductive (insulating) layer, with openings providing access to the conductive material to allow conduction of electrical signals through the openings to thereby define electrodes. In one example, the insulating layer extends over part of a surface of the microstructure, including a proximal end of the microstructure adjacent the substrate. The insulating layer could extend over at least half of a length of the microstructure and/or about 60 μm, 90 μm or 150 μm of a proximal end of the microstructure, and optionally, at least part of a tip portion of the microstructure. In one specific example, this is performed so the non-insulating portion is provided in the epidermis and/or dermis, so stimulatory signals are applied to and/or response signals received from, the epidermis and/or dermis. The insulating layer could also extend over some or all of a surface of the substrate. In this regard, in some examples connections are formed on a surface of the substrate, in which case a coating could be used to isolate these from the subject. For example, electrical tracks on a surface of the substrate could be used to provide electrical connections to the electrodes, with an insulating layer being provided on top of the connections to ensure the connections do not make electrical contact with the skin of the subject, which could in turn adversely affect measured response signals.

[0290] In another example, at least some of microstructures include an electrode. The microstructures could be made from a metal or other conductive material, so that the entire microstructure constitutes the electrode, or alternatively the electrode could be coated or deposited onto the microstructure, for example by depositing a layer of gold to form the electrode. In a further example, the microstructure could include an electrically conductive core covered by a non-conductive layer, with openings providing access to the core to allow conduction of electrical signals through the openings. The electrode material could include any one or more of gold, silver, colloidal silver, colloidal gold, colloidal carbon, carbon nano materials, platinum, titanium, stainless steel, or other metals, or any other biocompatible conductive material.

[0291] The electrodes could be used to apply electrical signals to a subject, measure intrinsic or extrinsic response electrical signals, for example measuring ECG or impedances. In another example, the one or more microstructure electrodes interact with one or more analytes of interest such that a response signal is dependent on a presence, absence, level or concentration of one or more analytes of interest, thereby allowing the level or concentration of one or more analytes to be quantified.

[0292] In one example, the microstructures include plates having a substantially planar face having an electrode thereon. The use of a plate shape maximizes the surface area of the electrode, whilst minimizing the cross sectional area of the microstructure, to thereby assist with penetration of the microstructure into the subject. This also allows the electrode to act as a capacitive plate, allowing capacitive sensing to be performed. In one example, the electrodes have a surface area of at least at least 10 mm^2, at least 1 mm^2, at least 100,000 μm^2, 10,000 μm^2, at least 7,500 μm^2, at least 5,000 μm^2, at least 2,000 μm^2, at least 1,000 μm^2, at least 500 μm^2, at least 100 μm^2, or at least 10 μm^2. In one example, the electrodes have a width or height that is up to 2500 μm, at least 500 μm, at least 200 μm, at least 100 μm, at least 75 μm, at least 50 μm, at least 20 μm, at least 10 μm or at least 1 μm. In the case of electrodes provided on blades, the electrode width could be less than 50000 μm, less than 40000 μm, less than 30000 μm, less than 20000 μm, less than 10000 μm, or less than 1000 μm, as well as including widths outlined previously. In this regard, it will be noted that these dimensions apply to individual electrodes, and in some examples each microstructure might include multiple electrodes.

[0293] In one specific example, the electrodes have a surface area of less than 200,000 μm^2, at least 2,000 μm^2 and about 22,500 μm^2 , with the electrodes extending over a length of a distal portion of the microstructure, optionally spaced from the tip, and optionally positioned proximate a distal end of the microstructure, again proximate the tip of the microstructure. The electrode can extend over at least 25% and less than 50% of a length of the microstructure, so that the electrode typically extends over about 60μm, 90 μm or 150 μm of the microstructure and hence is positioned in a viable epidermis and/or dermis of the subject in use.

[0294] In one example, at least some of the microstructures are arranged in groups, such as pairs, with response signals or stimulation being measured from or applied to the microstructures within the group. The microstructures within the group can have a specific configuration to allow particular measurements to be performed. For example, when arranged in pairs, a separation distance can be used to influence the nature of measurements performed. For example, when performing bioimpedance measurements, if the separation between the microstructures is greater than a few millimetres, this will tend the measure properties of interstitial fluid located between the electrodes, whereas if the distance between the microstructures is reduced, measurements will be more influenced by surface properties, such as the presence of materials bound to the surface of the microstructures. Measurements are also influenced by the nature of the applied stimulation, so that for example, current at low frequencies will tend to flow though extra-cellular fluids, whereas current at higher frequencies is more influenced by intra-cellular fluids.

[0295] In one particular example, plate microstructures are provided in pairs, with each pair including spaced apart plate microstructures having substantially planar electrodes in opposition. This can be used to generate a highly uniform field in the subject in a region between the electrodes, and/or to perform capacitive or conductivity sensing of substances between the electrodes. However, this is not essential, and other configurations, such as circumferentially spacing a plurality of electrodes around a central electrode, can be used. Typically the spacing between the electrodes in each group is typically less than 50 mm; less than 20 mm, less than 10 mm, less than 1 mm, less than 0.1 mm or less than 10 μm, although it will be appreciated that greater spacings could be used, including spacing up to dimensions of the substrate and/or greater, if microstructures are distributed across multiple substrates.

[0296] Thus, in one specific example, at least some of the microstructures are arranged in pairs, with response signals being measured between microstructures in the pair and/or stimulation being applied between microstructures in the pair. Each pair of microstructures typically includes spaced apart plate microstructures having substantially planar electrodes in opposition and/or spaced apart substantially parallel plate microstructures. This arrangement allows each pair to function as a separate sensor, and through the use of suitable connections to the signal generator and/or sensors, can be used to perform independent sensing via each pair.

[0297] However, this is not essential, and alternatively, response signals can be measured between microstructures in different groups and/or stimulation can be applied between microstructures in different groups. In this example, each group can include multiple microstructures, or multiple pairs of microstructures. For example, each group could include multiple spaced apart plate microstructures having substantially planar electrodes or could include multiple pairs of microstructures including spaced apart plate microstructures having substantially planar electrodes in opposition.

[0298] Furthermore, microstructures or pairs of microstructures within each group can be electrically connected, so that each group functions collectively as a single electrode. In this example, a number of different groups, such as two groups, three groups, or more than three groups can be provided depending on the type of measurement being performed. For example, the groups can include a counter group including a plurality of counter microstructures defining a counter electrode, a reference group including a plurality of reference microstructures defining a reference electrode and one or more working groups, each working group including a plurality of working microstructures defining a respective working electrode. This allows measurements, such as cyclic voltammetry measurements to be performed.

[0299] In general, where reference, counter and working groups are provided, the reference group is smaller than the working and counter groups, or includes fewer microstructures than the working and counter groups, and can be positioned adjacent each working groups.

[0300] In these examples, the groups can be provided on a common substrate, although this is not essential, and one or more groups could alternatively be provided on different substrates.

[0301] In one example, at least some microstructures or pairs of microstructures are angularly offset, and in one particular example, are orthogonally arranged. Thus, in the case of plate microstructures, at least some pairs of microstructures extend in different and optionally orthogonal directions. In this regard, it will be understood that the terms angularly offset and orthogonal refer to an orientation of plate like microstructures about an axis extending perpendicularly from the substrate, and that in general, each microstructure extends perpendicularly from the substrate. This distributes stresses associated with insertion of the patch in different directions, and also acts to reduce sideways slippage of the patch by ensuring plates at least partially face a direction of any lateral force. Reducing slippage either during or post insertion helps reduce discomfort, erythema, or the like, and can assist in making the patch comfortable to wear for prolonged periods. Additionally, this can also help to account for any electrical anisotropy within the tissue, for example as a result of fibrin structures within the skin, cellular anisotropy, or the like.

[0302] In one specific example, adjacent microstructures or pairs of microstructures are angularly offset, and/or orthogonally arranged, and additionally and/or alternatively, microstructures or pairs of microstructures can be arranged in rows, with the microstructures or pairs of microstructures in one row being orthogonally arranged or angularly offset relative to microstructures or pairs of microstructures in other rows.

[0303] In one specific example, when pairs of microstructures are used, a spacing between the microstructures in each pair is typically less than 0.25 mm, more than 10 μm and about 0.1 mm, whilst a spacing between groups of microstructures is typically less than 1 mm, more than 0.2 mm and about 0.5 mm. Such an arrangement helps ensure electrical signals are primarily applied and measured within a pair and reduces cross talk between pairs, allowing independent measurements to be recorded for each pair of microstructures / electrodes.

[0304] The microstructures can be configured in order to interact with, and in particular, bind with one or more analytes of interest, allowing these to be detected. Specifically, in one

example, binding of one or more analytes to the microstructures can alter the charge carrying capability, in turn leading to changes in capacitance of electrode pairs, which can then be monitored, allowing analyte levels or concentrations to be derived. Binding of analytes can be achieved using a variety of techniques, including selection of mechanical properties of the microstructure, such as the presence of pores or other physical structures, the material from which the microstructures are manufactured, the use of coatings, or otherwise influencing the microstructure properties, such as by using magnetic microstructures.

[0305] Additionally, the microstructures and/or substrate can incorporate one or more materials or other additives, either within the body of the microstructure, or through addition of a coating containing the additive. The nature of the material or additive will vary depending on the preferred implementation and could include a bioactive material, a reagent for reacting with analytes in the subject, a binding agent for binding with analytes of interest, a material for binding one or more analytes of interest, a probe for selectively targeting analytes of interest, a material to reduce biofouling, a material to attract at least one substance to the microstructures, a material to repel or exclude at least one substance from the microstructures, a material to attract at least some analytes to the microstructures, or a material to repel or exclude analytes. In this regard, substances could include any one or more of cells, fluids, analytes, or the like. Example materials include polyethylene, polyethylene glycol, polyethylene oxide, zwitterions, peptides, hydrogels and self assembled monolayers.

[0306] The material can be contained within the microstructures themselves, for example by impregnating the microstructures during manufacture, can be the material from which the microstructures are formed, or could be provided in a coating. Accordingly, it will be appreciated that at least some of the microstructures can be coated with a coating such as a material for binding one or more analytes or interest, which can be used in order to target specific analytes of interest, allowing these to bind or otherwise attach to the microstructure, so that these can then be detected in situ using a suitable detection mechanism, such as by detecting changes in optical or electrical properties.

[0307] In some embodiments, the material or additive is a material for binding one or more analytes of interest. In particular embodiments, the material is an aptamer, especially a

plurality of aptamers. In particular embodiments, the aptamer is a coating on the microstructure.

[0308] The identity of the aptamer will depend on the specific analyte of interest and the method of detection. A skilled person will readily be able to identify and use suitable aptamers for each analyte of interest and method of detection. The aptamer is one which interacts or binds with an analyte of interest, and undergoes a conformational change upon analyte binding. For example, in some embodiments, the aptamer has a first conformation in the absence of analyte binding and a second conformation upon analyte binding.

[0309] In some embodiments, the second conformation results in a portion of the aptamer (e.g. a first end of the aptamer, such as the 3' or 5' end) being closer to the microstructure (and electrode) than in the first confirmation (i.e. the spacing between the portion of the aptamer and the microstructure is decreased in the second conformation). In alternative embodiments, the second confirmation results in a portion of the aptamer being further from the microstructure (and electrode) than in the first conformation (i.e. the spacing between the portion of the aptamer and the microstructure is increased in the second confirmation). Such change in proximity between the portion of the aptamer and the microstructure may then be detected using, for example, a labelling moiety such as a redox moiety or fluorescent label attached to or close to the relevant portion of the aptamer, such as a first end. In particular embodiments, the portion of the aptamer is a first end of the aptamer (e.g. the 5' end), preferably when a second end of the aptamer (e.g. the 3' end) is conjugated or otherwise attached, either directly or indirectly to the microstructure. Thus, in some embodiments, the second conformation results in a first end of the aptamer being closer to the microstructure than in the first conformation, or alternatively, results in a first end of the aptamer being further from the microstructure than in the first conformation. This may, for example, result in a first signal when the aptamer is in the first conformation and a second signal when the aptamer is in the second conformation, wherein the first signal is other than the second signal (i.e. the first and second signals are different).

[0310] While aptamers of any structure are contemplated, in particular embodiments, the aptamer comprises or consists of a stem-loop hairpin structure.

[0311] Suitable aptamers are well known in the art or may be identified using various methods well known in the art of aptamer selection.

[0312] For example, suitable aptamers may include, but are not limited to an aptamer described in Negahdary *et al.* (2018) *J Biomed Phys Eng*, 8(2): 167-178; Jo *et al.* (2015) *Anal Chem*, 87: 9869-9875; US 2012/0316326 A1; CN 102703455 A; KR 20160021488 A; US 2019/0219595 A1; Pfefiffer and Mayer (2016) *Front Chem*, 4:25; WO 2017/210683 A1; CN 102660547 A; WO 2017/210683 A1; CN 105136754 A; WO 2012/130948 A1; US 5582981 A; US 5595877 A; US 2018/0327746 A1; EP 2532749 B1; US 2012/0135540 A1; CN 105349545 A; US 2011/0318846 A1; CN 104745585 A; Stojanovic *et al.* (2000) *J Am Chem Soc*, 122: 11547-11548; WO 2015/197706 A1; WO 2019/094315 A1; or US 2017/0233738 A1; the entire contents of which are incorporated by reference herein.

[0313] In some embodiments, the aptamer is a troponin selective aptamer, representative examples of which include one described in Negahdary *et al.* (2018) *J Biomed Phys Eng*, 8(2): 167-178; Jo *et al.* (2015) *Anal Chem*, 87: 9869-9875; US 2012/0316326 A1; CN 102703455 A; KR 20160021488 A; and US 2019/0219595 A1; the entire contents of which are incorporated herein by reference. Troponin selective aptamers may bind to troponin or a subunit or complex thereof, such as troponin I, troponin C, troponin T, troponin I-C complex and/or troponin I-C-T complex, including cardiac troponin I, cardiac troponin C, cardiac troponin T, cardiac troponin I-C complex and/or cardiac troponin I-C-T. Such aptamers may bind a subunit alone (such as troponin I) and/or the subunit as part of a complex (such as troponin I as part of a troponin I-C or I-C-T complex).

[0314] In some embodiments, the aptamer comprises, consists or consists essentially of a nucleotide sequence selected from the group consisting of:

AGTCTCCGCTGTCCTCCCGATGCACTTGACGTATGTCTCACTTTCTTTTCATTGAC ATGGGATGACGCCGTGACTG [SEQ ID NO: 1];

CGTGCAGTACGCCAACCTTTCTCATGCGCTGCCCCTCTTA [SEQ ID NO: 2];

AGTCTCCGCTGTCCTCCCGATGCACTTGACGTATGTCTCACTTTCTTTTCATTGAC ATGGGATGACGCCGTGACTG [SEQ ID NO: 3];

CGTGCAGTACGCCAACCTTTCTCATGCGCTGCCCCTCTTA [SEQ ID NO: 4];

CGCATGCCAAACGTTGCCTCATAGTTCCCTCCCCGTGTCC [SEQ ID NO: 5];
TCACACCCTCCCTCCCACATACCGCATACACTTTCTGATT [SEQ ID NO: 6];
CCCGACCACGTCCCTGCCCTTTCCTAACCTGTTTGTTGAT [SEQ ID NO: 7];
ATGCGTTGAACCCTCCTGACCGTTTATCACATACTCCAGA [SEQ ID NO: 8];
CGTGCAGTACGCCAACCTTTCTCATGCGCTGCCCCTCTTA [SEQ ID NO: 9];
CAACTGTAATGTACCCTCCTCGATCACGCACCACTTGCAT [SEQ ID NO: 10];
CGCATGCCAAACGTTGCCTCATAGTTCCCTCCCCGTGTCC [SEQ ID NO: 11];
AGTCTCCGCTGTCCTCCCGATGCACTTGACGTATGTCTCACTTTCTTTTCATTGAC
ATGGGATGACGCCGTGACTG [SEQ ID NO: 12];
TCACACCCTCCCTCCCACATACCGCATACACTTTCTGATT [SEQ ID NO: 13];
CCCGACCACGTCCCTGCCCTTTCCTAACCTGTTTGTTGAT [SEQ ID NO: 14];
ATGCGTTGAACCCTCCTGACCGTTTATCACATACTCCAGA [SEQ ID NO: 15];
CGTGCAGTACGCCAACCTTTCTCATGCGCTGCCCCTCTTA [SEQ ID NO: 16];
CAACTGTAATGTACCCTCCTCGATCACGCACCACTTGCAT [SEQ ID NO: 17];
CGCATGCCAAACGTTGCCTCATAGTTCCCTCCCCGTGTCC [SEQ ID NO: 18];
TCACACCCTCCCTCCCACATACCGCATACACTTTCTGATT [SEQ ID NO: 19];
CCCGACCACGTCCCTGCCCTTTCCTAACCTGTTTGTTGAT [SEQ ID NO: 20];
ATGCGTTGAACCCTCCTGACCGTTTATCACATACTCCAGA [SEQ ID NO: 21];
CAACTGTAATGTACCCTCCTCGATCACGCACCACTTGCAT [SEQ ID NO: 22];
CGTGCAGTACGCCAACCTTTCTCATGCGCTGCCCCTCTTA [SEQ ID NO: 23];
CGCATGCCAAACGTTGCCTCATAGTTCCCTCCCCGTGTCC [SEQ ID NO: 24];
GGGATGGGGTGGGTGGCCAGCGATT [SEQ ID NO: 25];
and

TTAGGGGTGGTGTGGTTGGCAATTC [SEQ ID NO: 26];
especially SEQ ID NO: 1.

[0315] In some embodiments, the aptamer is an IL-6 selective aptamer, representative examples of which include, but are not limited to, an aptamer described in Hirota *et al.* (2016) *Nucleic Acid Therapeutics*, 26 (1): 10-19; WO 2014/159669 A1; Gupta *et al.* (2014) *J Biol Chem*, 289(12): 8706-8719; and Kumar *et al.* (2016) *Anal Methods,* 8(17): 3440-3444; the entire contents of which are incorporated by reference.

[0316] In exemplary embodiments, the aptamer comprises, consists or consists essentially of a nucleotide sequence selected from the group consisting of:

GG-ZZZ-GG-Q_a-GG-Q_b-GG [SEQ ID NO: 27];

GG-Q_a-GG-ZZZ-GG-Q_b-GG [SEQ ID NO: 28];

GG-Q_a-GG-Q_b-GG-ZZZ-GG [SEQ ID NO: 29];

wherein each Z is independently selected from U, T, and a modified pyrimidine; each Q is independently selected from a linker, a modified nucleotide, and an unmodified nucleotide; a is 1 to 50; and/ or b is 1 to 50; or

GGCAGGZZZGGZQ_aGZGG [SEQ ID NO: 30];

GGGYXAXGYAGCL$_b$GZGCGYAAGGCGGY [SEQ ID NO: 31];

wherein each Z is independently selected from U, T, and a modified pyrimidine (such as a 5'-modified pyrimidine); each Q is independently selected from a linker, a modified nucleotide, and an unmodified nucleotide; a is 1 to 50; each Y is independently selected from a modified pyrimidine (such as a 5'-modified pyrimidine); each X is independently selected from a modified pyrimidine (such as a 5'-modified pyrimidine); each L is independently selected from a linker, a modified nucleotide, and an unmodified nucleotide; and b is 1 to 20; or

YXAXGYARQ_aMGYAAGSCGRY [SEQ ID NO: 32];

MGYAAGSCGRYQ_bYXAXGYAR [SEQ ID NO: 33];

wherein each Y is independently selected from a modified pyrimidine (such as a 5'-modified pyrimidine); each X is independently selected from a modified pyrimidine (such as a 5'-modified pyrimidine); M is selected from C and A; S is selected from C and G; each R is independently selected from G and A; each Q is independently selected from a linker, a modified nucleotide, and an unmodified nucleotide; a is 1 to 30; and/or b is 1 to 30; or

GG[mC]AG[mG]Z^1Z^1EGGPAZ^1Z^1[mA]AC[mA][mC]GZ^1Z^1AAGZ^1[mC]GZ^1GG-idT

[SEQ ID NO: 34];

wherein Z^1 is benzyl modified 5-dU, P is naphthyl modified 5-dU, E is phenethyl modified 5-dU, mC is 2'-OMe C, mG is 2'-OMe G, mA is 2'-OMe A; or

GGCAGGZ^{1}Z^{1}Z^{1}GGZ^{1}AZ^{1}Z^{1}AACACGZ^{1}Z^{1}AAGZ^{1}CGZ^{1}GG [SEQ ID NO: 35];

GGGGZ^{1}Z^{1}AZ^{1}GZ^{1}AGCGAGZ^{1}GCGZ^{1}AAGGCGGZ^{1}GGGCGAGGGA [SEQ ID NO: 36]; or

LGGGZ^{1}Z^{1}AZ^{1}GZ^{1}AGCLLGZ^{1}GCGZ^{1}AAGGCGGZ^{1}G [SEQ ID NO: 37];

wherein Z^1 is benzyl modified 5-dU; and L is a C3-spacer (3-carbon alkyl linker).

[0317] The invention also contemplates variants of the sequences provided herein. Accordingly, in some embodiments, the aptamer comprises, consists or consists essentially of a nucleotide sequence which has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the nucleotide sequence of any one of SEQ ID NOs: 1-37, especially any one of SEQ ID NOs: 1-26, most especially SEQ ID NO: 1.

[0318] To determine the percentage sequence identity between two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g. gaps can be introduced in one or both of a first and a second nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In some embodiments, the length of a reference sequence aligned for comparison purposes is at least 40%, more usually at least 50% or 60%, and even more usually at least 70%, 80%, 90% or 100% of the length of the reference sequence. The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by the same nucleotide at the corresponding position in the second sequence, then the molecules are identical at that position.

[0319] The comparison of sequences and determination of percent identity between sequences can be accomplished using a mathematical algorithm. In certain embodiments, the percent identity between nucleic acid sequences is determined using the Needleman and Wünsch, (1970, *J. Mol. Biol.*, 48: 444-453) algorithm which has been incorporated into the

GAP program in the GCG software package (Devereaux *et al.* (1984) *Nucleic Acids Research*, 12: 387-395), using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In some embodiments, the percent identity between nucleic acid sequences can be determined using the algorithm of Meyers and Miller (1989, *Cabios*, 4: 11-17) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

[0320] Alternatively, a suitable aptamer may be identified and prepared using various methods known in the art of aptamer selection, including Systematic Evolution of Ligands by Exponential Enrichment (SELEX) techniques (e.g. as described in US 5475096 A and US 5270163 A), and the methods described in WO 2019/067383 A1, US 5582981 A, US 5595877 A, and US 5637459 A, the entire contents of which are incorporated herein by reference. In particular embodiments, an aptamer may be identified and prepared using SELEX techniques. In brief, the method may comprise systematically subjecting a large random pool of oligonucleotides to negative and positive rounds of selection against a target, e.g., an analyte, such as a protein, to filter out low affinity or nonspecific binders. The remaining aptamers may be collected and propagated, e.g., PCR amplified, and used in subsequent rounds of selection.

[0321] In some embodiments, it may be desirable to improve the stability of the aptamer. Several approaches are known in the art, including capping the terminal ends of the aptamer, substituting naturally occurring nucleotides with unnatural nucleotides (e.g. 2′-F, 2′-OCH_3, 2'-H, 2'-OH or 2′-NH_2 modified nucleotides such as 2′-fluorine-substituted pyrimidines, 2′-amino pyrimidines, and 2′-O-methyl ribose purines and pyrimidines), using unnatural internucleotide linkages such as phosphorothioate, methylphosphonate or triazole linkages, using altered sugar moieties, conjugating a molecule such as biotin to the 3' end, 3' end capping with inverted thymidine (dT), conjugating protein-like side chains e.g. to the nucleotides such as the 5-position of deoxyuridine (dU) (e.g. 5-(N-benzylcarboxyamide)-2-deoxyuridine), develop "spiegelmers" which are composed entirely of unnatural L-ribonucleic acid backbone, and the like. Further approaches are discussed in, for example, Shuaijian *et al.* (2017) *Int J Mol Sci*, 18(8): 1683, the content of which is incorporated herein by reference in its entirety.

[0322] The aptamer may also be modified to increase the sensitivity and binding kinetics of the aptamer for the analyte of interest. It is noted that one or more of the approaches for

improving the stability of the aptamer may have this result, particularly conjugating protein-like side chains e.g. to the nucleotides such as the 5-position of deoxyuridine (dU) (e.g. 5-(N-benzylcarboxyamide)-2-deoxyuridine). Additional modifications to increase the sensitivity and binding kinetics of the aptamer for the analyte of interest may be achieved using methods described in Ricci *et al.* (2016) *Acc Chem Res*, 49(9): 1884-1892, including population shift, allostery, matched receptor sets, sequestration and cooperativity. Further approaches contemplated by the invention may include attaching retaining structures which retain the aptamer in the second configuration to increase the aptamer recovery time, such as complementary primers attached to the ends of the aptamer, which bind together upon analyte binding to retain the aptamer in the second configuration beyond a recovery interval and at least one blocker bound to the aptamer which prevents the primers from binding together prior to analyte binding, or functional groups which interact with each other upon analyte binding to retain the aptamer in the second configuration beyond a recovery interval. Such approaches are discussed in WO 2018/031559 A1, the entire content of which is incorporated herein by reference.

[0323] In some embodiments, the aptamer comprises a moiety for attaching or immobilising the aptamer on the surface of the microstructure, such as a functional group or compound, preferably via a covalent bond. Suitable moieties for attaching or immobilising the aptamer on the surface of the microstructure include, but are not limited to, a thiol, amine, carboxylic acid, alcohol, carbodiimide, nafion, avidin, biotin, azide and the like; especially a thiol. While the moiety may be directly attached to the aptamer, in some embodiments, the moiety is attached to the aptamer via a linker, such as an alkyl chain, including a C_1-C_{20} alkyl, especially a C_6 or C_{11} alkyl, most especially a C_6 alkyl linker (i.e. $(CH_2)_6$ linker), a polymer, such as polyethylene glycol (PEG); or a nucleic acid sequence, including DNA and RNA sequences. In particular embodiments, the linker is an alkyl chain, such as a C_1-C_{20} alkyl, especially a C_6 or C_{11} alkyl, most especially a C_6 alkyl linker (i.e. $(CH_2)_6$ linker). Suitable linkers and synthetic routes for producing such linkers are known in the art, such as Lai *et al.* (2006) *Langmuir*, 22: 10796–10800, the entire contents of which is incorporated herein by reference.

[0324] Aptamers may be prepared using oligonucleotide synthetic techniques standard in the art, such as chemical synthesis (refer to, e.g. Itakura *et al.* (1984) *Ann Rev Biochem*, 53: 323-356). The aptamers may also be prepared by amplification (e.g. PCR) of aptamers prepared using SELEX techniques, as described in US 5475096 A and US 5270163 A, and the methods described in WO 2019/067383 A1, US 5582981 A, US 5595877 A, and US 5637459 A. Aptamers are also commercially available from a number of sources including Bioneer Pacific, Bio-synthesis Inc., Base Pair Biotechnologies, Inc. and TriLink Biotechnologies.

[0325] The aptamer is selective for binding the one or more analytes of interest or a subunit thereof. The aptamer is preferably selective for binding the one or more analytes of interest, such as troponin or a subunit or complex thereof, especially troponin I or cardiac troponin I-C complex, over at least one other substances present in the sample, preferably the majority of other substances present in the sample.

[0326] In some embodiments, the aptamer comprises a label or labelling moiety, such as a redox moiety, a fluorescent label and the like. Such moieties are useful for detecting the conformational change of the aptamer upon analyte binding as discussed herein.

[0327] In some embodiments, the aptamer comprises a redox moiety. Suitable redox moieties include any redoxable chemical moiety that can be conjugated or otherwise attached to an aptamer. For example, suitable redox moieties include, but are not limited to, methylene blue, ferrocene, vinylferrocene, anthraquinone, nile blue, thionine, anthraquinone-C5, dabcyl, 2,6-dichlorophenal-indophenol, gallocyanine, ROX, pentamethylferrocene, ferrocene-C5, neutral red and horseradish peroxidase; especially methylene blue, ferrocene, anthraquinone or nile blue; most especially methylene blue.

[0328] The redox moiety may be attached at any suitable point on the aptamer, provided that the conformational change which occurs upon analyte binding to the aptamer results in a detectable change in the spacing between the redox moiety and the electrode of the microstructure on which the aptamer is immobilised. In some embodiments, the redox moiety is closer to the electrode of the microstructure on which the aptamer is immobilised in the second conformation (i.e. upon analyte binding) compared to the first conformation (i.e. the spacing has decreased in the second conformation). In alternative embodiments, the redox

moiety is further from the electrode of the microstructure on which the aptamer is immobilised in the second conformation (i.e. upon analyte binding) compared to the first conformation (i.e. the spacing has increased in the second conformation). For example, in some embodiments, the redox moiety is attached at the 3' end or 5' end of the aptamer; especially at the 3' end of the aptamer, and the aptamer is attached to the microstructure through the opposite end, such as the 5' end and vice versa, preferably the 5' end. Without wishing to be bound by theory, it is proposed that electron transfer from the redox moiety to the electrode of the microstructure on which the aptamer is immobilised is increased when the spacing between the redox moiety and electrode is decreased and vice versa, thereby resulting in a detectable change which may be correlated to the presence, absence, level or concentration of analyte.

[0329] In some embodiments, the aptamer comprises a fluorescent label. Suitable fluorescent labels include, but are not limited to, fluorescein, 6-carboxyflourescein (FAM), coumarins, rhodamines, 5-TMRIA (tetramethylrhodamine-5-iodoacetamide), (9-(2(or 4)-(N-(2-maleimdylethyl)-sulfonamidyl)-4(or 2)-sulfophenyl)-2,3,6,7,12,13,16,17-octahydro-(1-H,5H,11H,15H-xantheno(2,-3,4-ij:5,6,7-i′j′)diquinolizin-18-ium salt) (Texas Red), 2-(5-(1-(6-(N-(2-maleimdylethyl)-amino)-6-oxohexyl)-1,3-dihydro-3,3-dimethyl-5-sulfo-2H-indol-2-ylidene)-1,3-propyldienyl)-1-ethyl-3,3-dimethyl-5-sulfo-3H-indolium salt (Cy3), N,N′-dimethyl-N-(iodoacetyl)-N′-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)ethylenediamine (IANBD amide), N-((2-(iodoacetoxy)ethyl)-N-methyl)amino-7-nitrobenz-2-oxa-1,3-diazole (IANBD ester), 6-acryloyl-2-dimethylaminonaphthalene (acrylodan), pyrene, 6-amino-2,3-dihydro-2-(2-((iodoacetyl)amino)ethyl)-1,3-dioxo-1H-benz(de)isoquinoline-5,8-disulfonic acid salt (lucifer yellow), 2-(5-(1-(6-(N-(2-maleimdylethyl)-amino)-6-oxohexyl)-1,3-dihydro-3,3-dimethyl-5-sulfo-2H-indol-2-ylidene)-1,3-pentadienyl)-1-ethyl-3,3-dimethyl-5-sulfo-3H-indolium salt (Cy5), 4-(5-(4-dimethylaminophenyl)oxazol-2-yl)phenyl-N-(2-bromoacetamidoethyl)sulfonamide (Dapoxyl®(2-bromoacetamidoethyl)sulfonamide)), (N-(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene-2-yl)iodoacetamide (BODIPY 507/545 IA), N-(4,4-difluoro-5,7-diphenyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)-N′-iodoacetylethylene diamine (BODIPY 530/550 IA), 5-((((2-iodoacetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid (1,5-IAEDANS), carboxy-X-rhodamine, 5/6-iodoacetamide (XRIA 5,6), BODIPY-FL-hydrazide, 6-carboxytetramethylrhodamine (TAMRA), cyan fluorescent protein, green fluorescent protein

and yellow fluorescent protein. Fluorescent quantum dots are also contemplated. Other suitable fluorescent labels include those described in ThermoFisher Scientific (2019) *The Molecular Probes Handbook – A Guide to Fluorescent Probes and Labeling Technologies*, accessed 29 September 2019, <https://www.thermofisher.com/au/en/home/references/molecular-probes-the-handbook.html>.

[0330] The fluorescent label may be attached at any suitable point on the aptamer. For example, in some embodiments, the fluorescent label is attached at the 3' end or 5' end of the aptamer; especially at the 3' end of the aptamer.

[0331] A skilled person will be well aware of suitable methods for attaching a labelling moiety to an aptamer, including chemical means, such as reduction, oxidation, conjugation, and condensation reactions. For example, a thiol-reactive group can be used to attach a labelling moiety, e.g., a fluorescent label or redox moiety, to a naturally occurring or engineered thiol group present in the aptamer. In a further example, reactive groups present in the aptamer can be labelled using succinimide ester derivatives of fluorescent labels. For example an amine may be introduced at the desired location of the aptamer for attachment of the labelling moiety, and an NHS-labelled redox moiety (e.g. NHS-labelled methylene blue) may be conjugated to the aptamer using, for example, succinimide ester coupling. Suitable methods are well known in the art, such as Liu *et al.* (2010) *Anal Chem,* 82(19): 8131-8136; Xiao *et al.* (2005) *Angew Chem Int Ed,* 44: 5456-5459; and US 2016/0278638 A1, the entire contents of which are incorporated herein by reference.

[0332] The labelling moiety may also be an autofluorescent or luminescent label.

[0333] While the labelling moiety may be directed attached to the aptamer, in some embodiments, the labelling moiety is attached to the aptamer via a linker. For example, in some embodiments, the moiety is attached to the aptamer via a linker, such as an alkyl chain, including a C_1-C_{20} alkyl, especially a C_6 or C_{11} alkyl, most especially a C_6 alkyl linker (i.e. $(CH_2)_6$ linker); a polymer, such as polyethylene glycol (PEG); or a nucleic acid sequence, including DNA and RNA sequences.

[0334] In some embodiments, the fluorescent label may be the only labelling moiety attached to the aptamer. Without wishing to be bound by theory, in such embodiments, it is proposed that analyte binding results in a conformational change in the aptamer, which causes a detectable change in the fluorescence of the fluorescent label (e.g. by changing the conjugation of the fluorescent label), such as an increase in fluorescence, a wavelength shift, and/or an increase in the fluorescence lifetime. Alternatively, the fluorescent label may interact with the bound analyte, resulting in a decrease in fluorescence of the fluorescent label.

[0335] In alternative embodiments, the aptamer comprises two labelling moieties, such as two fluorescent labels. Such embodiments are particularly suitable when generating an optical output, such as Förster resonance energy transfer (FRET). Such embodiments may utilise a pair of labelling moieties (e.g. a pair of fluorescent labels) attached at different points on the aptamer, where one label acts as a donor molecule (a first labelling moiety) and the other acts as an acceptor molecule (i.e. a quencher) (a second labelling moiety), wherein the absorption spectrum of the acceptor molecule overlaps the fluorescence emission spectrum of the donor molecule. Without wishing to be bound by theory, it is proposed that analyte binding results in a conformational change in the aptamer, which causes the proximity of the first and second labelling moieties to change and, thus, the fluorescence intensity of the first labelling moiety and emission intensity of the second labelling moiety to change. In some embodiments, the first and second labelling moieties may be closer to each other in the second conformation (i.e. upon analyte binding) compared to the first conformation (i.e. the spacing has decreased in the second conformation). In such embodiments, the fluorescence intensity of the first labelling moiety will decrease, and the emission intensity of the second labelling moiety will increase in the second conformation compared to the first conformation. In alternative embodiments, the first and second labelling moieties may be further from each other in the second conformation (i.e. upon analyte binding) compared to the first conformation (i.e. the spacing has increased in the second conformation). In such embodiments, the fluorescence intensity of the first labelling moiety will increase, and the emission intensity of the second labelling moiety will decrease in the second conformation compared to the first conformation.

[0336] In particular embodiments, both labelling moieties are preferably fluorescent labels, suitable examples of which are described *supra*. Exemplary combinations of which

include cyan fluorescent protein and yellow fluorescent protein, Cy3 and Cy5, FAM and TAMRA, and the like. In alternative embodiments, the first labelling moiety (i.e. donor molecule) is a fluorescent label and the second labelling moiety (i.e. acceptor molecule) is a non-fluorescent moiety. Non-limiting examples of suitable non-fluorescent moieties include 4-([4-(dimethylamino)phenyl]-azo)-benzoic acid (DABCYL), Iowa black RQ, 4-(4-dimethylaminophenylazo)benzenesulfonic acid (DABSYL), Iowa black FQ, IRDye QC-1, QXL quenchers, black hole quenchers including BHQ-1, BHQ-2 and BHQ-3, and the like, including the moieties described in Le Reste *et al.* (2012) *Biophysical Journal*, 11(6): 2658-2668, and Crisalli and Kool (2011) *Bioconj Chem*, 22(11): 2345-2354), the entire contents of which are incorporated herein by reference.

[0337] The first and second labelling moieties may be attached at any point on the aptamer, wherein the spacing between the first and second labelling moieties is different in the first and second aptamer conformations. In some embodiments, the spacing between the first and second labelling moiety is less than or equal to 10 nm in the first conformation and greater than 10 nm in the second conformation. In other embodiments, the spacing between the first and second labelling moiety is greater than 10 nm in the first conformation and less than or equal to 10 nm in the second conformation. For example, the first and second labelling moieties may be attached at or towards each end of the aptamer, e.g. at or towards the 3' and 5' ends. In some embodiments, the first labelling moiety is attached at the 3' end, and the second labelling moiety is attached at the 5' end or, alternatively, the first labelling moiety is attached at the 5' end and the second labelling moiety is attached at the 3' end.

[0338] The invention also contemplates embodiments wherein the acceptor molecule is the material from which the microstructure is formed, or a coating on the microstructure, such as graphene, graphene oxide, and the like.

[0339] In preferred embodiments, the aptamer is a coating on the microstructure (also referred to herein as an aptamer coating). The number of aptamers and/or aptamer density in the coating will depend on the analyte of interest (including analyte size and expected levels or concentration to be detected), application of the system of the invention and detection method. The aptamer density in the coating should be a density which results in a measurable response upon analyte binding, such as a change in impedance or fluorescence, especially upon analyte

binding at analyte concentrations or levels of interest. In some embodiments, the aptamer density in the coating is in the range of from about 1 x 10^{10} to about 1 x 10^{14} aptamer molecules/cm^2, about 5 x 10^{10} to about 5 x 10^{13} aptamer molecules/cm^2, about 1 x 10^{11} to about 1 x 10^{13} aptamer molecules/cm^2, about 5 x 10^{11} to about 5 x 10^{12} aptamer molecules/cm^2 (and all integers therebetween).

[0340] When applied as a coating on the microstructure, the aptamer may be coated using any suitable technique routine in the art, such as chemisorption, or chemical cross-linking. For example, the technique may include contacting the surface of the microstructure with the aptamer for a time period sufficient for a moiety for attaching or immobilising the aptamer on the surface of the microstructure to attach to the surface of the microstructure, such as via a covalent bond. Suitable, non-limiting methods may include chemisorption of thiolated aptamers on a gold microstructure; attachment of biotinylated aptamer to avidin-modified microstructure; immobilisation of an azide-ended aptamer to alkyne-modified microstructure; covalent immobilisation of amine-ended aptamer by amine coupling to carboxyl groups in functionalised microstructure; covalent immobilization of amine-ended aptamer to functionalized microstructure containing amine groups using glutaraldehyde, and the like. Exemplary methods are described in Xiao *et al.* (2007) *Nat Protocols*, 2(11): 2875-2880; Negahdary *et al.* (2018) *J Biomed Phys Eng*, 8(2): 167-178; and Mishra *et al.* (2018) *Biosensors*, 8(2): 28, the contents of which are incorporated herein by reference. The aptamer may be attached to the microstructure through any suitable point of the aptamer, especially the 3' or 5' end, most especially the 5' end of the aptamer.

[0341] The microstructure, such as a gold coated microstructure, may be prepared for aptamer functionalisation using methods standard in the art, such as cleaning using cyclic voltammetry in H_2SO_4; cleaning by soaking in acetone and sonicating for 5 minutes, soaking in isopropanol and sonicating for 5 minutes, drying using a stream of nitrogen gas, soaking in deionised water and sonicating for 5 minutes, and drying using a stream of nitrogen gas; or cleaning using an oxygen plasma clean. Without wishing to be bound by theory, plasma cleaning removes organic contamination through chemical reaction or physical ablation of hydrocarbons on the surface of gold coated electrodes, producing a protective gold oxide layer without creating defects in the gold surface. The chemical cleaning of the surface is thought

to occur by reactive oxygen ions and changes the surface energy through the introduction of polar functional groups.

[0342] The analyte may be any compound able to be detected in the epidermis and/or dermis. In particular embodiments, the analyte is a marker of a condition, disease, disorder or a normal or pathologic process that occurs in a subject, or a compound which can be used to monitor levels of an administered substance in the subject, such as a medicament (e.g., drug, vaccine), an illicit substance (e.g. illicit drug), a non-illicit substance of abuse (e.g. alcohol or prescription drug taken for non-medical reasons), a poison or toxin, a chemical warfare agent (e.g. nerve agent, and the like) or a metabolite thereof. Suitable analytes include, but are not limited to a:

- nucleic acid, including DNA and RNA, including short RNA species including microRNA, siRNA, snRNA, shRNA and the like;
- antibody, or antigen-binding fragment thereof, allergen, antigen or adjuvant;
- chemokine;
- cytokine;
- hormone;
- parasite, bacteria, virus, or virus-like particle, or a compound therefrom, such as a surface protein, an endotoxin, and the like;
- epigenetic marker, such as the methylation state of DNA, or a chromatin modification of a specific gene/region;
- peptide;
- polysaccharide (glycan);
- polypeptide;
- protein; and
- small molecule.

[0343] In particular embodiments, the analyte of interest is selected from the group consisting of a nucleic acid, antibody, peptide, polypeptide, protein and small molecule; especially a polypeptide and protein; most especially a protein.

[0344] In particular embodiments, the analyte is a cytokine, such as IL-6, IL-10 or TNF-α; especially IL-6 or TNF-α; most especially IL-6.

[0345] The analyte may be a biomarker, which is a biochemical feature or facet that can be used to measure the progress of a disease, disorder or condition or the effects of treatment of a disease, disorder or condition. The biomarker may be, for example, a virus or a compound therefrom, a bacterium or a compound therefrom, a parasite or a compound therefrom, a cancer antigen, a cardiac disease indicator, a stroke indicator, an Alzheimer's disease indicator, an antibody, a mental health indicator, an inflammatory marker and the like.

[0346] Alternatively, the analyte may be a compound which can be used to monitor levels of an administered or ingested substance in the subject, such as a medicament (e.g., drug, vaccine), an illicit substance (e.g. illicit drug), a non-illicit substance of abuse (e.g. alcohol or prescription drug taken for non-medical reasons), a poison or toxin, a chemical warfare agent (e.g. nerve agent, and the like) or a metabolite thereof.

[0347] In some embodiments, the analyte is a protein selected from the group consisting of troponin or a subunit thereof, an enzyme (e.g. amylase, creatinine kinase, lactate dehydrogenase, angiotensin II converting enzyme), a hormone (e.g. follicle-stimulating hormone or luteinising hormone), cystatin C, C-reactive protein, TNFα, IL-6, ICAM1, TLR2, TLR4, presepsin, D-dimer, a viral protein (e.g. non-structural protein 1 (NS1)), a bacterial protein, a parasitic protein (e.g. histone rich protein 2 (HRP2)), an antibody (e.g. an antibody produced in response to an infection, such as a bacterial or viral infection including an influenza infection) and botulinum toxin or a metabolite or subunit thereof; especially troponin or a subunit thereof, amylase, creatinine kinase, lactate dehydrogenase, angiotensin II converting enzyme, follicle-stimulating hormone, luteinising hormone, cystatin C, C-reactive protein, TNFα, IL-6, ICAM1, TLR2, TLR4, presepsin, D-dimer, botulinum toxin or a metabolite or subunit thereof. In particular embodiments, the analyte is troponin or a subunit thereof; especially troponin I, troponin C or troponin T; most especially troponin I. In alternative embodiments, the analyte is IL-6.

[0348] In particular embodiments, the analyte is troponin or a subunit or complex thereof; especially cardiac troponin or a subunit or complex thereof. In some embodiments, the analyte

is troponin I, troponin C, troponin T, troponin I-C complex or troponin I-T-C complex; especially cardiac troponin I (cTnI), cardiac troponin troponin I-C (cTnIC) complex or cardiac troponin I-T-C (cTnITC) complex; most especially cTnI or cTnIC.

[0349] In some embodiments, the analyte is an inflammatory marker selected from the group consisting of C-reactive protein, TNFα, IL-6, ICAM1, TLR2, TLR4, presepsin, IL-10 and procalcitonin.

[0350] The analyte may be a small molecule, non-limiting examples of which include a hormone (e.g. cortisol or testosterone), neurotransmitter (e.g. dopamine), amino acid, creatinine, an aminoglycoside (e.g. kanamycin, gentamicin and streptomycin), an anticonvulsant (e.g. carbamazepine and clonazepam), an illicit substance (e.g. methamphetamine, amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), *N*-ethyl-3,4-methylenedioxyamphetamine (MDEA), 3,4-methylenedioxy-amphetamine (MDA), cannabinoids (e.g. delta-9-tetrahydrocannabinol, 11-hydroxy-delta-9-tetrahydrocannabinol, 11-nor-9-carboxydelta-9-tetrahydrocannabinol), cocaine, benzoylecgonine, ecgonine methyl ester, cocaethylene, ketamine, and the opiates (e.g. heroin, 6-monoacetylmorphine, morphine, codeine, methadone and dihydrocodeine), an anticoagulant (e.g. warfarin), a chemical warfare agent, poison or toxin such as blister agents (e.g. cantharidin, furanocoumarin, sulfur mustards (e.g. 1,2-bis(2-chloroethylthio)ethane, 1,3-bis(2-chloroethylthio)-n-propane, 1,4-bis(2-chloroethylthio)-n-butane, 1,5-bis(2-chloroethylthio)-n-pentane, 2-chloroethylchloromethylsulfide, bis(2-chloroethyl)sulfide, bis(2-chloroethylthio)methane, bis(2-chloroethylthiomethyl)ether, bis(2-chloroethylthioethyl)ether), nitrogen mustards (e.g. bis(2-chloroethyl)ethylamine, bis(2-chloroethyl)methylamine and tris(2-chloroethyl)amine) and phosgene oxime), arsenicals (e.g. ethyldichloroarsine, methyldichloroarsine, phenyldichloroarsine and 2-chlorovinyldichloroarsine) and urticants e.g. phosgene oxime), blood agents (e.g. cyanogen chloride, hydrogen cyanide and arsine), choking agents (e.g. chlorine, chloropicrin, diphosgene and phosgene), nerve agents (e.g. tabun, sarin, soman, cyclosarin, novichok agents, 2-(dimethylamino)ethyl-*N,N*-dimethylphosphoramidofluoridate (GV), (S)-(ethyl{[2-(diethylamino)ethyl]sulfanyl}(ethyl)phosphinate) (VE), *O,O*-diethyl-*S*-[2-(diethylamino)ethyl]phosphorothioate (VG), *S*-[2-(diethylamino)ethyl]-*O*-ethyl methylphosphonothioate (VM), ethyl({2-[bis(propan-2-

yl)amino]ethyl}sulfanyl)(methyl)phosphinate (VX), tetrodotoxin and saxitoxin), animal venom component (e.g. tetrodotoxin and saxitoxin), cyanide, arsenic, a tropane alkaloid (e.g. atropine, scopolamine and hyoscyamine), a piperidine alkaloid (e.g. coniine, *N*-methylconiine, conhydrine, pseudoconhydrine and gamma-coniceine), a curare alkaloid (e.g. tubocurarine), nicotine, caffeine, quinine, strychnine, brucine, aflatoxin), and the like or a metabolite thereof. In some embodiments the small molecule is selected from the group consisting of cortisol, testosterone, creatinine, dopamine, kanamycin, gentamicin, streptomycin, carbamazepine, clonazepam, methamphetamine, amphetamine, MDMA, MDEA, MDA, delta-9-tetrahydrocannabinol, 11-hydroxy-delta-9-tetrahydrocannabinol, 11-nor-9-carboxydelta-9-tetrahydrocannabinol, cocaine, benzoylecgonine, ecgonine methyl ester, cocaethylene, ketamine, heroin, 6-monoacetylmorphine, morphine, codeine, methadone, dihydrocodeine, warfarin, cantharidin, furanocoumarin, 1,2-bis(2-chloroethylthio)ethane, 1,3-bis(2-chloroethylthio)-n-propane, 1,4-bis(2-chloroethylthio)-n-butane, 1,5-bis(2-chloroethylthio)-n-pentane, 2-chloroethylchloromethylsulfide, bis(2-chloroethyl)sulfide, bis(2-chloroethylthio)methane, bis(2-chloroethylthiomethyl)ether, bis(2-chloroethylthioethyl)ether), bis(2-chloroethyl)ethylamine, bis(2-chloroethyl)methylamine and tris(2-chloroethyl)amine), phosgene oxime, ethyldichloroarsine, methyldichloroarsine, phenyldichloroarsine, 2-chlorovinyldichloroarsine, phosgene oxime, cyanogen chloride, hydrogen cyanide, arsine, chlorine, chloropicrin, diphosgene, phosgene, tabun, sarin, soman, cyclosarin, novichok agents, 2-(dimethylamino)ethyl-*N,N*-dimethylphosphoramidofluoridate (GV), (S)-(ethyl{[2-(diethylamino)ethyl]sulfanyl}(ethyl)phosphinate) (VE), *O,O*-diethyl-*S*-[2-(diethylamino)ethyl]phosphorothioate (VG), *S*-[2-(diethylamino)ethyl]-*O*-ethyl methylphosphonothioate (VM), ethyl({2-[bis(propan-2-yl)amino]ethyl}sulfanyl)(methyl)phosphinate (VX), tetrodotoxin, saxitoxin, cyanide, arsenic, atropine, scopolamine, hyoscyamine, coniine, *N*-methylconiine, conhydrine, pseudoconhydrine, gamma-coniceine, tubocurarine, nicotine, caffeine, quinine, strychnine, brucine, aflatoxin and metabolites thereof.

[0351] In some embodiments, the analyte is a peptide, non-limiting examples of which include a hormone (e.g. oxytocin, gonadotropin-releasing hormone and adrenocorticotropic hormone), B-type natriuretic peptide, N-terminal pro B-type natriuretic peptide (NT-proBNP) and an animal venom component (e.g. a peptidic component of spider, snake, scorpion, bee,

wasp, ant, tick, conesnail, octopus, fish (e.g stonefish) and jellyfish venom) or a metabolite thereof. In particular embodiments, the peptide is oxytocin, gonadotropin-releasing hormone, adrenocorticotropic hormone, B-type natriuretic peptide or NT-proBNP.

[0352] In some embodiments, the analyte is a polysaccharide (glycan), suitable non-limiting examples of which include inulin, endotoxins (lipopolysaccharides), anticoagulants (e.g. heparin) and metabolites thereof.

[0353] In some embodiments, the analyte is an illicit substance or a non-illicit substance of abuse or a metabolite thereof. Suitable illicit substances include, but are not limited to, methamphetamine, amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), *N*-ethyl-3,4-methylenedioxyamphetamine (MDEA), 3,4-methylenedioxy-amphetamine (MDA), cannabinoids (e.g. delta-9-tetrahydrocannabinol, 11-hydroxy-delta-9-tetrahydrocannabinol, 11-nor-9-carboxydelta-9-tetrahydrocannabinol), cocaine, benzoylecgonine, ecgonine methyl ester, cocaethylene, ketamine, and the opiates (e.g. heroin, 6-monoacetylmorphine, morphine, codeine, methadone and dihydrocodeine), or metabolites thereof. Non-limiting non-illicit substances of abuse include alcohol, nicotine, prescription medicine or over the counter medicine taken for non-medical reasons, a substance taken for a medical effect, wherein the consumption has become excessive or inappropriate (e.g. pain medications such as opiates, sleep aids, anti-anxiety medication, methylphenidate, erectile-dysfunction medications), and the like, or metabolites thereof.

[0354] In some embodiments, the analyte is a medicament or a component or metabolite thereof. A wide variety of medicaments are suitable analytes, including, but not limited to, cancer therapies, vaccines, analgesics, antipsychotics, antibiotics, anticoagulants, antidepressants, antivirals, sedatives, antidiabetics, contraceptives, immunosuppressants, antifungals, antihelmintics, stimulants, biological response modifiers, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, disease-modifying anti-rheumatic drugs (DMARDs), anabolic steroids, antacids, antiarrhythmics, thrombolytics, anticonvulsants, antidiarrheals, antiemetics, antihistamines, antihypertensives, anti-inflammatories, antineoplastics, antipyretics, barbiturates, β-blockers, bronchodilators, cough suppressants, cytotoxics, decongestants, diuretics, expectorants, hormones, laxatives, muscle relaxants,

vasodilators, sedatives, vitamins, and metabolites thereof. Various examples of these medicaments are described herein and are well known in the art.

[0355] In some embodiments, the analyte is a poison, toxin, chemical warfare agent, or metabolite thereof. Suitable poisons, toxins and chemical warfare agents include, but are not limited to, including blister agents (e.g. cantharidin, furanocoumarin, sulfur mustards (e.g. 1,2-bis(2-chloroethylthio)ethane, 1,3-bis(2-chloroethylthio)-n-propane, 1,4-bis(2-chloroethylthio)-n-butane, 1,5-bis(2-chloroethylthio)-n-pentane, 2-chloroethylchloromethylsulfide, bis(2-chloroethyl) sulfide, bis(2-chloroethylthio)methane, bis(2-chloroethylthiomethyl)ether, bis(2-chloroethylthioethyl)ether), nitrogen mustards (e.g. bis(2-chloroethyl)ethylamine, bis(2-chloroethyl)methylamine and tris(2-chloroethyl)amine) and phosgene oxime), arsenicals (e.g. ethyldichloroarsine, methyldichloroarsine, phenyldichloroarsine and 2-chlorovinyldichloroarsine) and urticants e.g. phosgene oxime), blood agents (e.g. cyanogen chloride, hydrogen cyanide and arsine), choking agents (e.g. chlorine, chloropicrin, diphosgene and phosgene), nerve agents (e.g. tabun, sarin, soman, cyclosarin, novichok agents, 2-(dimethylamino)ethyl-*N,N*-dimethylphosphoramidofluoridate (GV), (S)-(ethyl{[2-(diethylamino)ethyl]sulfanyl}(ethyl)phosphinate) (VE), *O,O*-diethyl-*S*-[2-(diethylamino)ethyl]phosphorothioate (VG), *S*-[2-(diethylamino)ethyl]-*O*-ethyl methylphosphonothioate (VM), ethyl({2-[bis(propan-2-yl)amino]ethyl}sulfanyl)(methyl)phosphinate (VX), tetrodotoxin, saxitoxin and botulinum toxin), animal venom component (e.g. tetrodotoxin, saxitoxin or other component of spider, snake, scorpion, bee, wasp, ant, tick, conesnail, octopus, fish (e.g stonefish) and jellyfish venom), cyanide, arsenic, a component of Atropa Belladonna (deadly nightshade) such as a tropane alkaloid (e.g. atropine, scopolamine and hyoscyamine), a component of hemlock such as a piperidine alkaloid (e.g. coniine, *N*-methylconiine, conhydrine, pseudoconhydrine and gamma-coniceine), a curare alkaloid (e.g. tubocurarine), nicotine, caffeine, alcohol, quinine, atropine, strychnine, brucine, aflatoxin and metabolites thereof. In some embodiments, the analyte is a chemical warfare agent such as a blister agent (e.g. cantharidin, furanocoumarin, a sulfur mustard (e.g. 1,2-bis(2-chloroethylthio)ethane, 1,3-bis(2-chloroethylthio)-n-propane, 1,4-bis(2-chloroethylthio)-n-butane, 1,5-bis(2-chloroethylthio)-n-pentane, 2-chloroethylchloromethylsulfide, bis(2-chloroethyl)sulfide, bis(2-chloroethylthio)methane, bis(2-chloroethylthiomethyl)ether or bis(2-chloroethylthioethyl)ether), a nitrogen mustard

(e.g. bis(2-chloroethyl)ethylamine, bis(2-chloroethyl)methylamine or tris(2-chloroethyl)amine) or phosgene oxime), an arsenical (e.g. ethyldichloroarsine, methyldichloroarsine, phenyldichloroarsine or 2-chlorovinyldichloroarsine) or an urticant e.g. phosgene oxime), a blood agent (e.g. cyanogen chloride, hydrogen cyanide or arsine), a choking agent (e.g. chlorine, chloropicrin, diphosgene or phosgene), a nerve agent (e.g. tabun, sarin, soman, cyclosarin, a novichok agent, 2-(dimethylamino)ethyl-*N,N*-dimethylphosphoramidofluoridate (GV), (S)-(ethyl{[2-(diethylamino)ethyl]sulfanyl}(ethyl)phosphinate) (VE), *O,O*-diethyl-*S*-[2-(diethylamino)ethyl]phosphorothioate (VG), *S*-[2-(diethylamino)ethyl]-*O*-ethyl methylphosphonothioate (VM), ethyl({2-[bis(propan-2-yl)amino]ethyl}sulfanyl)(methyl)phosphinate (VX), tetrodotoxin, saxitoxin or botulinum toxin) or a metabolite thereof.

[0356] Examples of suitable analytes, diseases, disorders or conditions, or applications for which they are relevant and known lowest clinically relevant serum concentration ranges are provided in Table 1.

Table 1

Analyte	**Relevant disease, disorder or condition, or application**	**Lowest clinically relevant concentration (where available)**	**Molecular weight**
Troponin or a subunit thereof, such as troponin I, troponin C or troponin T	Cardiac damage, myocardial infarction, acute coronary syndrome	Less than 30 ng/L	23 kDa, 18 kDa and 34 kDa, respectively for I, C and T subunits
Troponin subunit complex, such as cTnIC and cTnITC complexes	Cardiac damage, myocardial infarction, acute coronary syndrome	Less than 30 ng/L	~20-100 kDa (depending on complex)
Cortisol (serum)	Addison's disease, Cushing's disease,	Less than 650 nmol/L	362 Da

	adrenal and/or pituitary gland function, psychological stress (wellness applications)		
Creatinine	Renal failure, creatinine clearance estimates	Less than 100 µmol/L	113 Da
Dopamine	Parkinson's disease, brain cancers, depression	0-30 pg/mL	153 Da
Aminoglycosides (e.g. kanamycin, gentamicin, streptomycin)	Monitor dose of therapeutic for bacterial infection	5-10 mg/L	Varied ~300-600 Da
Anticonvulsants (e.g. carbamazepine and clonazepam)	Monitor dose of therapeutic for epilepsy	0.02-12 mg/L	Varied ~100 Da
Hormones such as follicle stimulating hormone, luteinising hormone, oxytocin, gonadotropin-releasing hormone and testosterone	Assisted fertility, calcium levels, substance abuse (doping)	Varied	Varied ~200-300 Da
Amylase	Pancreatitis, bile duct obstruction	Less than 100 U/L	50 kDa
Creatinine kinase	Skeletal muscle damage, which may be indicative of rhabdomyolysis, injury and/or drug side-effects (statins)	Less than 200 U/L	80 kDa
Lactate dehydrogenase	Hepatic damage	119-229 U/L	140 kDa
B-type natriuretic peptide (BNP)	Cardiac failure	100 ng/L	36 kDa (high molecular weight form) or 3.5 kDa (low molecular weight form)

NT-proBNP	Cardiac failure	300 ng/L	8.5 kDa
Angiotensin II converting enzyme	Essential hypertension	8- 100 U/L	60-170 kDa
Cystatin C	Renal failure	0.6-1 mg/L	13 kDa
Stress hormones e.g. adrenocorticotropic hormone (ACTH)	Adrenal insufficiency or overactivity	2-11 pmol/L	~4 kDa
Inflammatory markers (e.g. C-reactive protein (CRP), TNFα, IL-6, ICAM1, TLR2, TLR4, presepsin, IL-10)	Bacterial or viral infection, autoimmune disorders, rheumatological disorders, sepsis	Less than 10 mg/L (CRP)	Varied 120 kDa (CRP)
Inulin	Renal failure, creatinine clearance estimates	Varied (dependent on amount administered)	Varied
Illicit substances (e.g. methamphetamine, amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), *N*-ethyl-3,4-methylenedioxyamphetamine (MDEA), 3,4-methylenedioxy-amphetamine (MDA), cannabinoids (e.g. delta-9-tetrahydrocannabinol, 11-hydroxy-delta-9-tetrahydrocannabinol, 11-nor-9-carboxydelta-9-tetrahydrocannabinol), cocaine, benzoylecgonine, ecgonine methyl ester, cocaethylene, ketamine, and the opiates (e.g. heroin, 6-monoacetylmorphine, morphine, codeine, methadone and dihydrocodeine))	Drug abuse, compliance monitoring, rehabilitation, screening	Varied (dependent on application e.g. rehabilitation compared with screening or drug abuse, and identity of substance)	Varied ~200-300 Da
Anticoagulants (e.g. warfarin and heparin)	Monitor dose of therapeutic for blood clotting	Varied	Varied

	disorders and diseases		
Glycoproteins and glycans	Bacterial infection (i.e. bacterial endotoxins)	Varied	Varied ~10-20 kDa
Cellular components and breakdown products	Bacterial infection, exosome detection, cancer, platelet detection	Varied	Varied
D-dimer	Pulmonary embolism	0.4 mg/mL	180 kDa
Oligonucleotides and polynucleotides (e.g. DNA, RNA and fragments thereof)	Bacterial infection, viral infection, circulating tumour cell breakdown, solid tissue cancers	Varied	Varied ~200-300 Da
Chemical warfare agents (e.g. blister agents, blood agents, choking agents and nerve agents)	Chemical warfare, environmental contamination	Varied	Varied
Soluble urokinase plasminogen activator receptor	Asthma, chronic rhinosinusitis, nonspecific inflammatory marker in both acute and chronic illness	> 3.69 ng/mL (7000 ng/L)	20–50 kDa (three domains, depending on glycosylation)
Serum amyloid A	Blood biomarker for tissue injury and inflammation, various inflammatory diseases	5-20 mg/mL	11.7 kDa
Prostaglandins	Inflammation and vasodilation	1 ng/mL	Varied

[0357] In some embodiments, the analyte is a metabolite of any one of the above exemplary analytes.

[0358] In some embodiments, the analyte is part of a complex, e.g. cTnI, as part of the cTnIC complex. Accordingly, in particular embodiments, the analyte is a complex comprising any one of the above analytes. The binding agent (e.g. aptamer) may interact with all components of the complex or may interact with part of the complex, such as a subunit.

[0359] While the analyte preferably binds directly to the binding agent, the invention also contemplates detecting agents probative of the analyte of interest such as a specific binding pair member complementary to the analyte of interest, whose presence will be detected only when a particular analyte of interest is present in a sample. Thus, the agent probative of the analyte becomes the analyte that is detected.

[0360] In some embodiments, the microstructures are coated with a material that reduces absorption of analytes that are not of interest. Example materials include alkyl groups coated with BSA (bovine serum albumin), bifunctional polyethylene glycol (PEG) polymers, or the like. Such materials have the effect of reducing adsorption of non-specific analytes, which are effectively repelled from the microstructures.

[0361] It will be appreciated that multiple coatings could be used in conjunction, for example, to repel or exclude non-specific analytes and bind analytes of interest, thereby allowing specific analytes of interest to be selectively captured, whilst non-specific analytes remain uncaptured.

[0362] A polymer coating may be applied using a variety of techniques routinely used in the art. For example, the microstructures can be coated with a polymer using a variety of techniques, including dip coating, spray coating, deposition coating, electropolymerisation, drop casting, electrospinning, ink jet coating, spin coating, or the like; especially electropolymerisation. In one example, a coating solution is applied to the microstructures and allowed to dry in situ, optionally using a gas jet. Where the coating is a polymer coating, the polymer may, in some embodiments, be synthesised prior to coating using, for example, bulk polymerisation. In alternative embodiments, the polymer is synthesised and coated simultaneously, such as when synthesising and coating using electropolymerisation. A skilled person will be well aware of suitable techniques.

[0363] Furthermore, to optimise coating, properties of the coating can be controlled through the addition of one or more other agents such as a viscosity enhancer, a detergent or other surfactant, and an adjuvant. These ingredients can be provided in a range of different concentrations. For example, the viscosity enhancer or surfactant can form between 0% and 90% of the coating solution.

[0364] A range of different viscosity enhancers can be used and examples include methylcellulose, carboxymethylcellulose (CMC), gelatin, agar, and agarose and any other viscosity modifying agents. The solution typically has a viscosity of between 10^{-3} Pa·s and 10^{-1} Pa·s. In one example, using a coating solution containing 1-2% methylcellulose, which results in suitable uniform coatings, resulting in a viscosity within the range 0.011 (1%) - 0.055 (2%) Pa·s.

[0365] Similarly, a range of different surfactants can be used to modify the surface tension of the coating solution, such as any detergent or any suitable agent that decreases surface tension, and that is biocompatible at a low concentration. The solution properties are also typically controlled through the addition of one or more other agents such as a viscosity enhancer, a detergent, other surfactant, or anything other suitable material. These ingredients can be provided in a range of different concentrations. For example, the viscosity enhancer or surfactant can form between 0% and 90% of the coating solution.

[0366] As an alternative to using a coating technique, reagents can alternatively be embedded within the microstructures. Thus, for example, in the case of moulded patches manufactured using a polymer material, the reagent can be introduced into the mould together with the polymer material so that the reagent is distributed throughout the structures. In this example, the polymer can be arranged so that pores form within the structures during the curing process.

[0367] Using affinity surface coatings on each structure also allows a reduction of non-specific adsorption of ISF and/or blood components whilst facilitating specific extraction of the molecular targets of interest.

[0368] Thus, in one example, the one or more microstructures interact with one or more analytes of interest such that a response signal is dependent on a presence, absence, level or

concentration of analytes of interest. In one particular example, the analytes interact with a coating on the microstructures to change electrical and/or optical properties of the coating, thereby allowing the analytes to be detected.

[0369] For example, measurements can be performed by passing a current between electrodes, with measurements of the resulting signal between the electrodes being used to detect changes in the electrical properties and hence, the presence, absence, level or concentration of analytes. In this regard, the electrical output signal can be indicative of any one or more of a voltage, a current, a resistance, a capacitance, a conductance, or an impedance, or a change in any of these variables. Thus, signals could be potentiometric, amperometric, voltametric, impedimetric, or the like.

[0370] For example, impedance measurements, such as in electrochemical impedance spectroscopy (EIS), investigate the dynamics of the bound analyte or the charge transfer in the bulk or the interfacial region of the aptamer. In this regard, when an aptamer captures a target analyte, the captured analyte can change the structure of the aptamer changing the electrical properties. The measurement only requires ions in the samples and can be done without a redox moiety.

[0371] In this example, the electrodes can be arranged in pairs, although alternatively the system could measure impedances between different groups of electrodes, for example with one group acting as a working electrode, another group working as a counter electrode, and optionally a further group acting as a reference electrode. In this example, the microstructures operating as part of the working electrode are functionalised, for example using a coating including an aptamer, MIP or similar.

[0372] In a further example, voltametric/amperometric techniques can be used, including cyclic voltammetry (CV), liner sweep voltammetry (LSV), differential pulse voltammetry (DPV), square wave voltammetry (SWV), alternating current voltammetry (ACV), or chronoamperometry (CA).

[0373] In this example, a current output is generated from the redox reaction of the electroactive species (redox moiety) which takes place on the conductive material (e.g gold microstructures). When analyte of interest is captured in the aptamer, the structure of the

aptamer changes resulting in the redox moieties moving relative to the microstructure surface, thereby altering the current output.

[0374] Since a redox reaction is required in this type of transduction, some researchers attach a redox moiety to the aptamer.

[0375] In this example, reference electrodes might also be provided, in which case electrodes might be arranged in three groups, including working, counter and reference electrodes. The reference electrodes need only be in the vicinity of the working and counter electrodes, so that, for example, electrodes could be arranged in pairs of working and counter electrodes, with a row of pairs of electrodes being used as reference electrodes. Suitable reference electrode materials are known in the art and may include, for example, Ag/AgCl, iridium oxide (IrOx), platinum, graphite/Agl and Ag/AgI.

[0376] In a further example potentiometric measurements can be performed in which an electrical output is generated in response to binding of target analyte in the aptamer. Here the change in the voltage corresponding to the amount of analyte bound in the aptamer is measured. Potentiometric techniques can be found in sensor like ion selective electrodes (ISE) and field-effect transistors (FET).

[0377] Other measurement techniques include mass sensitive acoustic transducers such as surface-acoustic wave (SAW) oscillator, Love-wave oscillator, or quartz crystal microbalance. (QCM). In binding of analyte could be quantified via the change in the oscillation frequency resulting from the mass change at the oscillator surface.

[0378] In a further example, one or more microstructures include a treatment material, and wherein at least one treatment delivery mechanism is provided that controls release of the treatment material. In one preferred example, release of the treatment material is controlled by applying stimulation to the microstructure(s), for example by applying light, heat or electrical stimulation to release the treatment material.

[0379] In one preferred example, the treatment material is contained in a coating on the at least one microstructure and the stimulation is used to dissolve the coating on the microstructure and thereby deliver the treatment material. It will be appreciated that this

technique can be applied to any treatment material that can be incorporated into a coating, and which can be selectively released using stimulation, such as mechanical, magnetic, thermal, electrical, electromagnetic or optical stimulation.

[0380] The nature of the treatment material will vary depending on the preferred implementation and/or the nature of the treatment being performed, including whether the treatment is cosmetic or therapeutic. Example treatment materials include, but are not limited to, nanoparticles, a nucleic acid, an antigen or allergen, parasites, bacteria, viruses, or virus-like particles, metals or metallic compounds, molecules, elements or compounds, DNA, protein, RNA, siRNA, sfRNA, iRNA, synthetic biological materials, polymers, drugs, or the like.

[0381] It will be appreciated that the use of coatings is not essential however, and additionally and/or alternatively treatment materials can be incorporated into the microstructures themselves.

[0382] Irrespective of how treatment materials are provided, the substrate can include a plurality of microstructures with different microstructures having different treatment materials and/or different treatment doses. In this case, the processing devices can control the therapy delivery mechanism to release treatment material from selected microstructures, thereby allowing different treatments to be administered, and/or allowing differential dosing, depending on the results of measurements performed on the subject. In particular, as will be described in more detail below, the processing devices typically perform an analysis at least in part using the measured response signals; and, use results of the analysis to control the at least one therapy delivery mechanism, thereby allowing personalised treatment to be administered substantially in real time.

[0383] It will be appreciated that microstructures could be differentially coated, for example by coating different microstructures with different coatings, and/or by coating different parts of the microstructures with different coatings. This could be used to allow different analytes to be detected at different depths, so that for example a different coating is used for part of the microstructure that enters the dermis as opposed to the viable epidermis. This could also be used to allow for detection of different analytes, or different levels or

concentrations of the same analyte. Additionally, at least some microstructures could remain uncoated, for example, to allow these to be used as a control, some may be partially coated, or may include a porous structure with an internal coating. It will also be appreciated that multiple coatings could be provided. For example, an outer coating could be provided that gives mechanical strength during insertion, and which dissolves once in-situ, allowing an underlying functional coating to be exposed, for example to allow analytes to be detected.

[0384] The nature of the coating and the manner in which this is applied will vary depending on the preferred implementation and techniques such as dip coating, spray coating, jet coating or the like, could be used, as described above. The thickness of the coating will also vary depending on the circumstances and the intend functionality provided by the coating. For example, if the coating is used to provide mechanical strength, or contains a payload material to be delivered to the subject, a thicker coating could be used, whereas if the coating is used for sensing other applications, a thinner coating might be required.

[0385] In one example, stimulation, such as chemical, biochemical, electrical, optical or mechanical stimulation, can be used to release material from the coating on the microstructure, disrupt the coating, dissolve the coating or otherwise release the coating.

[0386] In another example, the microstructures can be coated with a selectively dissolvable coating. The coating could be adapted to dissolve after a defined time period, such as after the microstructures have been present within the subject for a set length of time, in response to the presence, absence, level or concentration of one or more analytes in the subject, upon breaching or penetration of the functional barrier, or in response application of a stimulatory signal, such as an electrical signal, optical signal or the like. Dissolving of the coating can be used in order to trigger a measurement process, for example by exposing a binding agent, or other functional feature, so that analytes are only detected once the coating has dissolved.

[0387] In a further example, dissolving of the coating could be detected, for example through a change in optical or electrical properties, with the measurement being performed after the coating has dissolved. Thus, dissolving of the coating could be detected based on a change in a response signal.

[0388] In one example, the coating can be used to provide mechanical properties. For example, the coating can provide a physical structure that can be used to facilitate penetration of the barrier, for example by providing a microstructure with a smooth tapered outer profile. The coating can strengthen the microstructures, to prevent microstructures breaking, fracturing, buckling or otherwise being damaged during insertion, or could be used to help anchor the microstructures in the subject. For example, the coating could include hydrogels, which expand upon exposure to moisture, so that the size of the microstructure and coating increases upon insertion into the subject, thereby it harder to remove the microstructure.

[0389] The coating can also be used to modify surface properties of the microstructures, for example to increase or decrease hydrophilicity, increase or decrease hydrophobicity and/or minimize biofouling. The coating can also be used to attract or repel or exclude at least one substance, such as analytes, cells, fluids, or the like. The coating could also dissolve to expose a microstructure, a further coating or material, allowing this to be used to control the detection process. For example, a time release coating could be used to enable a measurement to be performed a set time after the patch has been applied. This could also be used to provide stimulation to the subject, for example by releasing a treatment or therapeutic material, or the like.

[0390] Thus, in one example, the system includes a plurality of microstructures and wherein different microstructures are differentially responsive to analytes. For example, different microstructures could be responsive to different analytes, responsive to different combination of analytes, responsive to different levels or concentrations of analytes, or the like.

[0391] In one example, at least some of the microstructures attract at least one substance to the microstructures and/or repel or exclude at least one substance from the microstructures. The nature of the substance will vary depending on the preferred implementation and may include one or more analytes, or may include other substances containing analytes, such as ISF, blood or the like. This can be used to attract or repel or exclude analytes, for example attracting analytes of interest, allowing these to be concentrated and/or sensed, or repelling or excluding analytes that are not of interest.

[0392] The ability to repel or exclude substances can also assist with preventing biofouling. For example, the microstructures could contain a material, or include a coating, such as polyethylene glycol (PEG), which generally repels substances from the surface of the microstructure. Reduction in biofouling could also be achieved based on a choice of microstructure material or structure of the microstructure e.g. coating the binding agent in the pores of a porous microstructure, surface coatings that release to expose a sensing surface when sensing is to be performed, permeable coatings such as a porous polymer e.g. a nylon membrane, a polyvinylidenefluoride coating, a polyphenylenediamine coating, a polyethersulfone coating, or a hydrogel coating such as a poly(hydroxyethyl methacrylate) or PEG coating; an isoporous silica micelle membrane; a protein membrane, such as a fibroin membrane; a polysaccharide membrane, such as a cellulose membrane or a chitosan membrane; or a diol or silane membrane; releasable coatings that interfere with biofouling material, and/or porous coatings. In particular embodiments, the microstructure is porous, and the binding agent is coated in the pores of the microstructure.

[0393] In another example, biofouling can be accounted for using a control. For example, a patch could include functionalised microstructures for analyte detection as well as un-functionalised microstructures that act as a control. Assuming both sets of microstructures are subject to similar levels of biofouling, changes in response signals measured via the un-functionalised microstructures can be used to quantify a degree of biofouling that has occurred. This can then be accounted for when processing signals from the functionalised microstructures, for example by removing any change in response signals arising from the biofouling.

[0394] In one example, the system includes an actuator configured to apply force to the substrate, which in one example is used to help the microstructures to breach the barrier. The actuator could additionally and/or alternatively be used for other purposes.

[0395] For example, movement of the microstructures could be used to sense tissue mechanical properties. For example, a response of the actuator, such as an amount of current required to induce movement of the microstructures, could be used sense mechanical properties, such as a degree of elasticity, or the like, which can in turn be indicative of health issues, such as diseases or similar. This could also be used in conjunction with mechanical

response signals, for example measuring a stress or strain on the microstructures using a suitable sensing modality, allowing the transmission of actuator movements to be monitored. Other external mechanical stimulus could also be used, such providing a ring or other structure around the patch, which generates pressure waves within the tissue, allowing the responses to be measured.

[0396] The actuator can be used to provide mechanical stimulation, for example to trigger a biological response, such as inflammation, or to attract or repel or exclude substances. Additionally, physical movement can be used to release material from a coating on at least some microstructures, or could be used to disrupt, dissolve, dislodge or otherwise release a coating on at least some microstructures. This can be used to trigger a measurement process, for example, releasing a coating or material to trigger a reaction with analytes, allowing the analytes to be detected.

[0397] The actuator can also be used to cause the microstructures to penetrate the barrier, or retract the microstructures from the barrier and/or the subject. In one example, this allows the microstructures to be inserted and removed from the subject as needed, so that microstructures can be removed when measurements are not being performed. This can be used to comfort, to reduce the chance of infection, reduce biofouling, or the like.

[0398] As the microstructures are provided in a low-density configuration, the force required is typically minimal, in which case this could be achieved utilising an actuator that provides a small force, such as piezoelectric actuator, or a mechanical actuator, such as an offset motor, vibratory motor, or the like. Other actuators could however be used, including any one or more of an electric actuator, a magnetic actuator, a polymeric actuator, a fabric or woven actuator, a pneumatic actuator, a thermal actuator, a hydraulic actuator, a chemical actuator, or the like. For example, a chemical or biochemical reaction, including exposure to air, light, water or other substance, could trigger exothermic release of energy, which can be used for to provide a mechanical impulse to urge the substrate and hence microstructures into the subject. It will also be appreciated that actuation could also be achieved manually, by applying a force to the patch, or by using a strap or similar to urge the patch against the subject.

[0399] In one specific example, this is achieved using a biasing force, for example provided by a spring or electromagnetic actuator, together with a vibratory, periodic or repeated force, which can assist with penetration, for example by agitating the microstructures to overcome the elasticity of the stratum corneum and/or reduce friction for penetrating the epidermis and/or dermis, as well as to reduce the force required to pierce a barrier. This reduces the overall force required to penetrate the stratum corneum. However, this is not essential and single continuous or instantaneous forces could be used.

[0400] The frequency of vibration used will vary depending upon the preferred implementation and potentially the type of skin to which the microstructures are applied, and could include any one or more of at least 0.01 Hz, 0.1 Hz, 1 Hz, at least 10 Hz, at least 50 Hz, at least 100 Hz, at least 1 kHz, at least 1 kHz, or at least 100 kHz and potentially up to several MHz. In one example, a varying frequency could be used. The frequency could vary depending on a wide range of factors, such as a time of application, and in particular the length of time for which the application process has been performed, the depth or degree of penetration, a degree of resistance to insertion, or the like. In one example, the system uses response signals measured via the microstructures in order to detect when the barrier has been breached, such as when the microstructures have penetrated the stratum corneum. Thus, the frequency could be continuously varied, either increasing or decreasing, until successful penetration is achieved, or depending on a depth of penetration, which can be detected using response signals, at which point the actuator can be deactivated. In another example, the frequency starts high and progressively reduces as the microstructures penetrate the barrier, and in particular the stratum corneum.

[0401] In another example, the magnitude of the applied force can also be controlled. The force used will vary depending on a range of factors, such as the structure of the patch, the manner in which the patch is applied, the location of application, the depth of penetration, or the like. For example, patches with large numbers of microstructures typically require an overall higher force in order to ensure penetration, although for minimal numbers of microstructures, such as 10 or so, a larger force may be required to account for damping or loss from the substrate/skin. Similarly, the force required to penetrate the stratum corneum, would typically be higher than that required to penetrate the buccal mucosa. In one example, the

applied force could be any one or more of at least 0.1 μN, at least 1 μN, at least 5 μN, at least 10 μN, at least 20 μN, at least 50 μN, at least 100 μN, at least 500 μN, at least 1000 μN, at least 10 mN, or at least 100 mN, per microstructure and/or collectively. For example, if there are 1000 microstructures, the force could be 100 mN in total, or 100 mN per projection, leading to an overall 100 N force.

[0402] Again, the force could vary, either increasing or decreasing, depending on a time of application, a depth or degree of penetration, which could be determined based on response signals, for examining a change in measured impedance, or an insertion resistance, or the like. In one specific example, the force is progressively increased until a point of penetration, at which point the force decreases.

[0403] As mentioned above, the force could be applied as a single continuous or instantaneous force. However, more typically the force is periodic. In this instance the nature of the periodic motion could vary, this could for example, have any waveform, including square waves, sine waves, triangular waves, variable waveforms, or the like. In this case, the force could be an absolute magnitude, or could be a peak-to-peak or Root Mean Square (RMS) force.

[0404] Similarly, a magnitude of movement of the microstructures can also be controlled. The degree of magnitude will depend on factors, such as the length of the microstructures and the degree of penetration required. The magnitude could include any one or more of greater than 0.001 times a length of the microstructure, greater than 0.01 times a length of the microstructure, greater than 0.1 times a length of the microstructure, greater than a length of the microstructure, greater than 10 times a length of the microstructure, greater than 100 times a length of the microstructure or greater than 1000 times a length of the microstructure. The magnitude may also vary, either increasing or decreasing, depending a time of application, a depth of penetration, a degree of penetration or an insertion resistance. Again, the magnitude may increase until a point of penetration and then decrease after a point of penetration.

[0405] In the above example, the system can be configured to detect aspects of the insertion process. In one example, this can be achieved by monitoring the actuator, for example, monitoring the current required by the actuator to achieve a specific movement,

which can in turn be used to detect, a depth of penetration, a degree of penetration an insertion resistance, or the like, with this then being used to control the actuator.

[0406] The actuator can also be used to apply mechanical stimulation, which could be used for a variety of purposes. For example, the actuator can be configured to physically disrupt or dislodge a coating on the microstructures, physically stimulate the subject, cause the microstructures to penetrate the barrier, retract the microstructures from the barrier or retract the microstructures from the subject.

[0407] The actuator is typically operatively coupled to the substrate, which could be achieved using any suitable mechanism, such as mechanical, electromechanical, or the like.

[0408] In one specific example, the actuator includes a spring or electromagnetic actuator to provide a constant bias, and at least one of a piezoelectric actuator and vibratory motor to apply a vibratory force. The vibratory force is applied at a frequency that is at least 10 Hz, less than 1 kHz and about 100-200 Hz. The continuous force is typically greater than 1 N, less than 10 N, less than 20 N, or about 5 N, whilst the vibratory force is at least 1 mN, less than 1000 mN and about 200 mN. The actuator is typically configured to cause movement of the microstructures that is at least 10 μm, less than 300 μm and about 50 μm to 100 μm.

[0409] In one example, the system includes a housing containing at least the sensor and one or more electronic processing devices, and optionally including other components, such as a signal generator, actuator, power supply, wireless transceiver, or the like. In one particular example, the housing provides reader functionality that can be used to interrogate the microstructures, and which can be provided in an integrated device, or could be provided remote to the substrate and engaged or provided in proximity with the substrate when readings are to be performed.

[0410] In the integrated configuration, the reader is typically mechanically connected / integrated with the patch during normal use, allowing measurements to be performed automatically. For example, continual monitoring could be performed, with a reading being performed every 1 second to daily or weekly, typically every 2 to 60 minutes, and more typically every 5 to 10 minutes. The timing of readings can vary depending on the nature of the measurement being performed and the particular circumstance. So for example, an athlete

might wish to undergo more frequent monitoring while competing in an event, and then less frequent monitoring during post event recovery. Similarly, for a person undergoing medical monitoring, the frequency of monitoring may vary depending on the nature and/or severity of a condition. In one example, the frequency of monitoring can be selected based on user inputs and/or could be based on a defined user profile, or the like.

[0411] In the integrated arrangement, the reader can be connected to the patch using conventional resistance bridge circuitry, with analogue to digital conversion being used to perform measurements.

[0412] Alternatively, the reader can be separate, which allows the reader to be removed when not in use, allowing the user to wear a patch without any integrated electronics, making this less intrusive. This is particularly useful for applications, such as sports, geriatric and paediatric medicine, or the like, where the presence of a bulkier device could impact on activities. In this situation, the reader is typically brought into contact or proximity with the patch allowing readings to be performed on demand. It will be appreciated that this requires a user/person to drive the interrogation. However, the reader could include alert functionality to encourage interrogation.

[0413] Readings could be performed wirelessly, optionally using inductive coupling to both power the patch and perform the reading as will be described in more detail below, although alternatively, direct physical contact could alternatively be used. In this example, the microstructures and tissue form part of a resonant circuit with discrete inductance or capacitance, allowing the frequency to be used to determine the impedance and hence analyte level or concentration. Additionally, and/or alternatively, ohmic contacts could be used, where the reader makes electrical contact with connectors on the patch.

[0414] In either case, some analysis and interpretation of the analyte level or concentration may be performed in the reader, optionally allowing an indicator to be displayed on the reader using an output, such as an LED indicator, LCD screen, or the like. Additionally, and/or alternatively, audible alarms may be provided, for example providing an indication in the event that the subject has an analyte level or concentration outside an acceptable range. The reader can also incorporate wireless connectivity, such as Bluetooth, Wi-Fi or similar, allowing

reading events to be triggered remotely and/or to allow data, such as impedance values, analyte level or concentration indicators, or the like to be transmitted to remote devices, such as a client device, computer system, or cloud based computing arrangement.

[0415] In use, the housing typically selectively couples to the substrate, allowing the housing and substrate to be attached and detached as needed. In one example, this could be achieved utilising any appropriate mechanism, such as electromagnetic coupling, mechanical coupling, adhesive coupling, magnetic coupling, or the like. This allows the housing and in particular sensing equipment to only be connected to the substrate as needed. Thus, a substrate could be applied to and secured to a subject, with a sensing system only being attached to the substrate as measurements are to be performed. However, it will be appreciated that this is not essential, and alternatively the housing and substrate could be collectively secured to the subject for example using an adhesive patch, adhesive coating on the patch/substrate, strap, anchor microstructures, or the like. In a further example, the substrate could form part of the housing, so that the substrate and microstructures are integrated into the housing.

[0416] When the housing is configured to attach to the substrate, the housing typically includes connectors that operatively connect to substrate connectors on the substrate, to thereby communicate signals between the signal generator and/or sensor, and the microstructures. The nature of the connectors and connections will vary depending upon the preferred implementation and the nature of the signal, and could include conductive contact surfaces, that engage corresponding surfaces on the substrate, or could include wireless connections, such as tuned inductive coils, wireless communication antennas, or the like.

[0417] In one example, the system is configured to perform repeated measurements over a time period, such as a few hours, days, weeks, or similar. To achieve this, the microstructures can be configured to remain in the subject during the time period, or alternatively could be removed when measurements are not being performed. In one example, the actuator can be configured to trigger insertion of the microstructures into the skin and also allow for removal of the microstructures once the measurements have been performed. The microstructures can then be inserted and retracted as needed, to enable measurements to be performed over a prolonged period of time, without ongoing penetration of the skin. However, this is not essential and alternatively short term measurements can be performed, in which case the time

period can be less than 0.01 seconds, less than 0.1 seconds, less than 1 second or less than 10 seconds. It will be appreciated that other intermediate time frames could also be used.

[0418] In one example, once measurements have been performed, the one or more electronic processing devices analyse the measured response signals to determine an indicator indicative of a health and/or physiological status of the subject.

[0419] In one example, this is achieved by deriving at least one metric, which can then be used to determine an indicator. For example, the system could be configured to perform impedance measurements, with the metric corresponding to an impedance parameter, such as an impedance at a particular frequency, a phase angle, or similar. The metric can then be used to derive indicators, such as an indication of analyte level or concentration.

[0420] The manner in which this is performed will vary depending upon the preferred implementation. For example, the electronic processing devices could apply the metric to at least one computational model to determine the indicator, with the computational model embodying the relationship between a health status and the one or more metrics. In this instance, the computational model could be obtained by applying machine learning to reference metrics derived from subject data measured for one or more reference subjects having known health statuses. In this instance, the health status could be indicative of organ function, tissue function or cell function, could include the presence, absence, degree or severity of a medical condition, or could include one or more measures otherwise associated with a health status, such as measurements of the presence, absence, level or concentration of one or more analytes, such as one or more biomarkers.

[0421] The nature of the model and the training performed can be of any appropriate form and could include any one or more of decision tree learning, random forest, logistic regression, association rule learning, artificial neural networks, deep learning, inductive logic programming, support vector machines, clustering, Bayesian networks, reinforcement learning, representation learning, similarity and metric learning, genetic algorithms, rule-based machine learning, learning classifier systems, or the like. As such schemes are known, these will not be described in any further detail. In one example, this can include training a single model to determine the indicator using metrics from reference subjects with a combination of

different health states, or the like, although this is not essential and other approaches could be used.

[0422] Measured signals can also be used in other manners. For example, changes in metrics over time can be used to track changes in a health state or medical condition for a subject. Measured signals can also be analysed in order to generate images or to perform mapping. For example, tomography could be used to establish a 2D or 3D image of a region of the subject based on impedance measurements or similar. The signals could also be used in contrast imaging, or the like.

[0423] In one example, the system can include a transmitter that transmits measured subject data, metrics or measurement data such as response signals or values derived from measured response signals, allowing these to be analysed remotely.

[0424] In one particular example, the system includes a wearable patch including the substrate and microstructures, and a monitoring device (also referred to as a "reader") that performs the measurements. The monitoring device could be attached or integrally formed with the patch, for example mounting any required electronics on a rear side of the substrate. Alternatively, the reader could be brought into contact with the patch when a reading is to be performed. In either case, connections between the monitoring device could be conductive (ohmic) contacts, but alternatively could be indicative coupling, allowing the patch to be wirelessly interrogated and/or powered by the reader.

[0425] The monitoring device can be configured to cause a measurement to be performed and/or to at least partially process and/or analyse measurements. The monitoring device can control stimulation applied to at least one microstructure, for example by controlling the signal generator and /or switches as needed. This allows the monitoring device to selectively interrogate different microstructures, allowing different measurements to be performed, and/or allowing measurements to be performed at different locations. This also allows microstructures to be selectively stimulated, for example, allowing different therapies to be applied to the subject. Thus by selectively stimulating microstructures, to thereby selectively release therapeutic materials, this could be used in order to provide dosage control, or to deliver different therapeutic materials.

[0426] The monitoring device could also be used to generate an output, such as an output indicative of the indicator or a recommendation based on the indicator and/or cause an action to be performed. Thus, the monitoring device could be configured to generate an output including a notification or an alert. This can be used to trigger an intervention, for example, indicating to a user that action is required. This could simply be an indication of an issue, such as telling a user they are dehydrated or have elevated troponin levels and/or could include a recommendation, such as telling the user to rehydrate, or seek medical attention or similar. The output could additionally and/or alternatively, include an indication of an indicator, such as a measured value, or information derived from an indicator. Thus, a hydration level or analyte level or concentration could be presented to the user.

[0427] The monitoring device could also be configured to trigger other actions.

[0428] The output could be used to alert a caregiver that an intervention is required, for example transferring a notification to a client device and/or computer of the caregiver. In another example, this could also be used to control remote equipment. For example, this could be used to trigger a drug delivery system, such as an electronically controlled syringe injection pump, allowing an intervention to be triggered automatically. In a further example, a semi-automated system could be used, for example providing a clinician with a notification including an indicator, and a recommended intervention, allowing the clinician to approve the intervention, which is then performed automatically.

[0429] In one example, the monitoring device is configured to interface with a separate processing system, such as a client device and/or computer system. In this example, this allows processing and analysis tasks to be distributed between the monitoring device and the client device and/or computer system. For example, the monitoring device could perform partial processing of measured response signals, such as filtering and/or digitising these, providing an indication of the processed signals to a remote process system for analysis. In one example, this is achieved by generating subject data including the processed response signals, and transferring this to a client device and/or computer system for analysis. Thus, this allows the monitoring device to communicate with a computer system that generates, analyses or stores subject data derived from the measurement data. This can then be used to generate an indicator at least partially indicative of a health status associated with the subject.

[0430] It will also be appreciated that this allows additional functionality to be implemented, including transferring notifications to clinicians, or other caregivers, and also allowing for remote storage of data and/or indicators. In one example, this allows recorded measurements and other information, such as derived indicators, details of applied stimulation or therapy and/or details of other resulting actions, to be directly incorporated into an electronic record, such as an electronic medical record.

[0431] In one example, this allows the system to provide the data that will underpin the growing telehealth sector empowering telehealth systems with high fidelity and accurate clinical data to enable remote clinicians to gain the information they require, and they will be highly valued both in central hospitals and in rural areas away from centralized laboratories and regional hospitals. With time to treatment a strong predictor of improved clinical outcomes with heart attack patients, decentralized populations cannot rely solely on access to conventional large-scale hospitals. Accordingly, the system can provide a low cost, robust and accurate monitoring system, capable for example of diagnosing a heart attack, and yet being provided at any local health facility and as simple as applying a patch device. In this example, resources could be dispatched quickly for patients who test positive to troponin I, with no delay for cardiac troponin laboratory blood-tests. Similarly patients determined to be low-risk could be released earlier and with fewer invasive tests, or funnelled into other streams via their GP etc.

[0432] In a further example, a client device such as a smart phone, tablet, or the like, is used to receive measurement data from the wearable monitoring device, generate subject data and then transfer this to the processing system, with the processing system returning an indicator, which can then be displayed on the client device and/or monitoring device, depending on the preferred implementation.

[0433] However, this is not essential and it will be appreciated that some or all of the steps of analysing measurements, generating an indicator and/or displaying a representation of the indicator could be performed on board the monitoring device.

[0434] Again, it will be appreciated that similar outputs could also be provided to or by a remote processing system or client device, for example, alerting a clinician or trainer that a

subject or athlete requires attention, that an intervention should be performed, controlling equipment, such as drug delivery devices, or the like.

[0435] The reader could be configured to perform measurements automatically when integrated into or permanently / semi permanently attached to the patch, or could perform measurements when brought into contact with the patch if the reader is separate. In this latter example, the reader can be inductively coupled to the patch.

[0436] Thus, it will be appreciated that functionality, such as processing measured response signals, analysing results, generating outputs, controlling measurement procedures and/or therapy delivery could be performed by an on-board monitoring device, and/or could be performed by remote computer systems, and that the particular distribution of tasks and resulting functionality can vary depending on the preferred implementation.

[0437] In one example, the system includes a substrate coil positioned on the substrate and operatively coupled to one or more microstructure electrodes, which could include microstructures that are electrodes, or microstructures including electrodes thereon. An excitation and receiving coil is provided, typically in a housing of a measuring device, with the excitation and receiving coil being positioned in proximity to the substrate coil in use. This is performed to inductively couple the excitation and receiving coil to the substrate coils, so that when an excitation signal is applied to the drive coil, this induces a signal in the substrate coil, which, in association with the electrodes and other reactive components on the substrate, may form a resonant circuit. As a result, the signal frequency, amplitude and damping (Q) of the resonant circuit on the substrate will be reflected in signal observed in the excitation and receive coil, which in turn alters the drive signal applied to the excitation and receiving coil, for example by changing the frequency, phase or magnitude of the signal, allowing this to act as a response signal, for example allowing a bioimpedance or biocapacitance to be measured.

[0438] This can be used in a variety of manners, but in one example, the one or more microstructure electrodes are configured to bind one or more analytes of interest, such that the response signal is dependent on a presence, absence, level or concentration of analytes of interest. This can be achieved in a variety of ways as discussed *supra*, such as coating the microstructures with a binding agent or forming the microstructures from material comprising

a binding agent, so that analytes interact with the microstructure electrodes, hence changing their electrical properties and thereby changing the characteristics of the response signal. For example, this could include having the analytes bind to a coating or the material forming the microstructure, such as an aptamer.

[0439] Detection of analytes could be performed in any manner, and this could involve examining changes in the response signal over time, for example as a level or concentration of analytes in the vicinity of the microstructure electrodes changes. Alternatively, in another example, two sets of microstructure electrodes are used, which are driven independently, with one acting as a control, and others being selectively responsive to one or more analytes so differences in measured signals are indicative of changes in analyte level or concentration.

[0440] In this example, the system typically includes a first substrate coil positioned on a substrate and operatively coupled to one or more first microstructure electrodes, a second substrate coil positioned on a substrate and operatively coupled to one or more second microstructure electrodes, the second microstructure electrodes being configured to interact with analytes of interest. At least one drive coil is positioned in proximity to at least one of the first and second substrate coils such that alteration, such as attenuation, or a phase or frequency change, of a drive signal applied acts as a response signal. In this case, the one or more electronic processing devices use the first and second response signals, and in particular difference between the first and second response signals to determine a presence, absence, level or concentration of one or more analytes of interest.

[0441] In the case of multiple substrate coil and electrode combinations forming resonant circuits, each may be intentionally designed by selection of fixed reactive components either inductive or capacitive to possess a different resonant frequency, thereby permitting a means of frequency based multiplexing of an entire array with a single excitation and receive coil.

[0442] A further example of a system for performing measurements in the biological subject will now be described with reference to Figures 3A to 3K.

[0443] In this example, the system includes a monitoring device 320, including a sensor 321 and one or more electronic processing devices 322. The system further includes a signal generator 323, a memory 324, an external interface 325, such as a wireless transceiver, an

actuator 326, and an input/output device 327, such as a touchscreen or display and input buttons, connected to the electronic processing device 322. The components are typically provided in a housing 330, which will be described below.

[0444] The nature of the signal generator 323 and sensor 321 will depend on the measurements being performed, and could include a current source and voltage sensor, laser or other electromagnetic radiation source, such as an LED and a photodiode or CCD sensor, or the like. The actuator 326 is typically a spring or electromagnetic actuator in combination with a piezoelectric actuator or vibratory motor coupled to the housing, to bias and vibrate the substrate relative to an underside of the housing, to thereby urge the microstructures into the skin, whilst the transceiver is typically a short-range wireless transceiver, such as a Bluetooth system on a chip (SoC).

[0445] The processing device 322 executes software instructions stored in the memory 324 to allow various processes to be performed, including controlling the signal generator 323, receiving and interpreting signals from the sensor 321, generating measurement data and transmitting this to a client device or other processing system via the transceiver 325. Accordingly, the electronic processing device is typically a microprocessor, microcontroller, microchip processor, logic gate configuration, firmware optionally associated with implementing logic such as an FPGA (Field Programmable Gate Array), or any other electronic device, system or arrangement.

[0446] In use the monitoring device 320 is coupled to a patch 310, including a substrate 311 and microstructures 312, which are coupled to the sensor 321 and/or signal generator 323 via connections 313. The connections could include physical conductive connections, such as conductive tracks, although this is not essential and alternatively wireless connections could be provided, such inductive coupling or radio frequency wireless connections. In this example, the patch further includes anchor microstructures 314 that are configured to penetrate into the dermis and thereby assist in securing the patch to the subject.

[0447] An example of the patch 310 is shown in more detail in Figures 3B and 3C. In particular, in this example the substrate 311 is generally rectangular, with round corners to avoid discomfort when the substrate is applied to the subject's skin. The substrate 311 includes

anchor microstructures 314 are provided proximate corners of the substrate 311 to help secure the substrate, whilst measurement microstructures 312 are arranged in an array on the substrate. In this example, the array has a regular grid formation, with the microstructures 312 being in provided in equally spaced rows and columns, but this is not essential and alternative spacing configurations could be used, as will be described in more detail below.

[0448] For example, in the arrangement of Figures 3D and 3E, three anchor microstructures 314.1, 341.2, 314.3 are provided, surrounded by respective circumferentially spaced microstructures 312.1, 312.2, 312.3. This can be useful to maximise the effectiveness of the anchor, specifically providing the microstructures 312 in close proximity to the anchor microstructures 314 to avoid movement of the microstructures 312 within the subject. Additionally, in this example, the anchor microstructures 314 could be used in measuring or applying signals, for example by acting as a ground connection, or similar.

[0449] In this example, the substrate is also formed from multiple substrate layers 311.1, 311.2, which can assist in creating internal structures, such as connections to the microstructures, coils, or the like, as will be described in more detail below. In a manner similar to that described below with respect to a backing, the substrate could also include different regions or layers having different material properties, or the like.

[0450] In this example, the anchor microstructure 314.1 is circular and includes a single surrounding group of circumferentially spaced microstructures 312.1. However, it will be appreciated that this is not essential, and in the case of the anchor microstructure 314.2, the anchor microstructure 314.2 is surrounded by two or more concentric groups of microstructure 312.2, with the outer group including a larger number of microstructures. This allows a greater range of measurements to be performed. It will be appreciated that other arrangements are also possible, such as providing further concentric groups, different numbers of microstructures in each group, or the like. Additionally, whilst circular groups are shown, this is not intended to be limiting, and other shapes or distributions could be used including oval shaped, square shaped, or similar.

[0451] In the case of the anchor microstructure 314.3 this is hexagonal, with six plate microstructures 312.3, each being positioned radially outwardly from a respective face of the

hexagonal anchor microstructure 314.3. In this manner measurements can be performed between each face of the anchor microstructure 314.23 and a respective microstructure 312.3, which can be useful to maximise a surface area of electrodes on each face and plate, whilst maintaining equidistant separation between the anchor and surrounding microstructures.

[0452] Whilst the above configurations have been described with respect to anchor microstructures, this is not essential and it will be appreciated that similar arrangements could be used with any drive or sense microstructure. Thus, in one example, a single drive microstructure could be used with multiple surrounding sense microstructure, or a single sense microstructure could be used with multiple surrounding drive microstructures. This provides an effective master slave arrangement, in which a single master drive/sense microstructure is used with multiple sense/drive microstructures.

[0453] Such master/slave relationships can be used in wide range of applications, for example to use a single drive signal to induce responses in multiple sense microstructures. In this example, this could be used for mapping, for example to identify different responses at different locations, and hence localise an effect, so as the presence of analytes or specific objects, such as lesions or cancer. Alternatively, this could be used with sense microstructures used to detect different analytes, for example using different coatings or similar, so that a single stimulation signal can trigger detection of different analytes.

[0454] In the example of Figures 3B and 3C, four connectors 315 are provided which are connected to respective microstructures 312 via connections 313 to allow stimulation signals and response signals to be applied to and measured from two sets of respective microstructures. This can be used to allow for symmetric or differential application and detection of signals, as opposed to asymmetric or single-ended application or detection, which is typically performed relative to a ground reference, and which is in turn generally noisier. However, it will be appreciated that for some detection modalities, such as optical detection, or the like, this is not relevant and single connections 315 may be provided.

[0455] In the example of Figures 3F and 3G, the housing 330 is a generally rectangular housing. The measuring device can optionally have a form factor similar to a watch, or other wearable device, in which case a strap 331 is included that allows the housing to be secured to

the user. However, this is not essential and other securing mechanisms could be used. Alternatively, the housing could simply be brought into engagement with the patch and held in position each time a measurement is performed. In this example, the housing includes coupling members 332, such as magnets, or the like, which can engage with corresponding coupling members 316 on the substrate allowing the substrate to be secured to the housing. Whilst any form of coupling member could be used, the use of magnets is particularly advantageous as these can be contained within the housing 330, allowing the housing to be sealed, and can also act to ensure correct alignment of the substrate 310, for example by having polarities of the magnets guide a relative orientation of the substrate 310 and housing 330.

[0456] An alternative example of the patch 310 is shown in more detail in Figures 3N and 3O. In this example the substrate 311 includes three rows of microstructures 312A, 312B, 312C arranged thereon, with each group of microstructures 312A, 312B, 312C being connected to a respective contact 315A, 315B, 315C, via respective connections 313A, 313B,313C. This can be used, for example to allow each row of microstructures 312A, 312B, 312C to function as a respective group, for example providing counter, reference and working electrode functionality, as will be described in more detail below.

[0457] However, it will be appreciated that this configuration is for the purpose of illustration only, and other arrangements could be used. For example, the substrate could form part of an adhesive patch, which is applied to the subject and retained in place. The housing 330, could then be selectively attached to the patch, for example, using magnetic coupling, thereby allowing measurements to be performed as needed.

[0458] In this example, the substrate could be a flexible substrate, which can be achieved using a woven or non-woven fabric or other suitable material, with microstructures directly attached thereto. More typically however, flexibility is achieved using a number of individual substrates 311 mounted on a flexible backing 319, to form a segmented substrate, as shown in Figure 3H. It will be appreciated that such arrangements can be used in a wide variety of circumstances, including having the substrates mounted to a strap or the like, for attachment to the subject.

[0459] A number of further variations are shown in Figures 3I to 3K.

[0460] Specifically in the example of Figure 3I, the backing 319 is formed from multiple backing layers 319.1, 319.2, with two being shown in the example for the purpose of illustration only. The use of multiple layers can be beneficial in achieving desired properties, for example to provide adhesive, or waterproof layers, or the like.

[0461] In the example of Figure 3J, the backing layer has multiple interspersed regions 319.3, which can be used for particular purposes, such as to allow for easier attachment of the substrates 311, to provide connectivity to a measuring device 320, to allow for increased flexibility between the substrates 311, or the like. In this example, interspersed regions are substantially aligned with the substrates, although it will be appreciated that this is not essential, and they could be provided at other locations.

[0462] A further example shown in Figure 3K, includes a number of shape modifications, including thinner regions 319.4, located between substrates, which could be used to enhance flexibility, thicker regions 319.5 between the substrates, which could increase strength. Similarly thinner or thicker regions 319.5, 319.6 could be provided in line with the substrates, for example to enhance strength, flexibility, connection to a measuring device, or the like.

[0463] Whilst these features have been described with reference to a backing layer, it will be appreciated that similar approaches could be used for the substrate itself.

[0464] An example of an actuator configuration to assist with applying a patch will now be described with reference to Figure 3L.

[0465] In this example, the housing 330 includes a mounting 333 to which the actuator 326, such as a piezoelectric actuator, or vibrating motor, is attached. The actuator 326 is aligned with an opening 334 in an underside of the housing 330, with an arm 326.1 coupled to the actuator 326 extending through the opening 334, which may be sealed using an O-ring 334.1, or other similar arrangement.

[0466] The patch substrate 311 is positioned adjacent the underside of the housing 330, with magnets 316, 332 being arranged to urge the substrate 311 towards the housing 330. The arm 326.1 engages the substrate to thereby transmit forces from the actuator 326 to the substrate 311, allowing the substrate and hence microstructures 312, 314, to be vibrated to aid insertion

of the microstructures into the subject. Specifically, this arrangement transmits forces directly to the substrate 311, allowing forces in the substrate to be maximised, whilst minimising vibration of the housing 330.

[0467] In the example of Figure 3L, the substrate also includes coupling members 316, such as magnets, which can be used to attach the substrate to the housing 330.

[0468] A further example actuator arrangement will now be described with reference to Figure 3M.

[0469] In this example, the actuator arrangement includes an actuator housing 335 having a base 335.1 including an opening 335.2. The housing contains a spring 336 and mounting 337, which in use supports a patch 310 (and optional integrated reader). The mounting also optionally contains a piezoelectric actuator or offset motor 338.

[0470] In use, the actuator housing 335 is positioned so that a base 335.1 of the housing 335 abuts against the subject's skin, with the patch at least partially projecting through the opening 335.2. In one example, this is achieved by having an operator hold the actuator housing. However, this is not essential and additionally and/or alternatively, the actuator housing could be integrated into and/or form part of a monitoring device as described above.

[0471] In use, the spring 336 is configured to apply a continuous biasing force to the mounting 337, so the patch 310 is urged against the subject's skin. Additionally, the piezoelectric actuator or offset motor 338 can cause the mounting 337, and hence patch 310, to vibrate, thereby facilitating piercing and/or penetration of the stratum corneum by the microstructures.

[0472] Example microstructure arrangements will now be described in more detail with reference to Figures 4 to 8.

[0473] In the example of Figure 4A, different length microstructures are shown with a first microstructure 412.1 penetrating the stratum corneum and viable epidermis, but not breaching the dermis, a second microstructure 412.2 entering the dermis but only just passes the dermal boundary, whereas a third microstructure 412.3 penetrates the dermal layer at greater distance.

It will be appreciated that the length of structure used will vary depending upon the intended application of the device, and specifically the nature of the barrier to be breached.

[0474] In the example of Figure 4B, pairs of microstructures are provided with a first microstructure pair 412.4 having a closer spacing and a second microstructure pair 412.5 having a relatively large spacing, which can be used to enable different properties to be detected, or different forms of stimulation to be performed.

[0475] For example, a greater electrode spacing can be used to perform impedance measurements of interstitial fluid and other tissues and liquids between the electrodes, whereas closer spaced electrodes are more suited to performing capacitive sensing to detect different analytes present on a surface of the electrodes.

[0476] Additionally, the electrical field strength generated by applying a signal to the first and second microstructure pairs are shown in Figures 4C and 4D, highlighting that the field strength between the electrodes decreases as the spacing increases, which in turn impacts on the ability to perform stimulation. For example, by providing an array of closely spaced microstructures, this can be used to generate a highly uniform field within the subject, without requiring a large applied field. This can be used to allow the field to be used for stimulation, for example, to perform electroporation, or the like.

[0477] The microstructures can have a range of different shapes. Specifically, these illustrate circular, rectangular, octagonal, cruciform, and star shapes. The shapes used will vary depending on the intended application. For example, larger numbers of the microstructures can be useful to provide multiple different electrode surfaces, whilst a greater overall surface area can be useful to maximise the amount of coating. Similarly, acute angled surfaces can, such as the cruciform and star arrangements, can allow coating to be used to provide an overall circular profile, with different coating depths around the microstructure.

[0478] A specific example of a plate microstructure is shown is shown in Figures 5A to 5C.

[0479] In this example, the microstructure is a plate having a body 512.1 and a tip 512.2, which is tapered to facilitate penetration of the microstructure 512 into the stratum corneum.

In this example, electrode plates 517 are provided on each side of the microstructure, with these being coupled via a single connection 513 to a connector 515 for onward connection to a sensor 321 and/or signal generator 323. This allows a signal to be measured from or applied to the electrode plates collectively. It will be appreciated however that this is not essential and independent connections could be provided allowing each of the electrodes to be driven or sensed independently. Additionally, each electrode 517 could be subdivided into multiple independent segments 517.1, 517.2, 517.3, 517.4, such that each face includes multiple electrodes.

[0480] As shown in Figures 5C and 5D, different arrangements could be used but in general, pairs of microstructures are formed with the microstructures facing each other allowing signals to be applied between the microstructures or measured between the microstructures. Again, different separations between electrodes in pairs of electrodes can be used to allow different measurements to be performed and/or to alter the profile of stimulation of the tissue between the electrodes.

[0481] A further example of a blade microstructure is shown is shown in Figures 5E and 5F.

[0482] In this example, the microstructure is an elongate body 512.1 and tip 512.2, which is tapered to facilitate penetration of the microstructure 512. This is generally similar in profile to the plate arrangement described above, but in this example is significantly wider, and in one particular example, can extend substantially the entire distance across the substrate. In this example, the microstructures include multiple electrode plates 517 on each side of the microstructure. In this case, the substrate can include multiple spaced parallel blades, allowing signals to be applied across or measured between the electrodes on different blades. However, it will be appreciated that other configurations could be used, such as providing a single electrode, segmented electrodes, or having the entire microstructure act as an electrode.

[0483] In the example, shown the blade tip is parallel to the substrate, but this is not essential and other configurations could be used, such as having a sloped tip, so that the blade penetrates progressively along the length of the blade as it is inserted, which can in turn

facilitate penetration. The tip may also include serrations, or similar, to further enhance penetration.

[0484] As mentioned above, in one example, microstructures are provided in a regular grid arrangement. However, in another example, the microstructures are provided in a hexagonal grid arrangement as shown in Figure 5G. This is particularly advantageous as each microstructure is equally spaced to all of the nearest neighbour microstructures, as shown by the arrows, meaning measurements can be performed relative to any adjacent microstructure without requiring response or stimulation signals to be modified to account for different spacings.

[0485] A further example arrangement is shown in Figures 5H to 5K, in which microstructures 512 are arranged in pairs 512.3, and with pairs arranged in offset rows, 512.4, 512.5. In this example, pairs in different rows are arranged orthogonally, so that the microstructures extend in different directions. This avoids all microstructures being aligned, which can in turn render a patch vulnerable to lateral slippage in a direction aligned with the microstructures. Additionally arranging the pairs orthogonally reduces interference, such as cross talk, between different pairs of electrodes, improving measurement accuracy and accounting for tissue anisotropy, particularly when measurements are being performed via multiple microstructure pairs simultaneously.

[0486] In one example, pairs of microstructures in each row can be provided with respective connections 513.41, 513.42; 513.51, 513.52, allowing an entire row of microstructure pairs to be interrogated and/or stimulated simultaneously, whilst allowing different rows to be interrogated and/or stimulated independently.

[0487] A Scanning Electron Microscopy (SEM) image showing an array of pairs of offset plate microstructures is shown in Figure 5K.

[0488] Specific examples of microstructures for performing measurements in the epidermis are shown in Figures 5L and 5M.

[0489] In this example, the microstructures are plates or blades, having a body 512.1, with a flared base 512.11, where the body joins the substrate, to enhance the strength of the

microstructure. The body narrows at a waist 512.12 to define shoulders 512.13 and then extends to a tapered tip 512.2, in this example, via an untapered shaft 512.14. Typical dimensions are shown in Table 2 below.

Table 2

Parameter	Min.	Typical	Max.	Units
Length	50	150	300	microns
Width	50	150	300	microns
Thickness	10	25	50	microns
Density	100	600	5000	cm^{-2}
Tip radius	0.1	1	5	microns
Surface area per electrode	2,000	22,500	200,000	$micron^2$
Buttress width at base	30	75	150	microns

[0490] An example of a pair of the microstructures of Figures 5L and 5M on insertion into a subject is shown in Figure 5N.

[0491] In this example, the microstructures are configured so that the tip 512.2 penetrates the stratum corneum *SC* and enters the viable epidermis *VE*. The waist 512.12, and in particular the shoulders 512.13 abut the stratum corneum *SC* so that the microstructure does not penetrate further into the subject, and so that the tip is prevented from entering the dermis. This helps avoid contact with nerves, which can lead to pain.

[0492] In this configuration, the body 512.1 of the microstructure can be coated with a layer of insulating material (not shown), with only the tip exposed. As a result a current signal applied between the microstructures, will generate an electric field *E* within the subject, and in particular within the viable epidermis *VE*, so that measurements reflect analyte levels or concentrations in the viable epidermis *VE*.

[0493] However, it will be appreciated that other configurations can be used. For example, in the arrangement of Figure 5O, the shaft 512.14 is lengthened so the tip 512.2 enters the dermis, allowing dermal (and optional epidermal) measurements to be performed.

[0494] In this example, typical dimensions are shown in Table 3 below.

Table 3

Parameter	Min.	Typical	Max.	Units
Length	50	250	450	microns
Width	50	250	450	microns
Thickness	10	30	50	microns
Density	100	600	5000	cm^{-2}
Tip radius	0.1	1	5	microns
Surface area per electrode	10,000	62,500	427,000	$micron^2$
Buttress width at base	30	75	150	microns

[0495] An example of the inter and intra pair spacing for these configurations are shown in Table 4 below.

Table 4

Parameter	Min.	Typical	Max.	Units
Separation between microstructures in a group or pair	10	100	1000	microns
Separation between groups of microstructures	200	500	1000	microns

[0496] Further example arrangements are shown in Figures 5P to 5U, in which microstructures 512 are arranged in groups 512A, 512B, 512C, with each group acting as working, reference and counter electrodes respectively. In each case, the microstructures in each group provide respective electrodes that are electrically connected, so that the group acts as a single electrode that penetrates the stratum corneum (or other functional barrier) at multiple locations, thereby improving the electrical connection between the working, reference and counter electrodes and the subject. Additionally, microstructures within the working group are typically functionalised, using an aptamer, MIP or similar.

[0497] In the example of Figure 5P, the microstructures 512 are arranged as parallel rows of plate microstructures, with microstructures in each row in the same orientation. In contrast, in the example of Figure 5Q, the microstructures within each group are arranged in pairs, with

adjacent pairs of microstructures orientated orthogonally. In these examples the groups are shown as rectangular regions, provided in abutment, with the reference group 512B positioned between the working and counter groups 512A, 512C. However, this is not essential, and other configurations can be used.

[0498] In general, the groups are arranged according to some basic guiding principles. For example, the counter electrode defined by the counter group 513C serves as the current reservoir for the three-electrode system and hence needs to be as large as possible to ensure that the working electrode defined by the working group 512A is never starved for electrons. However, since the size of the signal in an electrochemical aptamer based sensor is related to the surface area of the working electrode provided by the working group 513A, the counter electrode typically needs to be nearly as large as the working electrode. Conversely, the reference electrode defined by the reference group 513B is only required to maintain a stable potential over the bias voltage range of the sensor, and hence does not need to be as large. The effective size of the working, reference and counter electrodes are governed by the size and number of microstructures in each region. Accordingly, the reference group 513B typically includes less microstructures, and by virtue of the constant microstructure spacing, has is smaller physical size on the substrate than the working or counter groups 513A, 513C, which are in turn of a similar size and include similar numbers of microstructures.

[0499] The potential applied to the working electrode is with respect to this reference electrode, and so the reference group 513B, typically needs to be placed close, and preferable adjacent to, the working group 513A, so that the potential can be controlled without any potential drops in between.

[0500] It will be appreciated that this leads to be some flexibility over the physical layout of the groups, and alternative examples are shown in Figures 5R and 5S, which show abutting rectangular working and reference groups 512A, 512B, with a counter group 512C extending around three sides of the working group 512A and along either side of the reference group 512B.

[0501] In the examples shown in Figures 5T and 5U, three abutting rectangular working groups 512A1, 512A2, 512A3 are provided, with a single reference group 512B running along

one end of the working groups 512A1, 512A2, 512A3, and a counter group 513C extending around three sides of the working groups 512A1, 512A2, 512A3 and reference group 512B. This arrangement provides multiple working electrodes, each of which could be functionalised differently, allowing different measurements to be performed. For example, working groups 512A1, 512A2, 512A3 could be functionalised using different aptamers, allowing different analytes to be detected. In this example, different measurements would typically be performed at different times, for example using multiplexer, or other switching arrangements, to selectively measurement potentials and/or currents at the working electrodes.

[0502] In the above examples, the patch is substantially rectangular, but it will be appreciated that this is not essential and any configuration of patch could be used. Examples, of this are shown in Figures 5V and 5W, in which circular patches are used, with the groups including a central circular working electrode group 512A, and partial annular reference and counter electrode groups 512B, 512C, positioned radially outwardly from the working electrode group 512A.

[0503] Similarly, it will also be appreciated that the microstructures could be of different shapes, and could include microneedles, or other shapes, or combinations thereof.

[0504] A further example arrangement is shown at Figure 6A and 6B, with the microstructure again including a generally similar plate like arrangement, with the microstructure including spaced apart prongs 612.2, each having an electrode 617 thereon, so that the electrodes are on faces between the prongs 612.2, again allowing for the application of a highly uniform field, or to allow capacitive sensing to be performed.

[0505] A further example of a microstructure is shown at Figure 7A and Figure 7B, which includes a body 512.1 containing a core 513 that is conductive, covered by an insulating layer 512.1, which in one example could be a polymer or other material. In this instance, the core 513 terminates at an opening 513.2 allowing electrical signals to be communicated via the outlet. Additionally, and/or alternatively, ports 513.3 may also be provided extending through the insulating layer, allowing electrical signals to be communicated midway along the structure as shown at Figure 7B, allowing measurements to be performed at targeted depths within the viable epidermis and/or dermis.

[0506] It will also be appreciated that when pairs of microstructures are used, electrodes could be provided on an inner face of the pair only, for example, by insulating an outer face of the pair, to thereby reduce electrical interference between different pairs of microstructures.

[0507] An alternative technique for manufacturing microstructures will now be described with reference to Figures 8A to 8C.

[0508] In this example, a carrier wafer 891 is provided and spin coated with a photopolymer layer 892. The photopolymer layer 892 is selectively exposed to UV illumination and crosslinked, to create structural regions 892.1, which in this example form a substrate. A second photopolymer layer 893 is spun coated onto the first layer 891, and exposed to UV illumination and cross linked to form second structural regions 893.1, which in this example form microstructures, extending from the substrate. The carrier wafer and non-crosslinked polymer are removed to create the microstructures shown in Figure 8D.

[0509] It will be appreciated that this layering technique can be used to create a wide range of different microstructure configurations, and alternative design is shown in Figure 8E.

[0510] In one example, the monitoring device operates as part of a distributed architecture, an example of which will now be described with reference to Figure 9.

[0511] In this example, one or more processing systems 910 are coupled via communications networks 940, and/or one or more local area networks (LANs), to a number of client devices 930 and monitoring devices 920. The monitoring devices 920 could connect direction to the networks, or could be configured to connect to a client device 930, which then provides onward connectivity to the networks 940. It will be appreciated that the configuration of the networks 940 are for the purpose of example only, and in practice the processing systems 910, client devices 930 and monitoring devices 930 can communicate via any appropriate mechanism, such as via wired or wireless connections, including, but not limited to mobile networks, private networks, such as an 802.11 networks, the Internet, LANs, WANs, or the like, as well as via direct or point-to-point connections, such as Bluetooth, or the like.

[0512] In one example, each processing system 910 is configured to receive subject data from a monitoring device 920 or client device 930, and analyse the subject data to generate one

or more health status indicators, which can then be provided to a client device 930 or monitoring device 920 for display. Whilst the processing system 910 is a shown as a single entity, it will be appreciated that the processing system 910 can be distributed over a number of geographically separate locations, for example by using processing systems 910 and/or databases that are provided as part of a cloud based environment. However, the above described arrangement is not essential and other suitable configurations could be used.

[0513] An example of a suitable processing system 910 is shown in Figure 10.

[0514] In this example, the processing system 910 includes at least one microprocessor 1000, a memory 1001, an optional input/output device 1002, such as a keyboard and/or display, and an external interface 1003, interconnected via a bus 1004 as shown. In this example the external interface 1003 can be utilised for connecting the processing system 910 to peripheral devices, such as the communications network 940, databases 1011, other storage devices, or the like. Although a single external interface 1003 is shown, this is for the purpose of example only, and in practice multiple interfaces using various methods (e.g. Ethernet, serial, USB, wireless or the like) may be provided.

[0515] In use, the microprocessor 1000 executes instructions in the form of applications software stored in the memory 1001 to allow the required processes to be performed. The applications software may include one or more software modules, and may be executed in a suitable execution environment, such as an operating system environment, or the like.

[0516] Accordingly, it will be appreciated that the processing system 910 may be formed from any suitable processing system, such as a suitably programmed client device, PC, web server, network server, or the like. In one particular example, the processing system 910 is a standard processing system such as an Intel Architecture based processing system, which executes software applications stored on non-volatile (e.g., hard disk) storage, although this is not essential. However, it will also be understood that the processing system could be any electronic processing device such as a microprocessor, microchip processor, logic gate configuration, firmware optionally associated with implementing logic such as an FPGA (Field Programmable Gate Array), or any other electronic device, system or arrangement.

[0517] An example of a suitable client device 930 is shown in Figure 11.

[0518] In one example, the client device 930 includes at least one microprocessor 1100, a memory 1101, an input/output device 1102, such as a keyboard and/or display, and an external interface 1103, interconnected via a bus 1104 as shown. In this example the external interface 1103 can be utilised for connecting the client device 930 to peripheral devices, such as the communications networks 940, databases, other storage devices, or the like. Although a single external interface 1103 is shown, this is for the purpose of example only, and in practice multiple interfaces using various methods (eg. Ethernet, serial, USB, wireless or the like) may be provided.

[0519] In use, the microprocessor 1100 executes instructions in the form of applications software stored in the memory 1101 to allow communication with the processing system 910 and/or monitoring device 920.

[0520] Accordingly, it will be appreciated that the client devices 1130 may be formed from any suitable processing system, such as a suitably programmed PC, Internet terminal, lap-top, or hand-held PC, and in one preferred example is either a tablet, or smart phone, or the like. Thus, in one example, the client device 1130 is a standard processing system such as an Intel Architecture based processing system, which executes software applications stored on non-volatile (e.g., hard disk) storage, although this is not essential. However, it will also be understood that the client devices 1130 can be any electronic processing device such as a microprocessor, microchip processor, logic gate configuration, firmware optionally associated with implementing logic such as an FPGA (Field Programmable Gate Array), or any other electronic device, system or arrangement.

[0521] Examples of the processes for performing measurements and generating indicators will now be described in further detail. For the purpose of these examples it is assumed that one or more processing systems 910 acts to analyse received subject data and generate resulting indicators. Measurements are performed by the monitoring devices 920, with subject data being transferred to the processing systems 910 via the client devices 230. In one example, to provide this in a platform agnostic manner, allowing this to be easily accessed using client devices 930 using different operating systems, and having different processing capabilities, input data and commands are received from the client devices 930 using via a webpage, with resulting visualisations being rendered locally by a browser application, or other similar

application executed by the client device 930. The processing system 910 is therefore typically a server (and will hereinafter be referred to as a server) which communicates with the client device 930 and/or monitoring device 920, via a communications network 940, or the like, depending on the particular network infrastructure available.

[0522] To achieve this the server 910 typically executes applications software for hosting webpages, as well as performing other required tasks including storing, searching and processing of data, with actions performed by the processing system 910 being performed by the processor 1000 in accordance with instructions stored as applications software in the memory 1001 and/or input commands received from a user via the I/O device 1002, or commands received from the client device 1030.

[0523] It will also be assumed that the user interacts with the server 910 via a GUI (Graphical User Interface), or the like presented on the client device 930, and in one particular example via a browser application that displays webpages hosted by the server 910, or an App that displays data supplied by the server 910. Actions performed by the client device 930 are performed by the processor 1100 in accordance with instructions stored as applications software in the memory 1101 and/or input commands received from a user via the I/O device 1102.

[0524] However, it will be appreciated that the above described configuration assumed for the purpose of the following examples is not essential, and numerous other configurations may be used. It will also be appreciated that the partitioning of functionality between the monitoring devices 920, client devices 930, and the server 910 may vary, depending on the particular implementation.

[0525] An example of process for performing measurements on a subject will now be described in more detail with reference to Figures 12A and 12B.

[0526] In this example, a process for applying a patch including the substrate and microstructures is shown in steps 1200 to 1230, whilst a measurement process is shown in steps 1235 to 1260. In this regard, it will be appreciated that for patches that are used for performing multiple measurements over a period of time, steps 1200 to 1230 would only be performed a single time, with steps 1235 to 1260 being repeated as needed.

[0527] Furthermore, for the purpose of this example, it is assumed that the system includes a reader formed by the housing 330 and associated signal generator, sensor and processing electronics. The reader could be integral with the patch 310 and/or separate from the patch 310 depending on the preferred implementation.

[0528] At step 1200, the substrate is provided in a desired position, with the substrate and microstructures in place against the subject. At step 1205, assuming the reader is not integrated into the patch 310, the housing 330 is attached to the substrate 311, for example, by magnetically or otherwise coupling the housing and substrate, or by holding the housing in contact with the patch 310.

[0529] At step 1210, the processing device 322 selects a frequency/magnitude for the actuator. This can be a standard value and/or might depend on the barrier to be breached, so that different values might be selected for different sites on a subject, and/or for different subjects.

[0530] At step 1215, the actuator 326 is controlled, to thereby begin vibration of the microstructures, and hence facilitate movement of the microstructures within the subject.

[0531] At step 1220 stimulation is optionally applied, with response signals being measured at step 1225, allowing the processing device 322 to monitor breaching of the functional barrier and/or a depth of penetration. The mechanism for achieving this will depend on the nature of the response signals and optional stimulation. For example, the stimulation and response could be used to derive an impedance, with the impedance value altering as the microstructures penetrate the stratum corneum and enter the viable epidermis.

[0532] At step 1230, the processing device 322 optionally determines if breaching or penetration are complete and if not the process returns to step 1210 to select a different frequency and/or magnitude. Thus, this process allows the frequency and/or magnitude of any applied force to be adjusted continuously as the substrate and microstructures are applied, and in particular as the microstructures breach and optionally penetrate the functional barrier. In one example, this is used to allow the frequency to decrease during insertion, whilst the force progressively increases until the barrier is breached, at which point the force decreases. In this regard, it has been found that this can facilitate penetration of the barrier.

[0533] Once the patch is applied, measurements can commence. In this regard, if the reader is integrated into the patch, measurements can be performed as needed. Alternatively, if the reader is separate, this may require the reader be brought into proximity and/or contact with the patch, to allow a measurement to be performed.

[0534] In this example, at step 1235 the monitoring device 920 applies one or more stimulatory signals to the subject, and then measures response signal at step 1240. The response signals are measured by the sensor 321, which generates measurement data that is provided to the processing device 322 at step 1245.

[0535] In one example, the monitoring device 920 then transfers the measurement data to a client device 930 for further processing. In particular, the client device 930 might perform preliminary pre-processing of data and may append additional information, for example derived from onboard sensors, such as GPS or other like, to thereby add time or location information, or the like. This information can be useful in circumstances, such as tracking spread of infectious diseases or similar.

[0536] The resulting data is collated, for example by creating subject data, which can then be transferred to a server 910 allowing this to be analysed at step 1250. However, it will also be appreciated that the analysis could be performed on board the reader, and an indicator derived by performing the analysis could be displayed on the reader.

[0537] The nature of the analysis will vary depending on the preferred implementation and a wide range of options are envisaged.

[0538] When performing analyte level or concentration measurements, alternating electrical current signals are applied to the subject via a pair of microstructures, with resulting voltage signals being measured via the same microstructures. The magnitude and phase of the applied current and resulting voltage can then be used to calculate an impedance or capacitance value, which depends on analyte level or concentration within the subject. Accordingly, the measured impedance value can be correlated with an analyte level or concentration, allowing the progression of a disease, disorder or condition to be monitored or a disease, disorder or condition to be diagnosed, or the presence, absence, level or concentration of a medicament, illicit substance or non-illicit substance of abuse, or chemical warfare agent, poison or toxin to

be determined. For example, the subject data could be used in conjunction with previously collected subject data in order to perform a longitudinal analysis, examining changes in measured values over time. Additionally and/or alternatively, the subject data could be analysed using a machine learning model or similar.

[0539] One or more indicators are generated at step 1255, with the nature of the indicators and the manner in which these are generated varying depending upon the preferred implementation and the nature of the analysis being performed.

[0540] At step 1260 data, such as the subject data, the indicators, or the measurement data, are recorded allowing this to be subsequently accessed as needed. The indicator may also be provided to the client device 930 and/or monitoring device 920, allowing this to be displayed.

[0541] In one example, monitoring devices are allocated to respective users, with this allocation being used to track measurements for the subject. An example of a process for allocating a monitoring device 920 to a subject will now be described with reference to Figure 13.

[0542] In this example, the subject initially undergoes an assessment at step 1300, with this process being performed by a clinician. The clinician will use the assessment to guide the type of monitoring that needs to be performed, for example to identify particular biomarkers that are to be measured, which in turn may depend on any symptoms or medical diseases, disorders or conditions suffered by the subject. As part of this process, the clinician will typically acquire subject attributes at step 1310, such as measurement of weight, height, age, sex, details of medical interventions, or the like. This can be performed using a combination or techniques, such as querying a medical record, asking questions, performing measurements or the like.

[0543] Once the assessment has been completed, a monitoring device type can be selected at 1320, with this being performed based on the measurements that are required. In this regard, it will be appreciated that different combinations of microstructure arrangement and sensing modalities can be used in order to allow a range of different measurements to be performed, and it is therefore important that the correct selection is made to enable the measurements to be collected. A specific monitoring device 920 is then allocated to the subject at step 1330. In

this regard, in each device will typically include a unique identifier, such as a MAC (Media Access Control) address or other identifier, which can be used to uniquely associate the monitoring device with the subject.

[0544] At step 1340 the monitoring device 920 can optionally be configured, for example to update firmware or the instruction set needed to perform the respective measurements. At step 1350, a subject record is created, which is used to store details associated with the subject, including subject attributes, subject data, indicators, or any other relevant information. Additionally, the subject record will also typically include an indication of the monitoring device identifier, thereby associating the monitoring device with the subject.

[0545] An example of the process of using the device to perform measurements will now be described with reference to Figures 14A and 14B.

[0546] In this example, at step 1400 one or more measurements are performed. The measurements are performed by utilising the process described above, for example by having the monitoring device apply stimulatory signals and measure response signals. Measurement data is recorded based on the response signals with this being uploaded to the client device 930 at step 1405, allowing the client device 930 to generate subject data at step 1410. The subject data could simply be the measurement data, but may also include additional information provided by the client device 930. This allows user inputs to be provided via the client device 930, for example providing details of symptoms, changes in attributes or the like. The subject data is then uploaded to the server 910 at step 1415. The server 910 then retrieves one more subject attributes at step 1420, for example from the subject record, with the server 910 then calculating one or more metrics at step 1425.

[0547] At step 1430, the server 910 analyses the metrics. The manner in which this is performed will vary depending on the preferred implementation. For example, this could be achieved by applying the metrics to a computational model that embodies a relationship between a relevant health status and the one or more metrics. Alternatively, the metrics could be compared to defined thresholds, which can be established from a population of reference subjects, and which are used to represent certain diseases, disorders or conditions, such as the presence or absence of a medical condition. As a further option, the metrics could be compared

to previous metrics for the subject, for example to examine changes in the metrics, which could in turn represent a change in health status. The results of the analysis can be used to generate one or more indicators at step 1435. In one example, the indicator can be in the form of a score representing a health status, or could be indicative of a presence, absence or degree of diseases, disorders or condition.

[0548] At step 1440 the indicator can be stored, with an indication of the indicator being transferred to the client device 930 at step 1445, allowing the indicator to be displayed, either by the client device 930 or the monitoring device 920 at step 1450.

[0549] Additionally, and/or alternatively, at step 1455 the indicator can be used to determine if an action is required, for example if an intervention should be performed. The assessment of whether an action is required could be performed in any one of a number of manners, but typically involves comparing the indicator to assessment criteria defining a predetermined threshold or range of acceptable indicator values. For example, comparing a hydration indicator to a range indicative of normal hydration, or comparing an analyte indicator indicative of a normal level or concentration of analytes.

[0550] The assessment criteria can also specify the action required if the indicator falls outside of the acceptable range, and any steps required to perform the action, allowing the action to be performed at step 1460. For example, if certain analytes are detected, this could be indicative of a medical situation, in which the processing system or monitoring device could generate a notification which is provided to a clinician, or other nominated person or system, allowing them to be alerted. The notification could include any determined indicator and/or measured response signals, allowing the clinician to rapidly identify any interventions needed. In a theranostic application, the action could involve causing the applying monitoring device to apply a stimulation signal to electrodes, thereby allowing one or more therapeutic agents to be released. This could be performed in accordance with a dosing regime, which could be specified as part of the assessment criteria or defined manually by a clinician, for example in response to a notification provided as described above. Alternatively, the action could involve notifying the user, so for example, if the subject is dehydrated, the action could include having the monitoring device provide a recommendation to the user to hydrate.

[0551] It will therefore be appreciated that this enables actions to be triggered as needed.

[0552] The above described processes describe transfer of data to remote systems for analysis, which can have a number of benefits. For example, this allows more complex analysis to be performed than would otherwise be the case with existing processing capabilities. This also allows remote oversight, for example, allowing a clinician to access records associated with multiple patients, in real-time, enabling the clinician to respond rapidly as needed. For example, in the event that measured data shows an indication of a deleterious health state, the clinician could be alerted or notified, allowing an intervention to be triggered. Additionally, collective monitoring provides public health benefits, for example to allow tracking of infectious diseases or similar. Furthermore, central analysis allows data mining to be used in order refine analysis processes, making this more accurate as more data is collected.

[0553] However, it will be appreciated that the distributed implementation is not essential, and additionally or alternatively, analysis could be performed in situ, for example, by having the monitoring device 920 and/or client device 930 perform steps 1425 to 1460 with resulting information being displayed locally, for example, using the client device 930 or a in-built display.

[0554] A further example of a microstructure arrangement and analysis technique will now be described with reference to Figures 15A to 15F.

[0555] In this example, a patch 1510 is provided, including a substrate 1511 having a number of microstructures 512 thereon. The form and configuration of the microstructures is not critical for the purpose of this example, and it will be appreciated that a range of different configurations could be used, as described above.

[0556] In this example, the substrate 1511 includes a substrate coil 1515, positioned on the substrate 1511, typically on a rear surface. The coil is operatively coupled to the one or more microstructure electrodes, which could be electrodes provided on microstructures, or conductive microstructures themselves. Typically the substrate coil includes two ends, with each end being coupled to different microstructure electrodes, as shown by the dotted lines, so that a signal in the substrate coil 1511 is applied between the microstructure electrodes. An excitation and receiving coil (not shown) is provided, typically in a housing of a measuring

device, so that the excitation and receiving coil is aligned with and placed in proximity to the substrate coil when a measurement is to be performed, for example, when the housing is attached to the substrate. This is performed to inductively couple the excitation and receiving coil to the substrate coil, so that when an excitation signal is applied to the excitation and receiving coil by the signal generator, this induces a corresponding signal in the substrate coil 1515, which is then applied across the microstructure electrodes.

[0557] The tissue and/or fluid surrounding the microstructure electrodes, and the electrodes, act as capacitors, as shown. As a result, the excitation and receiving coil and the substrate coil act as a tuned circuit, and an example circuit configuration is shown in Figure 15B. This includes a fixed inductance 1561 and capacitance 1562 and resistance 1563, representing the inherent responsiveness of the excitation and substrate coils. The circuit also includes a variable capacitance and variable resistance 1565, 1564, representing the responsiveness of the microstructure electrodes, and the tissue or other materials between the electrodes. Thus, it will be appreciated that the frequency response and damping (Q) of the tuned circuit will vary depending on the values of the variable capacitance and resistance, which in turn depends on the environment within which the microstructure electrodes are present.

[0558] In general, when a signal is applied to the excitation and receiving coil, the overall response will be a constant amplitude signal in the excitation and receiving coil, as shown in Figure 15C. When the drive signal is halted, the circuit will continue to resonant, with the resulting signal decaying over time as shown to the right of the dotted line. The rate and/or frequency of the decay depends on the values of the variable capacitance and resistance, so different responses 1581, 1582 will arise depending on conditions within the subject, which in turn allows information regarding conditions within the subject to be derived. For example, this can be influenced by binding of analytes to the microstructure electrode, fluid levels, or the like, so examining changes in the decay rate and frequency can be used to derive information regarding the presence of analytes, fluid levels, or the like.

[0559] However, as decay signals are transient, in another example the circuit's response at different frequencies is analysed and used to determine the resonant frequency and Q factor of the tuned circuit, which are in turn indicative of the resistance and capacitance values. In

this regard, a change in electrical conditions within the subject will result in a change in the frequency response, as shown in Figure 15D. For example, a response in absence of analytes might be as shown in solid lines, whereas the presence of analytes might result in an increase or decrease in the resonant frequency and/or Q factor, as shown in dotted lines.

[0560] In one particular example, in order to be able to more accurately interpret the response, it is preferable to provide a control reference. An example of this is shown in Figure 15E, in which two patches 1510.1, 1510.2, are provided, each having a respective substrate 1511 microstructures 1512 and substrate coils 1515. In this example, the patch 1510.2 is coated with a binding agent to attract analytes of interest, whilst the patch 1510.1 is uncoated and acts as a control.

[0561] In this case, each substrate coil is driven and alterations, including attenuation and/or frequency or phase changes of the signal are measured, which will depend on the resonant frequency and Q factor. Example altered drive signals are shown in Figure 15F, with the signals 1571 representing a control obtain for the patch 1510.2, and the signals 1571.11, 1571.12 and 1571.21, 1571.22 representing different response obtained for the patch 1510.2, respectively. In this regard, the signals 1571.11, 1571.21 represent applied signals with no analytes, highlighting how different patches can have different tuned frequency responses, and with the signals 1571.12, 1571.22, showing changes in frequency δ_1, δ_2, which highlight how different responses can be measured, which can in turn be used to derive information regarding the level or concentration of analytes in the vicinity of the microstructures of the second patch 1510.2.

[0562] The measurement of the changes in frequency occurring in response to different analyte levels or concentrations may also be performed in the frequency domain by use of a return-loss-bridge circuit in the excitation coil. In this manner, the absorption of rf electromagnetic signal while being swept over a range of frequencies will show a signal loss in decibels (dB) at the resonant frequency of the substrate coil. The frequency and depth of this absorption will be indicative of the analyte level or concentration.

[0563] It will be appreciated that this technique employs a patch with no electronically active sensing elements, whilst allowing measurements to be made regarding conditions within

the subject, such as the presence, absence, level or concentration of analytes to be easily determined. It will also be appreciated that suitably adapting the coating allows a range of different analytes to be sensed and that this can also be adapted for performing other suitable measurements.

[0564] However, this is not essential, and in some examples sensing electronics could be partially or wholly incorporated within the patch.

[0565] An example of a driving and sensing arrangement for a working / reference / counter electrode configuration will now be described with reference to Figure 15G.

[0566] In this example, the circuit includes a signal generator *A1*, reference amplifier *A2* and signal amplifier *A3*, which acts as the detector for a cyclic voltammetry system. In use, a ramp oscillator input *Vin* sweeps the desired voltage range to interrogate the redox moiety used in the aptamer sensor. This conditioned signal is applied to the counter electrode *CE*, formed from a respective counter group of microstructures. To correct for the impedance of the medium, a reference electrode *RE*, formed from a respective counter group of microstructures, senses the error, buffers this signal using the reference amplifier *A2* and applies negative feedback to the input drive signal. The loop gain of this feedback system is determined by the ratio of the resistor inputs to the inverting input of signal generator *A1*. Output current of the sensor obtained via the working group of microstructures is converted to a voltage by the transimpedance amplifier stage *A3*, with the resulting voltage *Vout* being used with the input to derive a current-voltage characteristics. The current amplitudes at predetermined voltages are proportional to the aptamer-target binding activity.

[0567] Further details exemplifying the above described arrangements will now be described.

Manufacture

[0568] Example process for manufacturing a substrate including microstructures will now be described in more detail.

[0569] In a first example, shown in Figures 17A to 17P, microstructures are made from an insulating polymer applied to a substrate, with electrodes patterned on the substrate through

selective etching to act acting as electrical connections for the polymer microstructures. It will be also be appreciated that conductive polymers could be used, for example through suitable doping of an insulating polymer.

[0570] In this example, a first step shown in Figures 17A to 17G is to selectively pattern an electrode architecture onto a flexible polyethylene terephthalate (PET) substrate 1701. An electrode design, upon which microstructures were to be defined, was patterned on the PET; in this case Indium Tin Oxide (ITO) 1702 layer deposited atop flexible PET substrate, and the electrode pattern selectively etched from the ITO layer. The substrate was prepared (Fig. 17A), before a positive photoresist, AZ1518 (MicroChemicals), was patterned on top of the ITO via photolithography (Fig. 17B), and soft baked (Fig. 17C). The photoresist is selectively exposed to UV (Fig. 17D) to define an electrode pattern, before the photoresist is baked and developed using a developer AZ 726MIF (MicroChemicals) (Fig. 17E) and the exposed ITO regions wet acid etched (Fig. 17F). The photoresist was removed to reveal the final etched ITO pattern that provides the conductive electrodes for the device (Fig. 17G).

[0571] In a second step, shown in Figures 17H to 17P, 3D microstructures were fabricated from photosensitive polymers onto the ITO electrodes. The patterned PET substrate with ITO electrodes was treated with an oxygen plasma (Fig. 17H), to improve wetting and resist adhesion, and a seed adhesion layer of SU-8 3005 (MicroChemicals) 1704 was spin-coated on to the ITO-PET substrate (Fig. 17I). After baking of the seed SU-8 layer lamination (Fig. 17J) an SUEX SU-8 film resist 1705 (DJ MicroLaminates) was bonded to the substrate (Fig. 17K) through thermal lamination. After alignment and exposure to UV through a mask aligner (Fig. 17L), the exposed SU-8 areas crosslinked to form rows of rectangular microstructures 1706 with vertical wall profile along the conductive ITO fingers 1702 (Fig. 17M). The structures are baked, with the SU-8 1704 and SUEX 1705 before being developed in PGMEA (Propylene glycol monomethyl ether acetate) (Sigma Aldrich), and then hard baked (Fig. 17N). A shadow mask 1708 is applied to the substrate 1701 with the microstructures 1706 being coated with gold 1707 (Fig. 17O) through selective deposition, before the mask is removed (Fig. 17P), leaving selectively metallized microstructures that act as electrodes.

[0572] In this example the microstructures have flat tips, but it will be appreciated that other UV lithography techniques such as greyscale lithography, backside diffraction lithography, 2 photon lithography etc. could be employed to define tapered microstructures.

[0573] Resulting microstructures are shown in Figures 18A to 18D, with further examples shown in Figures 18E to 18G.

[0574] In a second example, shown in Figures 19A to 19L, microstructures are made by molding.

[0575] In this example, a silicon wafer 1901 was deposited with a 90 nm layer 1902 of Nitride (Fig. 19A). AZ1505 (MicroChemicals) positive resist 1903 was then spun on at 4000 rpm (Fig. 19B). Rectangular pattern to define the blade outline was directly written using a mask writer 1904 (Fig. 19C). The written pattern was developed using AZ 726 MIF (MicroChemicals) for 30 secs (Fig. 19D). Reactive ion etching is used to remove the nitride layer 1902 (Fig. 19F), before the photoresist 1913 is removed (Fig. 1919E). The wafer is then held vertically in a bath of Potassium Hydroxide at 80 °C for 40 mins, to etch the silicon wafer along the crystal axis of the wafer (Fig. 19G). The etching stops at the axis 111 thus defining the sharp tips needed, this then acts as a mold for the devices that are fabricated.

[0576] Omni-Coat is used as a lift off resist and is coated onto the wafer to a thickness of about 20 nm, using a spin recipe of 3000 RPM for 1 min and then baking at 200 °C for 1 min. Following this a 5 micron layer 1905 of SU8 3005 is spun on to the wafer at 3000 RPM following by baking at 65 °C for 1 min, then at 95 °C for 20 secs followed by 65 °C again for 1 min (Fig. 19H). The thinner formulation of the SU8 3005 would allow it to flow more easily into the sharp triangular crevices etched into the silicon wafer mold. A layer 2016 of SU8 1900 is then spun on top of this layer to a thickness of 200 microns using a spin recipe of 2000 RPM for 60 secs (Fig. 19I). Following this the wafer was baked at 65 °C for 5 mins, then at 95 °C for 35 mins and then again at 65 °C for 5 mins. This layer of SU8 1900 would allow the sharp tips to stand on a solid layer.

[0577] Finally the wafer is flood exposed using an Ultra Violet source 1907 delivering 15mW/cm^2 of Power for 40 secs (Fig. 19J). The structures are released by soaking the wafer in an AZ 726 developer solution overnight (Fig. 19K) and exposed the wafer to a thermal shock

of 120°C for 15 secs. The structures are removed from the mold flipped and dried using Nitrogen gas (Fig. 19L).

[0578] Resulting microstructures are shown in Figures 20A, 20B, 20C and 20D, with additional examples shown in Figures 20E to 20F.

[0579] Figures 21A and 21B show silicon blades fabricated via etching. Figure 21A shows the blade coated with a nearly 1 micron thick layer of SU8 3005 which has been diluted in a ratio of 3:2 using SU8 thinner and spun at 5000 RPM for 40 secs. Figure 21B gives a depiction of the blade selectively coated at its base with the polymer coating. While the tip of the blade is bare and available for detection purposes only at this area. This selective coating is achieved by pressing and removing the coated blade in Figure 21A into a thin layer of Aluminium foil which mechanically removes the resist from the tip of the blade. This allows the blade to be partially covered with an insulative coating, so that only the tip portion acts as an electrode, thereby allowing measurements to be performed in the epidermis and/or dermis, as described above with respect to Figures 5L and 5M.

[0580] Further example microstructures are shown in Figures 21C and 21D. In this example, the microstructures are selectively coated with a dielectric coating on the base of microstructures, leaving an electrically conductive microstructure body exposed away from the base, allowing the body of the microstructure to act as an electrode.

Analyte Detection Examples – Aptamers

[0581] Analyte detection has been demonstrated using aptamers.

[0582] To demonstrate the effectiveness of aptamers, experiments were performed to detect troponin. All chemicals and reagents used are commercially available from, for example, Sigma-Aldrich Co. LLC, unless otherwise specified.

[0583] A troponin specific aptamer with the following sequence was obtained (Bioneer Pacific): 5'-(SH)-$(CH_2)_6$-AGT CTC CGC TGT CCT CCC GAT GCA CTT GAC GTA TGT CTC ACT TTC TTT TCA TTG ACA TGG GAT GAC GCC GTG ACT G-[Methylene blue]-3' as previously described in Negahdary *et al.* (2018) *J. Biomed. Phys. Eng.*, 8(2): 167. The methylene blue (MB) and the thiol group were covalently attached to the 5' and 3' ends of the

aptamer using standard techniques, such as those described in Liu *et al.* (2010) *Anal Chem*, 82(19): 8131-8136, the contents of which is incorporated herein by reference. The aptamers were immobilised to the gold electrode by forming thiol self assembled monolayers. This was achieved by drop casting 10 μM aptamer in 150 mM PBS on the electrode for 80 minutes, and removing excess solution. The electrode was washed with deionised water and dried with nitrogen, before the process was repeated with 1mM 6-mercaptohexanol in 150 mM PBS for 40 minutes, before rinsing and drying as above. The electrode was stored in PBS in the dark at 4°C for up to 7 days before use.

[0584] This aptamer is composed of three distinct elements as shown in Figure 22A and 22B. In this example, the aptamers include a thiol group 2202 for adhesion to gold electrodes 2201, a DNA section 2203 in the middle that interacts to specifically bind troponin I 2205, and a methylene blue (MB) moiety 2204 attached to the 3' end. The MB is electrochemically active, thus when it comes into proximity of the electrode at a certain potential it will oxidize or reduce, producing a measurable current. When in the presence of troponin I, as shown in Figure 22B, the aptamers adopt a significantly different spatial conformation to unbound aptamers shown in Figure 22A, with the result being the MB moieties are less able to interact with the electrode and the measurable redox current is therefore smaller.

[0585] An experiment was performed using cyclic voltammetry to detect electrical changes in aptamer-coated microstructures provided in perfused pig skin. The following steps were used:

- Microstructures were coated with gold on the front (protrusion side) and on one of the patch edges, with the protrusion side further coated in a layer of aptamers as described above.
- Copper wire was soldered to the gold covered edge to provide an electrical contact. Silver foil coated in AgCl was used as a pseudo-reference/counter electrode and was placed under the skin near the microstructure.
- 40 N of pressure was used to push the microstructures into the skin, which were held in place with surgical clamps during the measurements.
- Data was measured with alternating current voltammetry to boost the signal obtained from the redox of the MB groups.

- Starting at 25 minutes, 5 mL of perfusate containing 600 ng recombinant troponin I/mL was introduced over the course of 10 minutes, with massaging the vein in between measurements to help diffusion into the surrounding tissue.

[0586] Results in Figure 23 show the effect on an aptamer-functionalized microstructure of adding troponin I in perfusate to a vein in a pig ear. The 0 min and 20 min curves establish a baseline for the size of the MB redox peak, then at 25 minutes troponin I was introduced into the vein. Voltammograms measured at 30 mins, 60 mins, and 120 mins show the decreased current response of MB with troponin I exposure, indicating that the patch quickly responds to the analyte and maintains a constant signal.

[0587] It is possible that this consistency of the signal over the course of the experiment is due to saturation of the aptamer layer with troponin I, and therefore does not show the changing levels of troponin in the system as more perfusate is injected.

[0588] A further experiment was performed using aptamer-functionalized disk electrodes to establish specificity of the aptamer-functionalized electrodes to troponin I over a nonspecific protein. These data were measured *in vitro*, measuring the current response of aptamer-functionalized gold disk electrode in phosphate buffered saline (PBS) with increasing amounts of recombinant troponin I added to the solution. The response to bovine serum albumin (BSA) was also measured to assess selectivity by exposure to a possible confounding compound. The following steps were performed:

- Gold disk electrodes (4 mm diameter) were coated in a layer of aptamers (prepared as described above). A coiled platinum wire was used as a counter electrode, and an Ag/AgCl wire was used as a pseudo reference electrode.
- Data was measured with alternating current voltammetry to boost the signal obtained from the redox of the MB groups.
- 150 mM PBS was used (pH 7.4) as a proxy for interstitial fluid.

[0589] Results are shown in Figures 24A and 24B. Figure 24A shows current response of the MB in PBS as a baseline measurement, with the response decreasing with increasing concentrations of troponin I. The range of concentrations covers the 0.03-50 ng/mL clinically

relevant range of troponin I in solution and are differentiable. Concentration curve data were measured once 5, 10 and 15 minutes had passed after spiking the solution with troponin I, then were averaged. It was assumed that an aptamer-troponin I equilibrium was established in the first few minutes because there were no systematic changes in the voltammograms between 5, 10, and 15 min. Figure 24B shows the current response of the MB in PBS and a solution of 50 ng/mL BSA. There was no change in signal upon exposure to BSA, indicating that the troponin I aptamer functionalised electrode selectively detects troponin I.

[0590] A further *in vitro* experiment was performed to generate a response curve for troponin I detection. The current response of aptamer-functionalized gold disk electrode in phosphate buffered saline (PBS) with increasing amounts of recombinant troponin I (10 ng/mL to 1000 ng/mL) added to the solution was measured. The following steps were performed:

- A gold disk electrode (4 mm diameter) was cleaned using successive sonication in acetone, isopropanol and deionised water for 5 mins at each step, followed by a blow dry in a stream of nitrogen gas.
- 5 μL of 50 μM of the aptamer described above was mixed with 10 μL of 1 mM reducing agent (DTT or TCEP) and left for 20 mins at room temperature.
- 150 μL pure ethyl acetate was added, the solution was inverted 10 times and was allowed to separate for three mins.
- The ethyl acetate layer was discarded. The ethyl acetate addition and removal steps were repeated 4 times.
- 235 μL PBS was added (to result in a 1 μM aptamer solution), and the solution was mixed.
- 30 μL was dropped on the gold electrode surface, covering both the working and counter electrode, and the electrode was incubated for 80 mins at 4 °C in the dark.
- The electrode was washed with PBS and excess liquid was flicked off.
- 50 μL of 1 mM 6-mercaptohexanol (MCH) in PBS was dropped onto the aptamer functionalised electrode, covering both the working and counter electrode, and the electrode was incubated for 40 mins at room temperature in the dark.
- The aptamer functionalised electrode was rinsed with PBS, and was stored in PBS at 4 °C in the dark until use.

- A gold plate was used as a counter electrode, and Ag/AgCl (3 M KCl) was used as a pseudo reference electrode.
- Data was measured with square wave voltammetry to boost the signal obtained from the redox of the MB groups.
- 10 mM PBS (pH 7.4) containing 5 mM NaCl, 2 mM KCl and 1 mM $MgCl_2$ was used as a proxy for interstitial fluid.

[0591] Detection of troponin I was done via three electrode measurements where functionalised gold disks act as the working electrode, a gold plate as the counter electrode and Ag/AgCl (3M KCl) as the reference electrode. Data was measured once 5, 10 and 15 minutes had passed after spiking the solution with troponin I and three traces were averaged at each time point. The response to human serum albumin (HSA) was also measured to assess selectivity by exposure to a possible confounding compound using this method. The concentration of the HSA ranged from 10 to 1000 ng/mL, which was the same range as the troponin I protein spiked into the PBS.

[0592] The results are shown in Figures 28A, B and C, and demonstrate a troponin I concentration dependent change in the signal (Figures 28A and 28B). No significant change in signal was observed upon HSA addition (Figures 28A and 28C).

[0593] Further experiments were performed to exemplify IL-6 detection. A gold electrode (gold disk electrode, 4 mm diameter) was cleaned using successive sonication in acetone, isopropanol and deionised water for 5 mins at each step, followed by a blow dry in a stream of nitrogen gas.

[0594] A human IL-6 aptamer (catalogue no. ATW0035; https://www.basepairbio.com/il-6-aptamer-atw0035/) was purchased from Base Pair Biotechnologies, Inc. (Pearland, Texas, USA). A methylene blue group ($C_{16}H_{18}ClN_3S$) was covalently attached to the 3' end and a thiol linker was attached to the 5' end to give an aptamer with the following general sequence: 5'-SH-$(CH_2)_6$-[human IL-6 aptamer]-$C_{16}H_{18}ClN_3S$-3'. The thiol linker and methylene blue (MB) group were covalently attached to the 5' and 3' ends of the aptamer by Integrated DNA Technologies, Inc. (Coralville, Iowa, USA). For example, thiol linker and MB groups may be

attached using standard techniques, such as those described in Liu *et al.* (2010) *Anal Chem*, 82(19): 8131-8136, the entire contents of which is incorporated herein by reference.

[0595] The aptamers were immobilised to the gold electrode by forming thiol self assembled monolayers. In brief, an aliquot of IL-6 aptamer (5 µL of 50 µM aptamer solution) and 20 µL of folding buffer (provided by Base Pair Biotechnologies, Inc. with purchase of aptamer) were mixed and left to stabilise for five mins. The mixture was then placed in a water bath at 90-95 °C for five mins, followed by cooling to room temperature for 15 mins. 25 µL of reducing buffer (provided by Base Pair Biotechnologies, Inc with purchase of aptamer) was added to the mixture and the mixture was left to stand at room temperature for one hour in the dark. The solution was diluted to an aptamer concentration of 1 µM by adding 200 µL PBS with 1mM $MgCl_2$, followed by briefly vortexing.

[0596] The aptamer was attached to the gold electrode by dropping approximately 30 µL of the aptamer solution onto the electrode surface, covering both the working and counter electrode, followed by incubation for 2 hours at room temperature in the dark. The electrode was then washed with PBS containing 1 mM $MgCl_2$ and excess liquid was flicked off. Approximately 50 µL of 1 mM MCH in PBS was dropped onto the aptamer functionalised electrode, covering both the working and counter electrode, and was left for 1 hour at room temperature in the dark. The functionalised electrode was then rinsed with PBS with 1 mM $MgCl_2$ and stored in the dark in PBS with 1 mM $MgCl_2$ at 4 °C until use.

[0597] As per the above example, this IL-6 aptamer is composed of three distinct elements as shown in Figure 22A and 22B. In this example, the aptamers include a thiol linker 2202 at the 5' end for adhesion to gold electrodes 2201, a DNA section 2203 in the middle (aptamer) that specifically binds human IL-6 2205, and a methylene blue (MB) moiety 2204 attached to the 3' end. The MB is electrochemically active, thus when it comes into proximity of the electrode at a certain potential it will oxidize or reduce, producing a measurable current. When in the presence of IL-6, as shown in Figure 22B, the aptamers adopt a significantly different spatial conformation to aptamers in the absence of IL-6 shown in Figure 22A (i.e. the MB moiety is further away from the electrode), with the result being the MB moieties are less able to interact with the electrode and the measurable redox current is therefore smaller.

[0598] The ability of the functionalised electrode to detect human IL-6 was determined *in vitro* by submerging the electrode in a PBS solution containing 1 mM $MgCl_2$ (as a proxy for interstitial fluid). An Ag/AgCl wire was used as a pseudo reference electrode, and was also submerged into the solution. Increasing amounts of human IL-6 (R&D Systems, Minnesota, USA) was spiked into the solution to result in an IL-6 concentration from 25 to 1000 ng/mL and the change in current was measured at a frequency of 260 Hz using square wave voltammetry. An IL-6 concentration dependent change in the signal was observed (Figures 29A and 29B), with a negative signal change indicating that the MB moiety is further away from the electrode when IL-6 is bound (i.e. a reduced signal).

[0599] The selectivity of the human IL-6 aptamer-functionalised electrode for detection of human IL-6 was assessed by spiking the solution with increasing amounts of human IL-6 or recombinant troponin I. In brief, a human IL-6 aptamer-functionalised electrode prepared as above was submerged in a PBS solution containing 1 mM $MgCl_2$. Ag/AgCl (3M KCl) was used as a reference electrode, and was also submerged into the solution. Increasing amounts of recombinant troponin I or human IL-6 were separately spiked into the solution to result in an analyte concentration from 50 pg/mL to 10 ng/mL and the change in current was measured at a frequency of 240 Hz using square wave voltammetry. A concentration dependent change in the signal was observed in the presence of human IL-6, but no significant change of signal was observed in the presence of recombinant troponin I (Figure 30), indicating that the aptamer-functionalised electrode selectively detects human IL-6. The limit of detection was calculated as being 37 pg/mL IL-6, which is within the physiologically relevant range of IL-6.

Erythema

[0600] Studies have been performed to evaluate the tolerability and functionality of microstructure patches in humans.

[0601] In one example, a qualitative tolerability assessment was performed following microstructure patches application which noted a very mild local response at the application site immediately post-removal. This was characterized by slight indentation with no overt erythema or oedema, which was resolved within 15 minutes of removal. This is shown in Figure 25A. This shows the indentation was most prominent around the edges and corners of

the microstructure patch, with very mild redness at these locations, and with no redness associated with the microstructures themselves.

[0602] Scanning Electron Microscopy (SEM) was performed to confirm that the microstructures had, in fact, penetrated the skin, showing cellular debris remaining on the removed microstructures, as shown in Figure 25B, confirming successful microstructure penetration despite the absence of overt erythema.

[0603] To investigate this observation further, we two dedicated erythema studies were performed with multiple subjects. These studies investigated the local skin response to microstructure patch application to the skin of the anterior forearm over a time period of 2 hours. Microstructure patches were applied using a guided load cell mechanism, at a force of either 5N remaining in place for 30 minutes (Study 1) or 3N and remaining in place for 10 minutes (Study 2).

[0604] The first human erythema study was on five volunteers. In some cases, hair was removed from the skin using depilatory cream and a paper mask was fixed to the application area to avoid any effect due to sensitivity to surgical adhesives in tapes. Three separate non-functionalised microstructure patches were applied to skin exposed by windows in the paper mask, and a fourth window was untreated and used as a control for comparison.

[0605] Observations were made for local erythema and a scoring rubric was used as given in Table 5 below.

Table 5

eScore	**Observation**
0	No discernable difference relative to control
1	Very mild redness
2	Mild redness
3	Red region extending beyond 4mm^2 application area
4	Extensive redness and/or capillary rupture
5	Frank blood and/or oedema superficially

[0606] Results from the first study are shown in Figure 26A, which shows the eScores for Subjects 01-05 in this study, which were independently assessed at 10, 20, 30, 60 and 120 minutes post-application. Data points represent the average eScore from three Microwearables per subject per timepoint.

[0607] Results show that all volunteers experienced some mild or very mild erythema at the site of Microwearable application as observed immediately after removal, which quickly resolved within 60 minutes. No erythema was noted after this time point. Similar to the earlier single subject observation, the indentation/redness was localised around the edges of the Microwearable, with little or no effect seem from the microstructures themselves.

[0608] The second erythema study was performed on three volunteers. Two Microwearable devices were applied at 3N and were removed after 10 minutes of wearing. To investigate further the 'edge effect' observed in a first-in-human trial and in Study 1, a flat patch (i.e. without microstructures) was applied on the third skin site, for comparison. The fourth window remained untreated as a control. Results are shown in Figure 26B, which shows the eScore observations (data points are an average of 2 separate observations per subject per time point) over 120 minutes post-removal.

[0609] Results are similar to Study 1 in that no subject experienced erythema more extensive than 'mild redness' at the site immediately prior to removal of the Microwearable. This mild erythema resolved quickly within 60 minutes, with one subject with a score of 0.5 at 60 minutes, which subsequently resolved completely by 120 minutes. No erythema was observed following application of flat patches, which may suggest that the very mild/mild erythema observed following microstructure patch application is associated with skin barrier penetration (i.e. by the presence of microstructures).

[0610] Microstructure patch eScores were, in general, lower in Study 2 than Study 1, suggesting that lowering the application force of application reduces the extent of the mild erythema that occurs. As the erythema was observed immediately after the microstructure patches were removed and did not increase over time, it appears erythema is caused by the application event itself – driven by the corners and edges of the microstructure patches – and

is not exacerbated by continuous wearing. Future-generation microstructure patch can use different edges and corner configurations leading to negligible erythema.

[0611] As no local erythema was observed within the area covered by microstructures, SEM was performed to confirm that the structures had successfully penetrated the skin of the subjects in Study 1. Example images of individual or row of microstructures after application to two subjects are shown in Figures 27, including images of individual microstructures prior to application to the skin (Figs. 27A and 27D) and images post application (Figs. 27B, 27C and 27E, 27F).

[0612] Images from all subjects confirmed successful penetration of the skin, from the presence of biological material located on the upper portion of the microstructures (Figs. 27B and 27E), with arrows indicating examples of cellular debris extracted by the microstructures on removal.

[0613] Figs. 27C and 27F show rows of microstructures, and exhibit areas with dried interstitial fluid as indicated by the arrows. These observations confirm that the microstructures have successfully breached the outermost stratum corneum layer of the skin and are able to access cellular environments beneath to gain access to the interstitial fluid, which is the source of bio-signals including biomarkers of disease.

[0614] It is therefore apparent that microstructure patches are at worst only associated with very mild/mild erythema at the site of application. This mild local response is transient, and is completely resolved within 60-120 mins post-application. Any redness immediately occurs after application, and is not associated with continuous wearing of the microstructure patch.

[0615] Any erythema is focused around the edges and corners of the microstructure patch, with little/no erythema noted in the area covered by microstructures, but the observation that a flat patch had no effect suggests that the erythema after microstructure patch application is associated with a physical breach of the skin barrier.

[0616] Despite the observation that microstructures did not cause overt erythema, it was we confirmed that microstructure penetration was successful, with visible breaching of the stratum corneum and with confirmed access to skin compartments rich in interstitial fluid.

Use of the System

[0617] The system of the invention may be used to determine the presence, absence, level or concentration of one or more analytes in a wide range of applications as discussed herein, including, diagnosing or monitoring the progression of a disease, disorder or condition in a subject; the presence, absence, level or concentration of an illicit substance or non-illicit substance, or a chemical warfare agent, poison or toxin, or the level or concentration of a medicament.

[0618] Accordingly, in a further aspect, there is provided a method for diagnosing or monitoring the progression of a disease, disorder or condition in a subject, comprising determining the presence, absence, level or concentration of one or more analytes in the viable epidermis and/or dermis of the subject using the system of the invention, and determining the presence, absence and/or progression of the disease, disorder or condition based on whether the one or more analytes is present or absent, or whether the level or concentration of the one or more analytes is above or below a corresponding predetermined threshold that correlates with the presence, absence or progression of the disease, disorder or condition.

[0619] The invention also provides the use of the system of the invention for diagnosing or monitoring the progression of a disease, disorder or condition in a subject. There is further provided the system of the invention for use in diagnosing or monitoring the progression of a disease, disorder or condition in a subject. In particular embodiments of any one of the above aspects, the system determines the presence, absence, level or concentration of one or more analytes in the viable epidermis and/or dermis of the subject and the presence, absence and/or progression of the disease, disorder or condition is determined based on whether the one or more analytes is present or absent, or whether the level or concentration of the one or more analytes is above or below a corresponding predetermined threshold that correlates with the presence, absence or progression of the disease, disorder or condition.

[0620] Suitable diseases, disorders or conditions, analytes and exemplary concentration levels are discussed *supra*.

[0621] In some embodiments, the disease, disorder or condition is selected from cardiac damage, myocardial infarction and acute coronary syndrome, and the one or more analytes is

troponin or a subunit thereof. In particular embodiments, the one or more analytes is troponin I.

[0622] In some embodiments, the disease, disorder or condition is an infection, such as a viral or bacterial infection, and the one or more analytes is IL-6, IL-10, C-reactive protein and/or TNF-α; especially IL-6 or TNF-α; most especially IL-6. In particular embodiments, the disease, disorder or condition is a bacterial or viral infection, especially a viral infection and the one or more analytes is IL-6.

[0623] Suitable viral infections include, but are not limited to, infections caused by HIV, hepatitis, influenza virus, Japanese encephalitis virus, Epstein-Barr virus, herpes simplex virus (e.g. HSV-1 or HSV-2), filovirus, human papillomavirus, human T-cell lymphotropic virus, human retrovirus, cytomegalovirus, varicella-zoster virus, poliovirus, measles virus, rubella virus, mumps virus, adenovirus, enterovirus, rhinovirus, ebola virus, west nile virus, coronavirus, such as SARS-CoV-2, SARS-CoV or MERS-CoV, parvovirus, small pox virus, vaccinia virus, hepadnaviridae, polyoma virus, and respiratory syncytial virus; especially infections caused by a coronavirus, such as SARS-CoV-2. Bacterial infections include, but are not restricted to, those caused by *Neisseria* species, *Meningococcal* species, *Haemophilus* species, *Salmonella* species, *Streptococcal* species, *Legionella* species, *Mycoplasma* species, *Bacillus* species, *Staphylococcus* species, *Chlamydia* species, *Actinomyces* species, *Anabaena* species, *Bacteroides* species, *Bdellovibrio* species, *Bordetella* species, *Borrelia* species, *Campylobacter* species, *Caulobacter* species, *Chlrorbium* species, *Chromatium* species, *Chlostridium* species, *Corynebacterium* species, *Cytophaga* species, *Deinococcus* species, *Escherichia* species, *Francisella* species, *Helicobacter* species, *Haemophilus* species, *Hyphomicrobium* species, *Leptospira* species, *Listeria* species, *Micrococcus* species, *Myxococcus* species, *Nitrobacter* species, *Oscillatoria* species, *Prochloron* species, *Proteus* species, *Pseudomonas* species, *Rhodospirillum* species, *Rickettsia* species, *Shigella* species, *Spirillum* species, *Spirochaeta* species, *Streptomyces* species, *Thiobacillus* species, *Treponema* species, *Vibrio* species, *Yersinia* species, *Nocardia* species and *Mycobacterium* species.

[0624] In another aspect, there is provided a method of treating a disease, disorder or condition in a subject comprising determining the presence, absence, level or concentration of one or more analytes in the viable epidermis and/or dermis of the subject using the system of

the invention, determining the presence or progression of the disease, disorder or condition based on whether the one or more analytes is present, or whether the level or concentration of the one or more analytes is above or below a corresponding predetermined threshold that correlates with the presence or progression of the disease, disorder or condition, and administering a treatment for the disease, disorder or condition.

[0625] In a further aspect, there is provided a method of treating a disease, disorder or condition in a subject comprising exposing the subject to a treatment regimen for treating the disease, disorder or condition based on an indicator obtained from an indicator-determining method, said indicator-determining method comprising determining the presence, absence, level or concentration of one or more analytes in the viable epidermis and/or dermis of the subject using the system of the invention, and determining the presence or progression of the disease, disorder or condition based on whether the one or more analytes is present, or whether the level or concentration of the one or more analytes is above or below a corresponding predetermined threshold that correlates with the presence or progression of the disease, disorder or condition.

[0626] In a related aspect, the present invention provides a method for managing a disease, disorder or condition in a subject comprising exposing the subject to a treatment regimen for treating the disease, disorder or condition based on an indicator obtained from an indicator-determining method, said indicator-determining method comprising determining the presence, absence, level or concentration of one or more analytes in the viable epidermis and/or dermis of the subject using the system of the invention, and determining the presence or progression of the disease, disorder or condition based on whether the one or more analytes is present, or whether the level or concentration of the one or more analytes is above or below a corresponding predetermined threshold that correlates with the presence or progression of the disease, disorder or condition.

[0627] In any one of the above aspects, the predetermined threshold represents a level or concentration of the analyte in a corresponding sample from a control subject (e.g. in the viable epidermis and/or dermis of the control subject), or represents a level or concentration above or below the level or concentration of the analyte in a corresponding sample from a control subject, and levels or concentrations above or below said threshold indicates the presence,

absence or progression of a disease, disorder or condition. The control subject may be a subject who does not have the disease, disorder or condition; a subject who does have the disease, disorder or condition; or a subject who has a particular stage or severity of the disease, disorder or condition. When progression of the disease, disorder or condition is being monitored, the predetermined threshold may be a level or concentration of the analyte in a sample from the same subject taken at an earlier time (e.g. several minutes, hours, days, weeks or months earlier), and an increase or decrease in the analyte level or concentration may indicate the progression or regression of the disease, disorder or condition.

[0628] Suitable treatments for the disease, disorders or conditions discussed *supra* are well known in the art, and a skilled person will readily be able to select an appropriate treatment. For example, suitable disorders and exemplary treatments include, but are not limited to, renal failure and treatment with dialysis, a kidney transplant, an angiotensin-converting enzyme inhibitor (e.g. benazepril, zofenopril, perindopril, trandolapril, captopril, enalapril, lisinopril or ramipril), an angiotensin II receptor blocker (e.g. losartan, irbesartan, valsartan, candesartan, telmisartan or fimasartan), a diuretic (e.g. furosemide, bumetanide, ethacrynic acid, torsemide, chlorothiazide, hydrochlorothiazide, bendroflumethiazide or trichlormethiazide), a statin (e.g. atorvastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin or simvastatin), calcium, glucose or sodium polystyrene sulfonate, and/or a calcium infusion; cardiac failure and treatment with an angiotensin-converting enzyme inhibitor (e.g. benazepril, zofenopril, perindopril, trandolapril, captopril, enalapril, lisinopril or ramipril), an angiotensin II receptor blocker (e.g. losartan, irbesartan, valsartan, candesartan, telmisartan or fimasartan), a diuretic (e.g. furosemide, bumetanide, ethacrynic acid, torsemide, chlorothiazide, hydrochlorothiazide, bendroflumethiazide or trichlormethiazide), a beta blocker (e.g. carvedilol, metoprolol or bisoprolol), an aldosterone antagonist (e.g. spironolactone or eplerenone), and/or an inotrope (e.g. digoxin, berberine, levosimendan, calcium, dopamine, dobutamine, dopexamine, epinephrine, isoprenaline, norepinephrine, angiotensin II, enoximone, milrinone, amrinone, theophylline, glucagon or insulin); essential hypertension and treatment with a beta blocker (e.g. carvedilol, metoprolol or bisoprolol), a calcium channel blocker (e.g. amlodipine, felodipine, isradipine, nicardipine, nifedipine, nimodipine or nitrendipine), a diuretic (e.g. furosemide, bumetanide, ethacrynic acid, torsemide, chlorothiazide, hydrochlorothiazide, bendroflumethiazide or trichlormethiazide), angiotensin-

converting enzyme inhibitor (e.g. benazepril, zofenopril, perindopril, trandolapril, captopril, enalapril, lisinopril or ramipril), an angiotensin II receptor blocker (e.g. losartan, irbesartan, valsartan, candesartan, telmisartan or fimasartan), and/or a renin inhibitor (e.g. aliskiren); bacterial infection and treatment with antibiotics (e.g. quinolones (e.g. amifloxacin, cinoxacin, ciprofloxacin, enoxacin, fleroxacin, flumequine, lomefloxacin, nalidixic acid, norfloxacin, ofloxacin, levofloxacin, lomefloxacin, oxolinic acid, pefloxacin, rosoxacin, temafloxacin, tosufloxacin, sparfloxacin, clinafloxacin, gatifloxacin, moxifloxacin, gemifloxacin, or garenoxacin), tetracyclines, glycylcyclines or oxazolidinones (e.g. chlortetracycline, demeclocycline, doxycycline, lymecycline, methacycline, minocycline, oxytetracycline, tetracycline, tigecycline, linezolide or eperezolid), aminoglycosides (e.g. amikacin, arbekacin, butirosin, dibekacin, fortimicins, gentamicin, kanamycin, menomycin, netilmicin, ribostamycin, sisomicin, spectinomycin, streptomycin or tobramycin), β-lactams (e.g. imipenem, meropenem, biapenem, cefaclor, cefadroxil, cefamandole, cefatrizine, cefazedone, cefazolin, cefixime, cefmenoxime, cefodizime, cefonicid, cefoperazone, ceforanide, cefotaxime, cefotiam, cefpimizole, cefpiramide, cefpodoxime, cefsulodin, ceftazidime, cefteram, ceftezole, ceftibuten, ceftizoxime, ceftriaxone, cefuroxime, cefuzonam, cephacetrile, cephalexin, cephaloglycin, cephaloridine, cephalothin, cephapirin, cephradine, cefinetazole, cefoxitin, cefotetan, azthreonam, carumonam, flomoxef, moxalactam, amdinocillin, amoxicillin, ampicillin, azlocillin, carbenicillin, benzylpenicillin, carfecillin, cloxacillin, dicloxacillin, methicillin, mezlocillin, nafcillin, oxacillin, penicillin G, piperacillin, sulbenicillin, temocillin, ticarcillin, cefditoren, cefdinir, ceftibuten or cefozopran), rifamycins, macrolides (e.g. azithromycin, clarithromycin, erythromycin, oleandomycin, rokitamycin, rosaramicin, roxithromycin or troleandomycin), ketolides (e.g. telithromycin or cethromycin), coumermycins, lincosamides (e.g. clindamycin or lincomycin) or chloramphenicol); viral infection and treatment with antivirals (e.g. abacavir sulfate, acyclovir sodium, amantadine hydrochloride, amprenavir, cidofovir, delavirdine mesylate, didanosine, efavirenz, famciclovir, fomivirsen sodium, foscarnet sodium, ganciclovir, indinavir sulfate, lamivudine, lamivudine/zidovudine, nelfinavir mesylate, nevirapine, oseltamivir phosphate, ribavirin, rimantadine hydrochloride, ritonavir, saquinavir, saquinavir mesylate, stavudine, valacyclovir hydrochloride, zalcitabine, zanamivir or zidovudine); autoimmune disorders and treatment with immunosuppressants (e.g. prednisone, dexamethasone, hydrocortisone, budesonide,

prednisolone, tofacitinib, cyclosporine, cyclophosphamide, nitrosoureas, platinum compounds, methotrexate, azathioprine, mercaptopurine, fluorouracil, dactinomycin, anthracyclines, mitomycin C, bleomycin, mithramycin, antithymocyte globulin, thymoglobulin, Muromonab-CD3, basiliximab, daclizumab, tacrolimus, sirolimus, everolimus, infliximab, etanercept, IFN-β, mycophenolic acid or mycophenolate, fingolimod, azathioprine, leflunomide, abatacept, adalimumab, anakinra, certolizumab, golimumab, ixekizumab, natalizumab, rituximab, secukinumab, toclizumab, ustekinumab, vedolizumab or myriocin) and/or NSAIDs (e.g. acetylsalicylic acid (aspirin), diclofenac, diflusinal, etodolac, fenbufen, fenoprofen, flufenisal, flurbiprofen, ibuprofen, indomethacin, ketoprofen, ketorolac, meclofenamic acid, mefenamic acid, meloxicam, nabumetone, naproxen, nimesulide, nitroflurbiprofen, olsalazine, oxaprozin, phenylbutazone, piroxicam, sulfasalazine, sulindac, tolmetin, zomepirac, celecoxib, deracoxib, etoricoxib, mavacoxib or parecoxib); rheumatological disorders and treatment with NSAIDs as described *supra*, DMARDs (e.g. methotrexate, hydroxychloroquinone or penicillamine), prednisone, dexamethasone, hydrocortisone, budesonide, prednisolone, etanercept, golimumab, infliximab, adalimumab, anakinra, rituximab, abatacept, and/or other immunosuppressants described *supra*; sepsis and antibiotics as described *supra*, immunosuppressants as described *supra* and/or an antihypotensive agent (e.g. vasopressin, norepinephrine, dopamine or epinephrine); and pulmonary embolism and treatment with an anticoagulant (e.g. heparin, warfarin, bivalirudin, dalteparin, enoxaparin, dabigatran, edoxaban, rivaroxaban, apixaban or fondaparinux) and/or a thrombolytic/fibrinolytic (e.g. tissue plasminogen activator, reteplase, streptokinase or tenecteplase).

[0629] In some embodiments, the disease, disorder or condition is cardiac damage, myocardial infarction or acute coronary syndrome, the one or more analytes is troponin or a subunit thereof. Suitable treatments for cardiac damage, myocardial infarction or acute coronary syndrome may include, but are not limited to, aspirin, an anticoagulant (e.g. heparin, warfarin, bivalirudin, dalteparin, enoxaparin dabigatran, edoxaban, rivaroxaban, apixaban or fondaparinux), a beta-blocker (e.g. carvedilol or metoprolol), a thrombolytic/fibrinolytic (e.g. tissue plasminogen activator, reteplase, streptokinase or tenecteplase), an angiotensin-converting enzyme inhibitor (e.g. benazepril, zofenopril, perindopril, trandolapril, captopril, enalapril, lisinopril or ramipril), an angiotensin II receptor blocker (e.g. losartan, irbesartan, valsartan, candesartan, telmisartan or fimasartan), a statin (e.g. atorvastatin, fluvastatin,

lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin or simvastatin), an analgesic (e.g. morphine, etc.), nitroglycerin, and the like, or combinations thereof.

[0630] In some embodiments, the disease, disorder or condition is an infection, such as a viral or bacterial infection, the one or more analytes is IL-6, IL-10 and/or TNF-α; especially IL-6 or TNF-α; most especially IL-6; and the treatment is an antibiotic or antiviral, suitable examples of which are discussed *supra*. The treatment may, additionally or alternatively, include ventilation where appropriate, such as a SARS-CoV-2 infection, or an IL-6 blocking agent, such as tocilizumab, sarilumab or siltuximab.

[0631] The invention further contemplates the use of the system of the invention for determining the presence, absence, level or concentration of an illicit substance or non-illicit substance of abuse in a subject. Accordingly, in another aspect, there is provided a method of determining the presence, absence, level or concentration of an illicit substance or non-illicit substance of abuse in a subject, comprising determining the presence, absence, level or concentration of the illicit substance, non-illicit substance of abuse or a metabolite thereof in the viable epidermis and/or dermis of the subject using the system of the invention.

[0632] There is also provided the use of the system of the invention for determining the presence, absence, level or concentration of an illicit substance or non-illicit substance of abuse in a subject, and the system of the invention for use in determining the presence, absence, level or concentration of an illicit substance or non-illicit substance of abuse in a subject. In particular embodiments of any one of these aspects, the system determines the presence, absence, level or concentration of the illicit substance, non-illicit substance of abuse or metabolite thereof in the viable epidermis and/or dermis of the subject.

[0633] Suitable illicit substances are discussed *supra* and include, but are not limited to, methamphetamine, amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), *N*-ethyl-3,4-methylenedioxyamphetamine (MDEA), 3,4-methylenedioxy-amphetamine (MDA), cannabinoids (e.g. delta-9-tetrahydrocannabinol, 11-hydroxy-delta-9-tetrahydrocannabinol, 11-nor-9-carboxydelta-9-tetrahydrocannabinol), cocaine, benzoylecgonine, ecgonine methyl ester, cocaethylene, ketamine, and the opiates (e.g. heroin, 6-monoacetylmorphine, morphine, codeine, methadone and dihydrocodeine). Non-limiting non-illicit substances of abuse include

alcohol, nicotine, prescription medicine or over the counter medicine taken for non-medical reasons, a substance taken for a medical effect, wherein the consumption has become excessive or inappropriate (e.g. pain medications, sleep aids, anti-anxiety medication, methylphenidate, erectile-dysfunction medications), and the like.

[0634] The invention further contemplates the use of the system of the invention for determining the presence, absence, level or concentration of a chemical warfare agent, poison and/or toxin in a subject. Accordingly, in another aspect, there is provided a method of determining the presence, absence, level or concentration of a chemical warfare agent, poison and/or toxin in a subject, comprising determining the presence, absence, level or concentration of the chemical warfare agent, poison and/or toxin or a metabolite thereof in the viable epidermis and/or dermis of the subject using the system of the invention. In particular embodiments, the method is for determining the presence, absence, level or concentration of a chemical warfare agent.

[0635] There is also provided the use of the system of the invention for determining the presence, absence, level or concentration of a chemical warfare agent, poison and/or toxin in a subject, and the system of the invention for use in determining the presence, absence, level or concentration of a chemical warfare agent, poison and/or toxin in a subject; especially a chemical warfare agent. In particular embodiments of any one of these aspects, the system determines the presence, absence, level or concentration of the chemical warfare agent, poison and/or toxin or a metabolite thereof in the viable epidermis and/or dermis of the subject.

[0636] Suitable chemical warfare agents, poisons and/or toxins are discussed *supra*.

[0637] The system of the invention may also be used to determine and/or monitor the level or concentration of a medicament administered to a subject, for example, to optimise and/or adjust the dose of the medicament. The invention provides a method for determining and/or monitoring the level or concentration of a medicament administered to a subject, comprising determining the level or concentration of the medicament or a component or metabolite thereof in the viable epidermis and/or dermis of the subject using the system of the invention.

[0638] There is further provided the use of the system of the invention for determining and/or monitoring the level or concentration of a medicament administered to a subject, and

the system of the invention for use in determining and/or monitoring the level or concentration of a medicament administered to a subject. In particular embodiments, the system of the invention determines the level or concentration of the medicament or a component or metabolite thereof in the viable epidermis and/or dermis of the subject.

[0639] In some embodiments, the dose of the medicament is increased or decreased following determination of the level or concentration of the medicament or a component or metabolite thereof.

[0640] In a further aspect, there is provided a method of monitoring the efficacy of a treatment regimen in a subject with a disease, disorder or condition, wherein the treatment regimen is monitored for efficacy towards a desired health state (e.g. absence of the disease, disorder or condition. Such method generally comprises determining the presence, absence, level or concentration of one or more analytes indicative of the efficacy of the treatment regimen in the viable epidermis and/or dermis of the subject using the system of the invention after treatment of the subject with the treatment regimen, and comparing the level or concentration of the one or more analytes to a reference level or concentration of the one or more analytes which is correlated with a presence, absence or stage of the disease, disorder or condition to thereby determine whether the treatment regimen is effective for changing the health status of the subject to a desired health state. In some embodiments, the one or more analytes is a medicament administered during the treatment regimen, or a component or metabolite thereof.

[0641] In a related aspect, there is provided a method of monitoring the efficacy of a treatment regimen in a subject with a disease, disorder or condition, wherein the treatment regimen is monitored for efficacy towards a desired health state (e.g. absence of the disease, disorder or condition). Such method generally comprises determining an indicator according to an indicator-determining method, said indicator-determining method comprising determining the presence, absence, level or concentration of one or more analytes in the viable epidermis and/or dermis of the subject using the system of the invention after treatment of the subject with the treatment regimen, and assessing the likelihood of the subject having a presence, absence or stage of a disease, disorder or condition based on whether the one or more analytes is present, or whether the level or concentration of the one or more analytes is above

or below a corresponding predetermined threshold that correlates with the presence, absence or stage of the disease, disorder or condition, using the indicator to thereby determine whether the treatment regimen is effective for changing the health status of the subject to a desired health state. In some embodiments, the one or more analytes is a medicament administered during the treatment regimen, or a component or metabolite thereof.

[0642] In some embodiments of any one of the above aspects, the treatment regimen is adjusted following such methods. Suitable predetermined thresholds for such aspects are discussed *supra*.

[0643] The invention also provides the system of the invention for use in such methods, and the use of the system for such methods.

[0644] A skilled person will readily appreciate that the system of the invention may be used to determine and monitor the level or concentration of a wide range of medicaments and treatment regimens and will readily be able to use and select suitable medicaments and treatment regimens. For example, suitable medicaments include, but are not limited to, cancer therapies, vaccines, analgesics, antipsychotics, antibiotics, anticoagulants, antidepressants, antivirals, sedatives, antidiabetics, contraceptives, immunosuppressants, antifungals, antihelmintics, stimulants, biological response modifiers, NSAIDs, corticosteroids, DMARDs, anabolic steroids, antacids, antiarrhythmics, thrombolytics, anticonvulsants, antidiarrheals, antiemetics, antihistamines, antihypertensives, anti-inflammatories, antineoplastics, antipyretics, barbiturates, β-blockers, bronchodilators, cough suppressants, cytotoxics, decongestants, diuretics, expectorants, hormones, laxatives, muscle relaxants, vasodilators, tranquilizers and vitamins.

[0645] In particular embodiments, the medicament is one which has a narrow therapeutic window, such as particular antibiotics (e.g. aminoglycosides including kanamycin, gentamycin and streptomycin), anticonvulsants (e.g. carbamazepine and clonazepam), vasodilators, anticoagulants including heparin and warfarin, digoxin, and the like. In such embodiments, the methods and uses may further comprise increasing or decreasing the dose of the medicament administered to the subject.

[0646] In any one of the above aspects, the methods and uses further comprise attaching the system of the invention to the skin of the subject prior to determining the presence, absence, level or concentration of the one or more analytes. In such embodiments, the system of the invention breaches a stratum corneum of the subject.

[0647] The above described patches may also be used to test other forms of subjects, such as food stuffs, or the like. In this example, the patch could be used to test for the presence of unwanted contaminants, such as pathogens, such as bacteria, exotoxins, mycotoxins, viruses, parasites, or the like, as well as natural toxins. Additionally contaminants could include agrochemicals, environmental contaminants, pesticides, carcinogens, bacteria, or the like.

[0648] Accordingly, it will be appreciated that the term subject can include living subjects, such as humans, animals, or plants, as well as non-living materials, such as foodstuffs, packaging, or the like.

[0649] Accordingly, the above described arrangement provides a wearable monitoring device that uses microstructures that breach a barrier, such as penetrating into the stratum corneum in order to perform measurements on a subject. The measurements can be of any appropriate form, and can include measuring the presence of biomarkers or other analytes within the subject, measuring electrical signals within the subject, or the like. Measurements can then be analysed and used to generate an indicator indicative of a health status of the subject.

[0650] In one example, the above described system allows analytes to be detected in specific tissue sites in the skin, in situ. The microstructures can be coated with a material for binding one or more analytes of interest or may be formed by a binding agent as described *supra*, allowing analytes within the subject to bind to the microstructures in turn allowing these to be detected using suitable optical or electrical measurement techniques. The coatings and/or microstructures can be specifically designed to capture analytes with extremely high specificity. Such specificity allows specific analytes of interest to be detected without the need for purification or complex chemical analysis.

[0651] The length of the structures can be controlled during manufacture to enable targeting of specific layers in the target tissue. In one example, this is performed to target

analytes in the epidermal and/or dermal layers, although analytes in capillary blood can also be targeted.

[0652] Specific probes can be localized to individual structures or areas of structures, so that multiple targets can be analysed in a single assay simply by their location in a 2-dimensional array. This could facilitate the analysis of disease-specific analyte panels to increase the sensitivity/specificity of the diagnostic results.

[0653] The patches can therefore provide a measurement device which overcomes the need for traditional blood or ISF samples to be taken for diagnostic purposes representing an opportunity for a clinician to diagnose and avoid time and processing costs at centralised testing facilities. It may also open new markets since diagnostic equipment and blood sampling expertise is not needed e.g. in developing countries, 'in-field' military applications, medical countermeasures, emergency and triage.

[0654] This allows patches to be used as a non-invasive, pain-free measurement platform that can measure analytes in situ. The type of material detected by the patch may be controlled by the length of the structures, such that different regions can be targeted specifically. This embodiment does not include a specific analysis type; a number of established techniques can be used for fluid analysis including, but not limited to, mass spectrometry, microarrays, DNA/protein sequencing, HPLC, ELISA, Western Blots and other gel methods, etc.

[0655] Using affinity surface coatings on each structure allows a reduction of non-specific adsorption of substances whilst facilitating specific extraction of the molecular targets of interest.

[0656] By arranging the structures in a two-dimensional format, multiple probes can be attached to the same patch, with the results from the sandwich assay decoded based on the 2-D array position of the individual structures. This essentially allows array-style processing without the need for sample extraction, purification, labelling, etc.

[0657] Accordingly, in one example, the above described system provides a minimally-invasive and pain-free way to access blood-borne biomarkers of disease: by accessing the outer skin layers with devices applied to the skin that are also pain-free. Currently, blood is accessed

by a needle/lancet which is often painful and laborious. Alternatively, blood is accessed directly in the body by surgically implanting a sensor. Surgical implants are not likely to be used widely, as implanting is an invasive procedure, with limited choice of materials suitable for implantation.

[0658] The system can provide rapid "on the spot" disease detection on the person, rather than the delays of sending blood samples to pathology laboratories for processing. This is also an advance over the current point-of-care devices, which usually still require a blood sample (e.g. by a needle) to be analysed away from the body.

[0659] The system can provide high-fidelity, low power, low cost body signal (e.g. biopotential, optical) sensing for practical disease/health diagnostics. As one example, pre-clinical animal skin testing of microstructure patches show a 100 fold reduction of bioimpedance, compared to standard, approaches applied to the surface of skin, leading to improved signal to noise ratio.

[0660] The system can provide simple, semi-continuous or continuous monitoring: a low cost-device micro wearable would be applied to the skin and potentially be worn for days (or longer), and then simply replaced by another micro wearable component. Thus, micro wearables provide a route for monitoring over time – which can be particularly important in detecting sudden events (e.g. cardiac biomarkers for a heart attack) – without surgically implanting a sensor into the body.

[0661] In one example, the above described approach can allow wearables to provide widespread, low-cost healthcare monitoring for a multitude of health conditions that cannot be assayed by current devices, which are placed on the skin.

[0662] In one example, the microstructure patches penetrate the skin barrier and so unlike today's wearables, access blood-borne biomarkers of disease for rapid "on the spot" disease detection on the person. Contrast this to the current method of sending blood samples to pathology laboratories for processing. This is also an advance over the current point-of-care devices, which usually still require a blood sample (e.g. by a needle) to be analysed away from the body.

[0663] In one example, the system can provide a low-cost microstructure patches would be applied to the skin and potentially be worn for days (or longer) for simple and pain free semi-continuous or continuous monitoring, and then simply replaced by another microstructure patch component. Thus, microstructure patches provide a route for monitoring over time – which can be particularly important in detecting sudden events (e.g. cardiac biomarkers for a heart attack) – without surgically-implanting a sensor into the body.

[0664] Persons skilled in the art will appreciate that numerous variations and modifications will become apparent. All such variations and modifications which become apparent to persons skilled in the art, should be considered to fall within the spirit and scope that the invention broadly appearing before described.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1) A system for performing measurements on a biological subject, the system including:
   a) at least one substrate including one or more microstructures configured to breach a functional barrier of the subject, wherein the one or more microstructures include an aptamer for binding one or more analytes;
   b) at least one sensor operatively connected to at least one microstructure, the at least one sensor being configured to measure response signals from the at least one microstructure; and,
   c) one or more electronic processing devices that:
      i) determine measured response signals; and,
      ii) perform an analysis at least in part using the measured response signals to determine at least one indicator at least partially indicative of analyte presence, absence, level or concentration in the subject.
2) A system according to claim 1, wherein the aptamer is a coating on the microstructure.
3) A system according to claim 1 or claim 2, wherein the aptamer selectively bind the one or more analytes.
4) A system according to any one of claims 1 to 3, wherein the aptamer undergoes a conformational change upon analyte binding.
5) A system according to claim 4, wherein the aptamer has a first conformation in the absence of analyte binding and a second conformation upon analyte binding.
6) A system according to any one of claims 1 to 5, wherein the aptamer comprises a labelling moiety.
7) A system according to claim 6, wherein the labelling moiety is a redox moiety.
8) A system according to claim 7, wherein the redox moiety is selected from the group consisting of methylene blue, ferrocene, vinylferrocene, anthraquinone, nile blue, thionine, anthraquinone-C5, dabcyl, 2,6-dichlorophenal-indophenol, gallocyanine, ROX, pentamethylferrocene, ferrocene-C5, neutral red and horseradish peroxidase.
9) A system according to claim 8, wherein the redox moiety is methylene blue.
10) A system according to claim 6, wherein the labelling moiety is a fluorescent label.
11) A system according to any one of claims 1 to 10, wherein the aptamer comprises a moiety for attaching or immobilising the aptamer on the surface of the microstructure.

12) A system according to claim 11, wherein the moiety is a thiol, amine, carboxylic acid, alcohol, carbodiimide, nafion, avidin, biotin or azide.

13) A system according to claim 12, wherein the moiety is a thiol.

14) A system according to claim 13, wherein the one or more microstructures are porous.

15) A system according to any one of claims 1 to 14, wherein the one or more analytes are selected from the group consisting of a nucleic acid, an antibody or antigen-binding fragment thereof, an allergen, a chemokine, a cytokine, a hormone, a parasite, a bacteria, a virus or virus-like particle, an epigenetic marker, a peptide, a polypeptide, a protein and a small molecule.

16) A system according to claim 15, wherein the one or more analytes is a protein.

17) A system according to claim 16, wherein the protein is troponin or a subunit thereof.

18) A system according to claim 17, wherein the protein is troponin I.

19) A system according to claim 16, wherein the protein is cardiac troponin I-C complex (cTnIC).

20) A system according to claim 15, wherein the one or more analytes is a cytokine.

21) A system according to claim 20, wherein the cytokine is IL-6.

22) A system according to any one of the claims 1 to 21, wherein the system is at least partially wearable.

23) A system according to any one of the claims 1 to 22, wherein the system includes a signal generator operatively connected to at least one microstructure to apply a stimulatory signal.

24) A system according to claim 23, wherein the one or more processing devices are configured to at least one of:

a) control the signal generator to cause a measurement to be performed; and

b) control the signal generator in accordance with measured response signals.

25) A system according to any one of the claims 1 to 24, wherein response and stimulatory signals include electrical signals, and wherein the substrate includes electrical connections to allow electrical signals to be applied to and/or received from respective microstructures.

26) A system according to any one of the claims 1 to 25, wherein response and stimulatory signals include optical signals, and wherein the substrate includes optical connections to allow optical signals to be applied to and/or received from respective microstructures.

27) A system according to any one of the claims 1 to 26, wherein the system includes one or more switches for selectively connecting at least one of at least one sensor and at least one signal generator to one or more of the microstructures.

28) A system according to claim 27, wherein the one or more processing devices are configured to control the switches to at least one of:

a) allow at least one measurement to be performed; and,

b) control which microstructures are used to measure response signals / apply stimulation.

29) A system according to any one of the claims 1 to 28, wherein at least one of the substrate and the microstructures include at least one of:

a) metal;

b) polymer; and,

c) silicon.

30) A system according to any one of the claims 1 to 29, wherein the substrate is at least one of:

a) at least partially flexible;

b) configured to conform to an outer surface of the functional barrier; and,

c) configured to conform to a shape of at least part of a subject.

31) A system according to any one of the claims 1 to 30, wherein the plate microstructures are at least partially tapered and have a substantially rounded rectangular cross sectional shape.

32) A system according to any one of the claims 1 to 31, wherein the microstructures include anchor microstructures used to anchor the substrate to the subject and wherein the anchor microstructures at least one of:

a) undergo a shape change;

b) undergo a shape change in response to at least one of substances in the subject and applied stimulation;

c) swell;

d) swell in response to at least one of substances in the subject and applied stimulation;

e) include anchoring structures;

f) have a length greater than that of other microstructures;

g) are rougher than other microstructures;

h) have a higher surface friction than other microstructures;

i) are blunter than other microstructures;

j) are fatter than other microstructures; and,

k) enter the dermis.

33) A system according to any one of the claims 1 to 32, wherein the microstructures are applied to skin of the subject, and wherein at least some of the microstructures at least one of:

a) penetrate the stratum corneum;

b) enter the viable epidermis but not the dermis; and,

c) enter the dermis.

34) A system according to any one of the claims 1 to 33, wherein at least some of the microstructures have at least one of:

a) a length that is at least one of:

i) less than 2500 μm;

ii) less than 1000 μm;

iii) less than 750 μm;

iv) less than 450 μm;

v) less than 300 μm;

vi) less than 250 μm;

vii) about 250 μm;

viii) about 150 μm;

ix) greater than 100 μm;

x) greater than 50 μm; and,

xi) greater than 10 μm;

b) a maximum width that is at least one of:

i) less than 2500 μm;

ii) less than 1000 μm;

iii) less than 750 μm;

iv) less than 450 μm;

v) less than 300 μm;

vi) less than 250 μm;

vii) of a similar order of magnitude to the length;

viii) greater than the length;

ix) greater than the length;

x) about the same as the length;

xi) about 250 µm;

xii) about 150 µm; and,

xiii) greater than 50 µm; and,

c) a maximum thickness that is at least one of:

i) less than the width;

ii) significantly less than the width;

iii) of a smaller order of magnitude to the length;

iv) less than 300 µm;

v) less than 200 µm;

vi) less than 50 µm;

vii) about 25 µm; and,

viii) greater than 10 µm.

35) A system according to any one of the claims 1 to 34, wherein at least some of the microstructures include at least one of:

a) a shoulder that is configured to abut against the stratum corneum to control a depth of penetration; and,

b) a shaft extending from a shoulder to the tip, the shaft being configured to control a position of the tip in the subject.

36) A system according to any one of the claims 1 to 35, wherein the microstructures have at least one of:

a) a density that is at least one of:

i) less than 5000 per cm^2;

ii) greater than 100 per cm^2; and,

iii) about 600 per cm^2; and,

b) a spacing that is at least one of:

i) less than 1 mm;

ii) about 0.5 mm;

iii) about 0.2 mm;

iv) about 0.1 mm; and,

v) more than 10 μm.

37) A system according to any one of the claims 1 to 36, wherein at least some of microstructures include an electrode.

38) A system according to claim 37, wherein at least one electrode at least one of:

a) extends over a length of a distal portion of the microstructure;

b) extends over a length of a portion of the microstructure spaced from the tip;

c) is positioned proximate a distal end of the microstructure;

d) is positioned proximate a tip of the microstructure;

e) extends over at least 25% of a length of the microstructure;

f) extends over less than 50% of a length of the microstructure;

g) extends over about 60 μm of the microstructure;

h) is configured to be positioned in a viable epidermis of the subject in use; and,

i) has a surface area of at least one of:

i) less than 200,000 μm^2;

ii) about 22,500 μm^2;

iii) at least 2,000 μm^2.

39) A system according to any one of the claims 1 to 38, wherein at least some of microstructures include at least part of an active sensor.

40) A system according to any one of the claims 1 to 39, wherein at least some of the microstructures include an electrically conductive material.

41) A system according to any one of claims 1 to 40, wherein at least some of the microstructures include an insulating layer extending over at least one of:

a) part of a surface of the microstructure;

b) a proximal end of the microstructure;

c) at least half of a length of the microstructure;

d) about 90 μm of a proximal end of the microstructure; and,

e) at least part of a tip portion of the microstructure.

42) A system according to any one of the claims 1 to 41, wherein at least some of the microstructures include plates having a substantially planar face including at least one electrode.

43) A system according to any one of the claims 1 to 42, wherein at least some of the microstructures are arranged in groups, and wherein at least one of:

a) response signals are measured between microstructures in different group;

b) stimulation is applied between microstructures in different groups;

c) response signals are measured between microstructures in a group; and,

d) stimulation is applied between microstructures in a group.

44) A system according to claim 43, wherein at least one of:

a) there are at least one of:

i) two groups;

ii) three groups; and,

iii) more than three groups;

b) electrodes of the microstructures within each group are electrically connected;

c) the groups are at least one of:

i) provided on a common substrate; and,

ii) provided on different substrates;

d) each group is a pair of microstructures including spaced apart plate microstructures having substantially planar electrodes in opposition;

e) each group includes multiple spaced apart plate microstructures having substantially planar electrodes; and,

f) each group includes multiple pairs of microstructures including spaced apart plate microstructures having substantially planar electrodes in opposition.

45) A system according to claim 43 or claim 44, wherein the groups include:

a) a counter group including a plurality of counter microstructures defining a counter electrode;

b) a reference group including a plurality of reference microstructures defining a reference electrode; and,

c) at least one working group, each working group including a plurality of working microstructures defining a respective working electrode.

46) A system according to claim 45, wherein at least one of:

a) the reference group is smaller than the working and counter groups;

b) the reference group includes fewer microstructures than the working and counter groups; and,

c) the reference group is positioned adjacent each working groups.

47) A system according to any one of the claims 44 to 46, wherein at least one of:

a) at least some microstructures are angularly offset;

b) at least some microstructures are orthogonally arranged;

c) adjacent microstructures are orthogonally arranged;

d) microstructures are arranged in rows, and microstructures in one row are angularly offset relative to microstructures in other rows;

e) microstructures are arranged in rows, and the microstructures in one row are orthogonally arranged relative to microstructures in other rows;

f) at least some pairs of microstructures are angularly offset;

g) at least some pairs of microstructures are orthogonally arranged;

h) adjacent pairs of microstructures are orthogonally arranged;

i) pairs of microstructures are arranged in rows, and the pairs of microstructures in one row are angularly offset relative to pairs of microstructures in other rows;

j) pairs of microstructures are arranged in rows, and the pairs of microstructures in one row are orthogonally arranged relative to pairs of microstructures in other rows.

48) A system arrangement according to any one of the claims 43 to 47, wherein at least one of:

a) the spacing between the electrodes in each group are at least one of:

i) less than 10 mm;

ii) less than 1 mm;

iii) about 0.1 mm; and,

iv) more than 10 μm; and,

b) a spacing between groups of microstructures is at least one of:

i) less than 50 mm;

ii) more than 20 mm;

iii) less than 20 mm;

iv) less than 10 mm;

v) more than 10 mm;

vi) less than 1 mm;

vii) more than 1 mm;

viii) about 0.5 mm; and,

ix) more than 0.2 mm.

49) A system according to any one of the claims 1 to 48, wherein the one or more microstructures interact with one or more analytes of interest such that a response signal is dependent on a presence, absence, level or concentration of the one or more analytes of interest.

50) A system according to claim 49, wherein the one or more analytes interact with a coating on the microstructures to change electrical and/or optical properties of the coating, thereby allowing the one or more analytes to be detected.

51) A system according to any one of the claims 1 to 50, wherein the microstructures include a material including at least one of:

a) a bioactive material;

b) a reagent for reacting with analytes in the subject;

c) a binding agent for binding with one or more analytes of interest;

d) a material for binding one or more analytes of interest;

e) a probe for selectively targeting one or more analytes of interest;

f) an insulator;

g) a material to reduce biofouling;

h) a material to attract at least one substance to the microstructures;

i) a material to repel or exclude at least one substance from the microstructures;

j) a material to attract at least some analytes to the microstructures; and,

k) a material to repel or exclude at least some analytes from the microstructures.

52) A system according to any one of the claims 1 to 51, wherein the substrate includes a plurality of microstructures and wherein different microstructures are at least one of:

a) differentially responsive to analytes;

b) responsive to different analytes;

c) responsive to different combination of analytes; and,

d) responsive to different levels or concentrations of analytes.

53) A system according to any one of the claims 1 to 52, wherein at least some of the microstructures at least one of:

a) attract at least one substance to the microstructures;

b) repel or excludes at least one substance from the microstructures;

c) attract at least one analyte to the microstructures; and,

d) repel or excludes at least one analyte from the microstructures.

54) A system according to any one of the claims 1 to 53, wherein at least some of the microstructures are at least partially coated with a coating.

55) A system according to claim 54, wherein at least one of:

a) at least some microstructures are uncoated;

b) at least some microstructures are porous with an internal coating;

c) at least some microstructures are partially coated;

d) different microstructures have different coatings;

e) different parts of microstructures include different coatings; and,

f) at least some microstructures include multiple coatings.

56) A system according to claim 54 or claim 55, wherein stimulation is used to at least one of:

a) release material from the coating on the microstructure;

b) disrupt the coating;

c) dissolve the coating; and,

d) release the coating.

57) A system according to any one of the claims 54 to 56, wherein at least some of the microstructures are coated with a selectively dissolvable coating.

58) A system according to any one of the claims 54 to 57, wherein the coating at least one of:

a) interacts with one or more analytes;

b) undergoes a change in properties upon exposure to one or more analytes;

c) undergoes a shape change to selectively anchor microstructures;

d) modifies surface properties to at least one of:

i) increase hydrophilicity;

ii) increase hydrophobicity; and,

iii) minimize biofouling;

e) attracts at least one substance to the microstructures;

f) repels or excludes at least one substance from the microstructures;

g) provides a physical structure to at least one of:

i) facilitate penetration of the barrier;

ii) strengthen the microstructures; and,

iii) anchor the microstructures in the subject;

h) dissolves to at least one of:

i) expose a microstructure;

ii) expose a further coating; and,

iii) expose a material;

i) provides stimulation to the subject;

j) contains a material;

k) selectively releases a material;

l) acts as a barrier to preclude at least one substance from the microstructures; and,

m) includes at least one of:

i) polyethylene;

ii) polyethylene glycol;

iii) polyethylene oxide;

iv) zwitterions;

v) peptides;

vi) hydrogels; and,

vii) self-assembled monolayer.

59) A system according to any one of the claims 1 to 58, wherein the system includes an actuator configured to apply a force to the substrate to at least one of pierce and penetrate the stratum corneum.

60) A system according to claim 59, wherein the actuator is at least one of:

a) an electromagnetic actuator;

b) a vibratory motor;

c) a piezoelectric actuator; and,

d) a mechanical actuator.

61) A system according to claim 59 or claim 60, wherein the actuator is configured to apply at least one of:

a) a biasing force;

b) a vibratory force; and,

c) a single continuous force.

62) A system according to any one of the claims 59 to 61, wherein the force at least one of:

a) includes a continuous force that is at least one of:

i) greater than 1 N;

ii) less than 10 N;

iii) less than 20 N; and,

iv) about 2.5 to 5 N; and,

b) includes a vibratory force that is at least one of:

i) at least 1 mN;

ii) about 200 mN; and,

iii) less than 1000 mN; and,

c) is applied at a frequency that is at least one of:

i) at least 10 Hz;

ii) about 100 to 200 Hz; and,

iii) less than 1 kHz.

63) A system according to any one of the claims 59 to 62, wherein at least one of a force and frequency are at least one of:

a) varying;

b) varying depending on at least one of:

i) a time of application;

ii) a depth of penetration;

iii) a degree of penetration; and,

iv) an insertion resistance; and,

c) increasing with an increasing depth of penetration;

d) decreasing with an increasing depth of penetration;

e) increasing until a point of penetration; and

f) decreasing after a point of penetration.

64) A system according to claim 62 or claim 63, wherein the one or more electronic processing devices control the actuator.

65) A system according to any one of the claims 1 to 64, wherein the system includes a housing containing the at least one sensor and at least one electronic processing device.

66) A system according to claim 65, wherein the housing selectively couples to the substrate.

67) A system according to claim 66, wherein the housing couples to the substrate using at least one of:

a) electromagnetic coupling;

b) mechanical coupling;

c) adhesive coupling; and,

d) magnetic coupling.

68) A system according to any one of the claims 65 to 67, wherein at least one of the housing and substrate are at least one of:

a) secured to the subject;

b) secured to the subject using anchor microstructures;

c) secured to the subject using an adhesive patch; and,

d) secured to the subject using a strap.

69) A system according to any one of the claims 65 to 68, wherein the housing includes housing connectors that operatively connect to substrate connectors on the substrate to communicate signals with the microstructures.

70) A system according to any one of the claims 1 to 69, wherein the system is configured to perform repeated measurements over a time period and wherein the microstructures are configured to remain in the subject during the time period.

71) A system according to claim 70, wherein the time period is at least one of:

a) at least one minute;

b) at least one hour;

c) at least one day; and,

d) at least one week.

72) A system according to claim 70 or claim 71, wherein the system is configured to perform repeated measurements with a frequency that is at least one of:

a) substantially continuously;

b) every second;

c) every minute;

d) every 5 to 10 minutes;

e) hourly;

f) daily; and,

g) weekly.

73) A system according to any one of the claims 1 to 72, wherein the one or more electronic processing devices analyse measured response signals to determine at least one indicator at least partially indicative of a physiological status associated with the subject.

74) A system according to any one of the claims 1 to 73, wherein the one or more electronic processing devices:

a) analyse measured response signals to determine at least one metric; and,

b) use the at least one metric to determine at least one indicator, the at least one indicator being at least partially indicative of a physiological status associated with the subject.

75) A system according to claim 74, wherein the one or more electronic devices apply the at least one metric to at least one computational model to determine the indicator, the at least one computational model embodying a relationship between a health status and the at least one metric.

76) A system according to claim 75, wherein the at least one computational model is obtained by applying machine learning to reference metrics derived from subject data measured for one or more reference subjects.

77) A system according to any one of the claims 1 to 76, wherein the one or more electronic devices are configured to determine an indicator by performing at least one of:

a) pattern matching;

b) a longitudinal analysis; and

c) comparison to a threshold.

78) A system according to any one of the claims 1 to 77, wherein the one or more processing devices are configured to determine a physiological status indicative of at least one of:

a) a presence, absence or degree of a medical condition;

b) a prognosis associated with a medical condition;

c) a presence, absence, level or concentration of a biomarker;

d) a presence, absence, level or concentration of an analyte;

e) fluid levels in the subject;

f) blood oxygenation; and,

g) bioelectric activity.

79) A system according to any one of the claims 1 to 78, wherein the one or more electronic devices are configured to generate an output at least one of:

a) including a notification;

b) including an alert;

c) indicative of an indicator;

d) derived from an indicator; and,

e) including a recommendation based on an indicator.

80) A system according to any one of the claims 1 to 79, wherein the system includes a transmitter that transmits at least one of:

a) subject data derived from the measured response signals;

b) at least one metric derived from measured response signals;

c) an indication of measured response signals; and,

d) at least one metric derived from the subject data.

81) A system according to any one of the claims 1 to 80, wherein the one or more electronic processing devices:

a) generate subject data indicative of the measured response signals; and,

b) at least one of:

i) at least partially process measured response signals;

ii) at least partially process the subject data;

iii) at least partially analyse the subject data; and,

iv) store an indication of the subject data.

82) A system according to any one of the claims 1 to 81, wherein the system includes a monitoring device and a patch including the substrate and microstructures.

83) A system according to claim 82, wherein the monitoring device is at least one of:

a) inductively coupled to the patch;

b) attached to the patch; and

c) brought into contact with the patch when a reading is to be performed.

84) A system according to any one of the claims 1 to 83, wherein the monitoring device is configured to at least one of:

a) cause a measurement to be performed;

b) at least partially analyse measurements;

c) control stimulation applied to at least one microstructure;

d) generate an output;

e) provide an output indicative of the indicator;

f) provide a recommendation based on the indicator; and,

g) cause an action to be performed.

85) A system according to any one of the claims 1 to 84, wherein the system includes at least one of:

a) a wearable monitoring device that performs the measurements; and,

b) a processing system that:

i) receives subject data derived from the measured response signals; and,

ii) analyses the subject data to generate at least one indicator, the at least one indicator being at least partially indicative of a health status associated with the subject.

86) A system according to claim 85, wherein the system includes a client device that:

a) receives measurement data from the wearable monitoring device;

b) generates subject data using the measurement data;

c) transfer the subject data to the processing system;

d) receive an indicator from the processing system; and,

e) displays a representation of the indicator.

87) A system according to any one of the claims 1 to 86, wherein the system includes:

a) a substrate coil positioned on the substrate and operatively coupled to one or more microstructure electrodes; and,

b) an excitation and receiving coil positioned in proximity to the substrate coil such that alteration of a drive signal applied to the excitation and receiving coil acts as a response signal.

88) A system according to any one of the claims 1 to 87, wherein one or more microstructure electrodes interact with one or more analytes of interest such that the response signal is dependent on a presence, absence, level or concentration of the one or more analytes of interest.

89) A system according to claim 88, wherein the system includes:

a) a first substrate coil positioned on a substrate and operatively coupled to one or more first microstructure electrodes;

b) a second substrate coil positioned on a substrate and operatively coupled to one or more second microstructure electrodes, the second microstructure electrodes being configured to interact with one or more analytes of interest; and,

c) at least one excitation and receiving coil positioned in proximity to at least one of the first and second substrate coils such that alteration of a drive signal applied to the at least one excitation and receiving coil acts as a response signal, and wherein the one or more electronic processing devices use the first and second response signals to a presence, absence, level or concentration of one or more analytes of interest.

90) A system according to claim 89, wherein first and second excitation and receiving coils are positioned in proximity to respective ones of the first and second substrate coils such that alteration of a drive signal applied to each excitation and receiving coil acts as a respective response signal.

91) A system for performing measurements on a biological subject, the system including:

a) at least one sensor configured to be operatively connected to one or more microstructures configured to breach a functional barrier of the subject in use, the at least one sensor being configured to measure response signals from the at least one microstructure, wherein the one or more microstructures include an aptamer for binding one or more analytes; and,

b) one or more electronic processing devices that:

i) determine measured response signals; and,

ii) at least one of:

(1) perform an analysis at least in part using the measured response signals; and,

(2) store data at least partially indicative of the measured response signals.

92) A method for performing measurements on a biological subject, the method including:

a) using at least one substrate including one or more microstructures to breach a functional barrier of the subject, wherein the one or more microstructures include an aptamer for binding one or more analytes;

b) using at least one sensor operatively connected to at least one microstructure to measure response signals from the at least one microstructure; and,

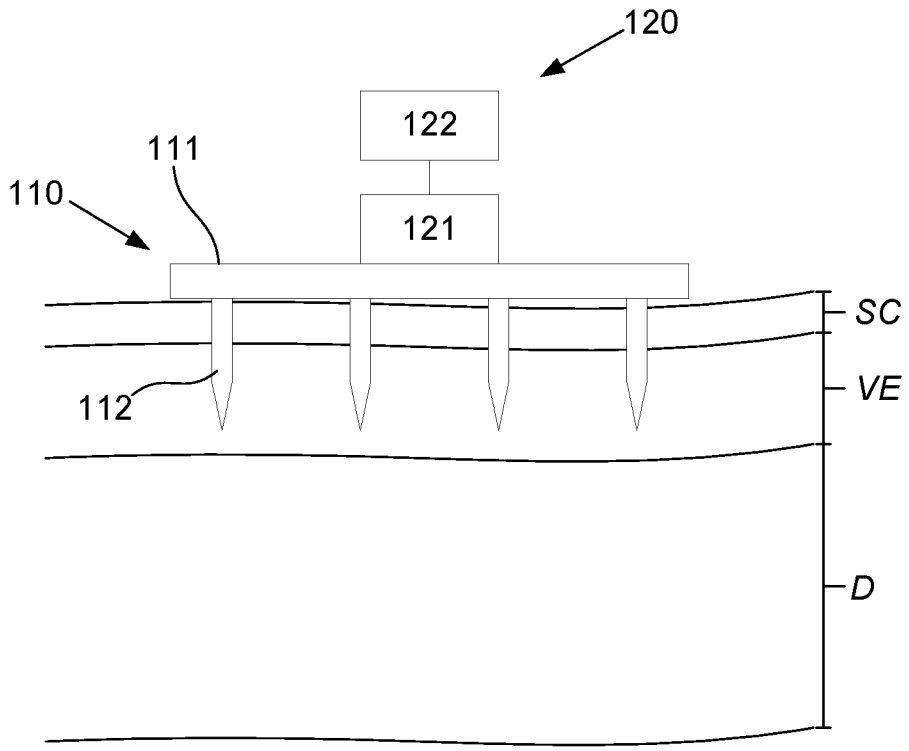
c) in one or more electronic processing devices:

i) determining measured response signals; and,

ii) at least one of:

(1) performing an analysis at least in part using the measured response signals; and,

(2) storing data at least partially indicative of the measured response signals.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112
D

Fig. 1

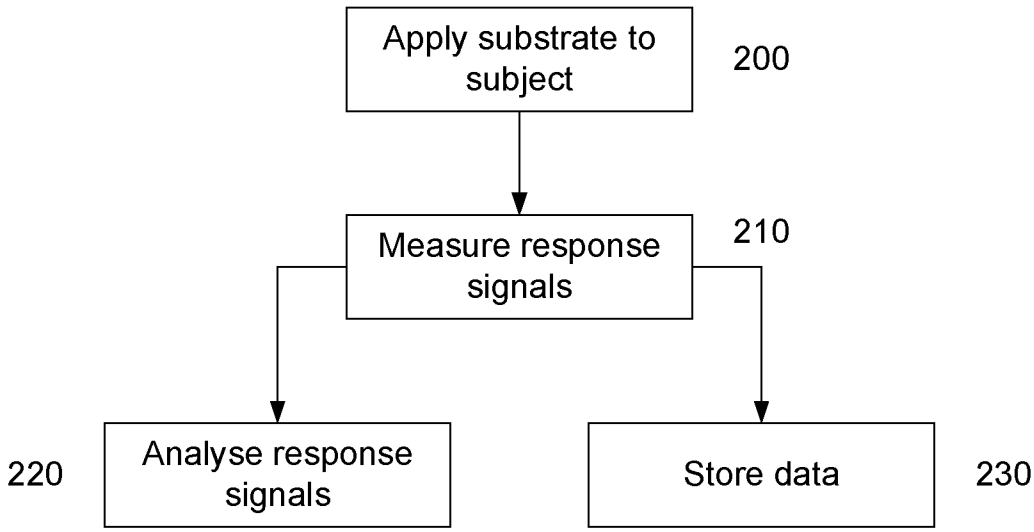
Apply substrate to subject
200
Measure response signals
210
220
Analyse response signals
Store data
230

Fig. 2

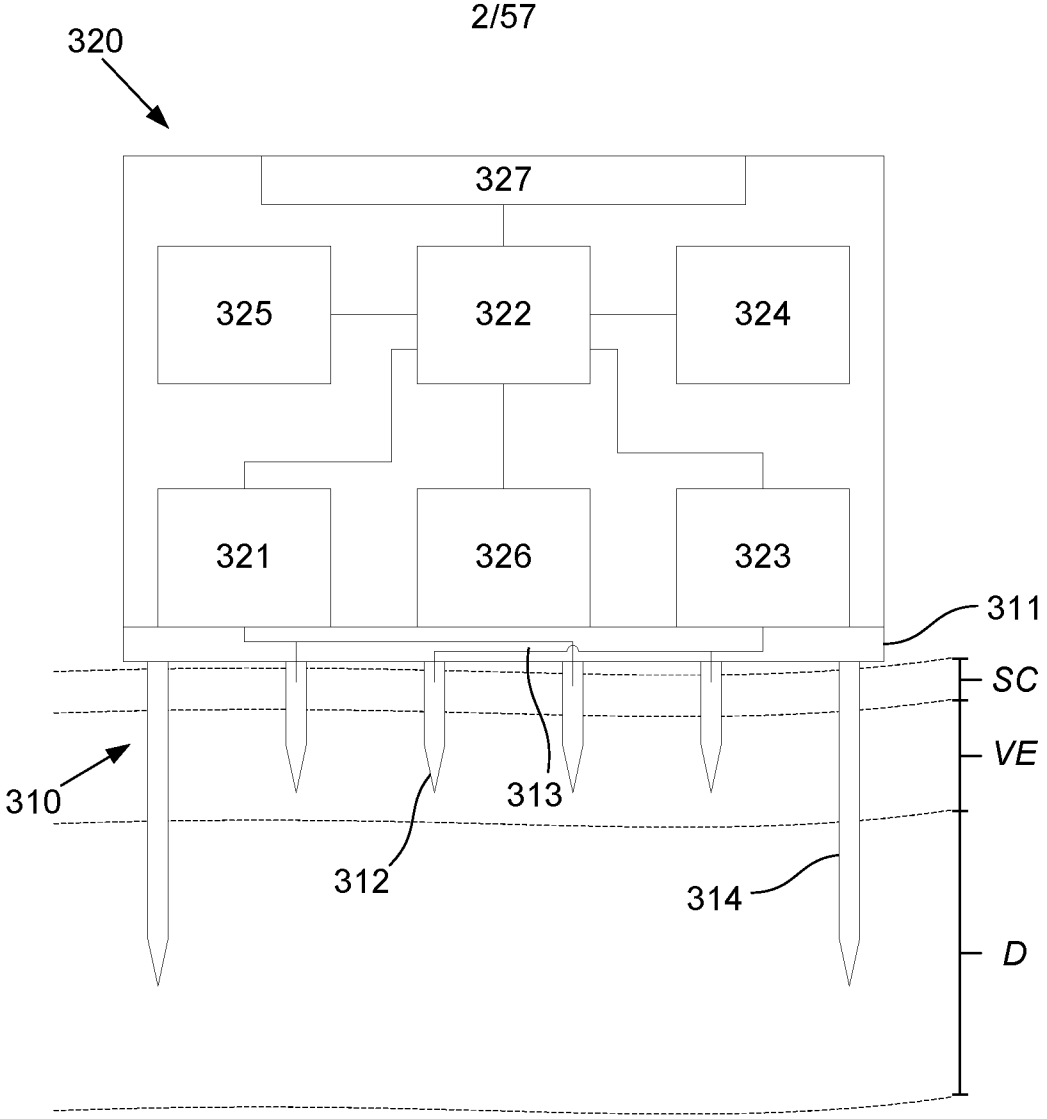

Fig. 3A

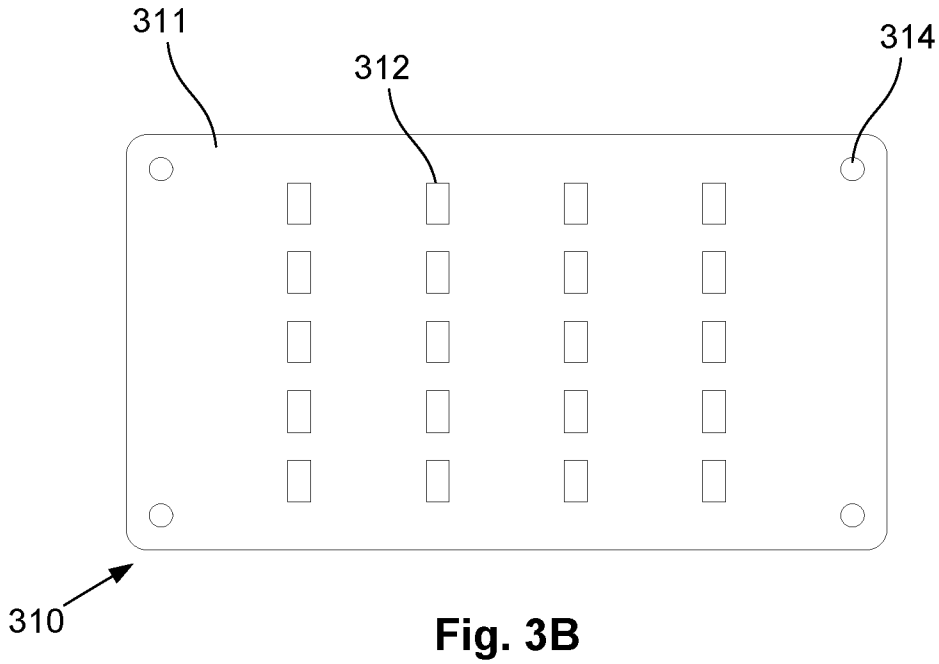

Fig. 3B

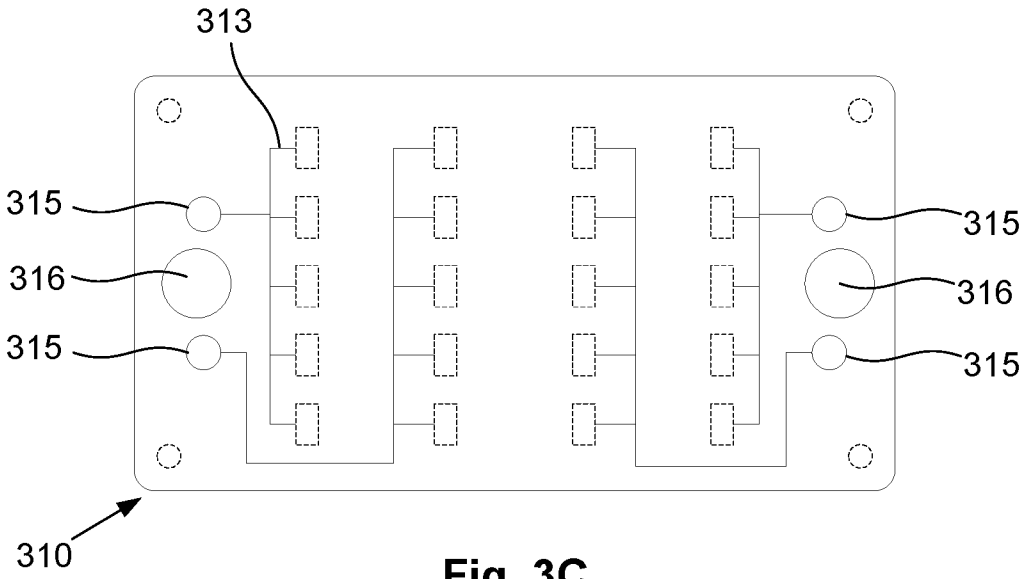

Fig. 3C

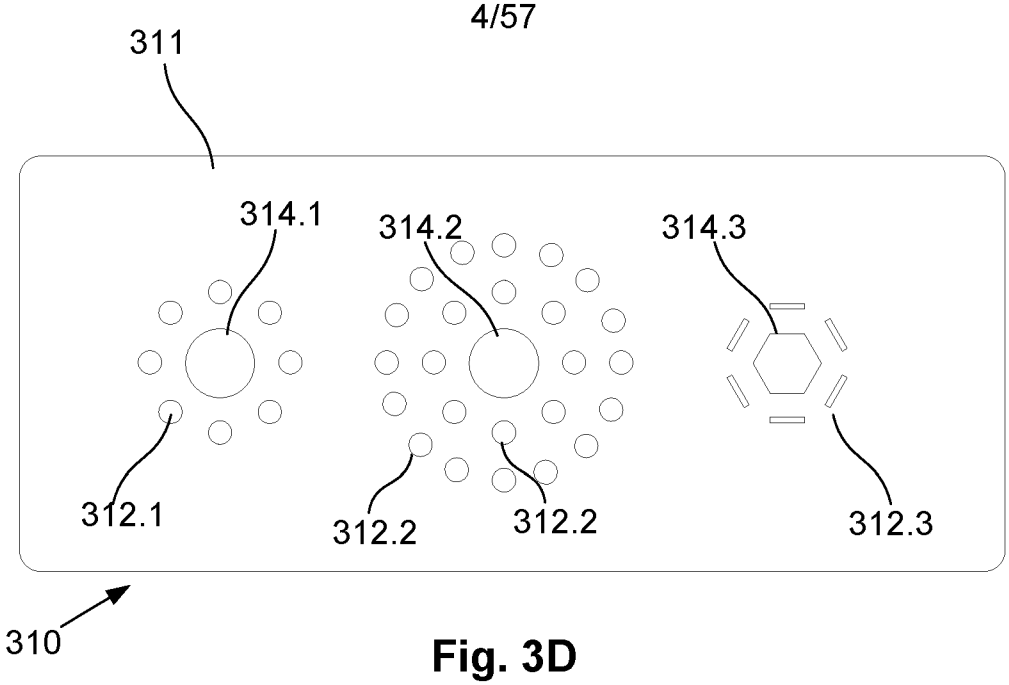
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Fig. 3D

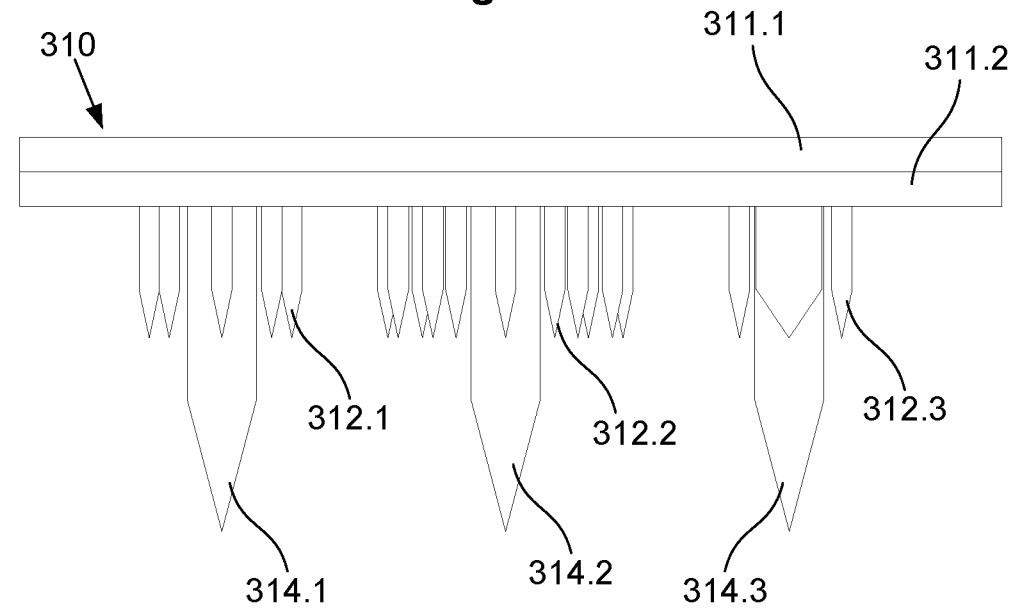
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Fig. 3E

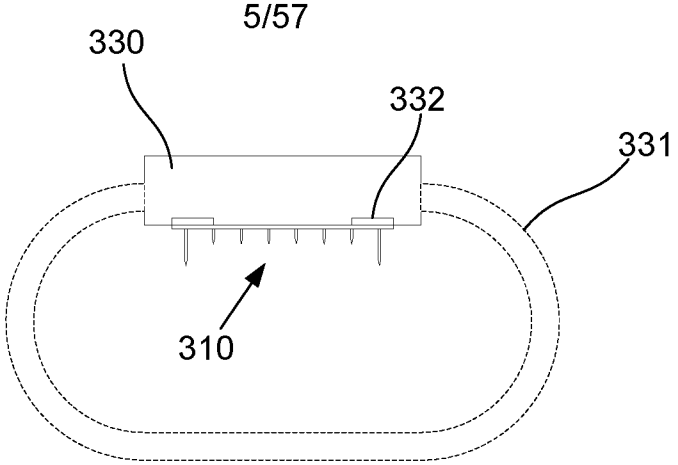


Fig. 3F

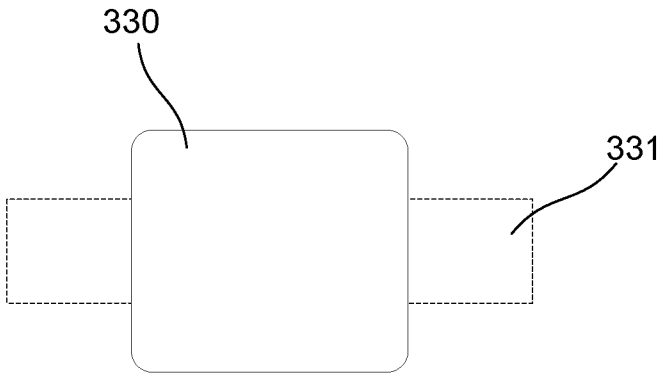


Fig. 3G

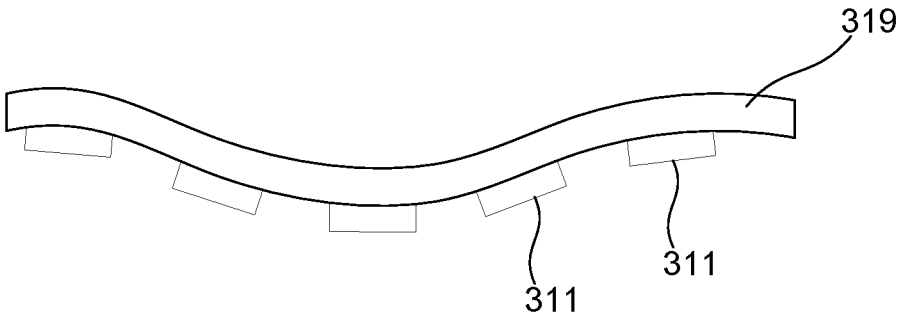


Fig. 3H

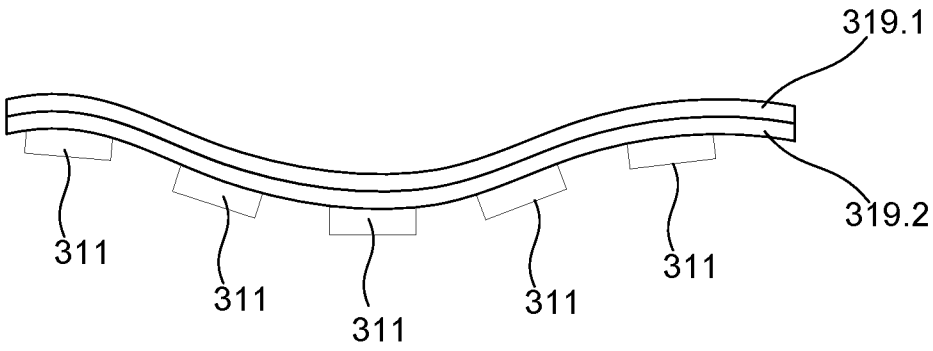


Fig. 3I

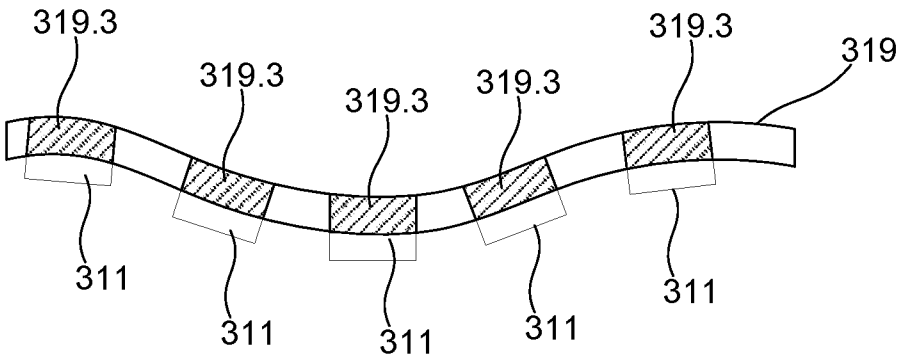
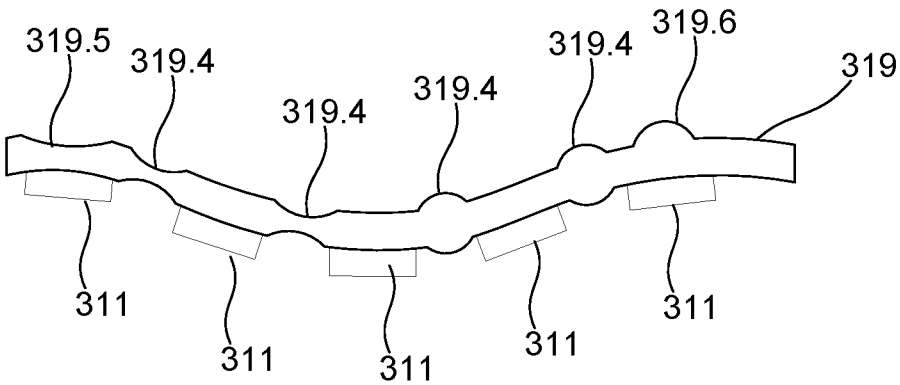


Fig. 3J



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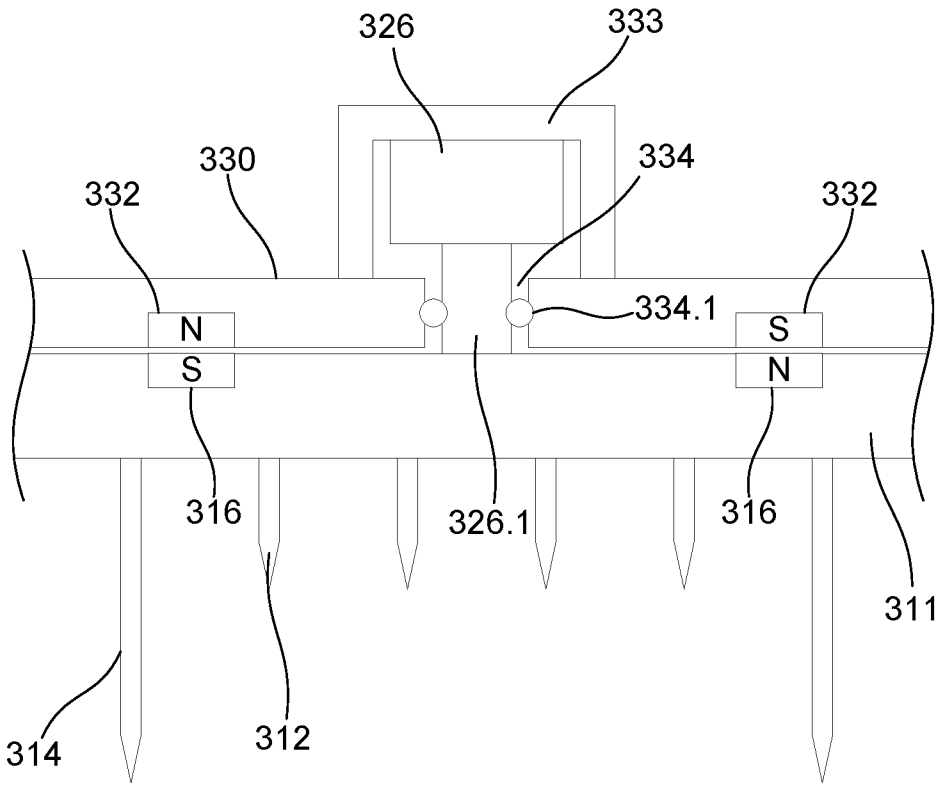
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Fig. 3L

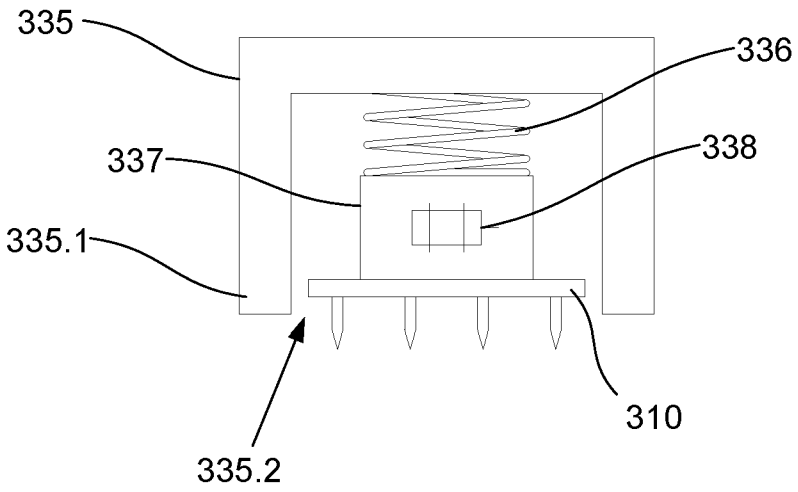
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Fig. 3M

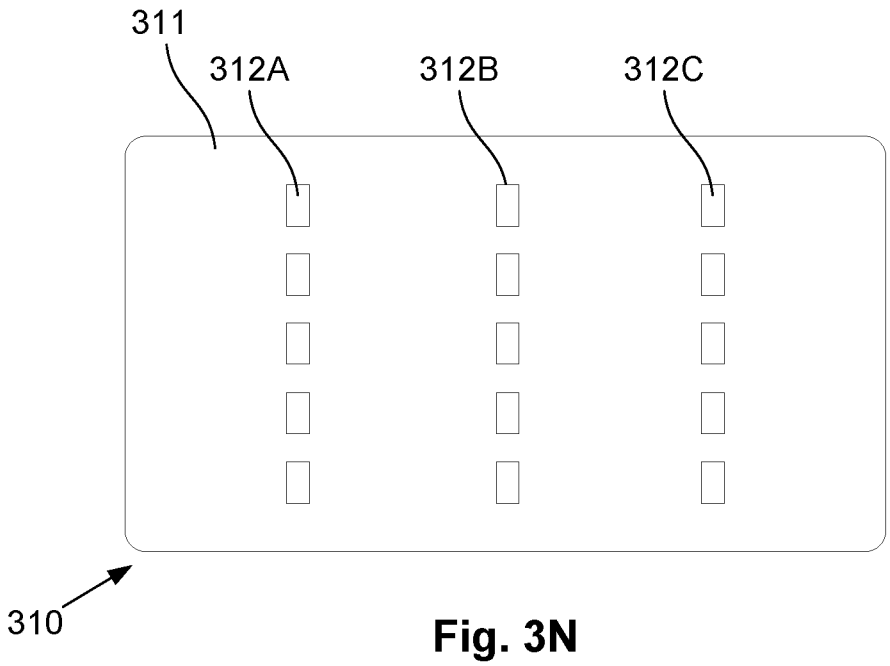


Fig. 3N

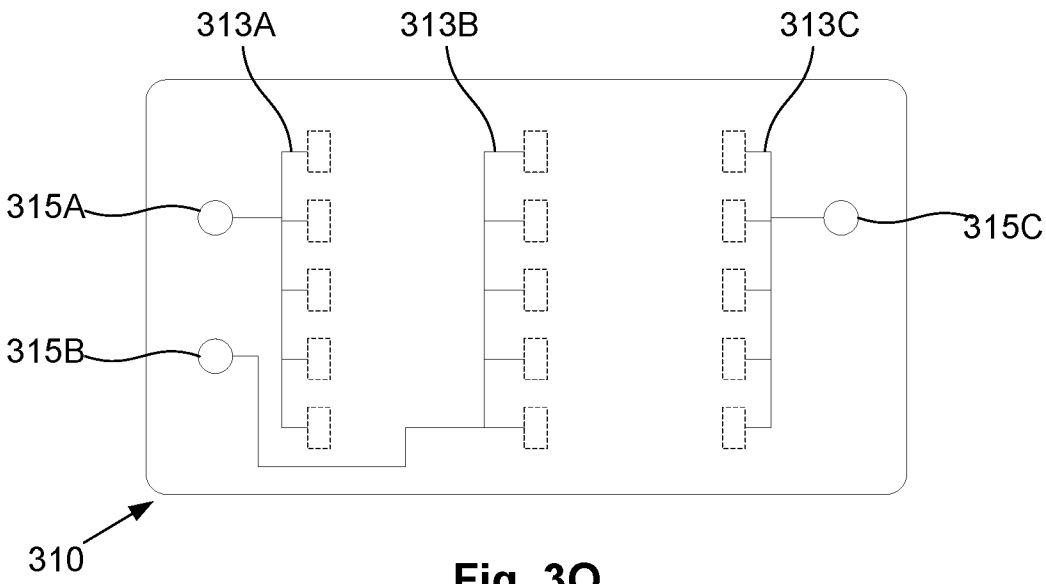


Fig. 3O

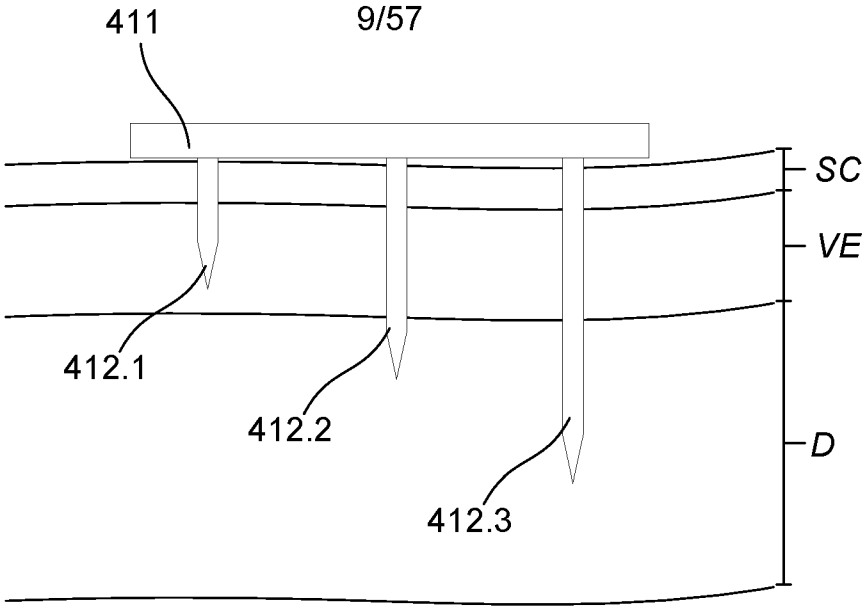


Fig. 4A

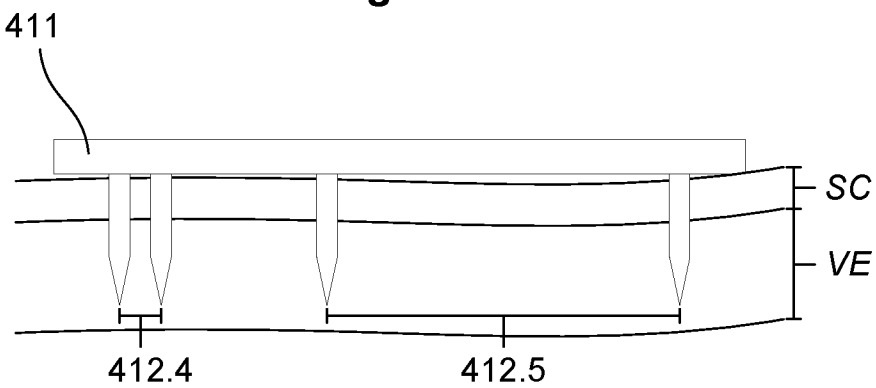


Fig. 4B

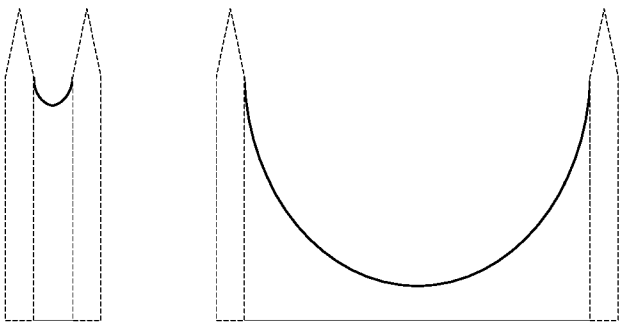


Fig. 4C Fig. 4D

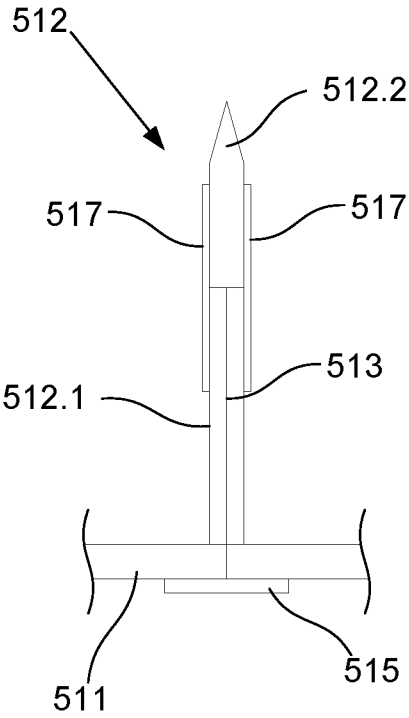


Fig. 5A

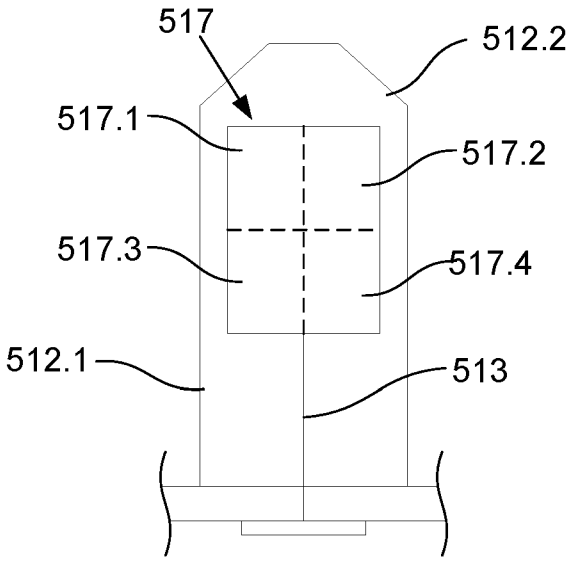


Fig. 5B

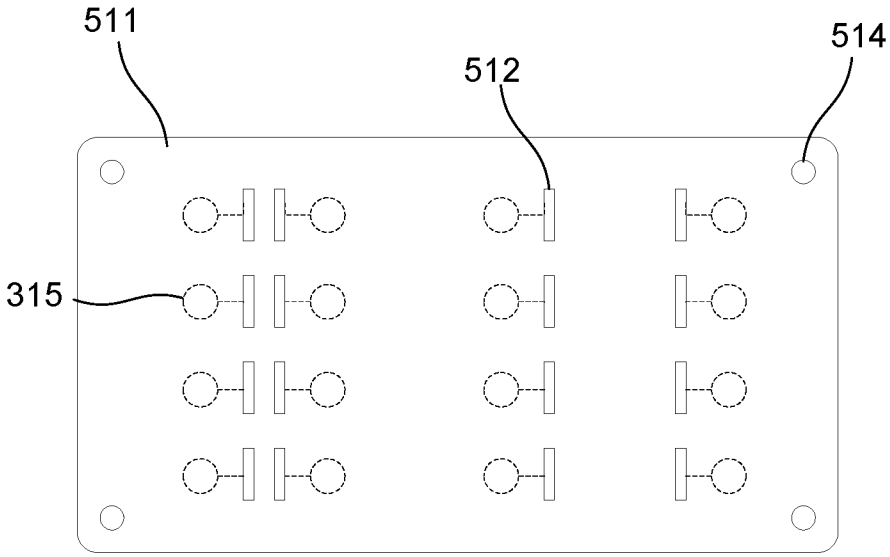


Fig. 5C

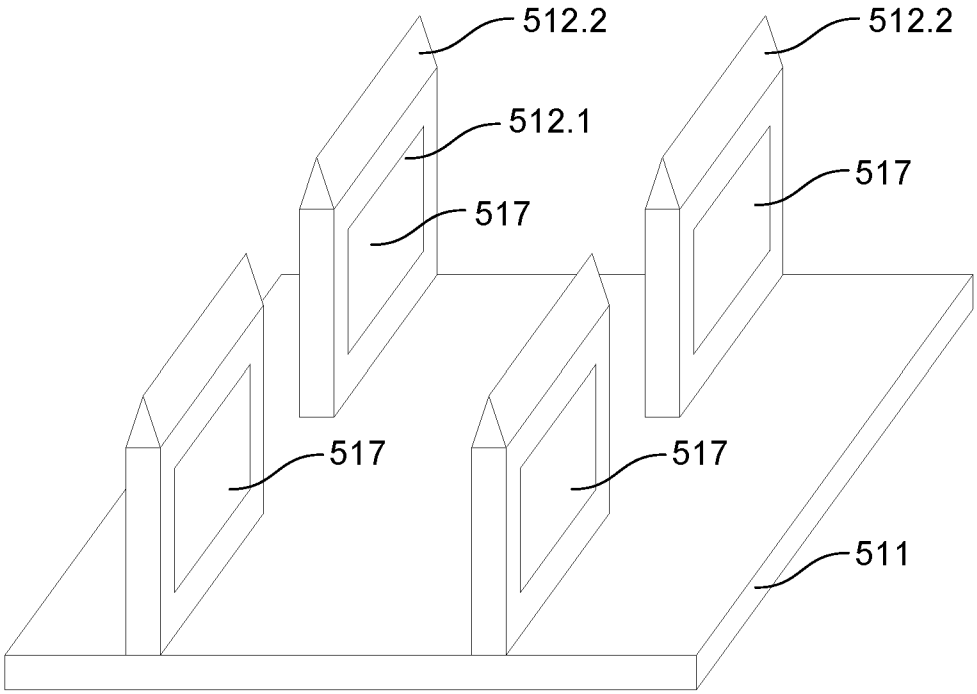


Fig. 5D

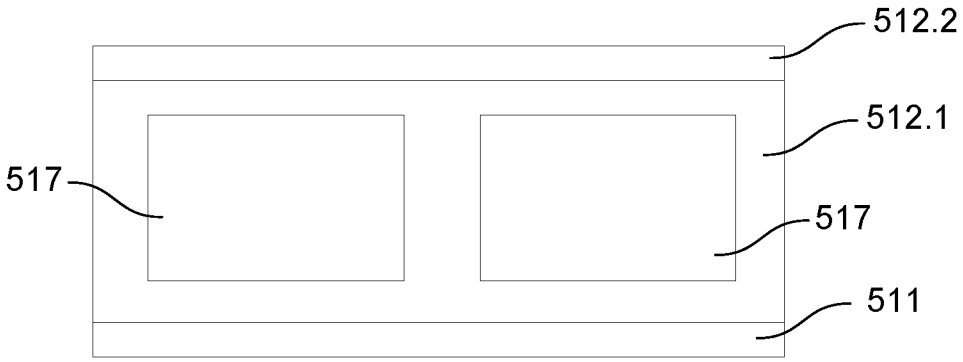
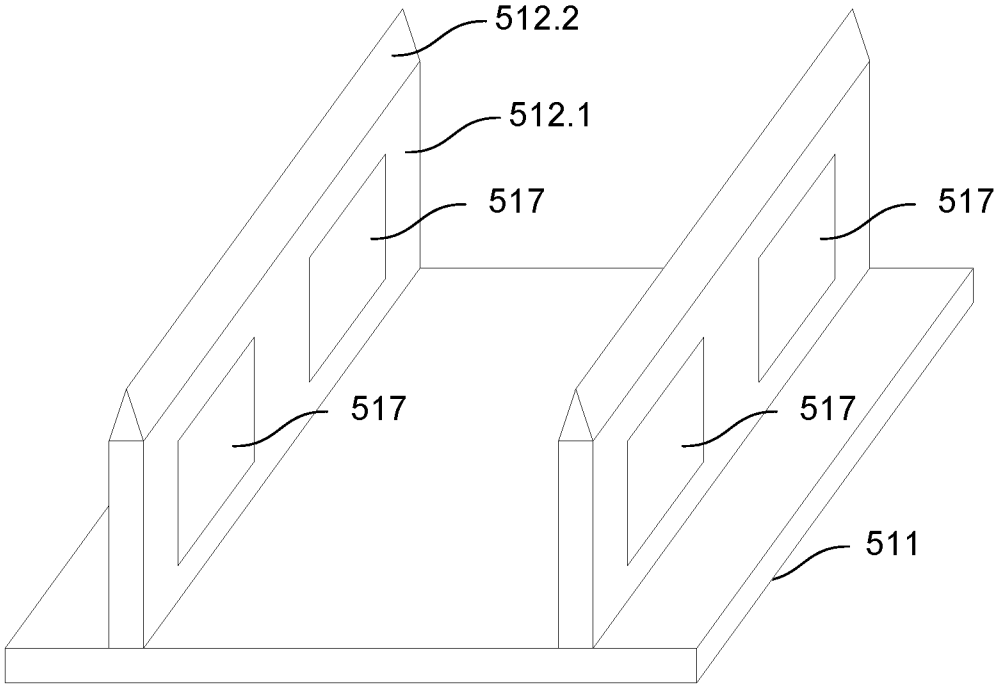


Fig. 5E



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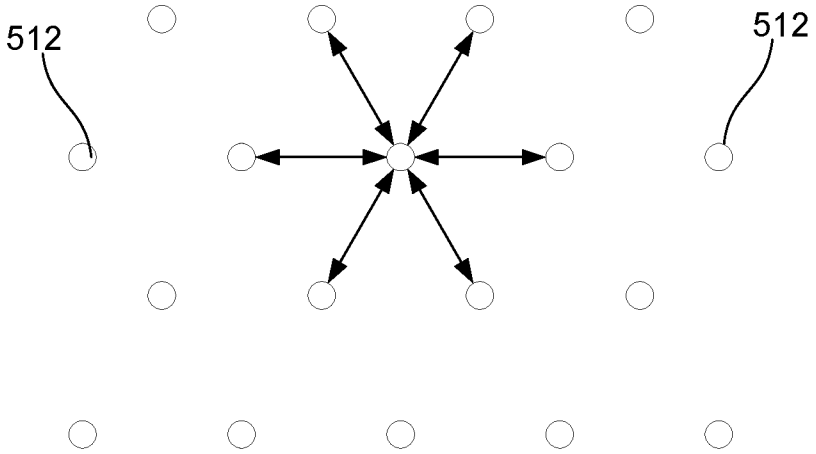


Fig. 5G

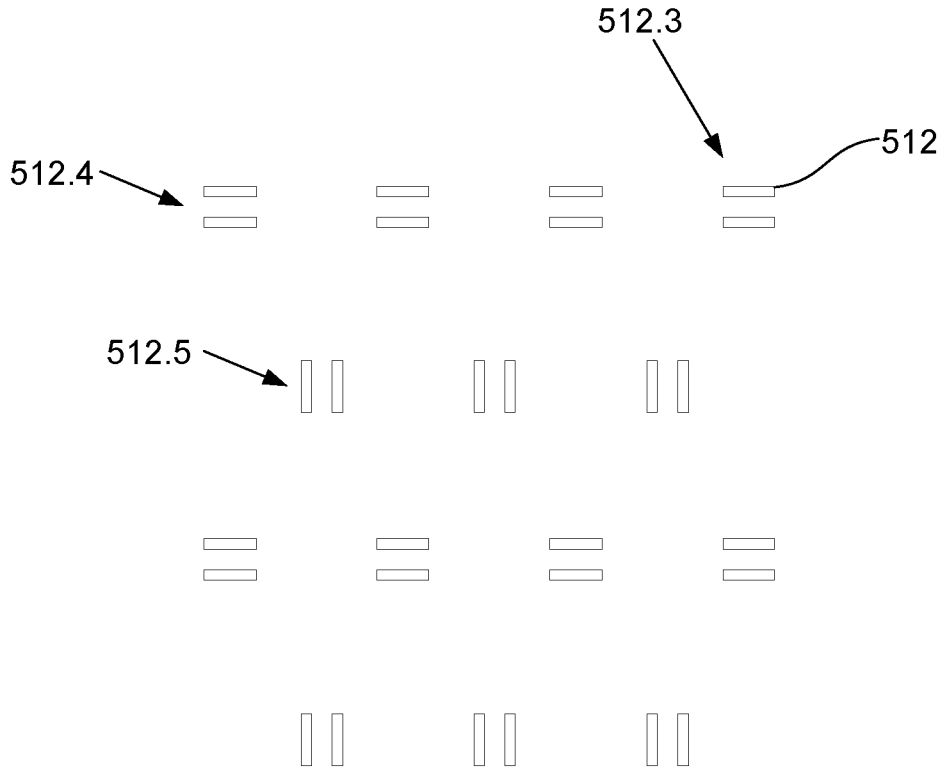


Fig. 5H

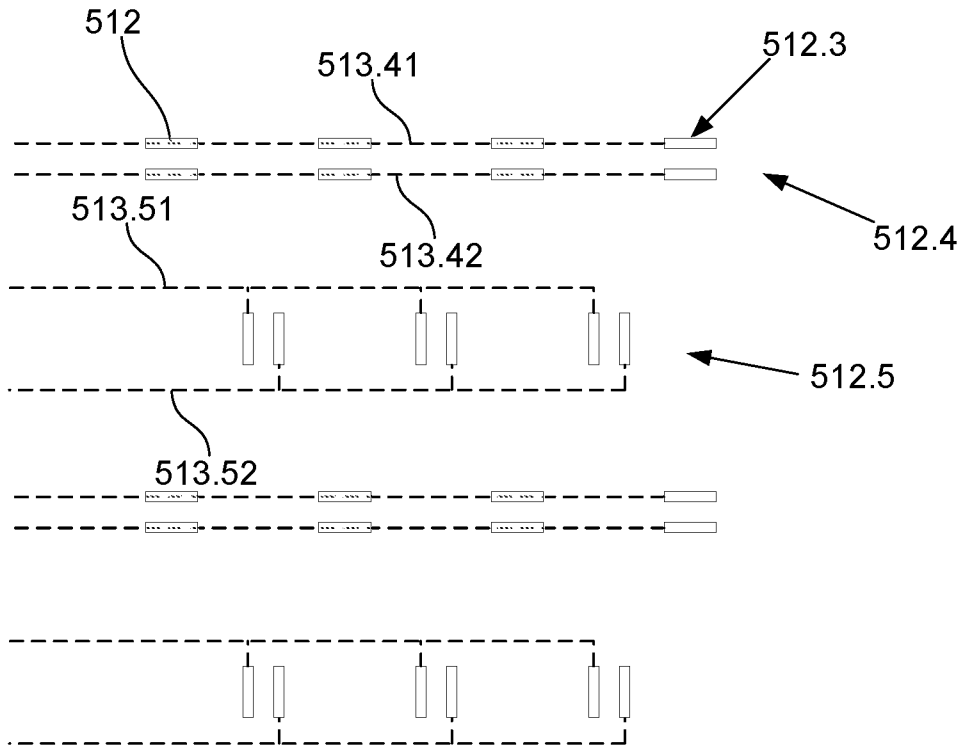
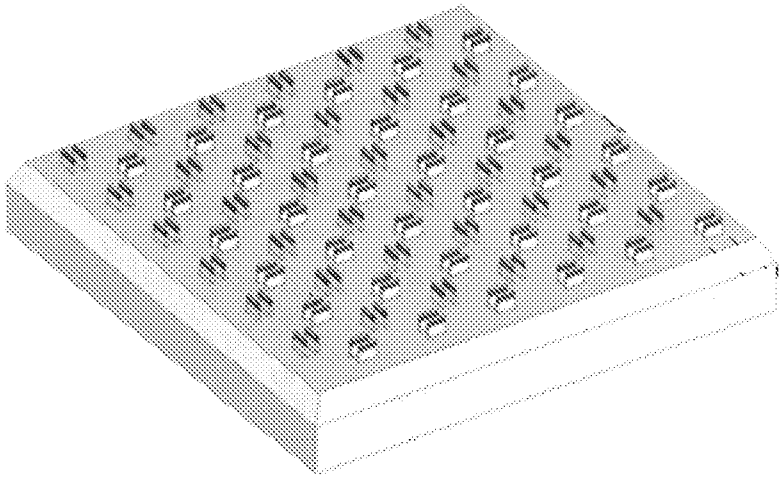


Fig. 5I



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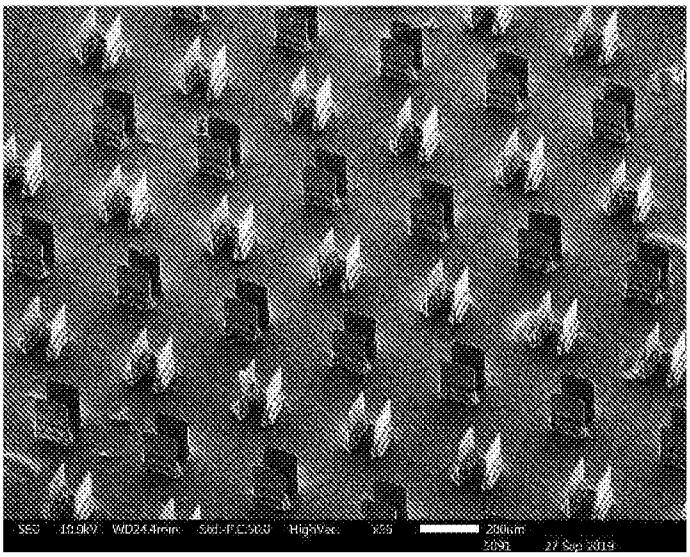


Fig. 5K

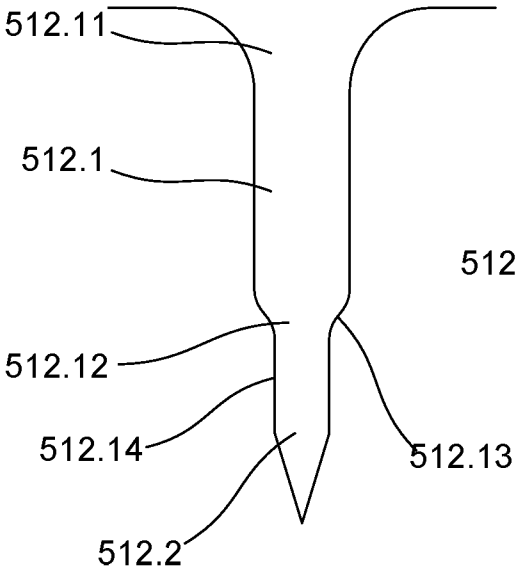
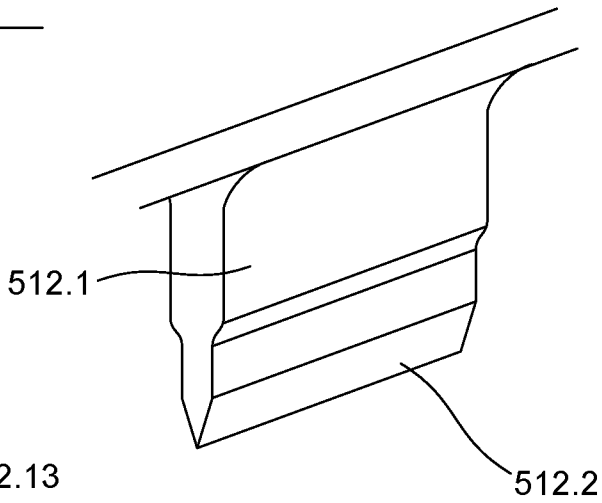


Fig. 5L



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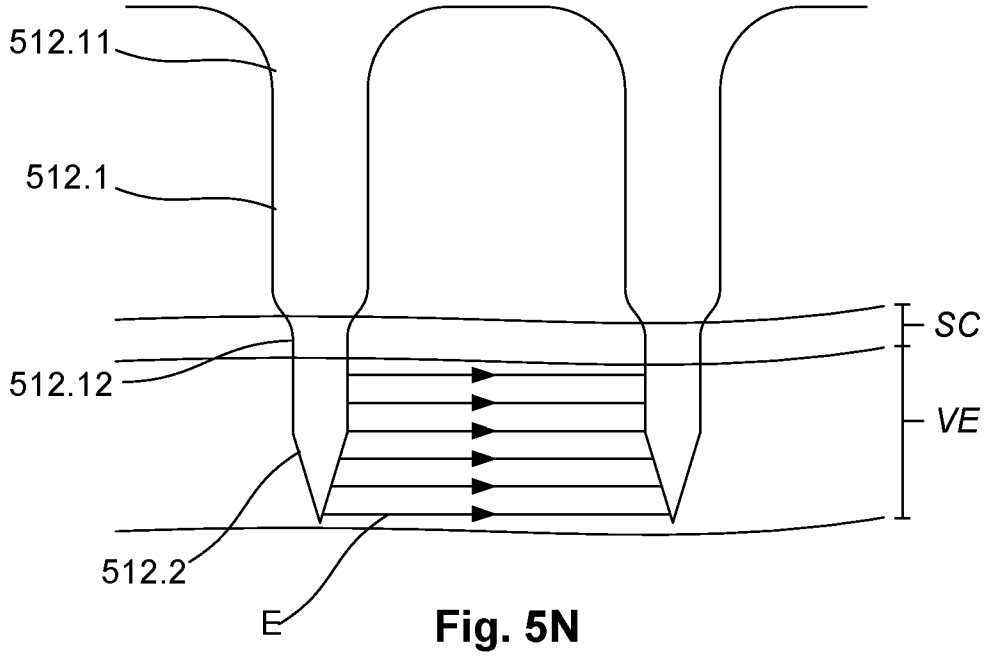
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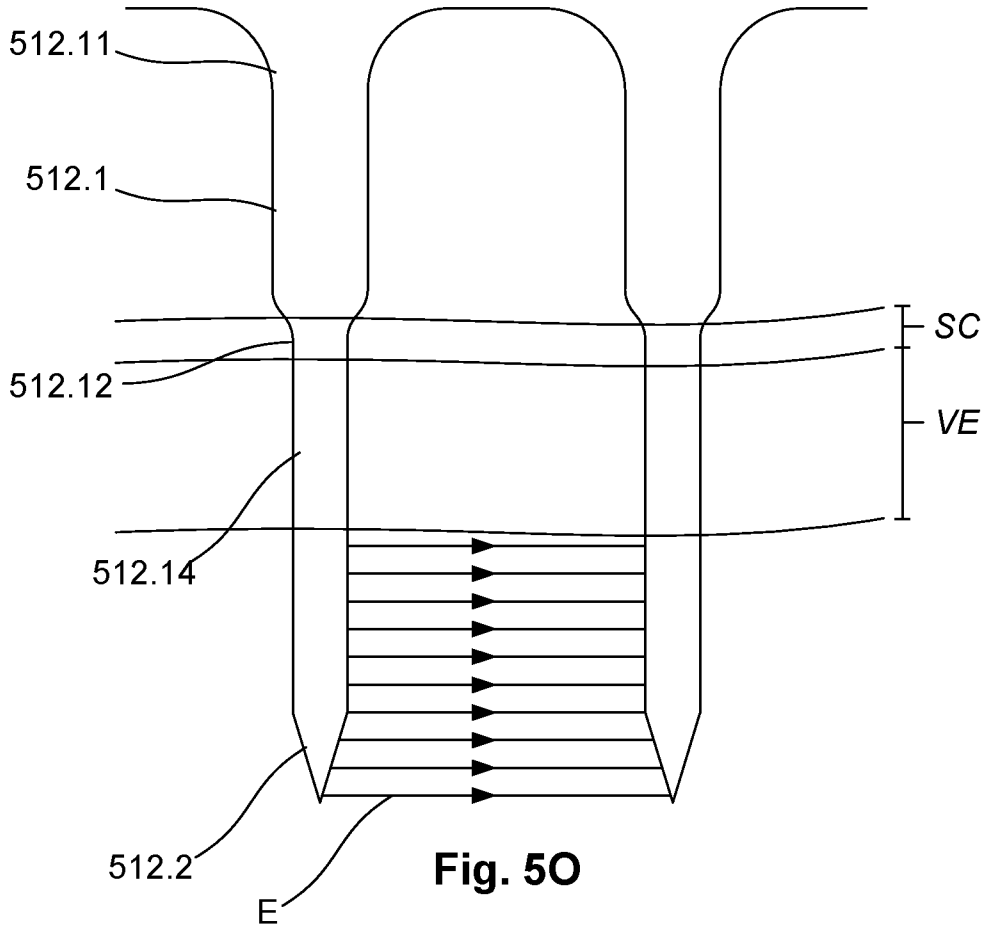
512.2
E
SC
VE

Fig. 5N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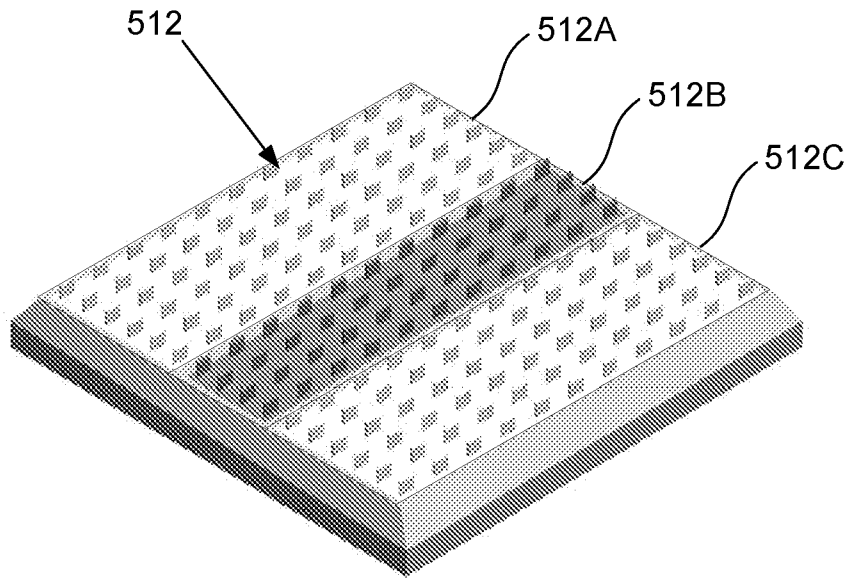
E
SC
VE

Fig. 5O



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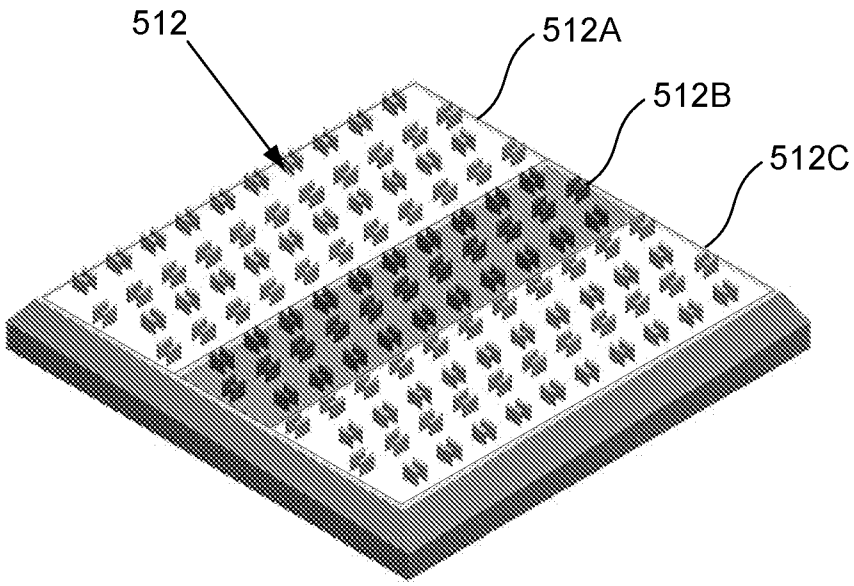

Fig. 5Q

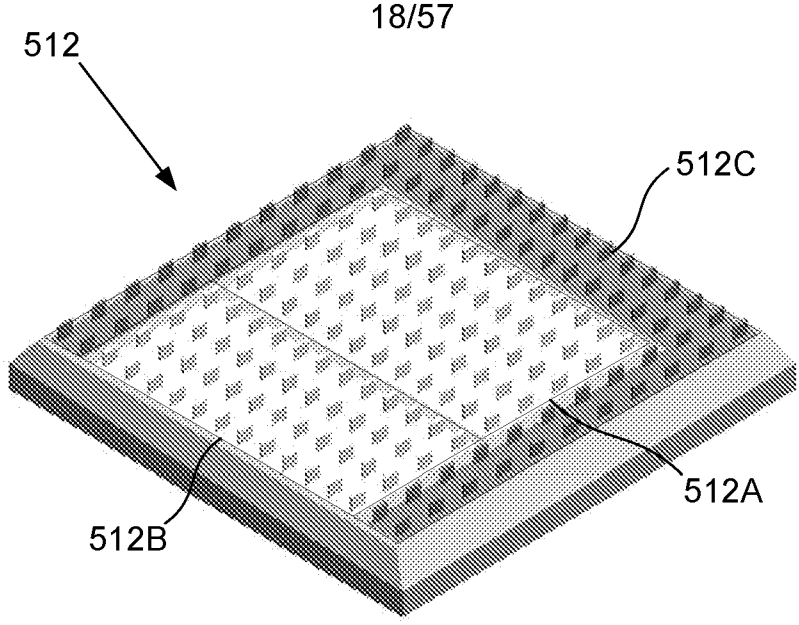

Fig. 5R

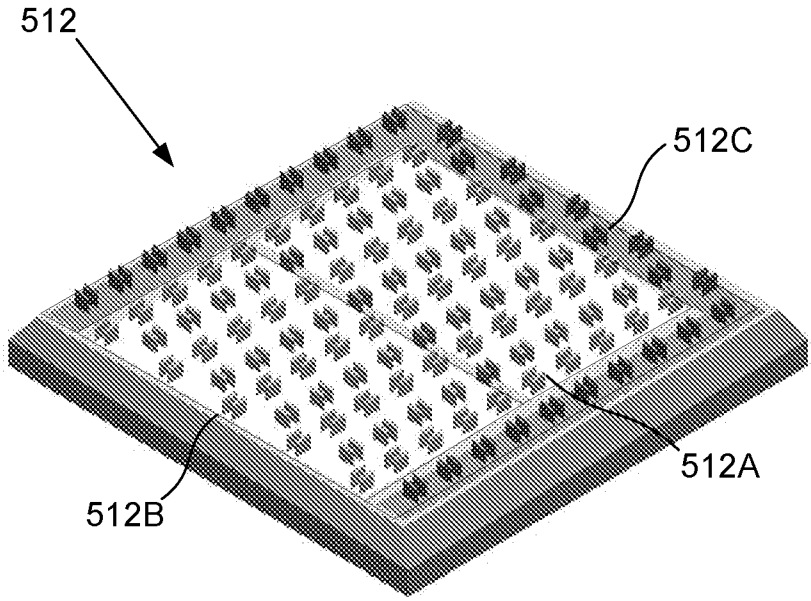

Fig. 5S

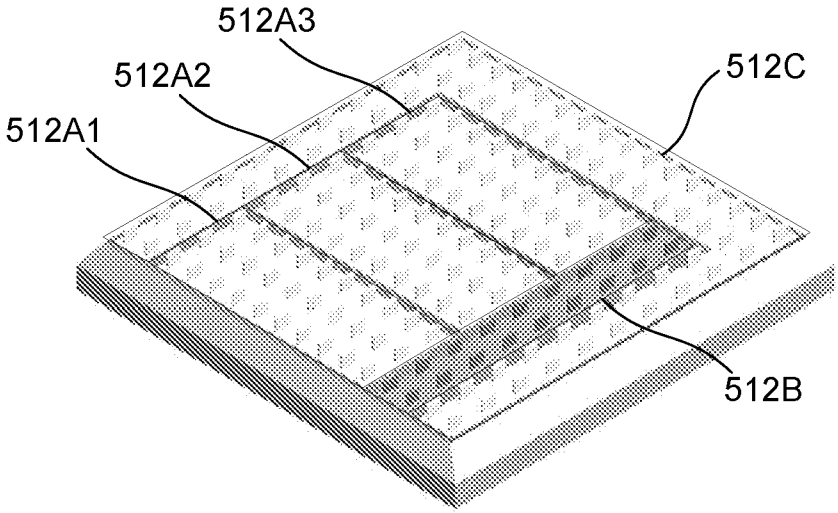

Fig. 5T

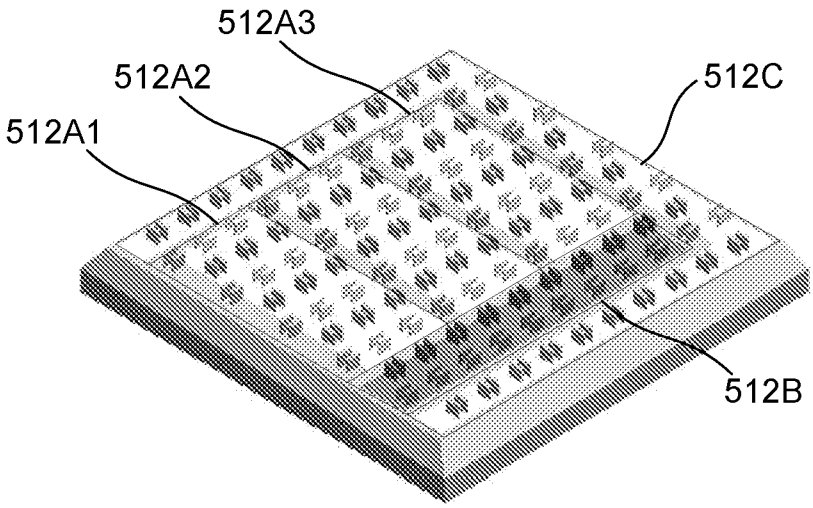

Fig. 5U

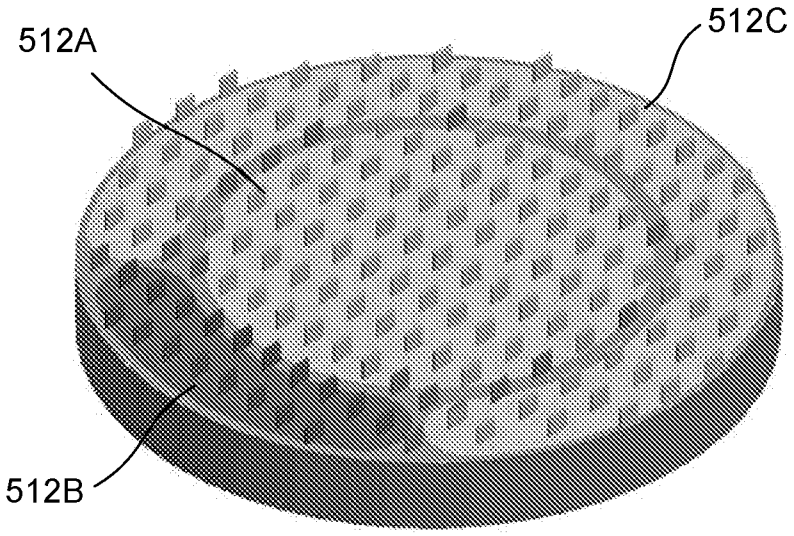

Fig. 5V

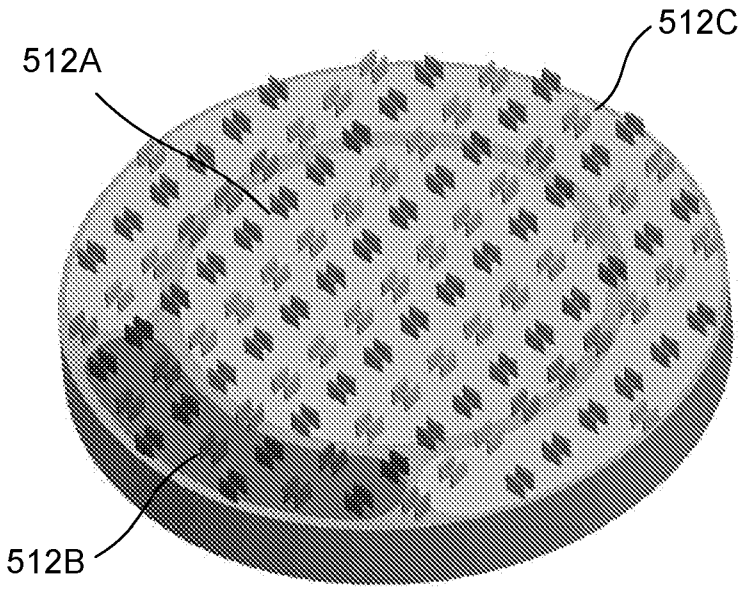

Fig. 5W

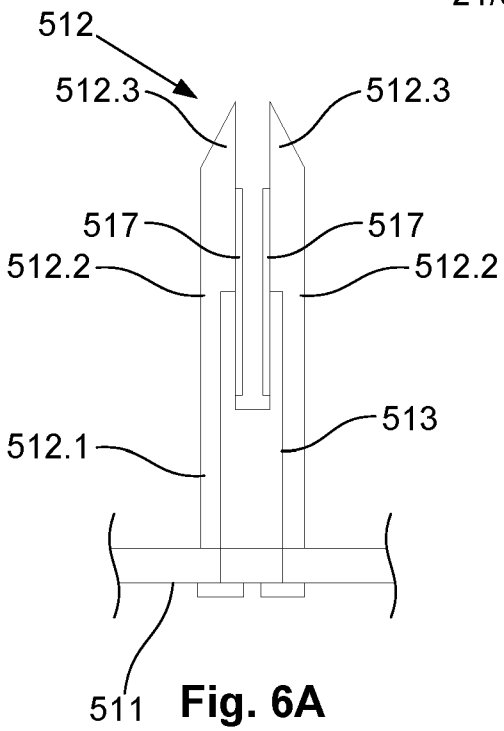

Fig. 6A

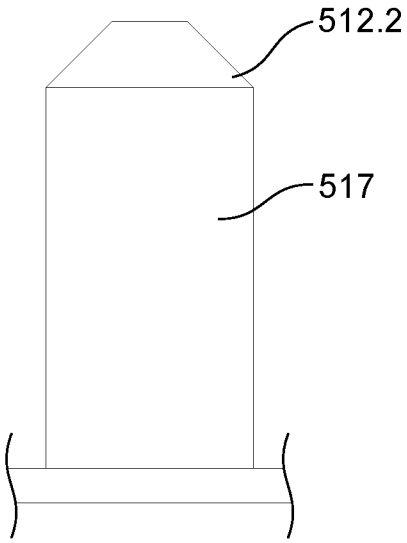

Fig. 6B

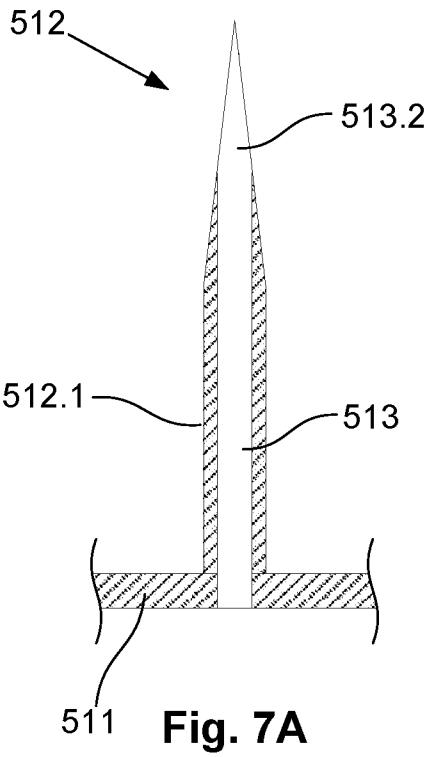

Fig. 7A

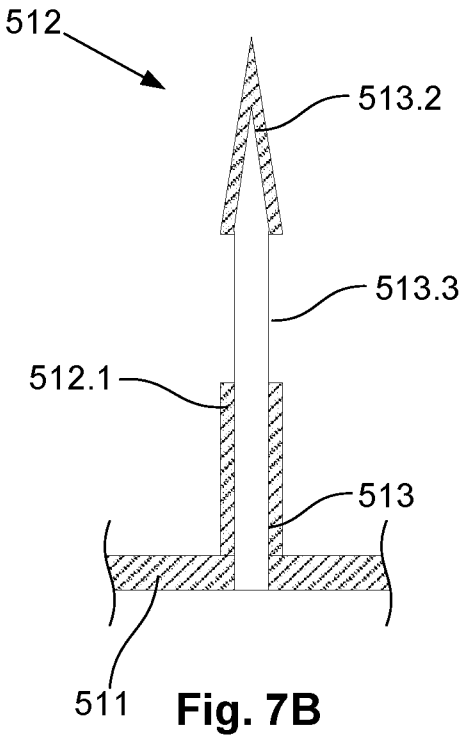

Fig. 7B

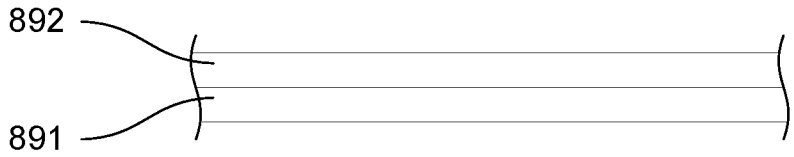

Fig. 8A

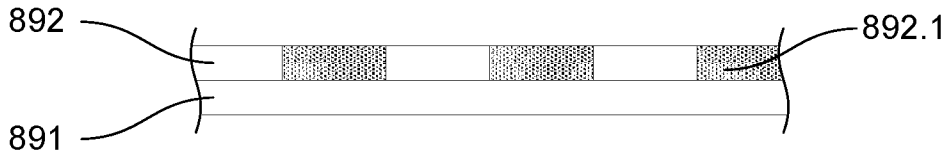

Fig. 8B

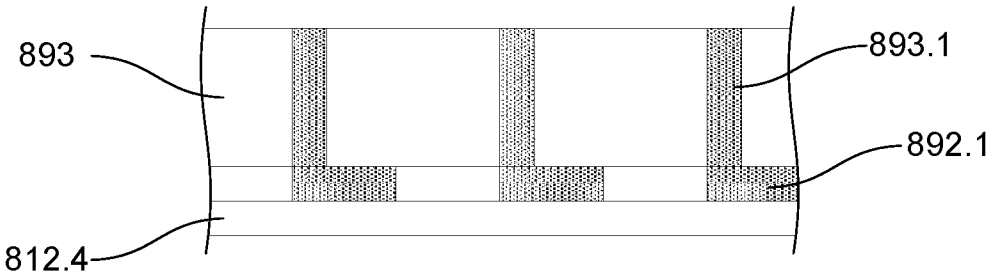

Fig. 8C

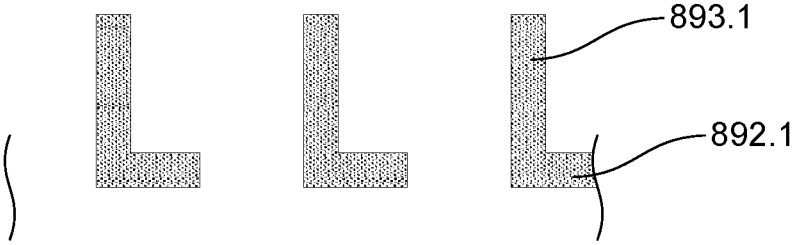

Fig. 8D

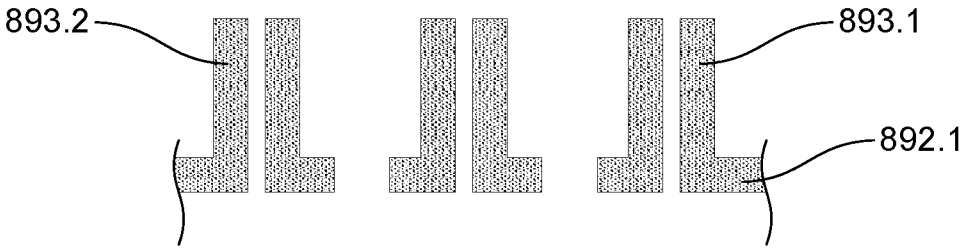

Fig. 8E

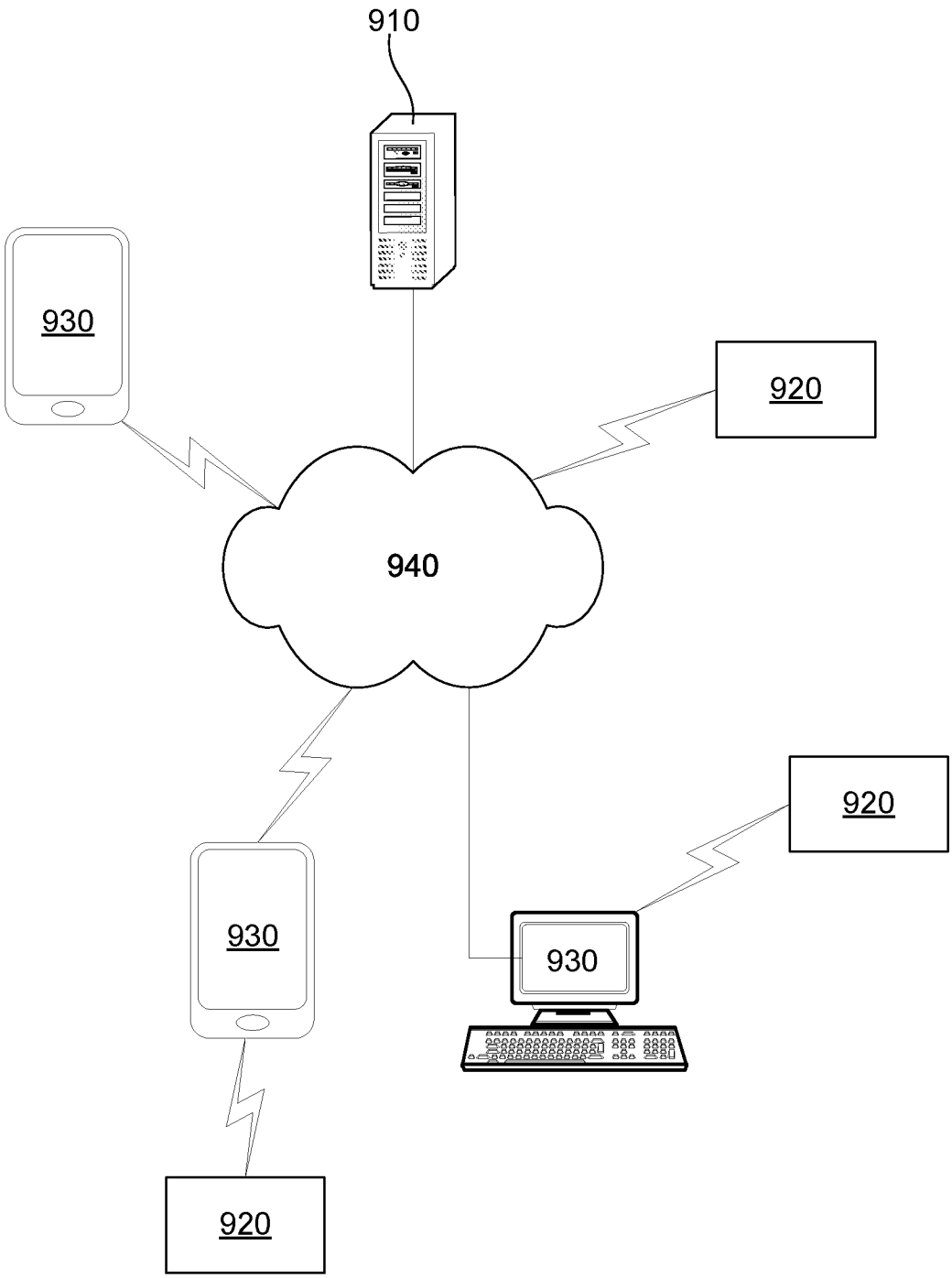

Fig. 9

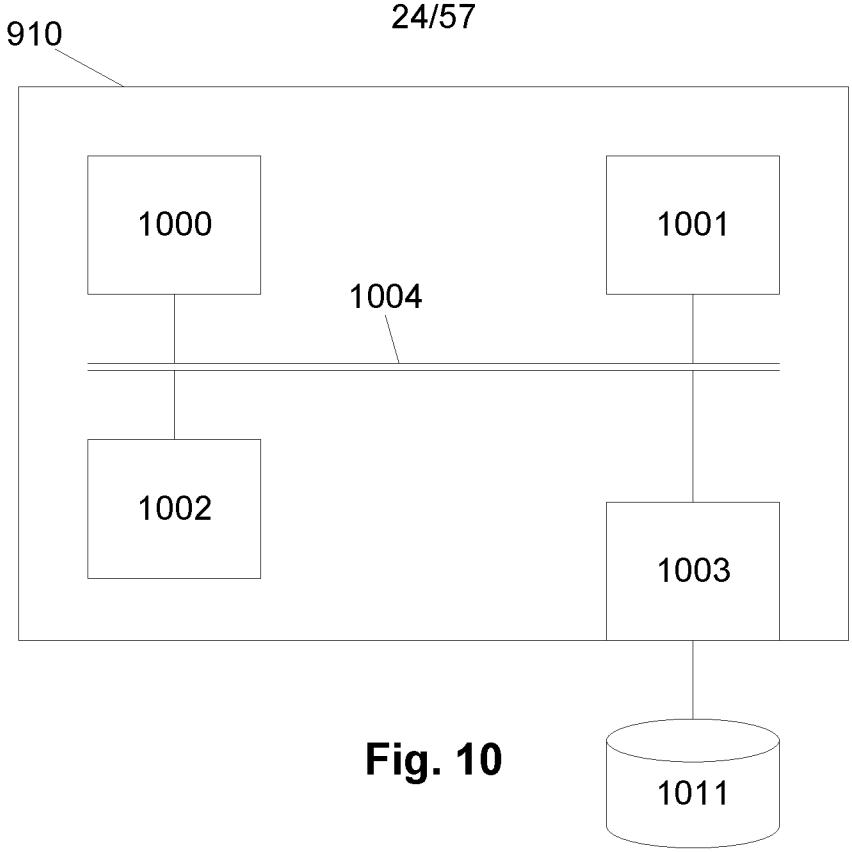
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Fig. 10

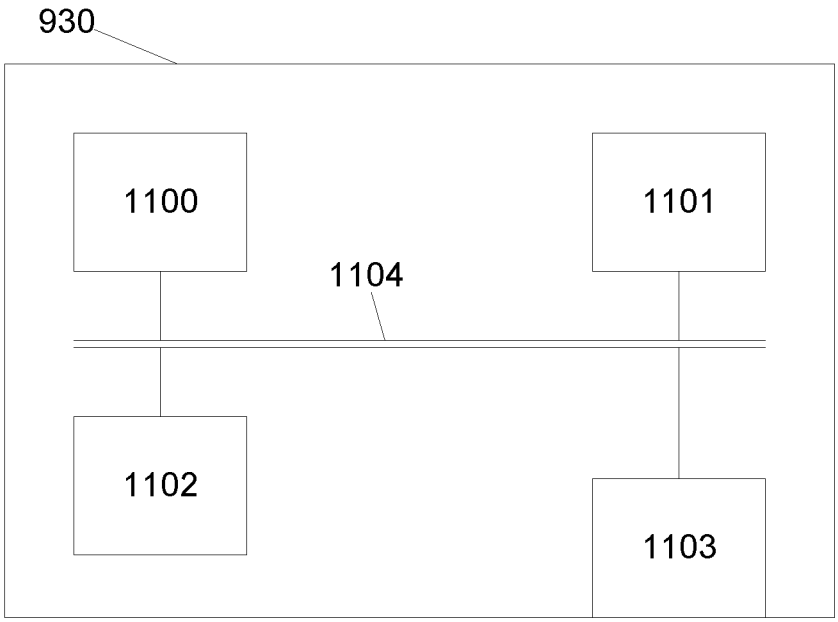
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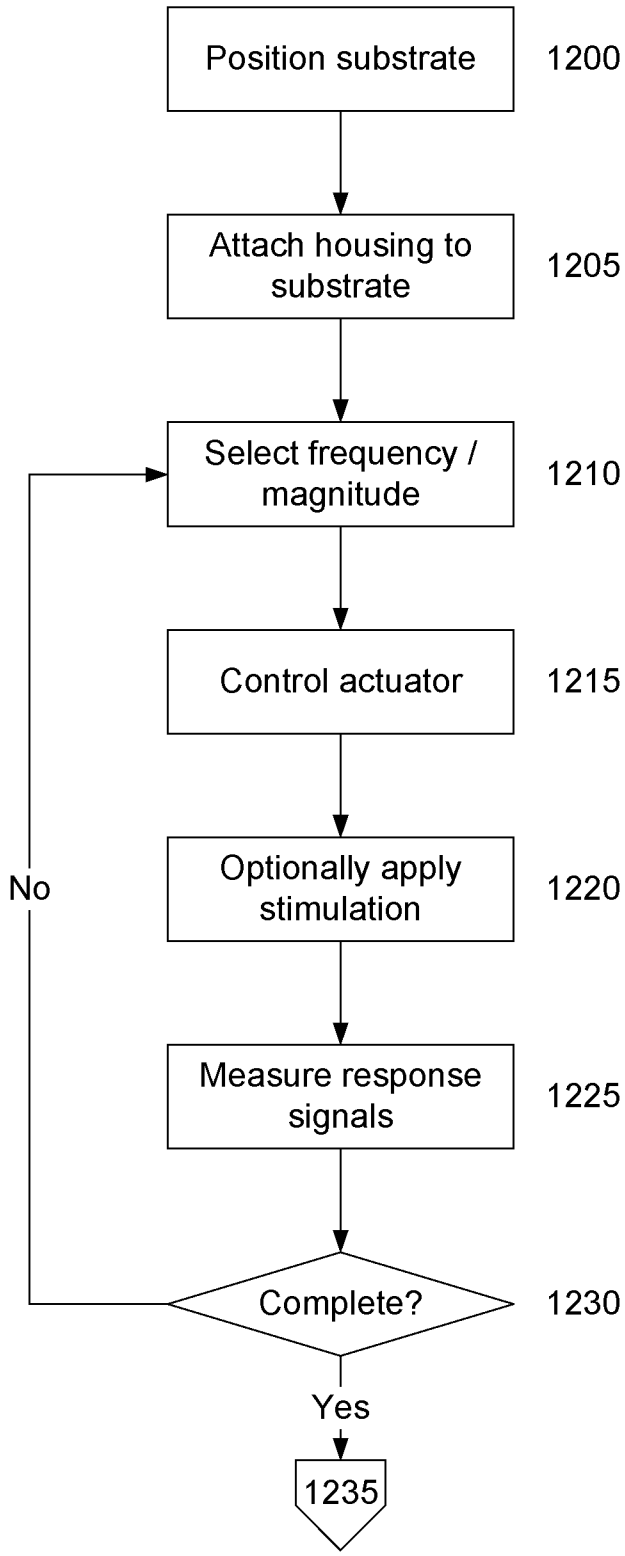
Fig. 11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1200
Attach housing to substrate
1205
Select frequency / magnitude
1210
Control actuator
1215
Optionally apply stimulation
1220
Measure response signals
1225
Complete?
1230
No
Yes
1235

Fig. 12A

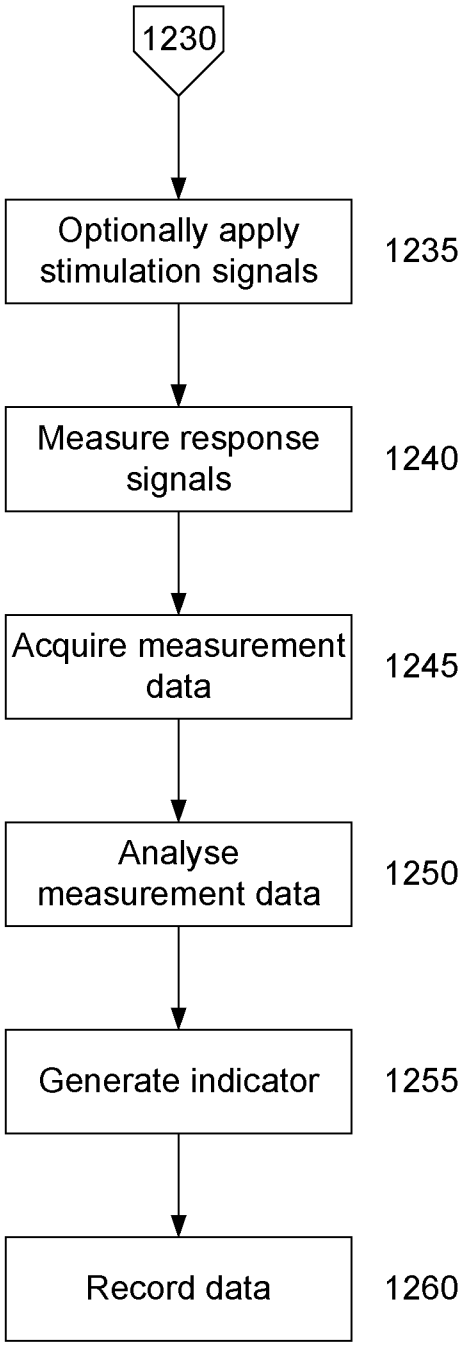

1230
Optionally apply stimulation signals
1235
Measure response signals
1240
Acquire measurement data
1245
Analyse measurement data
1250
Generate indicator
1255
Record data
1260

Fig. 12B

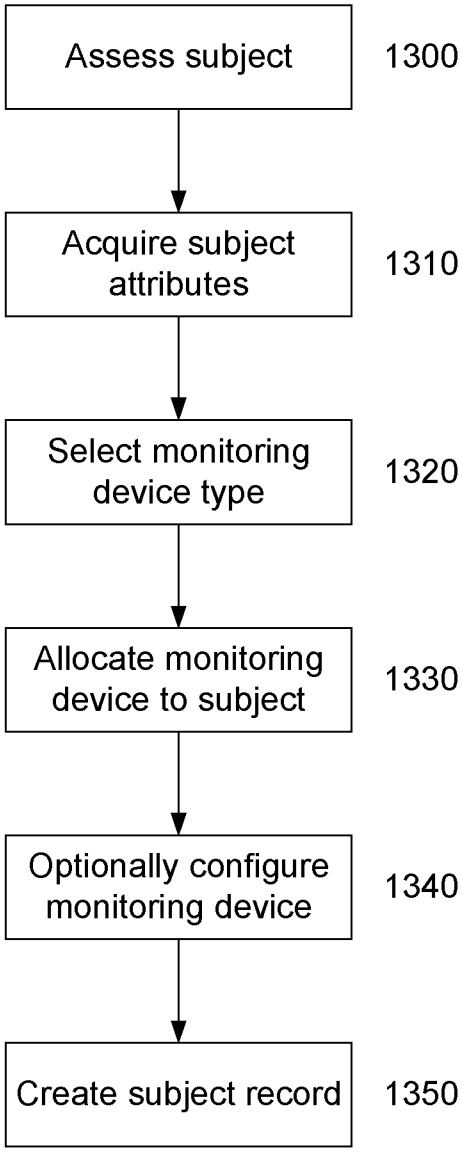

Assess subject
1300
Acquire subject attributes
1310
Select monitoring device type
1320
Allocate monitoring device to subject
1330
Optionally configure monitoring device
1340
Create subject record
1350

Fig. 13

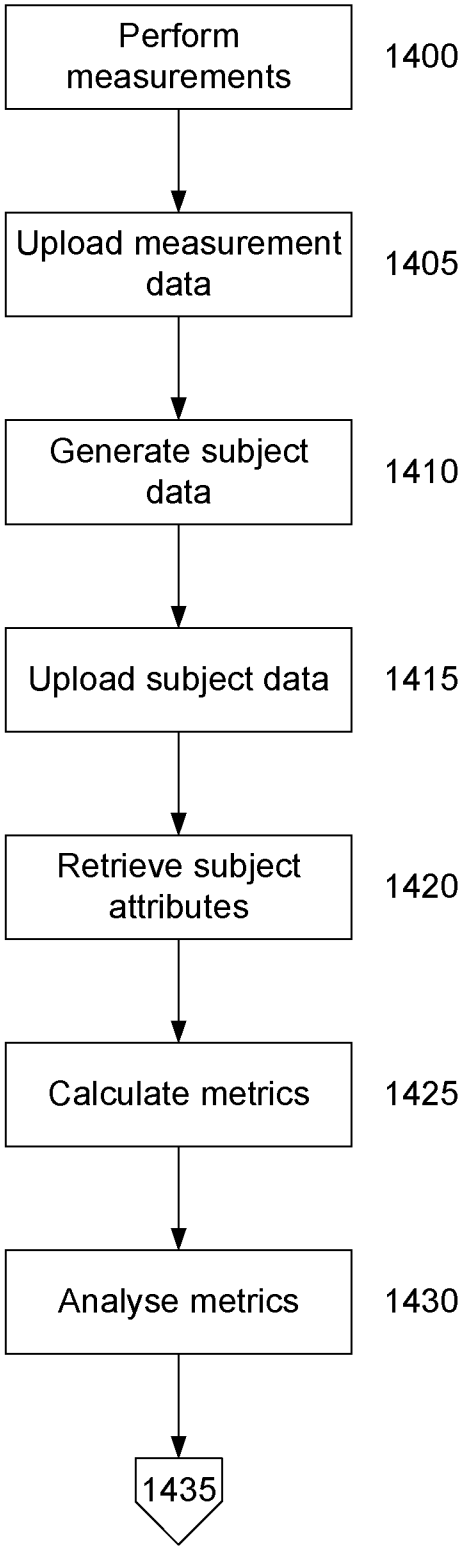
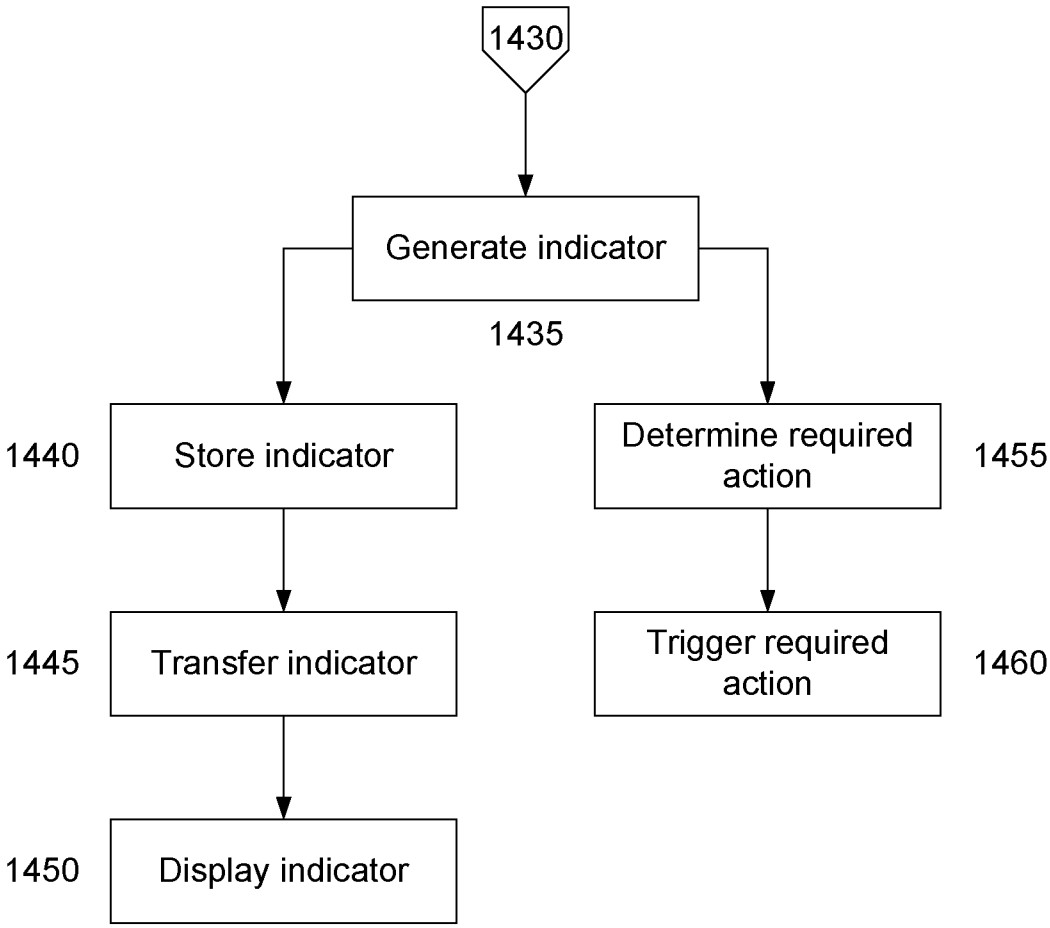

Perform measurements
1400
Upload measurement data
1405
Generate subject data
1410
Upload subject data
1415
Retrieve subject attributes
1420
Calculate metrics
1425
Analyse metrics
1430
1435

Fig. 14A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Generate indicator
1435
1440
Store indicator
1445
Transfer indicator
1450
Display indicator
Determine required action
1455
Trigger required action
1460

Fig. 14B

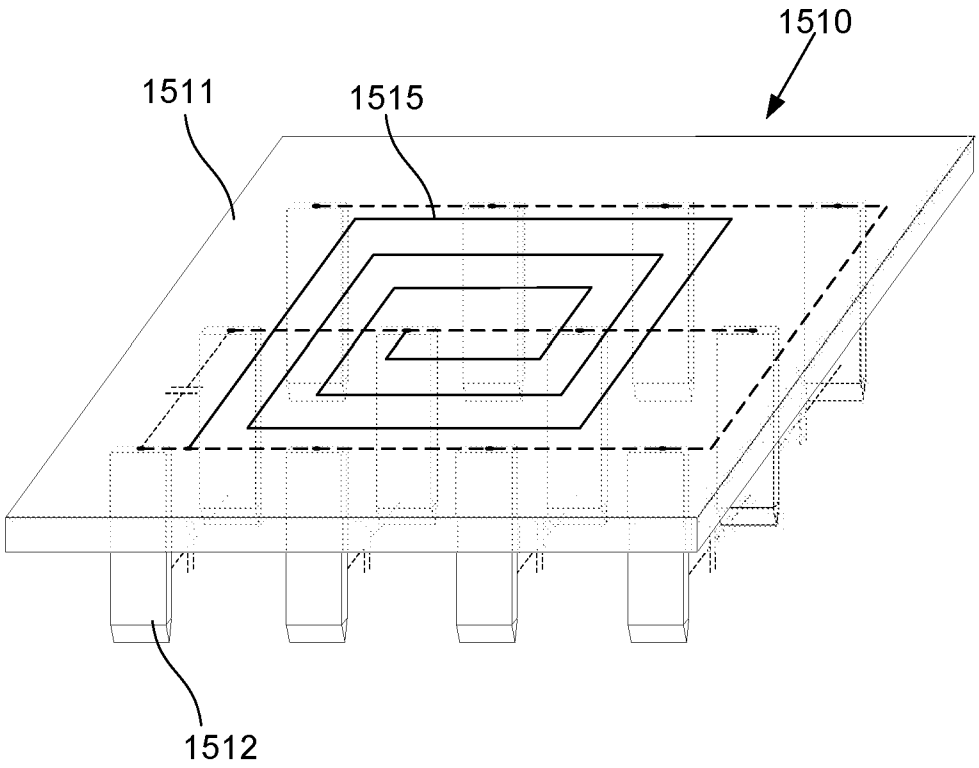

Fig. 15A

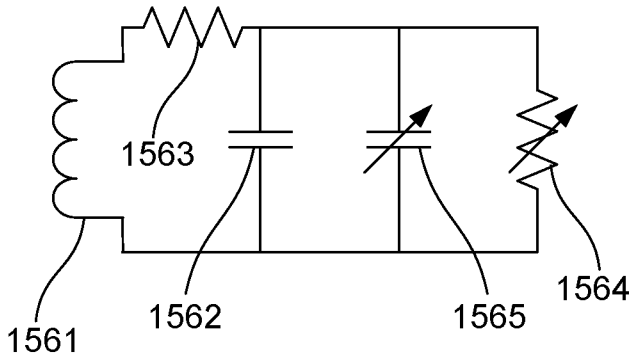

Fig. 15B

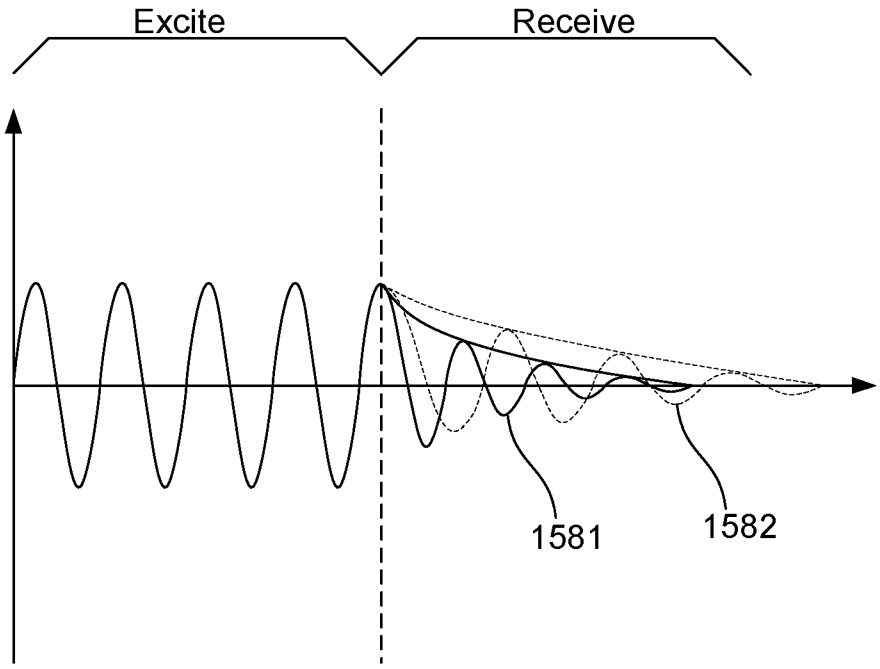

Fig. 15C

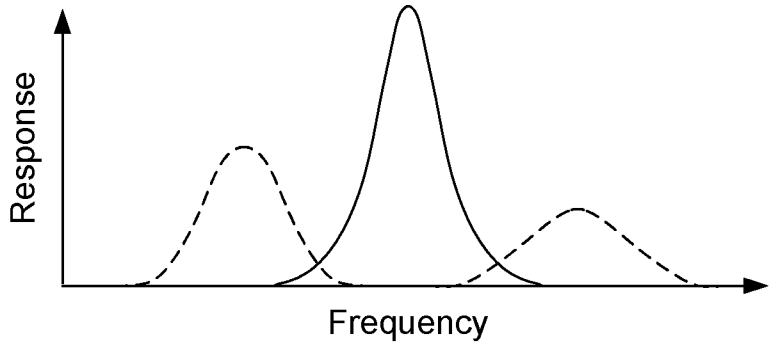

Fig. 15D

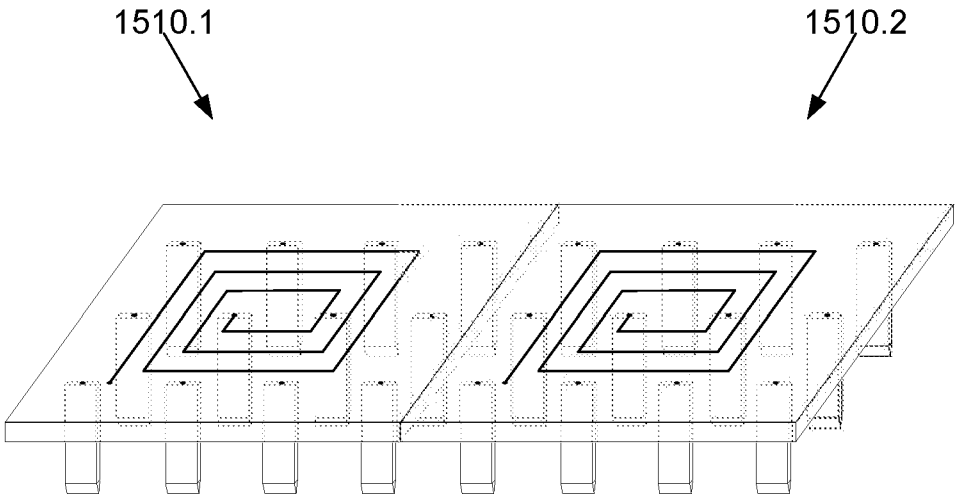

Fig. 15E

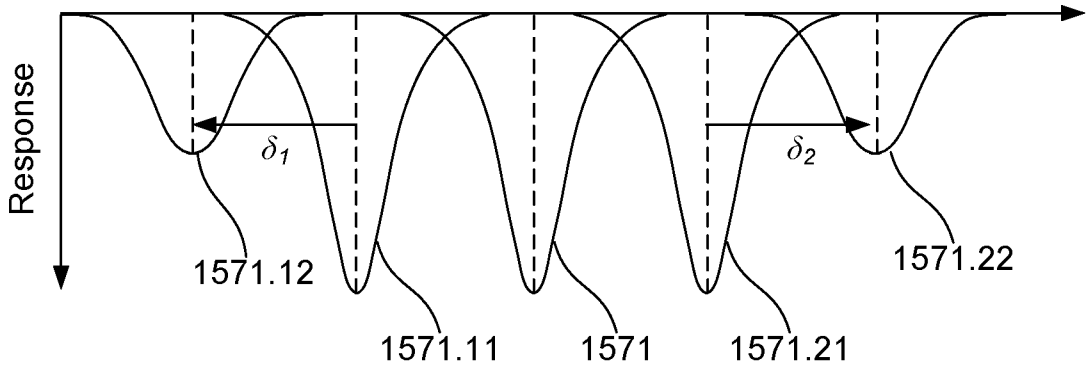

Fig. 15F

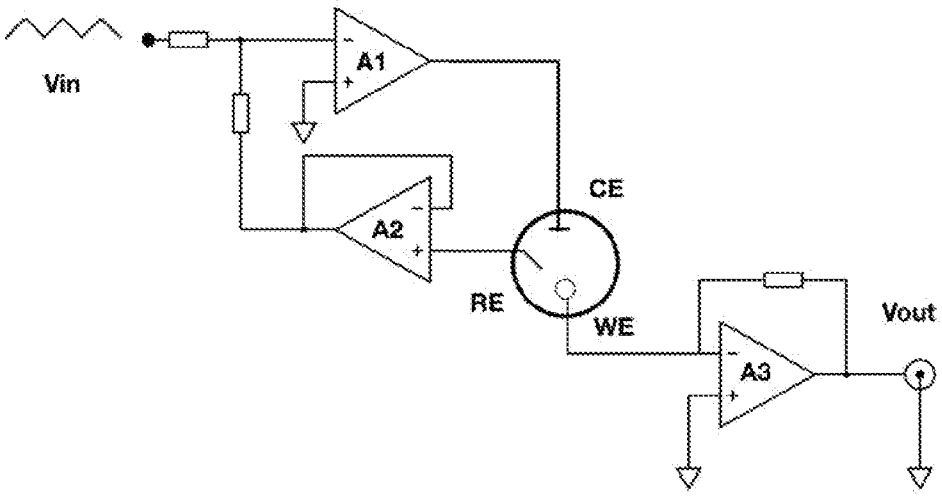

Fig. 15G

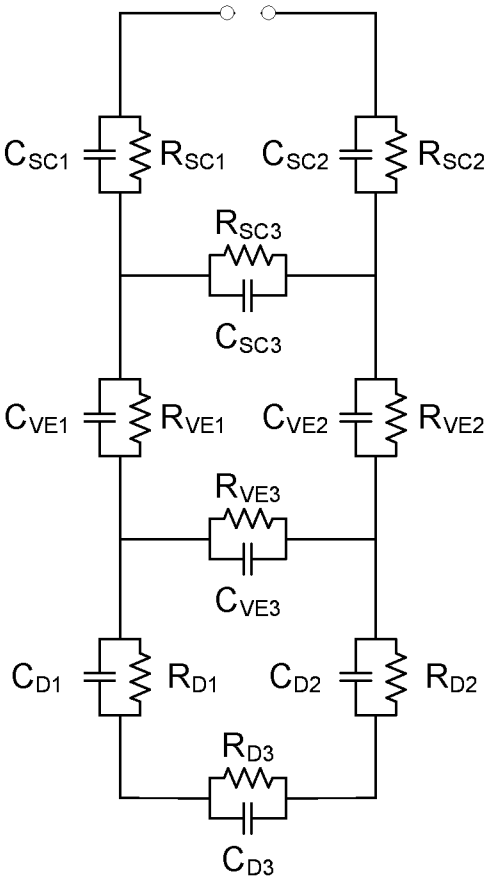

Fig. 16A

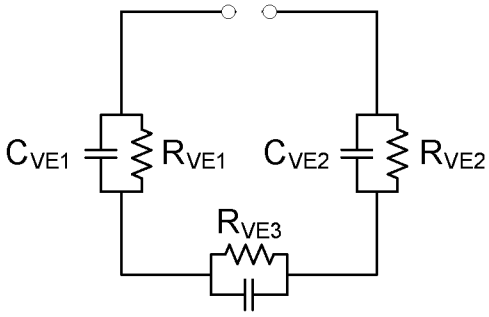

Fig. 16B

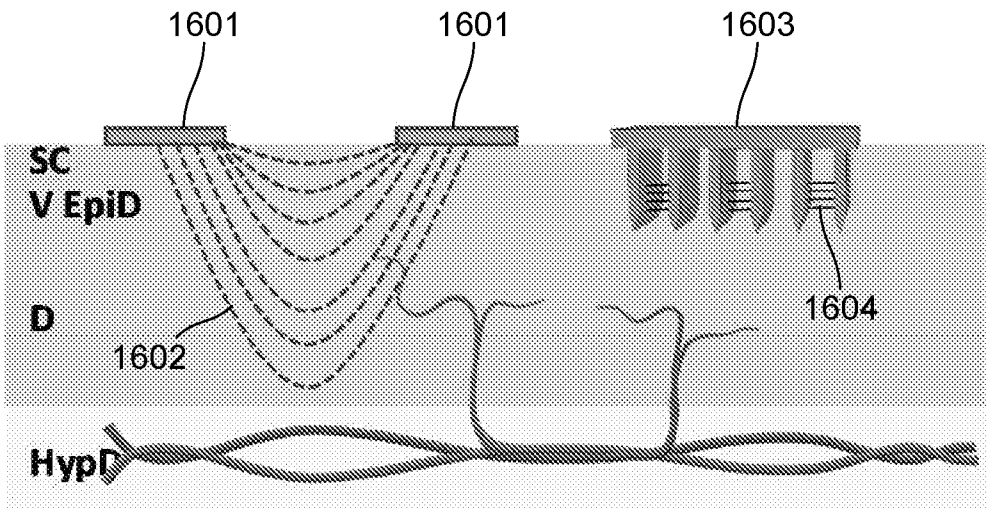

Fig. 16C

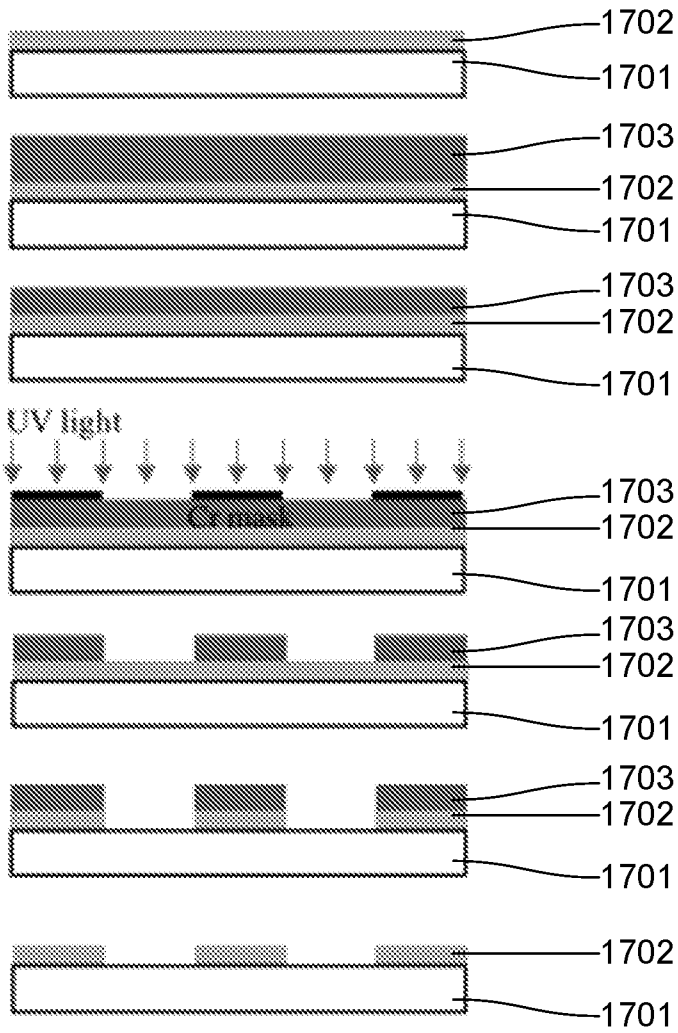

Fig. 17A

Fig. 17B

Fig. 17C

Fig. 17D

Fig. 17E

Fig. 17F

Fig. 17G

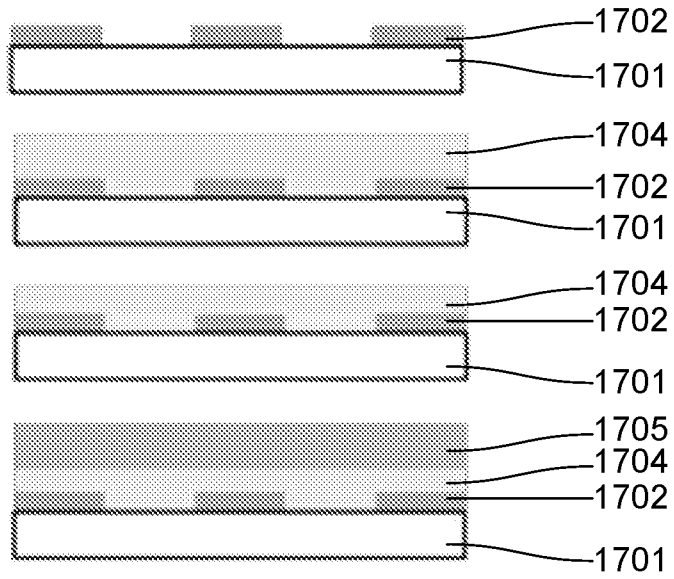

Fig. 17H

Fig. 17I

Fig. 17J

Fig. 17K

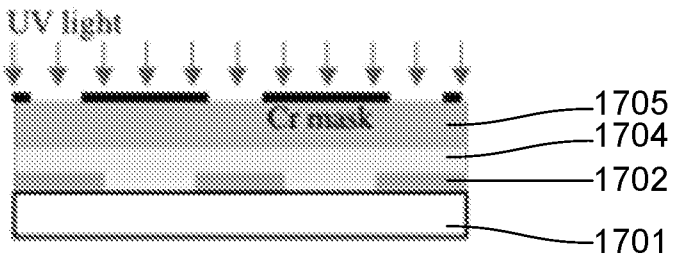

Fig. 17L

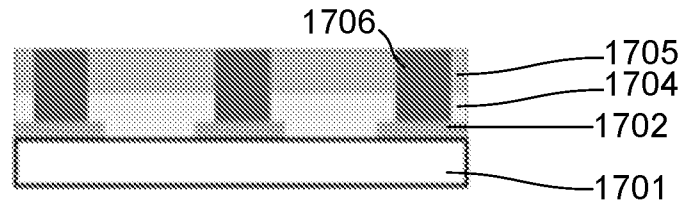

Fig. 17M

Fig. 17N

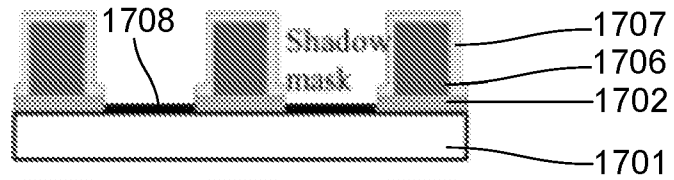

Fig. 17O

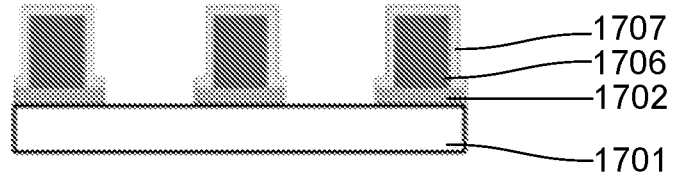

Fig. 17P

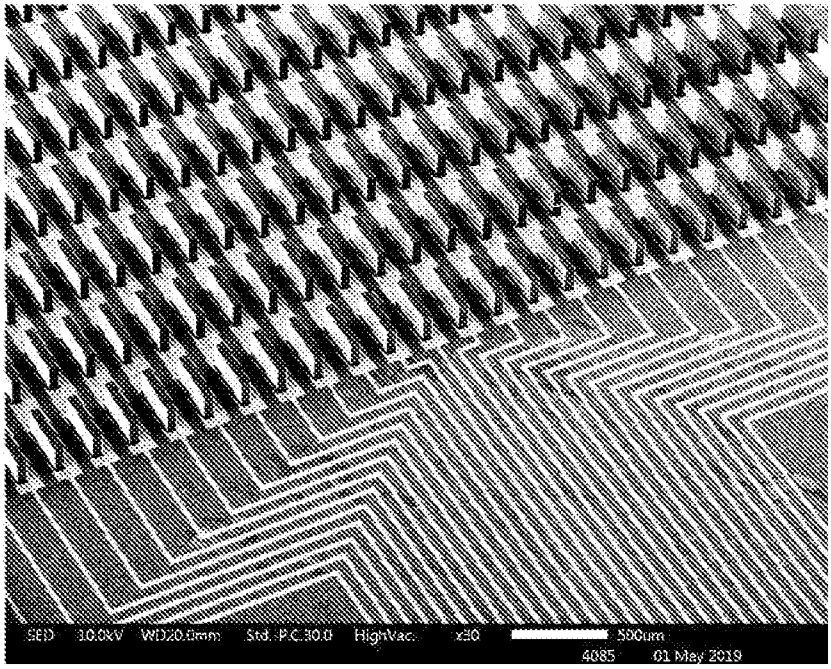

Fig. 18A

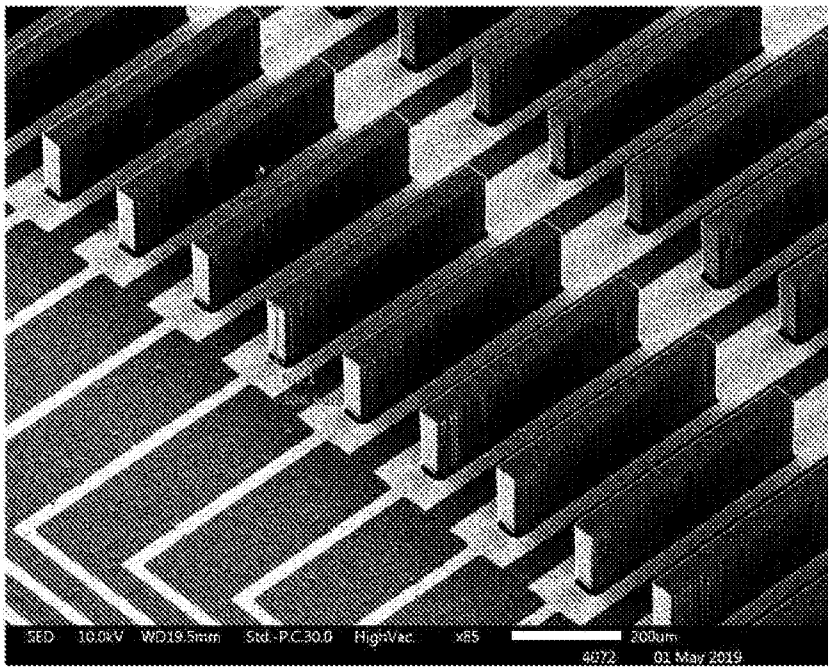

Fig. 18B

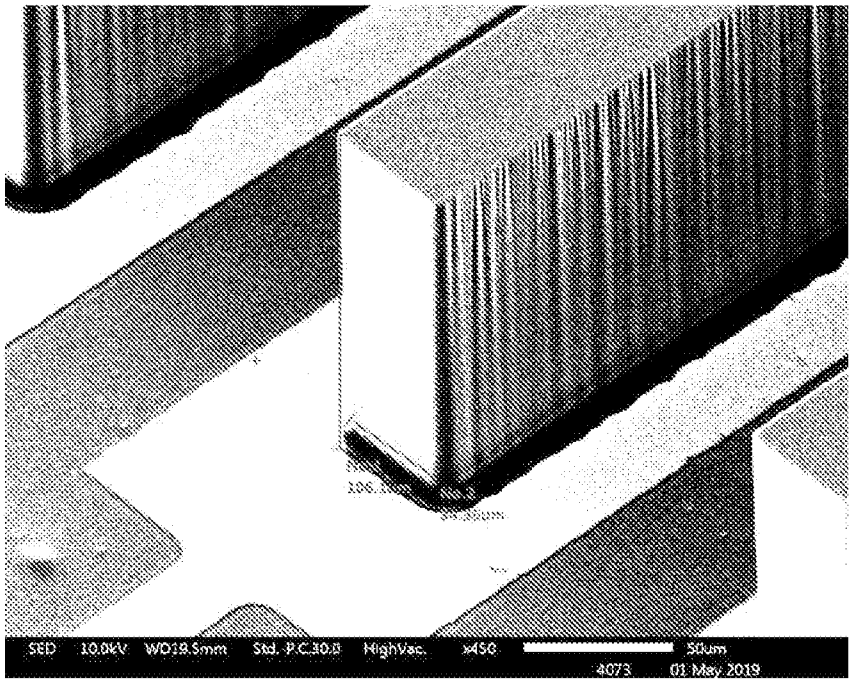

Fig. 18C

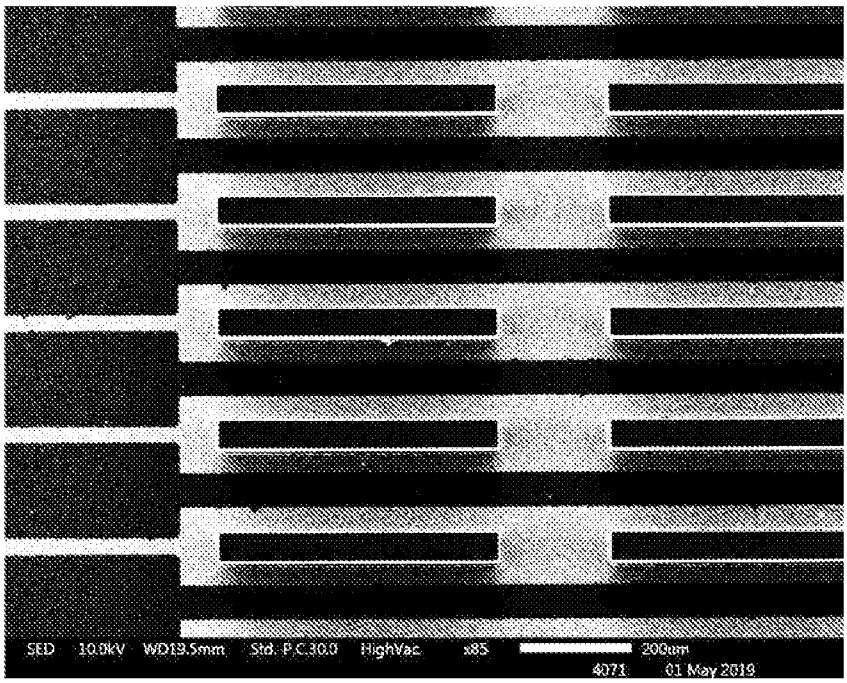

Fig. 18D

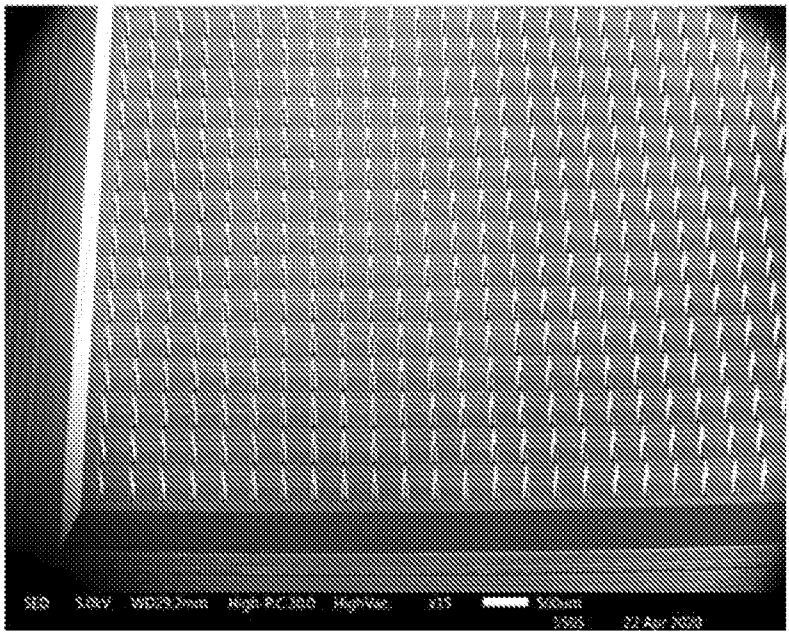

Fig. 18E

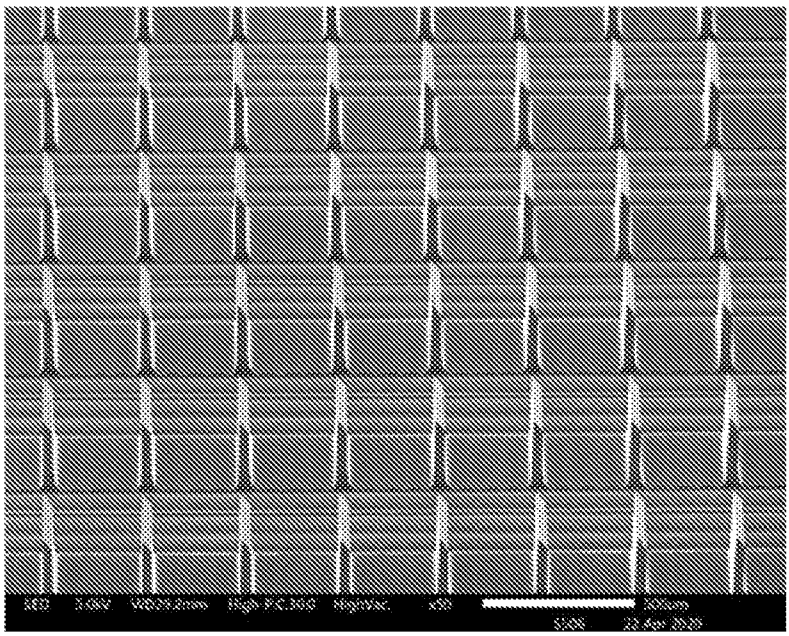

Fig. 18F

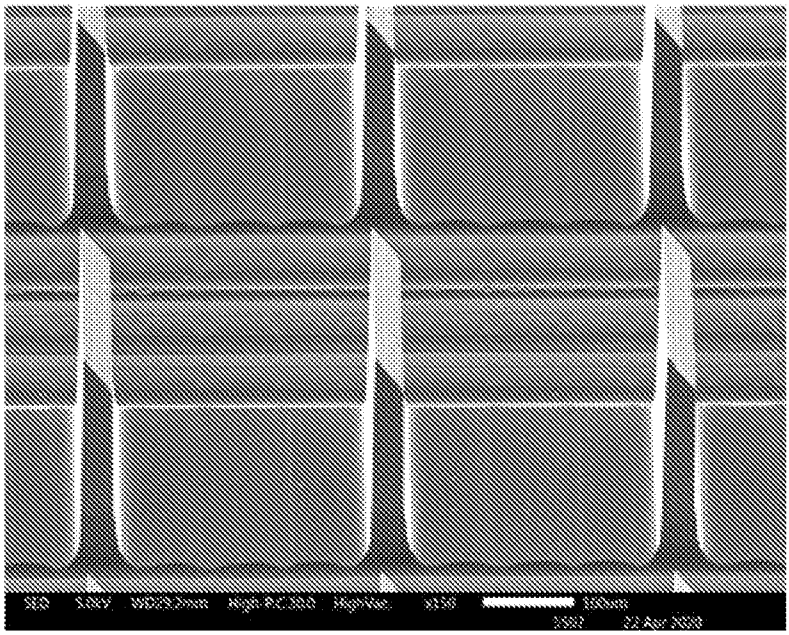

Fig. 18G

Fig. 19A

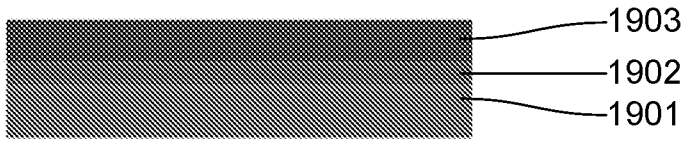

Fig. 19B

Fig. 19C

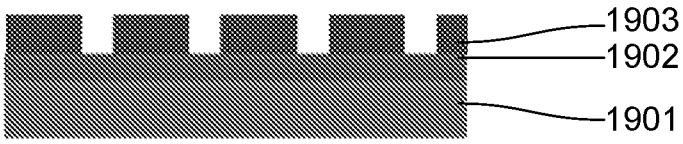

Fig. 19D

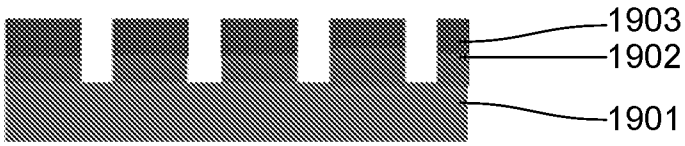

Fig. 19E

Fig. 19F

Fig. 19G

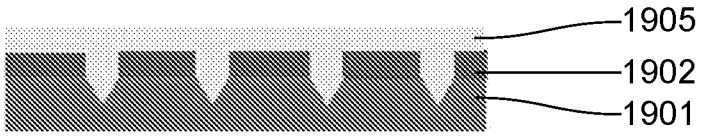

Fig. 19H

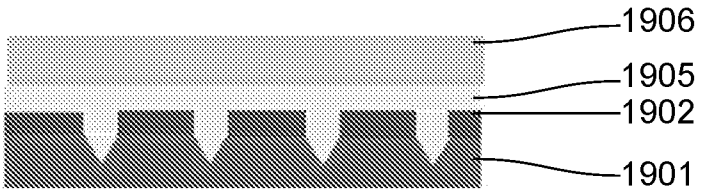

Fig. 19I

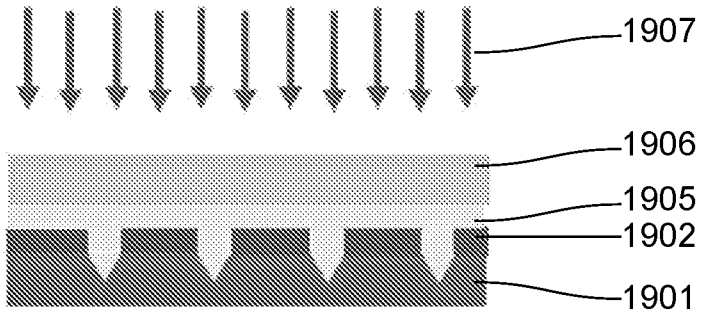

Fig. 19J

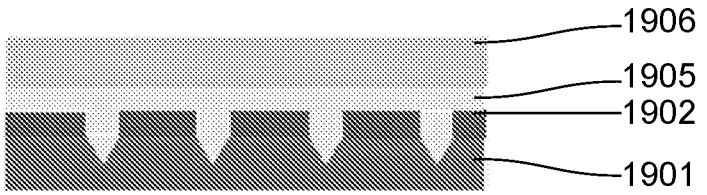

Fig. 19K

Fig. 19L

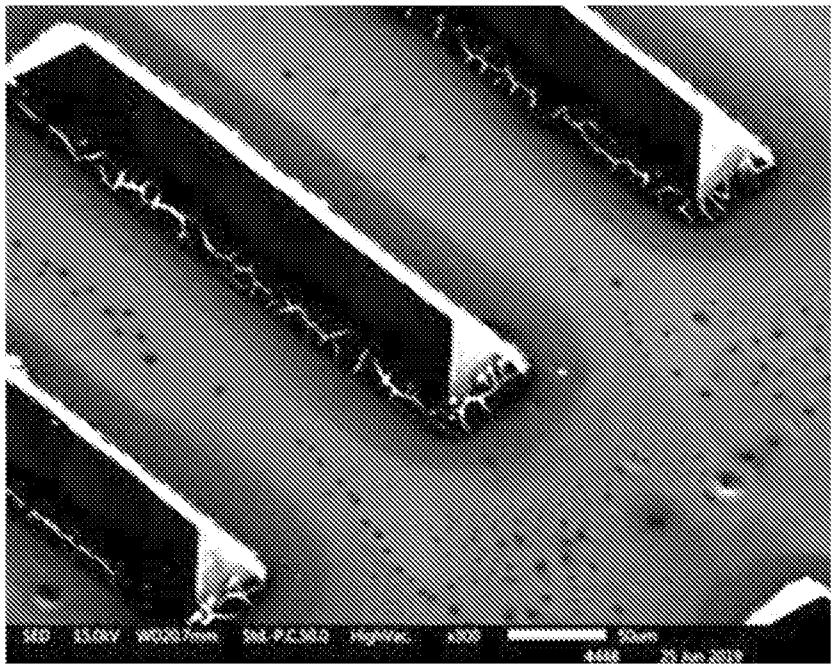

Fig. 20A

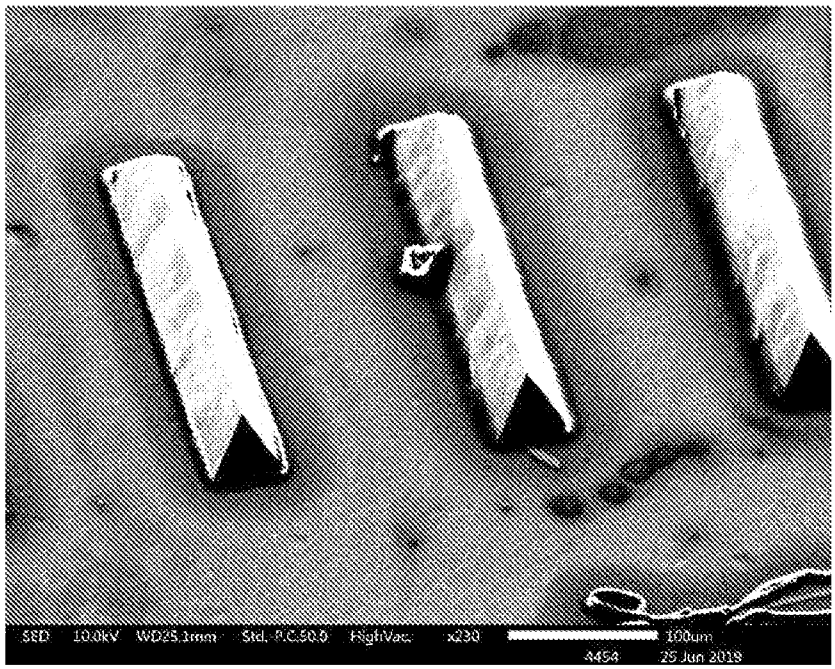

Fig. 20B

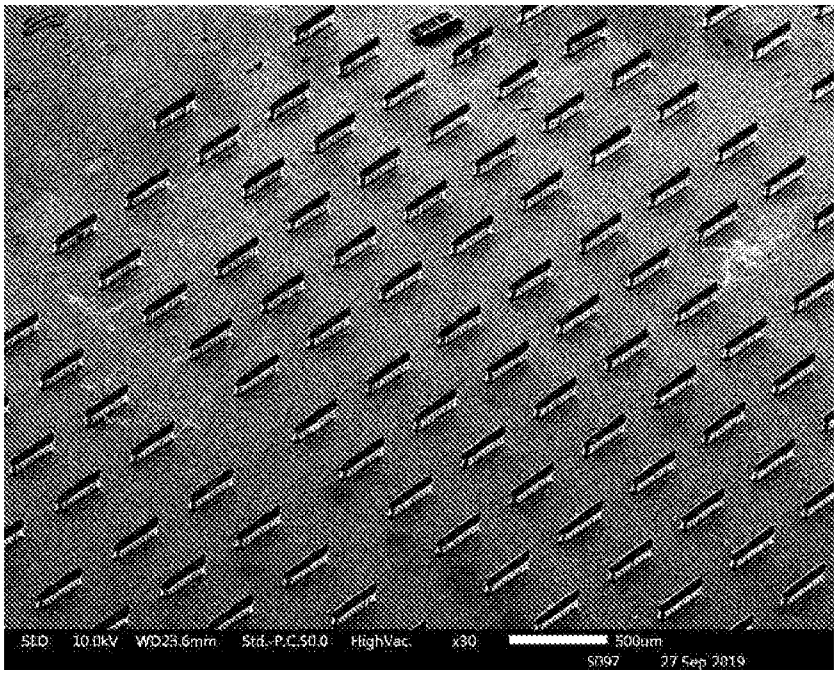

Fig. 20C

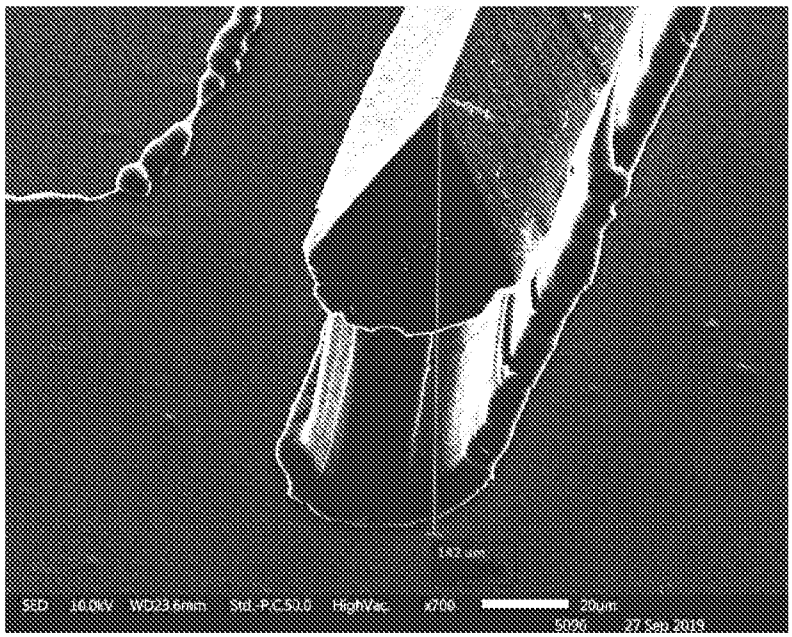

Fig. 20D

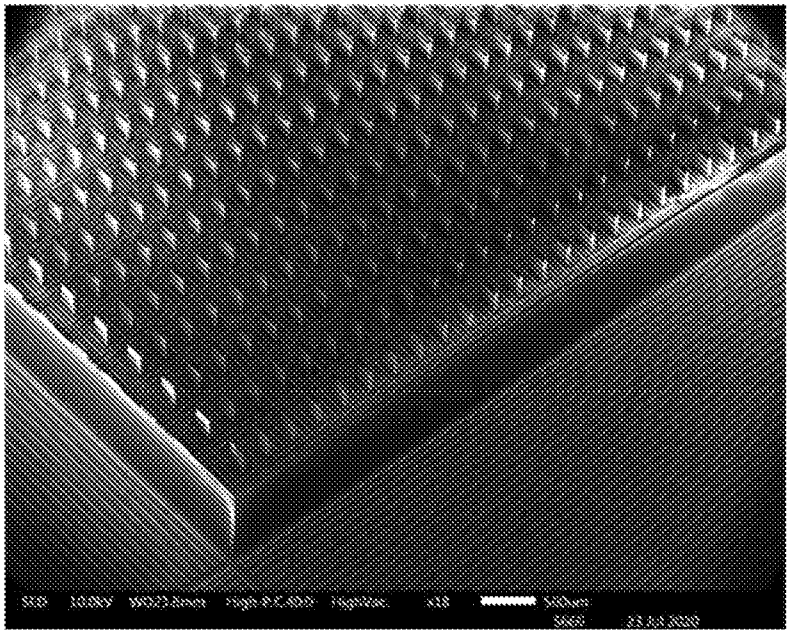

Fig. 20E

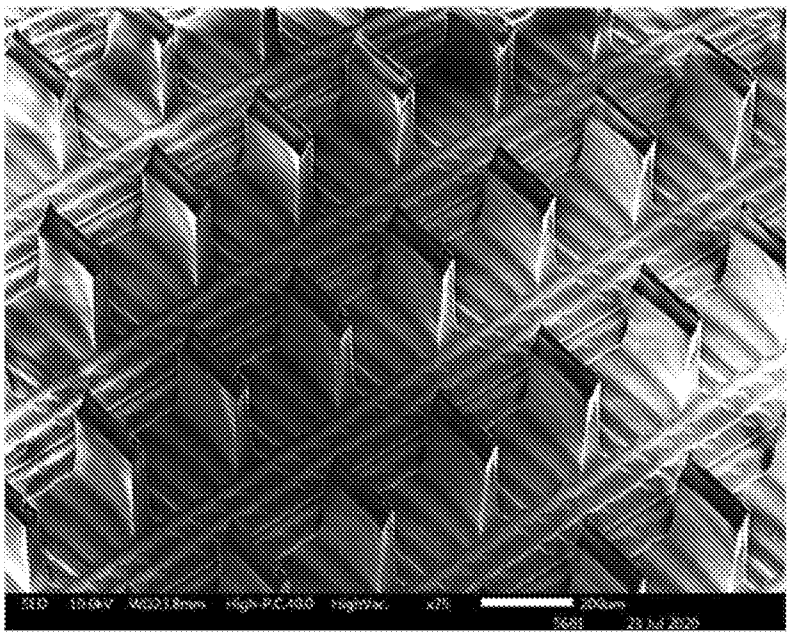

Fig. 20F

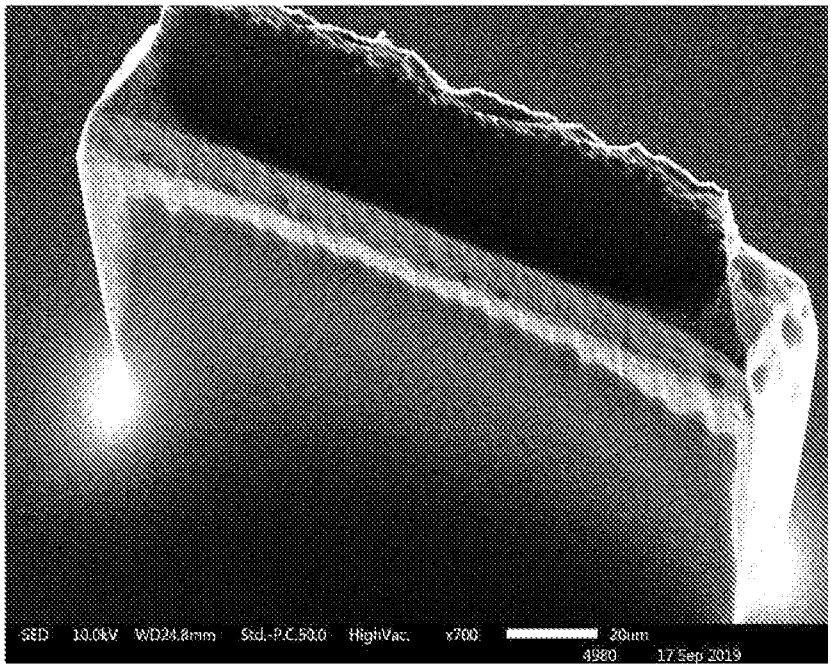

Fig. 21A

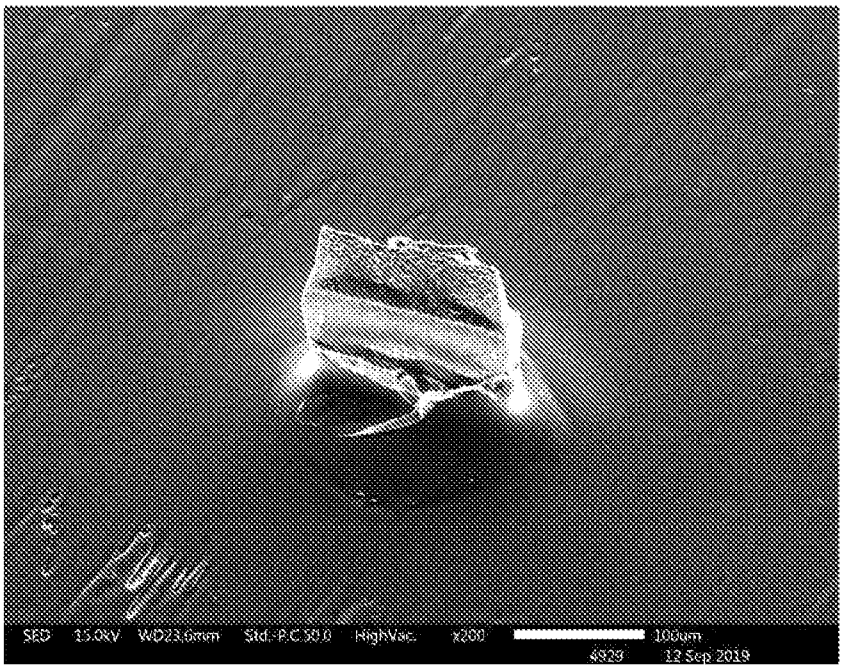

Fig. 21B

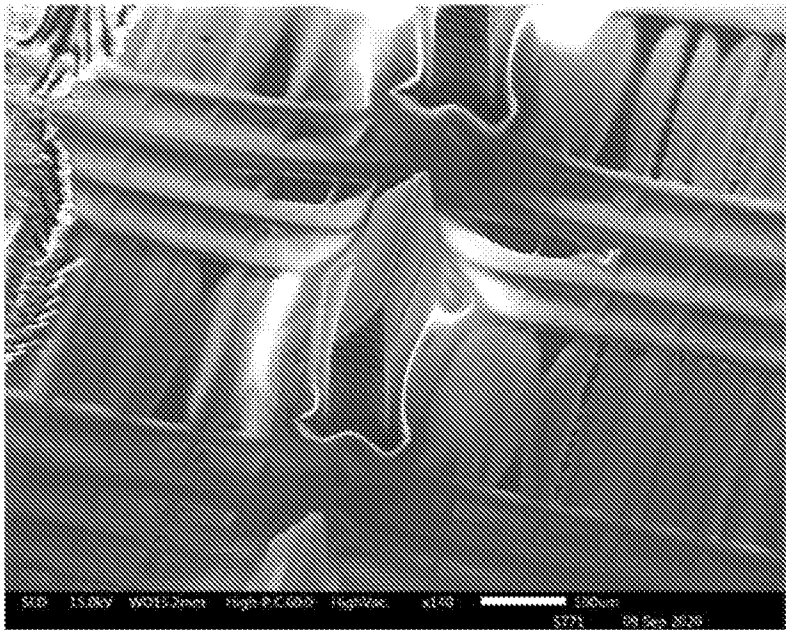

Fig. 21C

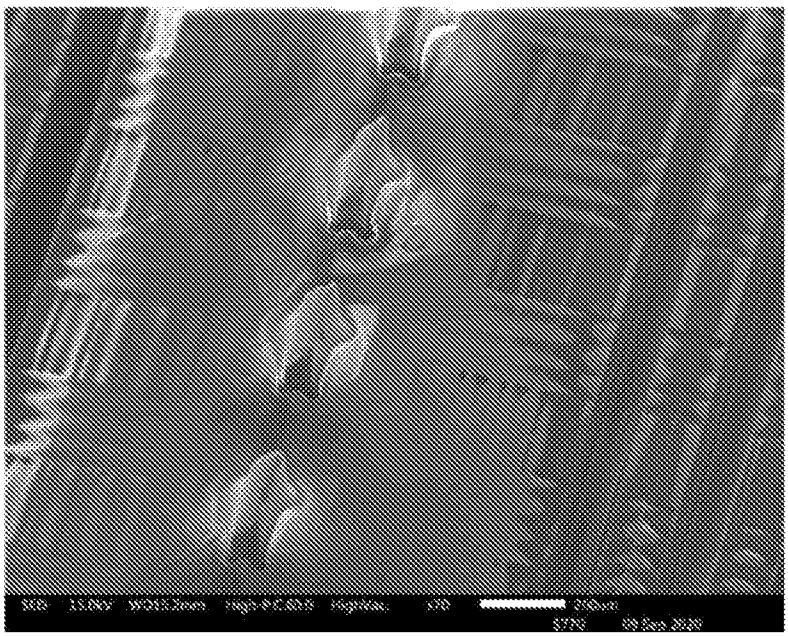

Fig. 21D

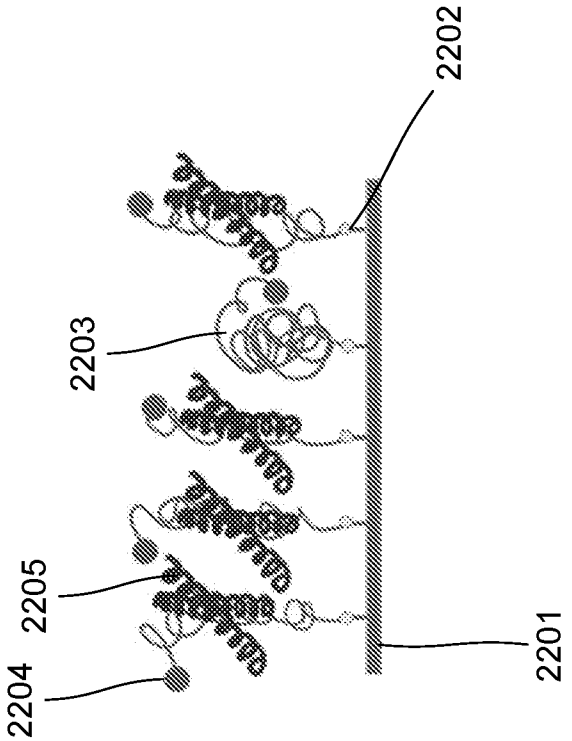

Fig. 22B

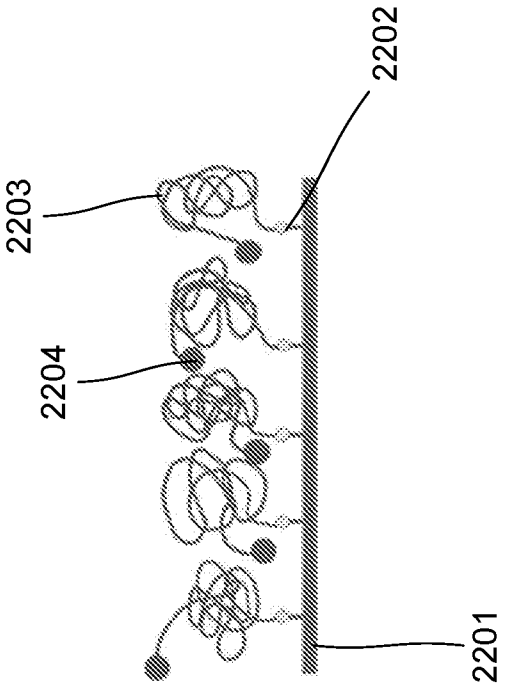

Fig. 22A

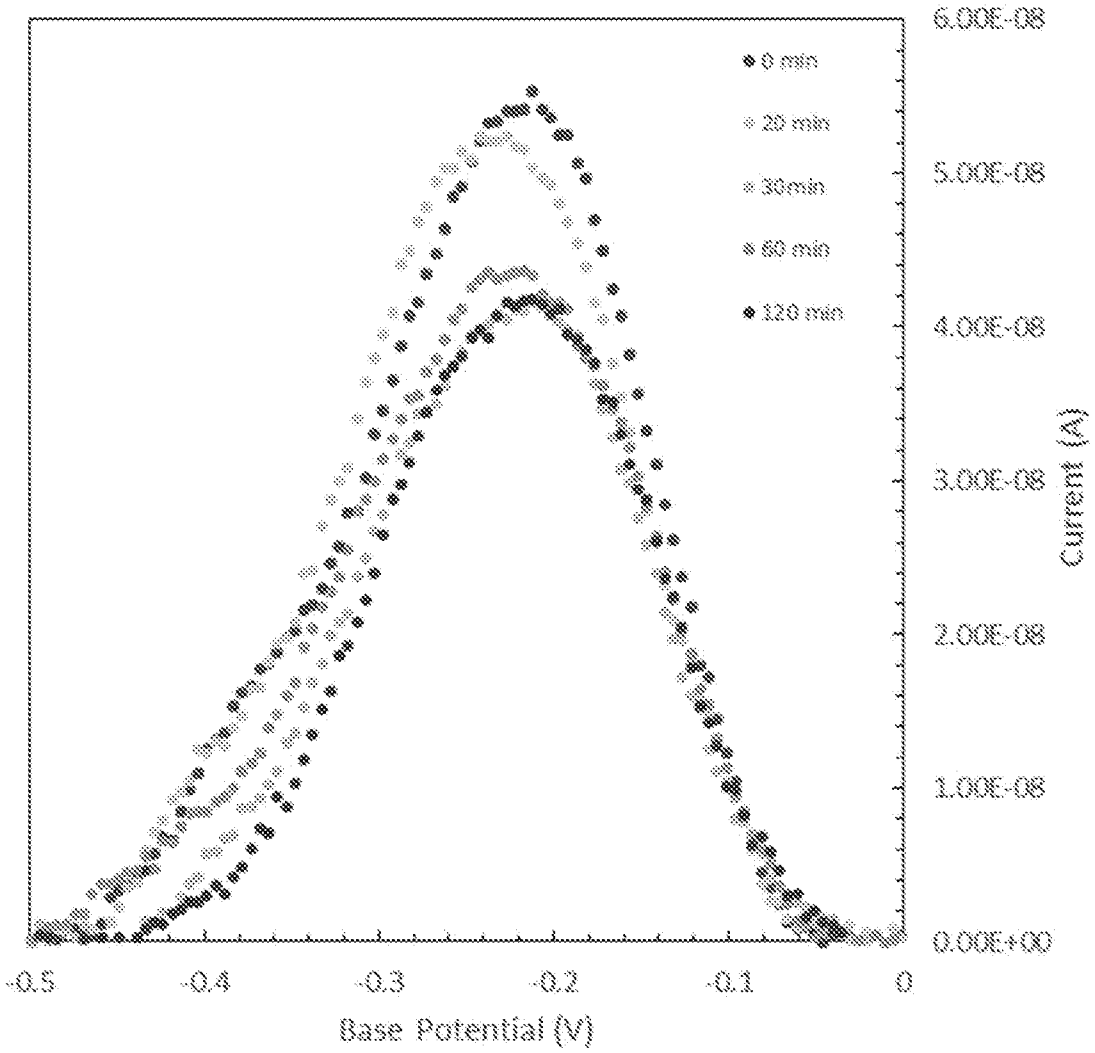
6.00E-08
5.00E-08
4.00E-08
3.00E-08
2.00E-08
1.00E-08
0.00E+00
Current (A)
0 min
20 min
30min
60 min
120 min
-0.5
-0.4
-0.3
-0.2
-0.1
0
Base Potential (V)

Fig. 23

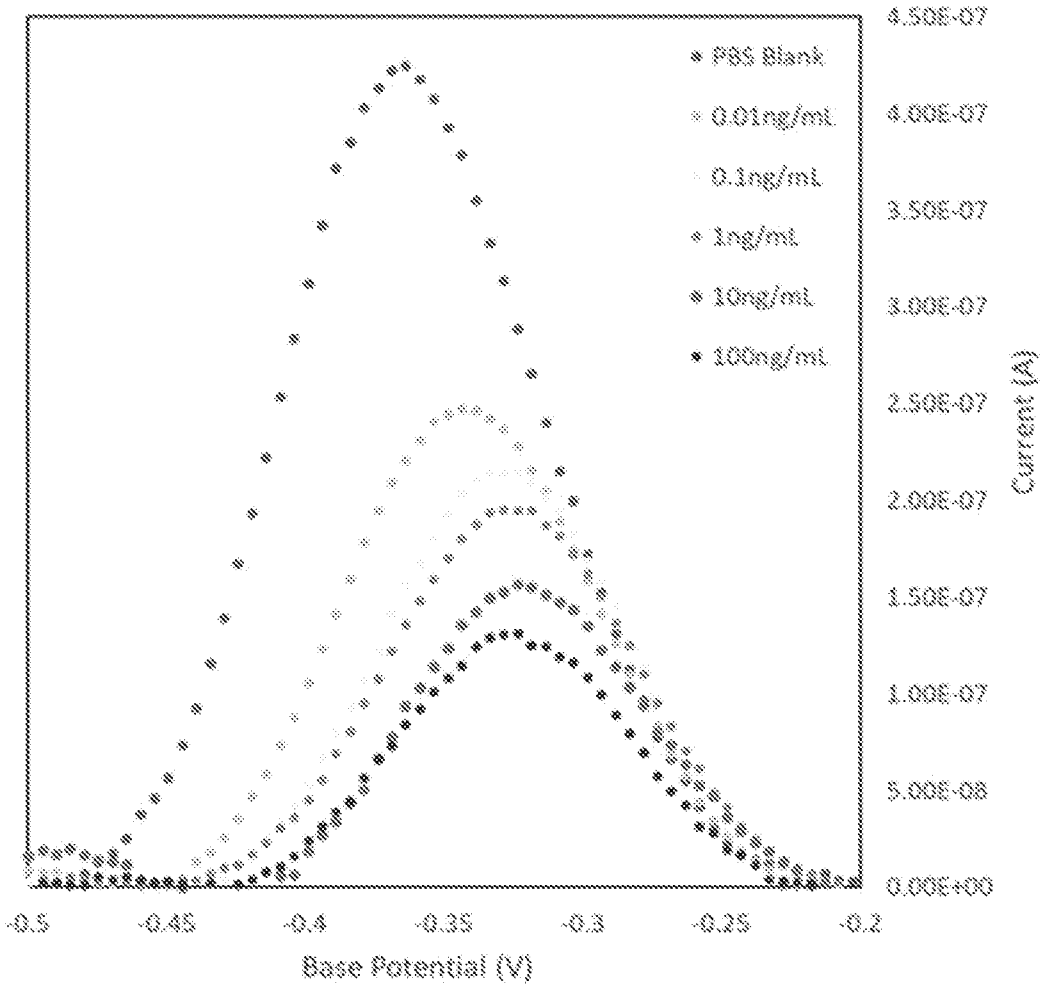
PBS Blank
0.01ng/mL
0.1ng/mL
1ng/mL
10ng/mL
100ng/mL
4.50E-07
4.00E-07
3.50E-07
3.00E-07
2.50E-07
2.00E-07
1.50E-07
1.00E-07
5.00E-08
0.00E+00
Current (A)
-0.5
-0.45
-0.4
-0.35
-0.3
-0.25
-0.2
Base Potential (V)

Fig. 24A

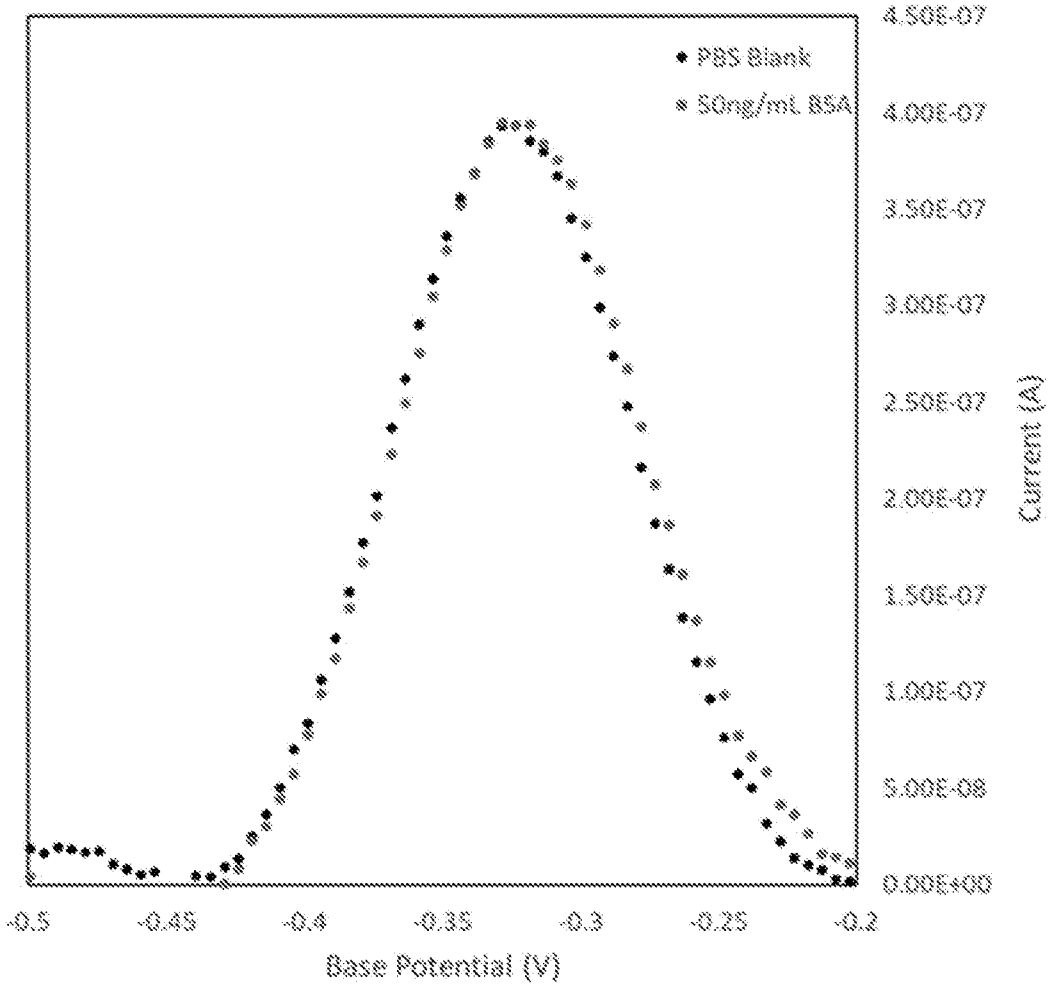
PBS Blank
50ng/mL BSA
4.50E-07
4.00E-07
3.50E-07
3.00E-07
2.50E-07
2.00E-07
1.50E-07
1.00E-07
5.00E-08
0.00E+00
Current (A)
-0.5
-0.45
-0.4
-0.35
-0.3
-0.25
-0.2
Base Potential (V)

Fig. 24B

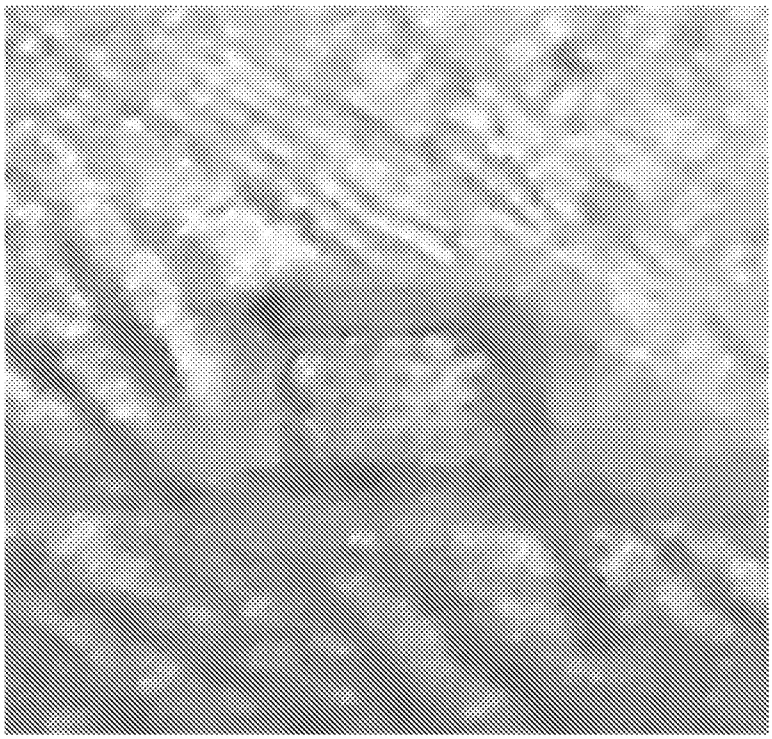

Fig. 25A

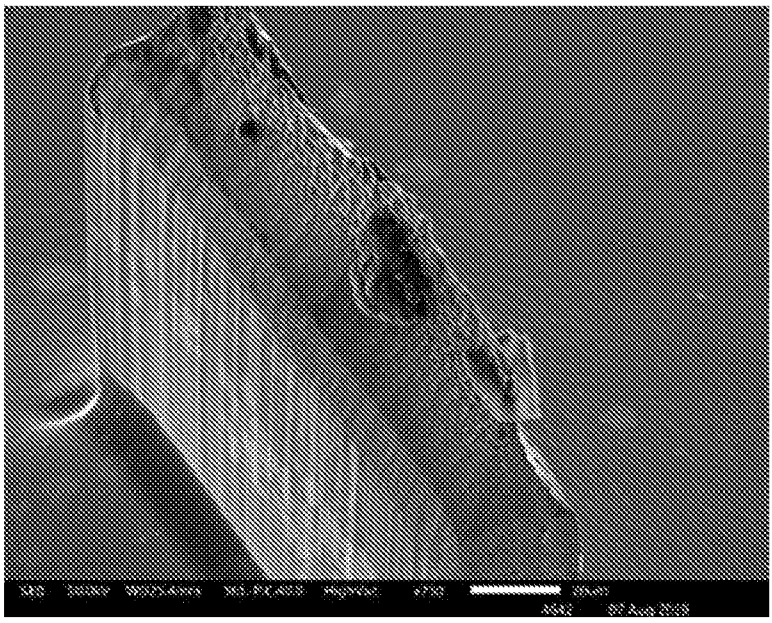

Fig. 25B

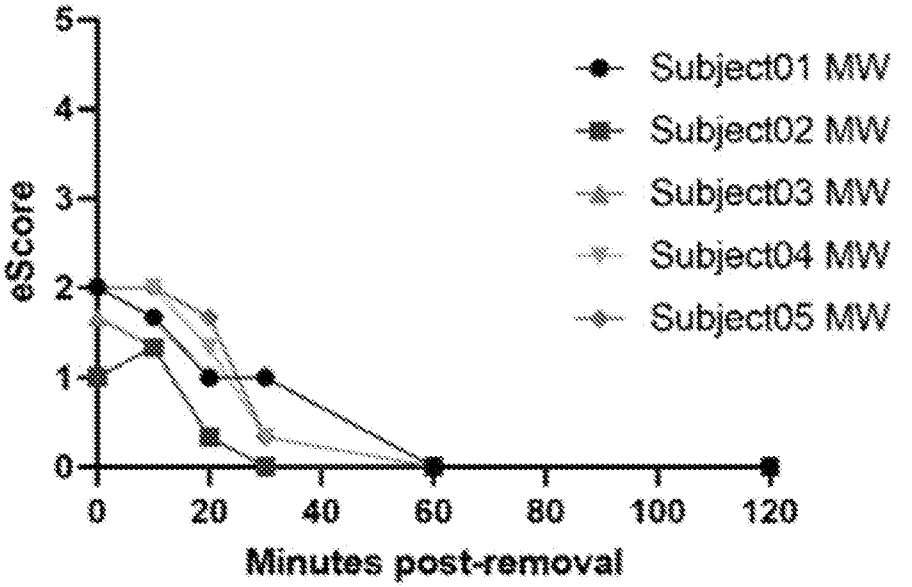

Fig. 26A

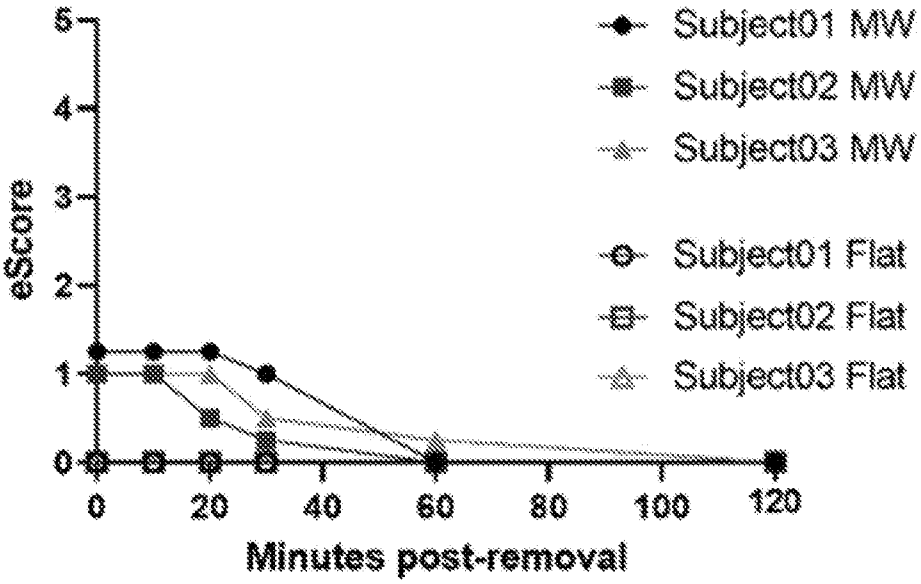

Fig. 26B

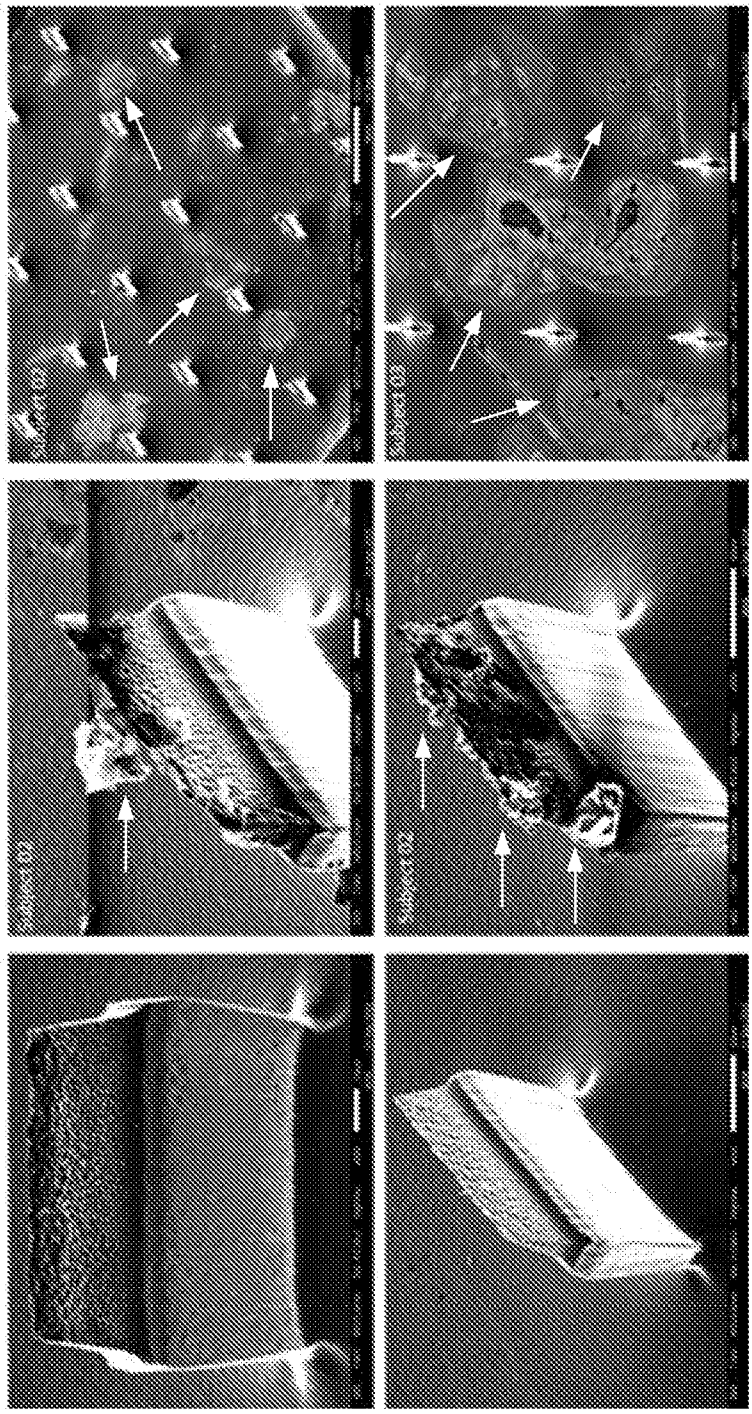

Fig. 27A Fig. 27B Fig. 27C

Fig. 27D Fig. 27E Fig. 27F

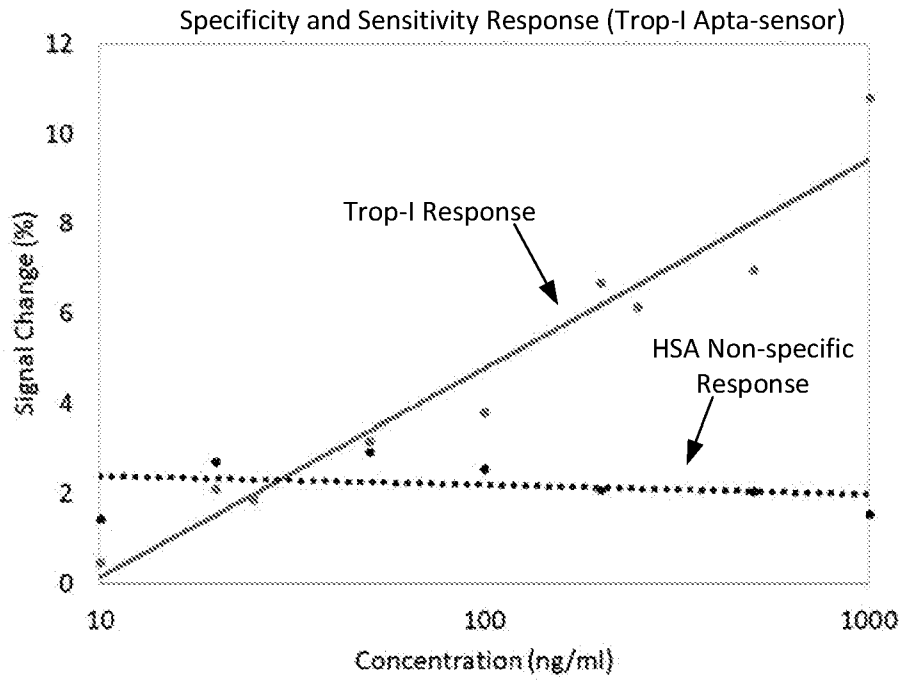

Fig. 28A

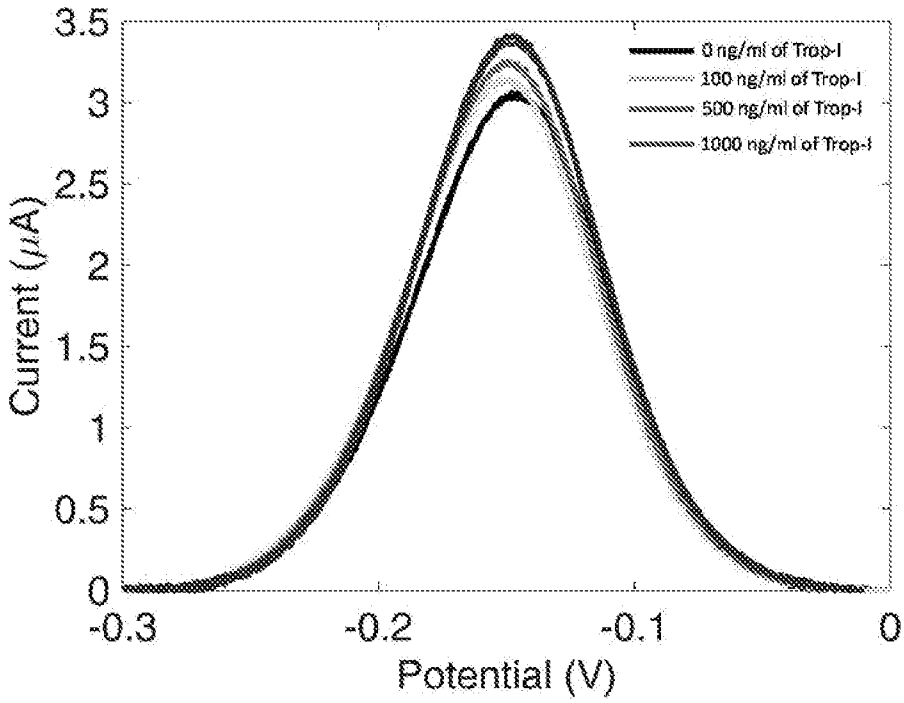

Fig. 28B

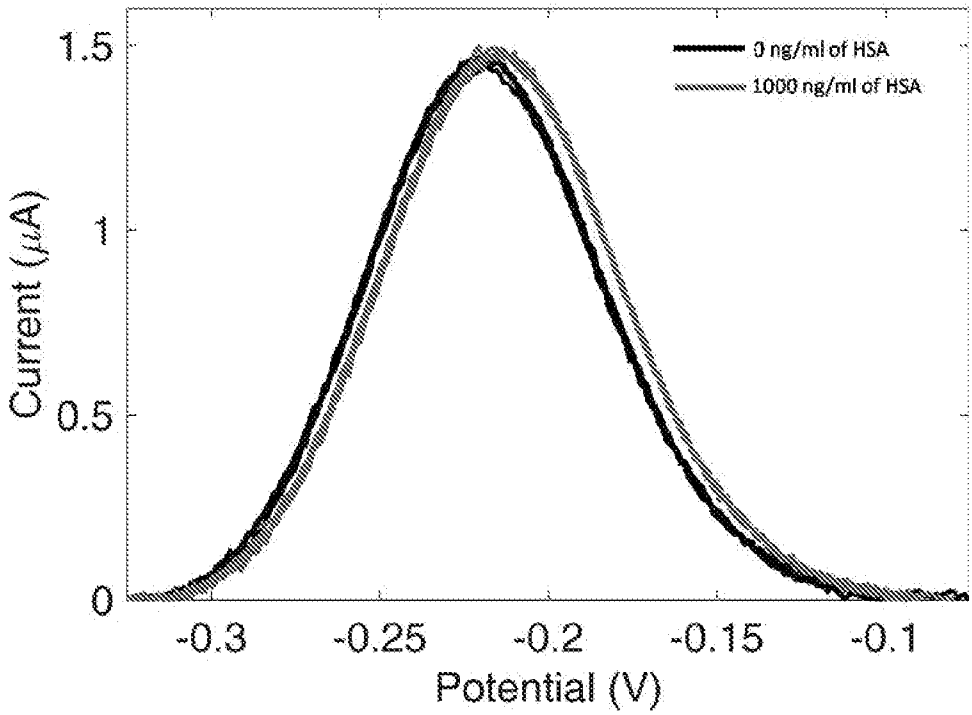

Fig. 28C

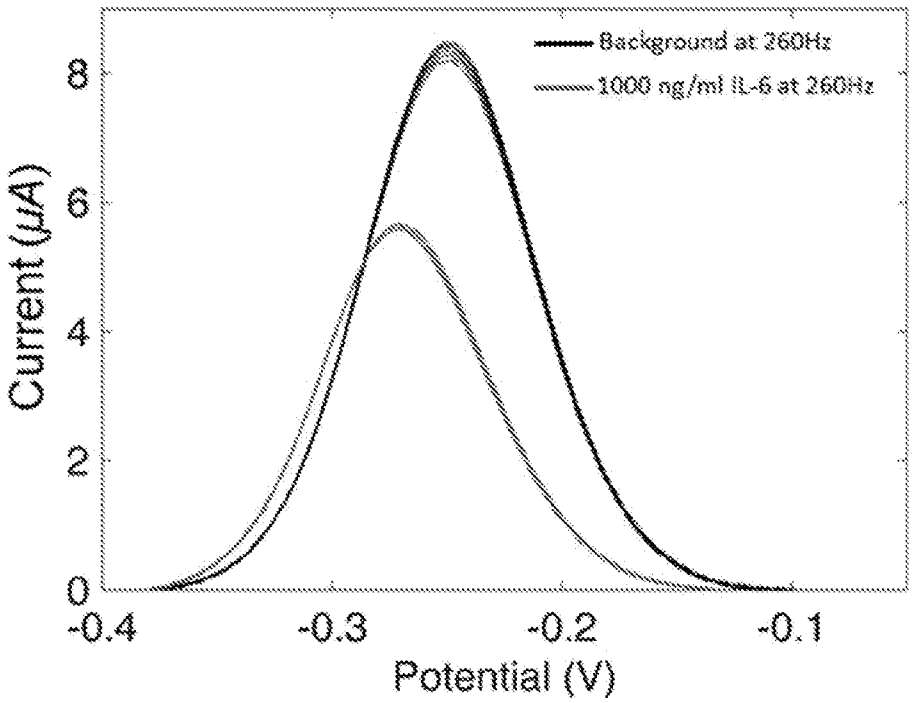

Fig. 29A

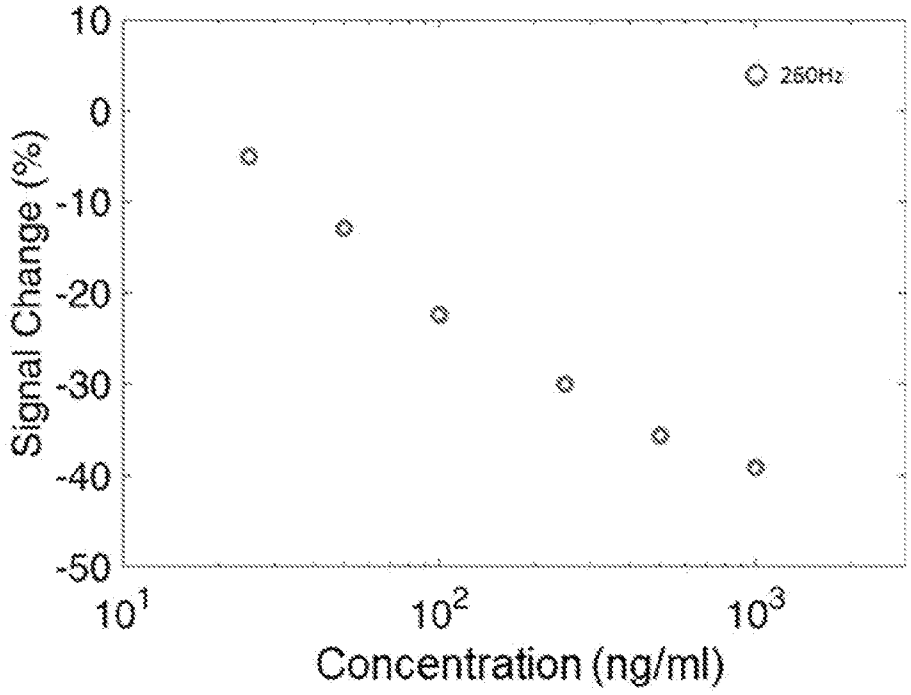

Fig. 29B

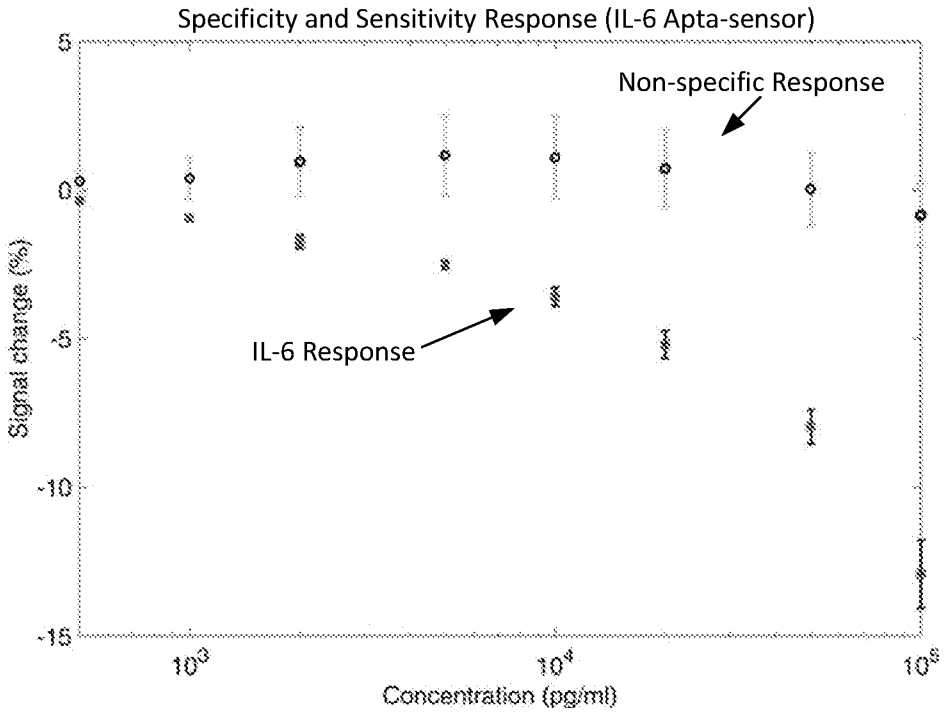

Fig. 30